

1. Question part 1/5 | 9700 Feb-March 2016 P2 V2 Q3

3 Erythropoietin, also known as EPO, is a large glycoprotein synthesised by specialised cells in the kidney. These cells are very sensitive to changes in oxygen concentration in the blood passing through the kidney and respond to a low oxygen concentration by increasing the synthesis of EPO.

EPO acts at the surface of particular target cells, such as cells in the bone marrow. These bone marrow cells are stimulated to produce red blood cells.

(a) (i) A low oxygen concentration also leads to an increase in the quantity of mRNA in the specialised cells in the kidney.

Suggest and explain why there is this increase in the quantity of mRNA.

.....

.....

.....

.....

.....

.....[2]

(ii) EPO is stored in secretory vesicles before being released from the specialised kidney cells.

Outline how EPO is released from the cells.

.....

.....

.....

.....

.....

.....[2]

1. Question part 2/5 | 9700 Feb-March 2016 P2 V2 Q3

- (b) All cells of the body are exposed to circulating blood plasma containing EPO, but only particular target cells respond.

- (i) Suggest and explain how EPO acts on target cells and why other cells are **not** affected.

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....[3]

- (ii) EPO cannot pass through the cell surface membrane to enter the bone marrow cells.

Suggest **one** reason why this is so.

.....

.....

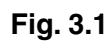
.....[1]

- (c) Red blood cells originate from undifferentiated cells in the bone marrow that are capable of continuous mitotic cell division.

State the name of this type of undifferentiated cell.

.....[1]

Fig. 3.1 shows the mean results for the subjects.



[4]

- (e) The concentration of EPO in the blood plasma of a person will increase when travelling from sea level to a high altitude. This is in response to the decrease in oxygen partial pressure in the atmosphere.

Explain why an increase in EPO blood plasma concentration will be of benefit if the person remains at high altitude.

.....

.....

.....

.....

.....

.....

.....

.....[3]

[Total: 16]

9700 Biology Paper 2 - Enzymes

Topical questions | 39 questions

1. Question part 5/5 | 9700 Feb-March 2016 P2 V2 Q3

BLANK PAGE

9700 Biology Paper 2 - Enzymes

Topical questions | 39 questions

2. Question | 9700 May-June 2016 P2 V2 Q1

- 1** Statements **A** to **E** are about the structure and functioning of enzymes.

State the correct term to match each of the statements **A** to **E**.

- A** The energy level, lowered by enzyme action, that needs to be overcome by reactants in order for products to be formed.

.....

- B** The mechanism of enzyme action that relies on the active site being partially flexible and changing shape in order to bind the substrate.

.....

- C** The term to describe a protein, such as an enzyme, with a tertiary or quaternary structure that results in an approximately spherical shape.

.....

- D** The term for enzymes that function outside cells.

.....

- E** The concentration of substrate that enables an enzyme to achieve half the maximum rate of reaction.

.....

[5]

[Total: 5]

3. Question part 1/2 | 9700 May-June 2016 P2 V3 Q2

- 2 Trypsin is a protease enzyme found in the digestive system.

Fig. 2.1 shows how the substrate concentration affects the rate of reaction of trypsin.

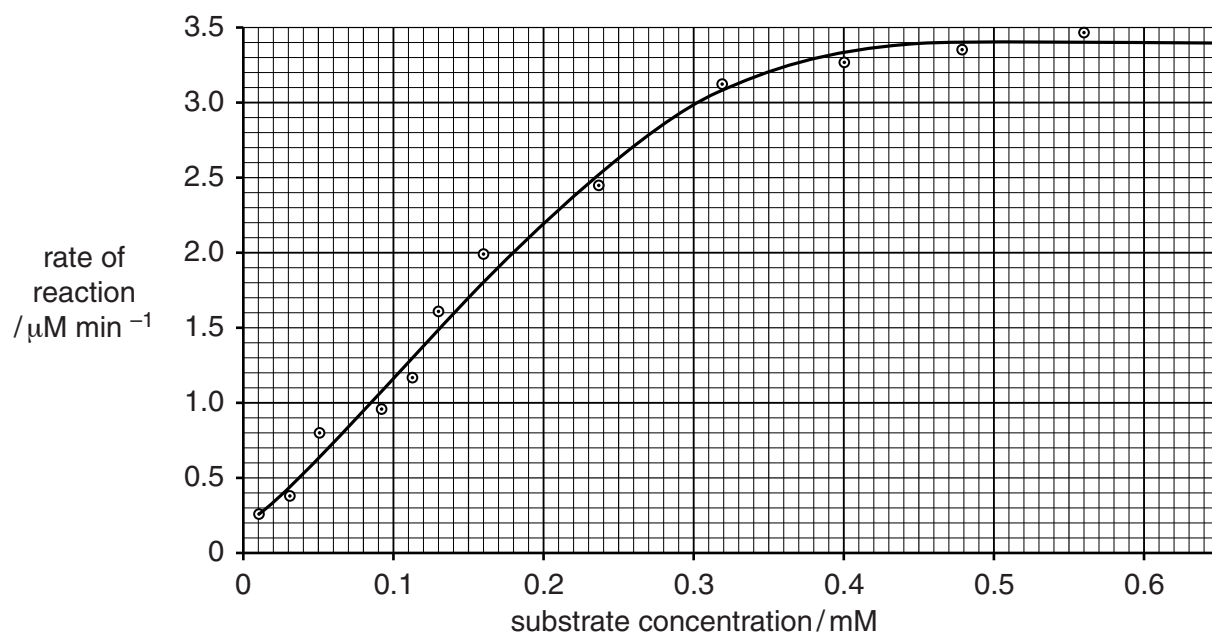


Fig. 2.1

(a) Use Fig. 2.1 to:

- (i) determine V_{max} for trypsin

.....[1]

- (ii) calculate K_m for trypsin.

Show your working.

.....[2]

(b) Describe **and** explain the shape of the curve in Fig. 2.1.

.....

.....

.....

.....

.....

.....

.....

.....

.....[4]

(c) Trypsin is composed of one polypeptide chain of 223 amino acids.

The active site of trypsin contains three amino acids which catalyse a hydrolysis reaction. These three amino acids occupy the following positions in the primary structure of trypsin:

- histidine, position 57
- aspartate, position 102
- serine, position 195.

(i) In the functioning enzyme, these three amino acids are close together in the active site.

Explain how the structure of the protein makes this possible.

.....

.....

.....

.....

.....

.....

.....

.....

.....[3]

(ii) When trypsin acts on a substrate, another substance is required as a reactant.

Name this other substance.

.....[1]

3 Fig. 3.1 shows the structure of the enzyme lysozyme.

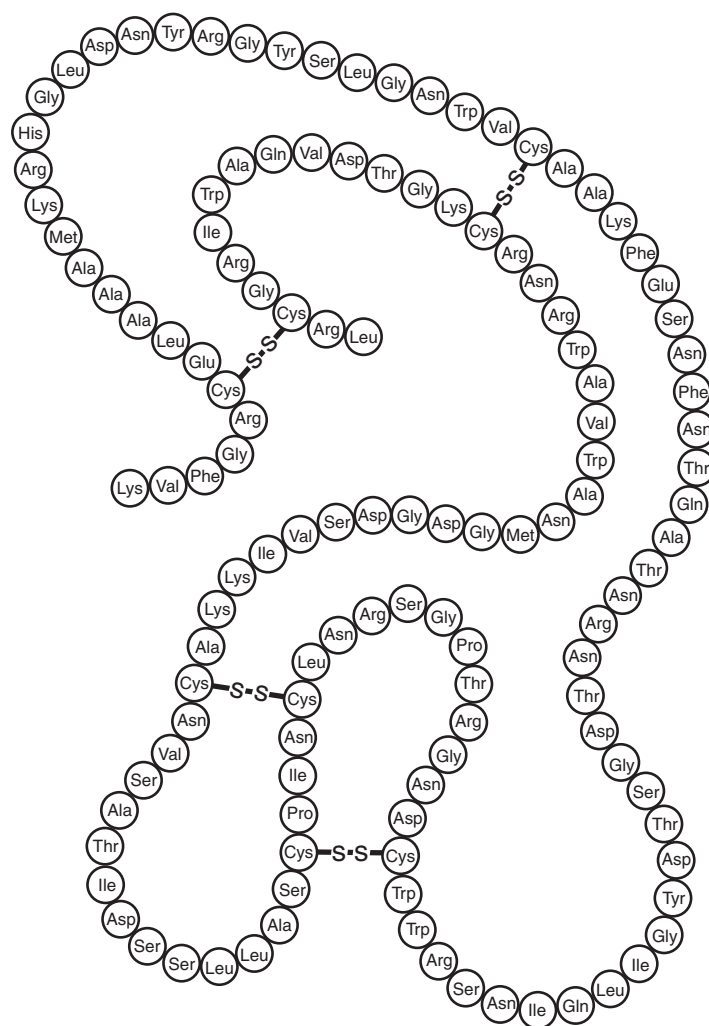


Fig. 3.1

(a) (i) Name the **two** types of covalent bond in the structure in Fig. 3.1.

.....[1]

(ii) The primary structure of lysozyme is shown in Fig. 3.1.

Explain the meaning of the term *primary structure*.

.....

[1]

(b) Lysozyme hydrolyses the β -1,4 glycosidic bonds present in compounds found in bacterial cell walls.

(i) State what is meant by the term *hydrolysis*.

.....
.....[1]

(ii) Suggest the type of biological molecule which is the substrate for lysozyme.

.....[1]

(iii) Lysozyme uses the induced fit mechanism.

Explain the mode of action of an enzyme that uses the induced fit mechanism.

.....
.....
.....
.....
.....
.....
.....
.....[4]

(c) In human tears and saliva, lysozyme acts as an extracellular enzyme.

State what is meant by the term *extracellular*.

.....
.....[1]

- (d) Fig. 3.2 shows the results of an investigation into the effect of substrate concentration on the rate of reaction catalysed by lysozyme.

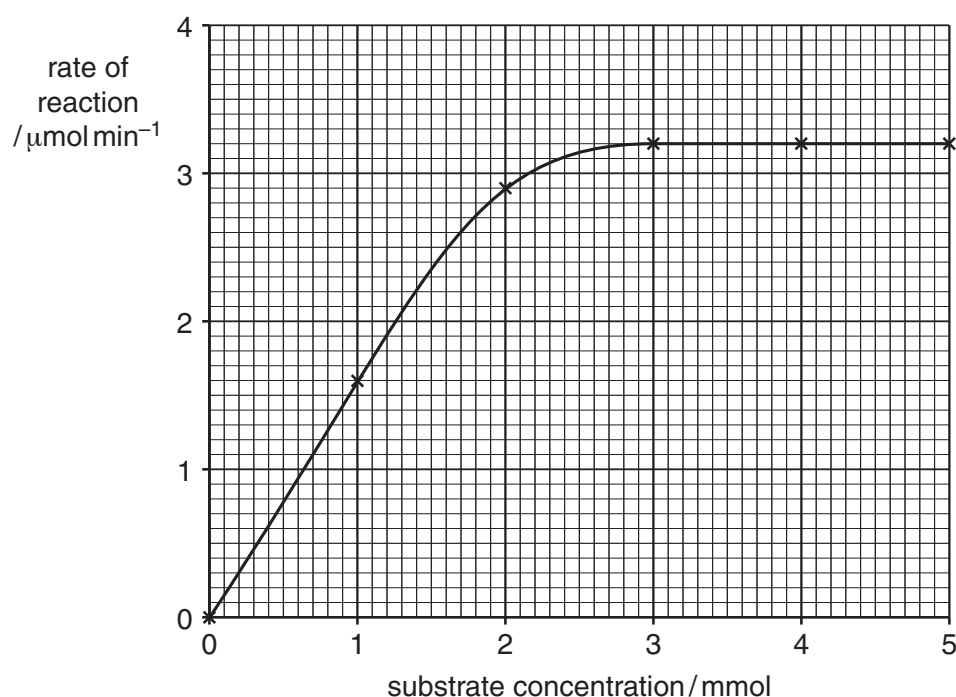


Fig. 3.2

Use Fig. 3.2 to:

- (i) state the lowest substrate concentration to give the maximum rate of reaction, V_{max}

.....[1]

- (ii) determine the Michaelis-Menten constant, K_m .

$K_m =$ [1]

- (e) The investigation was repeated in the presence of a competitive inhibitor of lysozyme.

Draw a curve **on Fig. 3.2** to show the expected results.

[2]

[Total: 13]

3 High fructose corn syrup, made from maize, can be used as a replacement for sucrose to sweeten food and drink products.

Commercial production of high fructose corn syrup involves the enzyme glucose isomerase, extracted from bacteria.

(a) Fructose and sucrose are both sugars.

State two structural differences between fructose and sucrose.

1

.....

.....

2

.....

.....[2]

(b) The glucose isomerase used in the production of high fructose corn syrup is extracted from a strain of a bacterium, *Thermus thermophilus*, which is found in hot springs. The enzyme has an optimum temperature of 95 °C.

Suggest **and** explain the advantages of using glucose isomerase from *T. thermophilus* to produce high fructose corn syrup, rather than using glucose isomerase that has an optimum temperature of 37 °C.

.....

.....

.....

.....

.....

.....

.....[3]

5. Question part 2/4 | 9700 Oct-Nov 2016 P2 V2 Q3

(c) The commercial production of high fructose corn syrup uses immobilised glucose isomerase.

Fig. 3.1 shows the effect of pH on the activity of immobilised glucose isomerase compared to glucose isomerase free in solution.

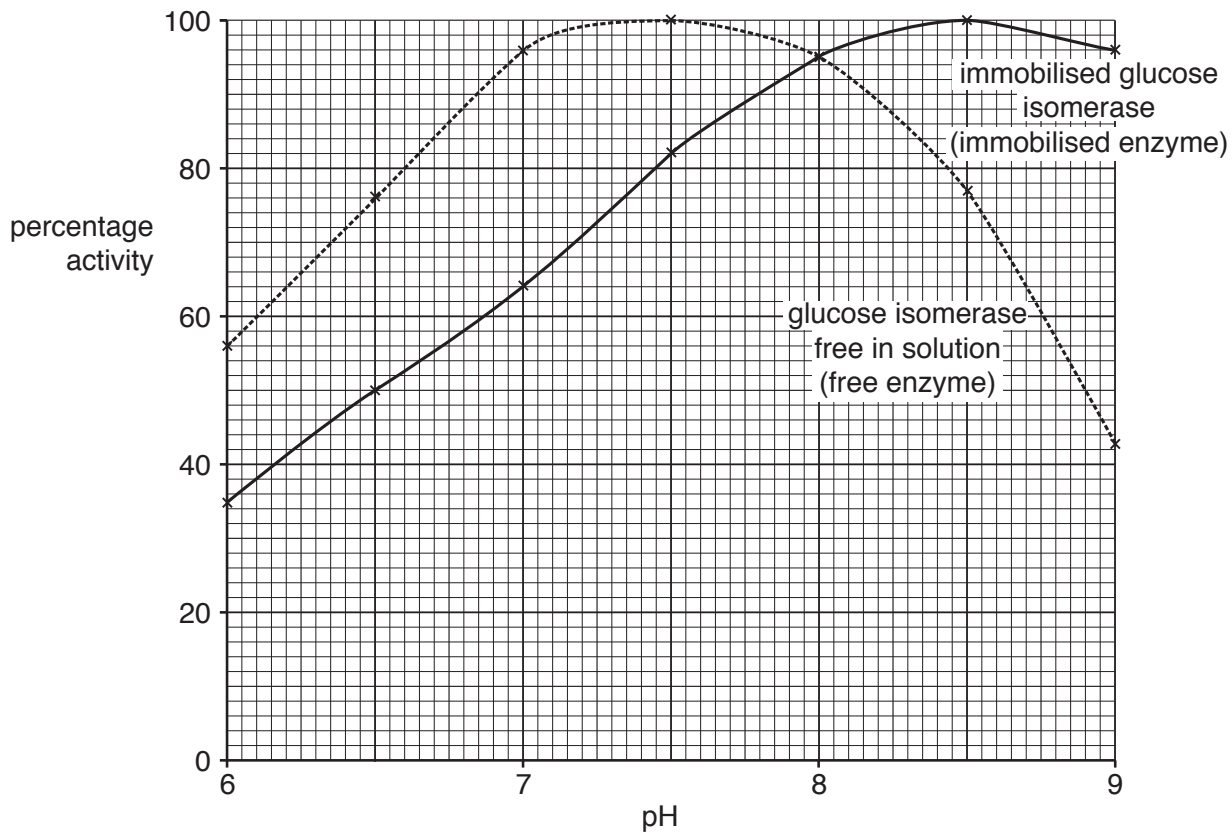


Fig. 3.1

With reference to Fig. 3.1, describe the differences shown between the immobilised enzyme and the free enzyme as pH changes.

[3]

- (d) The amino acid sequence of the enzyme glucose isomerase has been determined. The first five amino acids of this sequence are shown in Table 3.1.

Table 3.2 (on page 11) shows the genetic code (mRNA codons).

A student was asked to use Table 3.2 to work out an mRNA nucleotide sequence that would correspond to the first five amino acids of glucose isomerase. The student’s sequence is shown in Table 3.1.

Table 3.1

amino acid sequence	met	tyr	glu	pro	lys
student’s nucleotide sequence	AUG	UAU	GAC	CCU	UGU
correct = ✓ incorrect = ✗					

- (i) Complete Table 3.1 using a ✓ or a ✗ to indicate whether the student has used Table 3.2 correctly to identify the codons for each amino acid in the nucleotide sequence. [1]
- (ii) Discuss, with reasons, how an mRNA nucleotide sequence worked out to correspond to the first five amino acids using Table 3.2 may not be the same as the mRNA nucleotide sequence for those amino acids present in the bacterial cell.

.....

.....

.....

.....

.....

.....

.....

.....[3]

Table 3.2

first position	second position				third position
	U	C	A	G	
U	phe	ser	tyr	cys	U
	phe	ser	tyr	cys	C
	leu	ser	STOP	STOP	A
	leu	ser	STOP	trp	G
C	leu	pro	his	arg	U
	leu	pro	his	arg	C
	leu	pro	gln	arg	A
	leu	pro	gln	arg	G
A	ile	thr	asn	ser	U
	ile	thr	asn	ser	C
	ile	thr	lys	arg	A
	met	thr	lys	arg	G
G	val	ala	asp	gly	U
	val	ala	asp	gly	C
	val	ala	glu	gly	A
	val	ala	glu	gly	G

[Total: 12]

- 2 (a) Explain how enzymes lower the activation energy needed to allow reactions to proceed.

.....

.....

.....

.....

.....[2]

- (b) Folic acid is a molecule used by all cells for growth. Bacteria cannot absorb folic acid from their surroundings. Bacteria use an enzyme to make a molecule called PABA. PABA is used to make folic acid.

An investigation was carried out to determine the effect on the production of PABA when the concentration of an enzyme inhibitor is increased. Four different concentrations ($1\text{ }\mu\text{M}$ to $30\text{ }\mu\text{M}$) of the inhibitor were used, together with a control with no inhibitor.

The concentration of PABA produced in each reaction mixture was determined at 10 minute intervals.

The results are shown in Fig. 2.1.

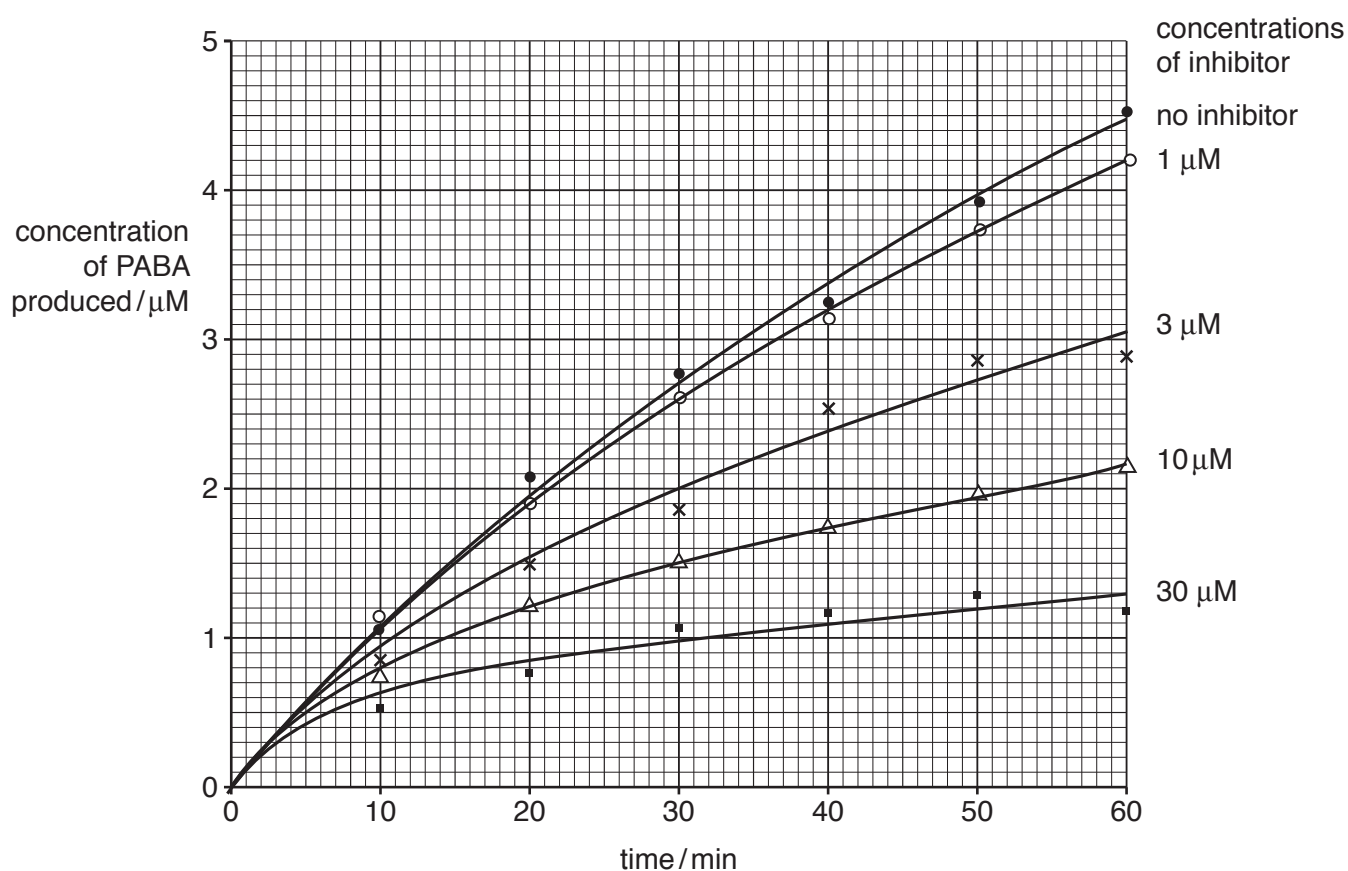


Fig. 2.1

(i) Use Fig. 2.1 to describe the results of the investigation.

.....

.....

.....

.....

.....

.....

.....

.....

.....[4]

(ii) Outline an experiment that could be carried out to determine whether the inhibitor of the enzyme that catalyses the reaction to produce PABA is competitive or non-competitive.

.....

.....

.....

.....

.....

.....

.....

.....

.....[3]

(iii) Folic acid from the diet is able to enter human cells, but is **not** able to cross bacterial cell walls. Human cells do **not** have an enzyme to make PABA.

Suggest why the inhibitor of this enzyme could be used as a drug to treat bacterial infections in humans.

.....

.....

.....[1]

9700 Biology Paper 2 - Enzymes

Topical questions | 39 questions

6. Question part 3/4 | 9700 Oct-Nov 2016 P2 V3 Q2

(iv) Suggest why there are few drugs that have any effect on viruses.

.....

.....

.....

.....

.....[2]

(c) The search for new antibiotics is important because there are many strains of bacteria that are resistant to antibiotics.

Suggest two ways to reduce the spread of antibiotic resistance.

1

.....

2

.....[2]

[Total: 14]

9700 Biology Paper 2 - Enzymes

Topical questions | 39 questions

6. Question part 4/4 | 9700 Oct-Nov 2016 P2 V3 Q2

BLANK PAGE

- 3 Catalase is an enzyme that catalyses the breakdown of hydrogen peroxide, a toxic waste product of metabolism.

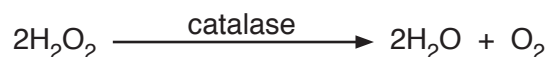


Fig. 3.1 shows the results of an investigation into the effect of hydrogen peroxide concentration on the rate of the catalase-controlled reaction, with and without the presence of two different inhibitors.

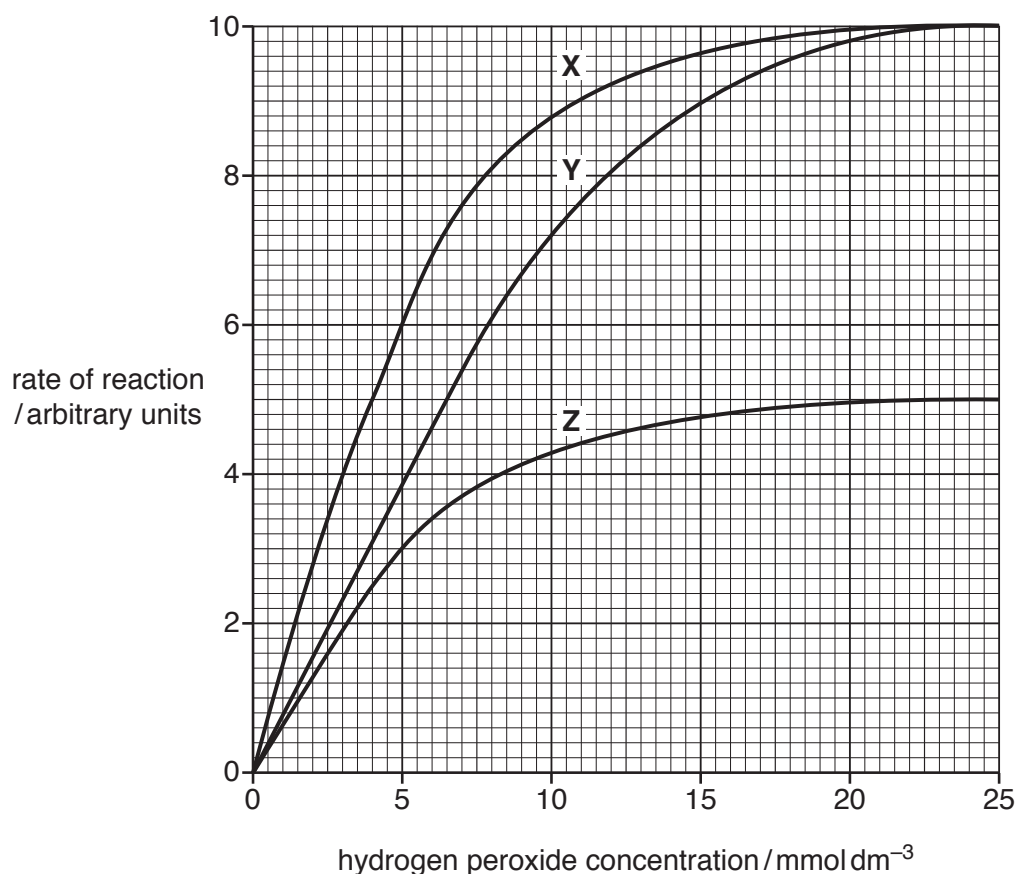


Fig. 3.1

- (a) The inhibitors used in the investigation have different modes of action.

Identify which of curves **X**, **Y** and **Z** are the results for:

- the reaction with the non-competitive inhibitor
- the reaction with the competitive inhibitor
- the reaction without any inhibitor.

non-competitive inhibitor

competitive inhibitor

without any inhibitor

(b) With reference to Fig. 3.1, compare the maximum rate of reaction, V_{max} , and the Michaelis-Menten constant, K_m , for curves **X**, **Y** and **Z**.

V_{max}

K_m

[4]

Hydrogen peroxide has a harmful effect on cells. One effect is to damage DNA.

(c) Describe the structure of DNA.

[4]

- (d) The cell has mechanisms to repair the damage to DNA caused by hydrogen peroxide. Errors in repair may cause a change to the structure of DNA.

Studies have investigated possible risks associated with foods and drinks that contain hydrogen peroxide. This is because the compound can be considered a mutagen. Mutagens cause mutations.

Explain why hydrogen peroxide can be considered a mutagen.

.....

.....

.....

.....

..... [2]

[Total: 11]

9700 Biology Paper 2 - Enzymes

Topical questions | 39 questions

7. Question part 4/4 | 9700 Feb-March 2017 P2 V2 Q3

Question 4 starts on page 10

8. Question part 1/2 | 9700 May-June 2017 P2 V1 Q2

- 2 Phosphatases are enzymes that catalyse the removal of phosphate groups from organic compounds.

Some students investigated the effect of substrate concentration on the rate of the reaction catalysed by an acid phosphatase (enzyme **A**). The results are shown in Fig. 2.1.

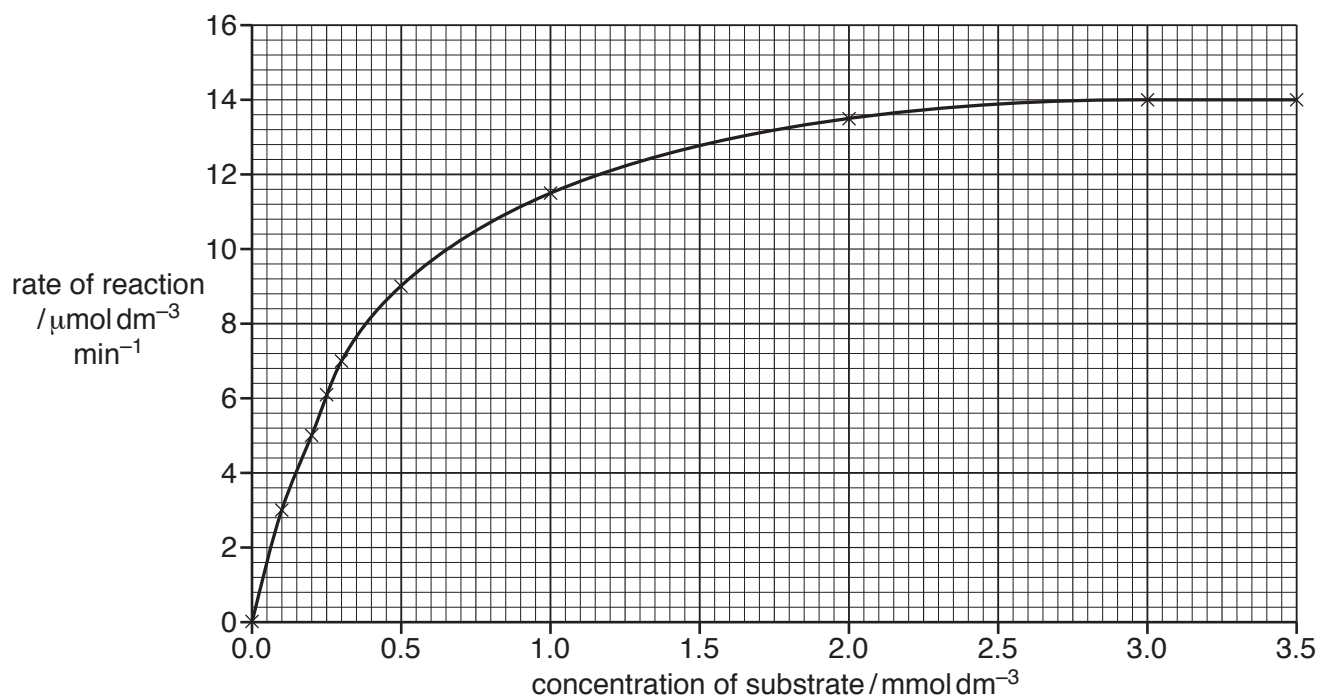


Fig. 2.1

- (a) The students used Fig. 2.1 to derive the Michaelis-Menten constant (K_m) for enzyme **A** as 0.3 mmol dm^{-3} .

Explain how they derived K_m .

.....

.....

.....

.....

.....

.....[2]

- (b) The students investigated a different phosphatase enzyme (enzyme **B**) and found the value of K_m to be higher than 0.3 mmol dm^{-3} .

Explain the difference between the values of K_m for these two phosphatase enzymes.

.....

.....

.....

.....

.....[2]

- (c) The students repeated their investigation on enzyme **A** with a competitive inhibitor.

They used the same concentrations of substrate as before, but added a competitive inhibitor to each reaction mixture.

They used the same concentration of the inhibitor in each reaction mixture.

The students found that V_{max} was the same as before, but K_m was higher than 0.3 mmol dm^{-3} .

Explain how the addition of the competitive inhibitor results in the same value for V_{max} but a higher value for K_m .

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....[4]

Topical questions | 39 questions

2 Lipase is an enzyme with many commercial uses. Some species of bacteria are of great interest as they produce large quantities of lipase.

triglyceride + ... $\xrightarrow{\text{lipase}}$

[2]

.....[3]

A line graph showing the relationship between pH and percentage activity. The x-axis is labeled 'pH' and ranges from 1.0 to 12.0 with major grid lines every 1.0 unit and minor grid lines every 0.2 units. The y-axis is labeled 'percentage activity' and ranges from 0 to 100 with major grid lines every 10 units and minor grid lines every 2 units. The data points are plotted as open circles connected by a solid line. The activity starts at 75% at pH 2.0, rises to 82% at pH 3.0, 90% at pH 4.0, 95% at pH 5.0, 96% at pH 6.0, 98% at pH 6.5, and reaches a maximum of 100% at pH 7.0. After pH 7.0, the activity drops sharply to 81% at pH 7.5, 76% at pH 8.0, 62% at pH 9.2, 57% at pH 9.5, 53% at pH 9.8, and finally 43% at pH 10.5.

pH	percentage activity
2.0	75
3.0	82
4.0	90
5.0	95
6.0	96
6.5	98
7.0	100
7.5	81
8.0	76
9.2	62
9.5	57
9.8	53
10.5	43

With reference to Fig. 2.2, describe the effect of pH on the activity of bacterial lipase.

This image shows a blank sheet of white paper with horizontal ruling lines. The lines are evenly spaced and extend across the width of the page. There are no margins, text, or other markings on the paper.

- (d) A separate investigation into the effect of pH on the same bacterial lipase compared the enzyme free in solution with the enzyme immobilised by physical attachment to a stable polymer.

At a temperature of 37°C, the optimum pH of the enzyme free in solution was the same as that shown in Fig. 2.2. The optimum pH of the immobilised enzyme was measured as pH 4.

- (i) Suggest **one** reason to explain why the enzyme free in solution has a different optimum pH compared to the immobilised enzyme.

.....
.....
.....[1]

- (ii) Suggest **one** advantage of immobilising the extracted lipase for commercial use.

.....
.....
.....[1]

[Total: 11]

10. Question part 1/3 | 9700 Oct-Nov 2017 P2 V2 Q3

3 Hydrolytic enzymes can function within the cell or can be secreted by the cell, where they are able to catalyse reactions.

(a) State the term used to describe an enzyme that functions within the cell.

.....[1]

(b) The rates of reaction of two different hydrolytic enzymes, enzyme **G** and enzyme **H**, were measured at different substrate concentrations. The results are shown in Fig. 3.1.

The two enzymes have different values of the Michaelis–Menten constant (K_m).

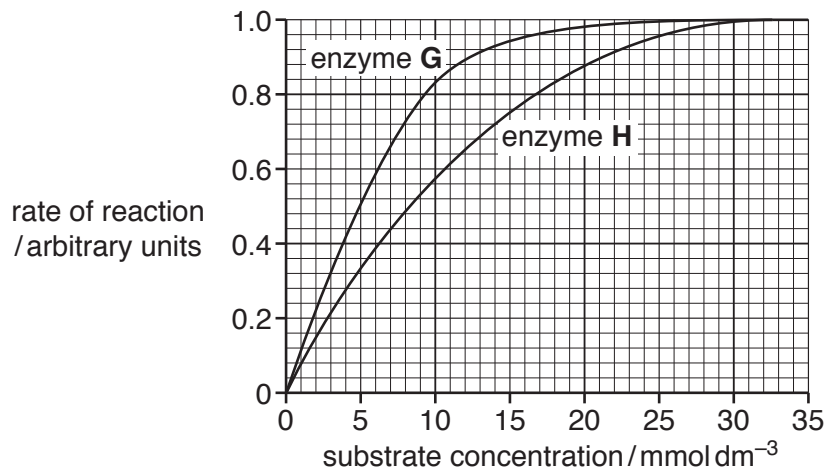


Fig. 3.1

(i) The K_m value of enzyme **G** is 5 mmol dm⁻³.

Use Fig. 3.1 to derive the K_m value for enzyme **H**.

Show your working.

.....[2]

(ii) With reference to Fig. 3.1, explain how the values of K_m for these enzymes provide information about the relationship between the enzyme and their substrates.

.....[2]

9700 Biology Paper 2 - Enzymes

Topical questions | 39 questions

10. Question part 2/3 | 9700 Oct-Nov 2017 P2 V2 Q3

Cells can break down an old or damaged organelle, such as a mitochondrion, by enclosing the organelle in a membrane formed from the endoplasmic reticulum. This forms a vacuole. Vesicles containing hydrolytic enzymes fuse with the vacuole and the organelle is then digested.

(c) Name the vesicles in the cell that contain hydrolytic enzymes.

.....[1]

(d) The cell has internal chemical messengers that signal when an old or damaged mitochondrion should be broken down.

Suggest **one** feature involving mitochondrial structure or function that could lead to the release of these internal signals.

.....
.....[1]

Question 3 continues on page 8

- The *GBA* gene codes for glucocerebrosidase. There are a number of different mutations of the *GBA* gene. Some mutations result in a single amino acid change in the enzyme molecule produced, and this causes a disorder known as Gaucher's disease. Two of these mutations, at different positions in the *GBA* gene, are shown in Table 3.1.

Table 3.1

amino acid change caused by mutation			severity of Gaucher's disease
	from	to	
mutation 1	asparagine (Asn)	serine (Ser)	mild
mutation 2	leucine (Leu)	proline (Pro)	severe

- outline how a mutation leads to a single amino acid change in the enzyme glucocerebrosidase **and**
- suggest why the two different amino acid changes, Asn to Ser and Leu to Pro, will result in differences in the tertiary structure of the enzyme.

.....[5]

[Total: 12]

Diagram illustrating the hydrolysis of lactose into galactose and glucose:

Lactose (a disaccharide composed of galactose and glucose) reacts with water (H_2O) in the presence of the enzyme lactase to produce galactose and glucose (labeled as product S).

Chemical structures shown:

- Lactose (left): A disaccharide consisting of galactose and glucose linked by a glycosidic bond.
- Galactose (middle): A monosaccharide product.
- Glucose (right, labeled product S): A monosaccharide product.

(a) Describe the reaction that is catalysed by lactase. Use Fig. 3.1 to help you.

In your answer, identify **R** and product **S**.

..... [4]

[4]

- (b) On a commercial scale, immobilised lactase can be used to produce lactose-free milk.

One of the products of the reaction shown in Fig. 3.1 acts as an inhibitor of lactase. This is an example of product inhibition.

- (i) Suggest why product inhibition is useful in *K. lactis* when lactase is acting as an intracellular enzyme, but can be a disadvantage when extracted lactase is used free in solution for the production of lactose-free milk.

.....
.....
.....
..... [2]

- (ii) Suggest how using immobilised lactase in a commercial application helps to reduce the problem of product inhibition.

.....
..... [1]

- (iii) The first large-scale production of lactose-free milk with an immobilised enzyme used lactase trapped in cellulose triacetate fibres.

Suggest **one** feature of cellulose triacetate that makes it useful as an immobilising material.

.....
..... [1]

- (c) For a commercial application using an enzyme, the progress of the enzyme-catalysed reaction needs to be studied.

Outline how the progress of an enzyme-catalysed reaction can be investigated experimentally.

.....
.....
.....
.....
.....
.....
..... [3]

[Total: 11]

The results are shown in Fig. 3.1.



- (a) With reference to Fig. 3.1, **compare** the effects of temperature on the activity of immobilised sucrase with the activity of sucrase free in solution.

[4]

percentage of maximum enzyme activity

key

- × — × immobilised sucrase
- — ○ sucrase free in solution

pH	immobilised sucrase (%)	sucrase free in solution (%)
3.0	95	92
4.5	100	100
5.5	98	95
6.0	95	22
7.0	78	4
8.0	40	0

pH

(b) Fig. 3.2 shows that immobilised sucrase remains active over a wider range of pH compared with sucrase free in solution.

Suggest reasons for the higher activity of immobilised sucrase over the range of pH between 5.5 and 8.0.

[2]

(c) State **one** variable that the researchers should keep constant in **both** investigations **and** explain your answer in terms of enzyme action.

variable
.....
explanation
.....
.....
.....[2]

(d) There are many advantages of using immobilised enzymes in industry.

Suggest **two** advantages of using immobilised enzymes in industry **other than** remaining active over a greater range of pH.
.....
.....
.....
.....
.....[2]

[Total: 10]

- Mevinolin acts as a competitive inhibitor of the enzyme HMG CoA reductase. This enzyme catalyses one of the first steps in the synthesis of cholesterol, as shown in Fig. 6.1.

Fig. 6.1

.....[3]

.....[2]

[Total: 5]

- Ficin, found in *F. carica*, and papain, found in *C. papaya*, are both cysteine protease enzymes. These enzymes have been extracted and purified for use commercially.

- (a) An investigation was carried out to compare the effect of temperature on the activity of ficin and papain.

The results are shown in Fig. 5.1.

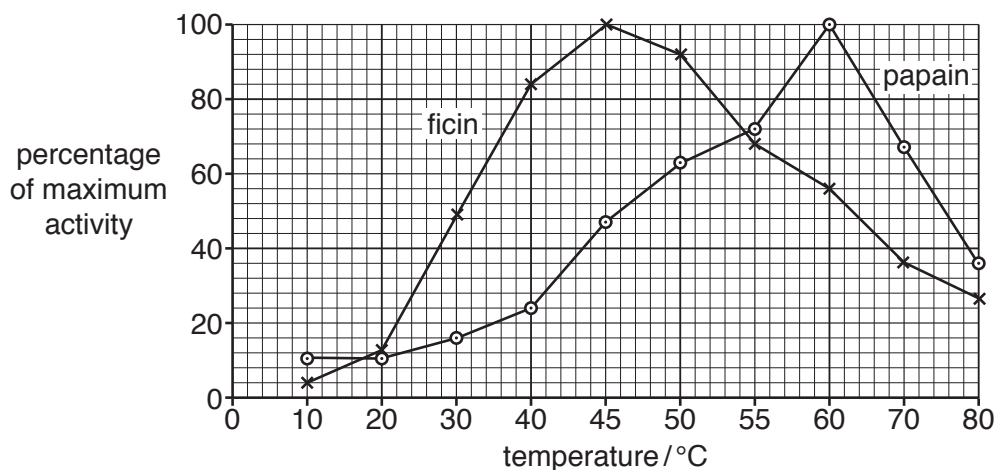


Fig. 5.1

- (i) With reference to Fig. 5.1, describe the differences between the activity of papain compared to the activity of ficin between 20°C and 80°C.

.....[3]

- (ii) Ficin and papain have been shown to be effective in the digestion of parasitic nematodes (roundworms).

With reference to Fig. 5.1, explain which enzyme you would select to use in an oral medication for the treatment of human intestinal parasitic nematodes.

.....

.....

.....

.....[1]

- (b) One commercial use of the enzyme ficin is the production of Fab fragments (antigen binding regions) of mouse IgG antibodies for use in immunological studies. The process uses immobilised ficin to cleave (cut) the antibodies in the hinge region.

Suggest **one** practical advantage of using immobilised ficin for this process, rather than ficin free in solution.

.....

.....

.....

.....[1]

- (c) *Streptococcus pyogenes* is a bacterium that can cause a range of diseases in humans.

S. pyogenes synthesises streptopain, a cysteine protease that hydrolyses structural proteins in human connective tissue.

- (i) Streptopain is secreted to the outside of the cell.

State the term given to an enzyme that is produced by a cell and is then secreted to the outside, where it has its action.

.....[1]

- (ii) Suggest **one** example of a structural protein in connective tissue that can be hydrolysed by streptopain.

.....[1]

[Total: 7]

15. Question part 1/4 | 9700 Oct-Nov 2018 P2 V1 Q2

- 2 Fig. 2.1 shows the disaccharide lactose, which is found in milk.

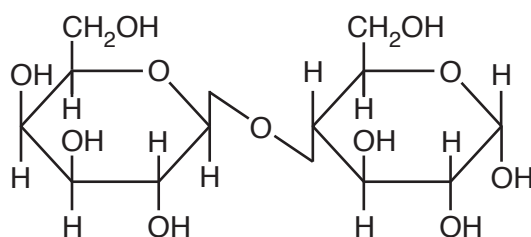


Fig. 2.1

- (a) Name the type of bond that joins the two monosaccharides in lactose.

..... [1]

- (b) The enzyme lactase catalyses the breakage of the bond between the two monosaccharides in lactose.

- (i) Name the type of reaction that breaks this bond.

..... [1]

- (ii) Some people do not produce the enzyme lactase, so cannot digest lactose.

The presence of lactose in the lumen of the intestine reduces the volume of water absorbed into the blood, resulting in diarrhoea.

Suggest why the presence of lactose in the intestine reduces the volume of water absorbed.

.....
.....
.....
.....
.....
..... [2]

(c) Enzymes, such as lactase, are often immobilised for use in the food industry.

A scientist carried out an investigation to determine the effects of temperature on the activity of lactase when it was immobilised and when it was free in solution.

The scientist produced alginate beads containing lactase for use in this investigation. The beads varied in size. The scientist selected small beads for the investigation and put them into a glass column.

(i) Suggest the advantage of using small beads rather than large beads.

.....

.....

.....

..... [2]

- (ii) Fig. 2.2 shows the results of the investigation to determine the effects of temperature on the activity of lactase when it was immobilised, **I**, and when it was free in solution, **F**.

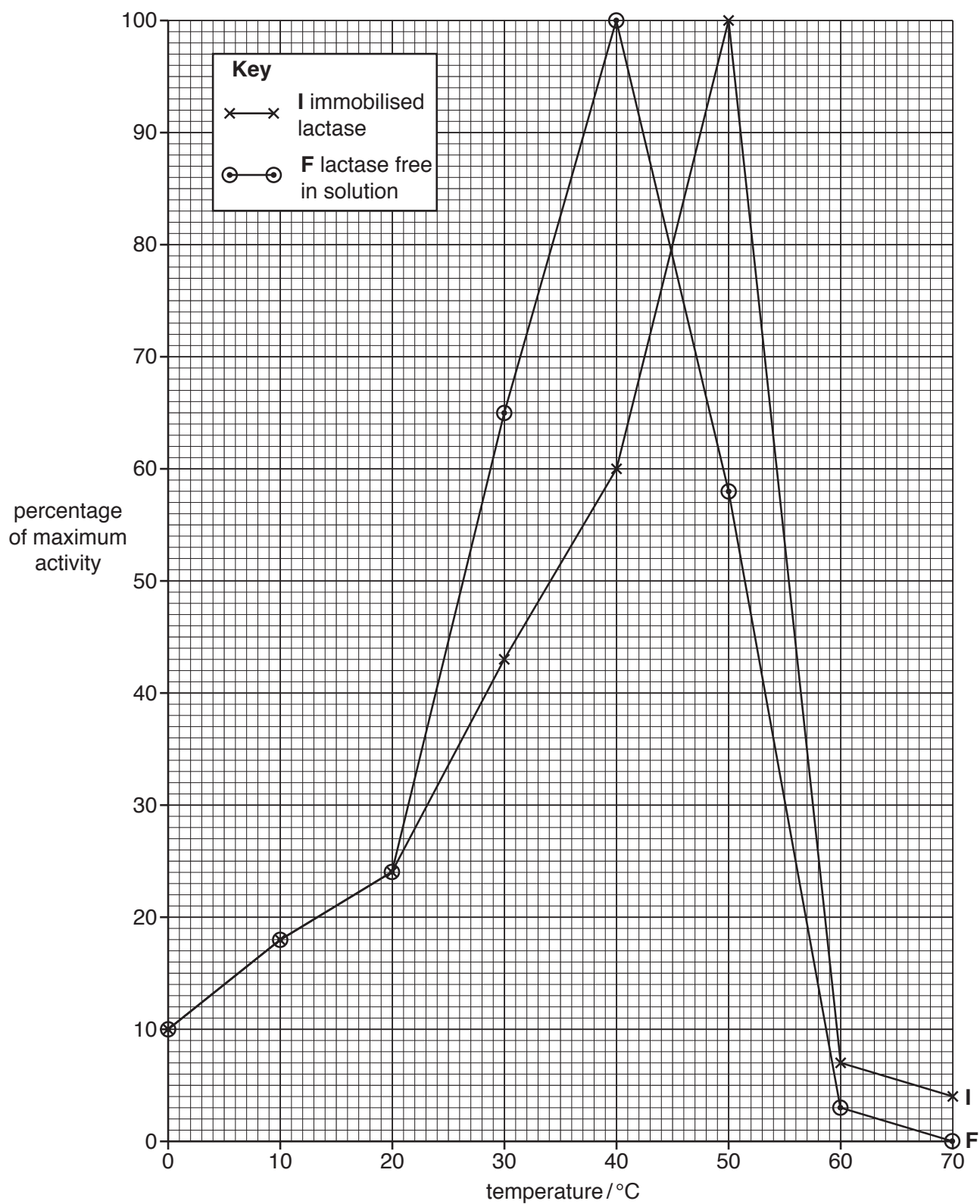


Fig. 2.2

15. Question part 4/4 | 9700 Oct-Nov 2018 P2 V1 Q2

With reference to Fig. 2.2, compare the effect of temperature on the activity of immobilised lactase, **I**, and lactase free in solution, **F**.

.....

.....

.....

.....

.....

.....

.....

.....

..... [3]

[Total: 9]

Topical questions | 39 questions

2 In mammalian red blood cells, carbonic anhydrase has an important role in the transport of carbon dioxide.

(a) Outline the features of an enzyme.

..... [4]

body tissues

lungs

[2]



Explain the extent to which Fig. 2.2 shows the primary, secondary and tertiary structure of carbonic anhydrase.

[4]

- 3** Neutrase® is an enzyme that is used to hydrolyse proteins in solution. When the enzyme is mixed with a 2% protein solution the reaction mixture changes from white to colourless.

A student carried out an experiment to find the effect of copper sulfate and potassium sulfate on the activity of Neutrase®.

The student made four reaction mixtures in test-tubes **A** to **D**. Test-tubes **A** to **C** contained equal volumes of protein solution and 0.1 cm^3 of solutions of copper sulfate or potassium sulfate. Test-tube **D** contained the same volume of protein solution and 0.1 cm^3 of water.

0.5 cm^3 of a 1% Neutrase® solution was added to test-tube **A** and immediately placed into a colorimeter. The colorimeter was used to measure the intensity of light that is absorbed by the solution (absorbance) over 100 seconds. The procedure was repeated with the other reaction mixtures, **B**, **C** and **D**.

The results are shown in Fig. 3.1.

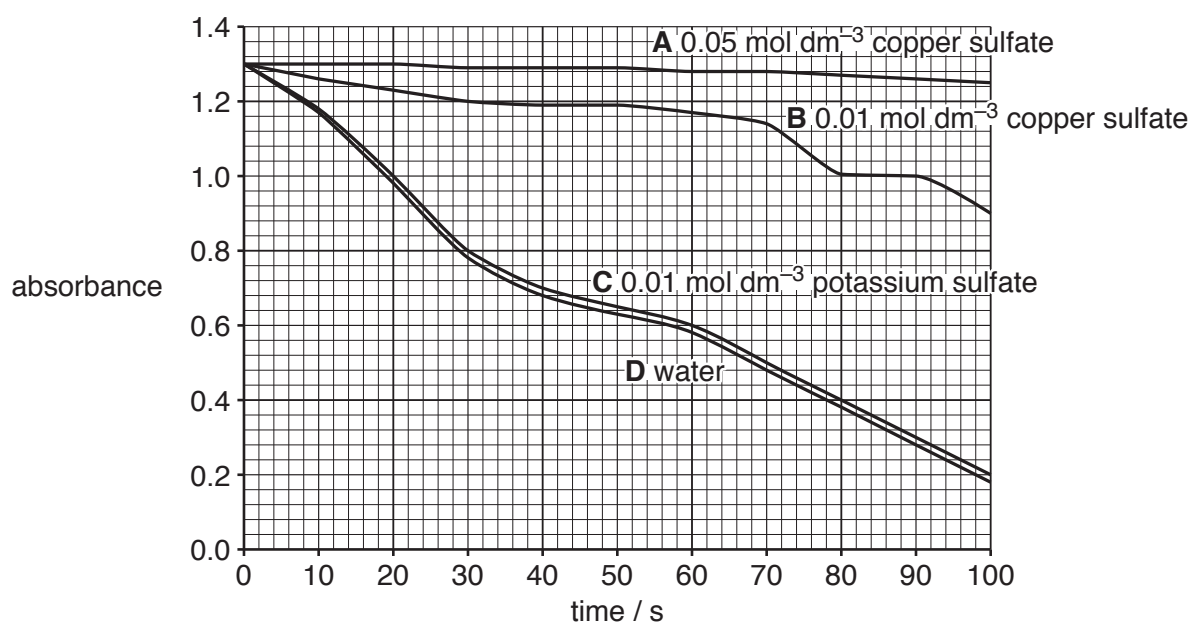


Fig. 3.1

- (a) (i)** Suggest **and** explain why measuring the absorbance of the reaction mixture over 100 s is a suitable method for determining the activity of Neutrase®.

.....

.....

.....

.....

..... [2]

- [5]

- [2]

- The reaction catalysed by catalase is shown in Fig. 6.1.

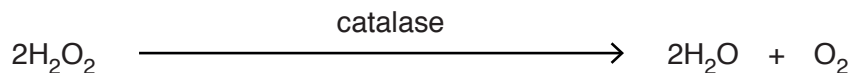


Fig. 6.1

- In the first experiment, hydrogen peroxide solution was added to three of the pieces of potato tissue and the progress of the reaction was measured.

Fig. 6.2 shows how the first experiment was set up.

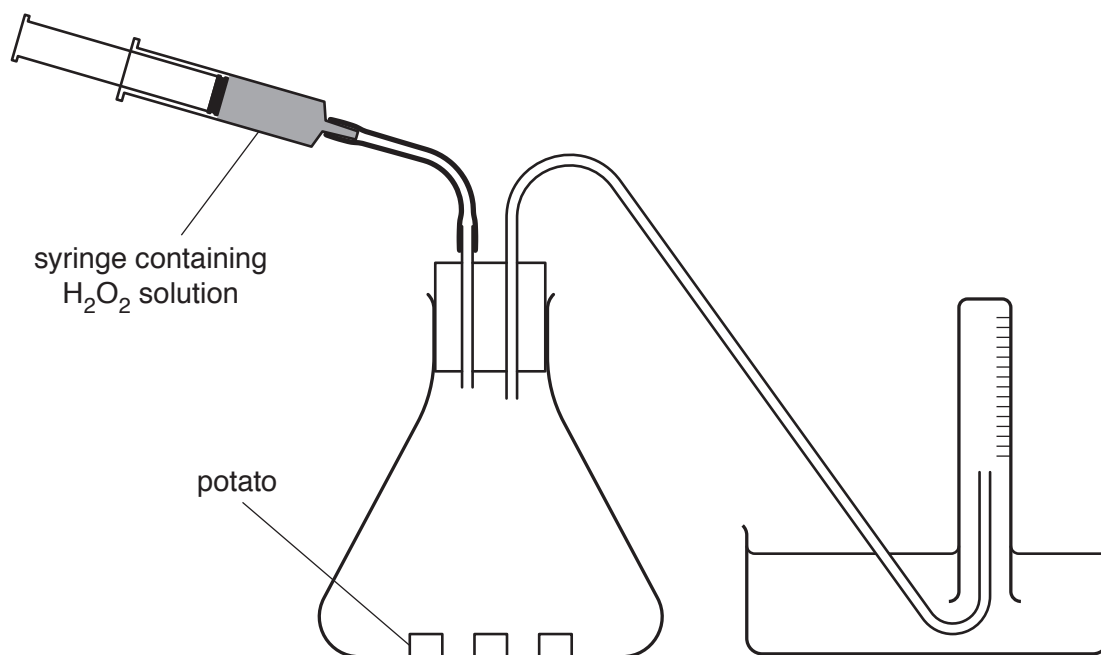


Fig. 6.2

- (i) Suggest how the progress of the reaction could be measured.

..... [2]

- (ii) In the second experiment, the student cut each of the three remaining pieces of potato to obtain six pieces, each measuring $10\text{ mm} \times 10\text{ mm} \times 10\text{ mm}$.

Using exactly the same conditions, the student measured the progress of the reaction and obtained different results to the first experiment.

Explain why the results of the second experiment were different from the results of the first experiment.

.....

.....

.....

.....

.....

..... [2]

- (b) The student then investigated the effect of temperature on the activity of catalase.

On Fig. 6.3, sketch a curve to show how temperature affects the activity of an enzyme such as catalase.

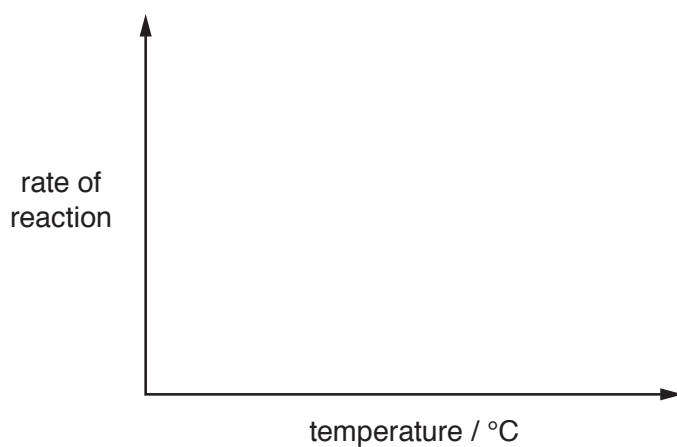


Fig. 6.3

[1]

[Total: 5]

9700 Biology Paper 2 - Enzymes

Topical questions | 39 questions

19. Question part 1/2 | 9700 Oct-Nov 2019 P2 V2 Q6

- 6** Telomerase is an enzyme that is important in maintaining the telomeres that are located at the ends of chromosomes.

(a) Explain the importance of telomeres.

.....

.....

.....

.....

..... [2]

(b) Suggest why telomerase is present in much higher quantities in stem cells and cancer cells than in other cell types.

.....

.....

..... [1]

(c) Studies suggest that telomerase is a flexible molecule when binding its substrate. The active site changes its shape slightly to allow the formation of the enzyme-substrate complex.

Name the theory that describes this mode of enzyme action.

..... [1]

- (d) An investigation was carried out into the effect of substrate concentration on the activity of telomerase.

The results are shown in Fig. 6.1.

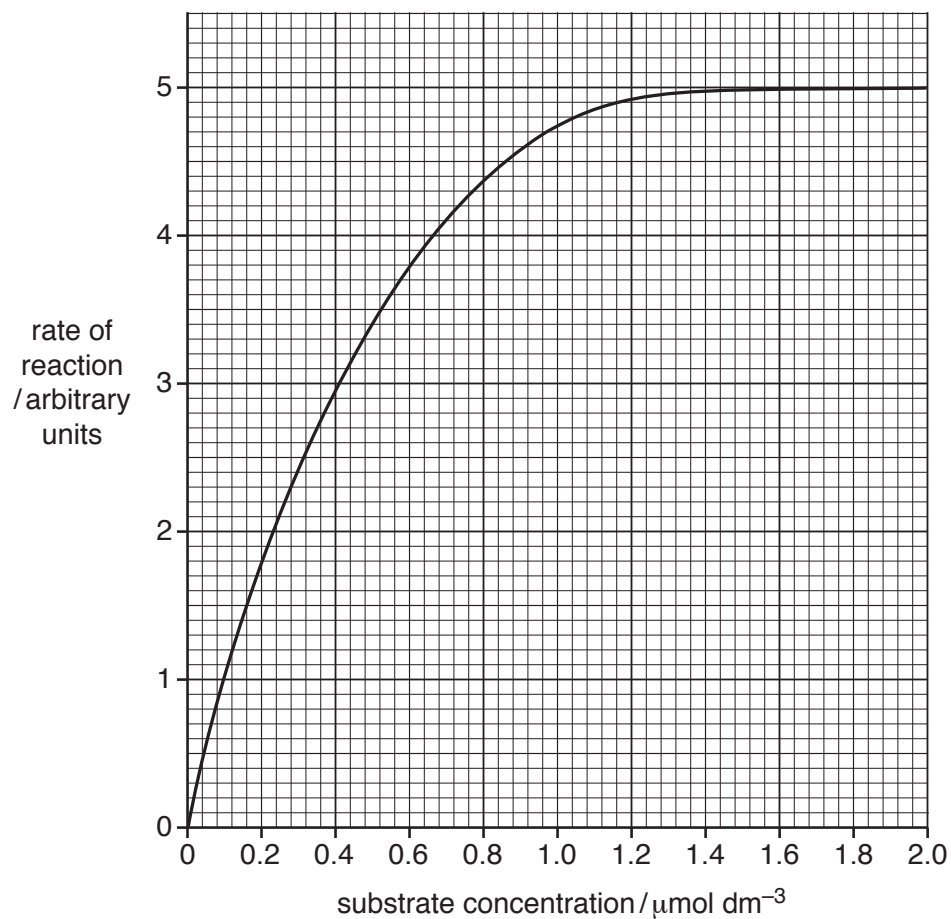


Fig. 6.1

With reference to Fig. 6.1, explain the difference in the rates of reaction obtained for telomerase at a substrate concentration of $0.2\mu\text{mol dm}^{-3}$ compared with a substrate concentration of $1.6\mu\text{mol dm}^{-3}$.

.....

.....

.....

.....

..... [2]

[Total: 6]

2 Phosphatidate phosphatase (PAP) enzymes have an important role in lipid metabolism.

The reaction catalysed by PAP is shown in Fig. 2.1.



Fig. 2.1

Experiments were carried out to investigate the activity of PAP extracted from the cotyledons (seed leaves) of bitter melon, *Momordica charantia*.

(a) There are two types of PAP enzymes:

- PAP1 enzymes need magnesium ions (Mg^{2+}) in the active site to function
- PAP2 enzymes do not need Mg^{2+} .

The effect of different concentrations of Mg^{2+} on the activity of PAP extracted from *M. charantia* was investigated.

The results are shown in Fig. 2.2.

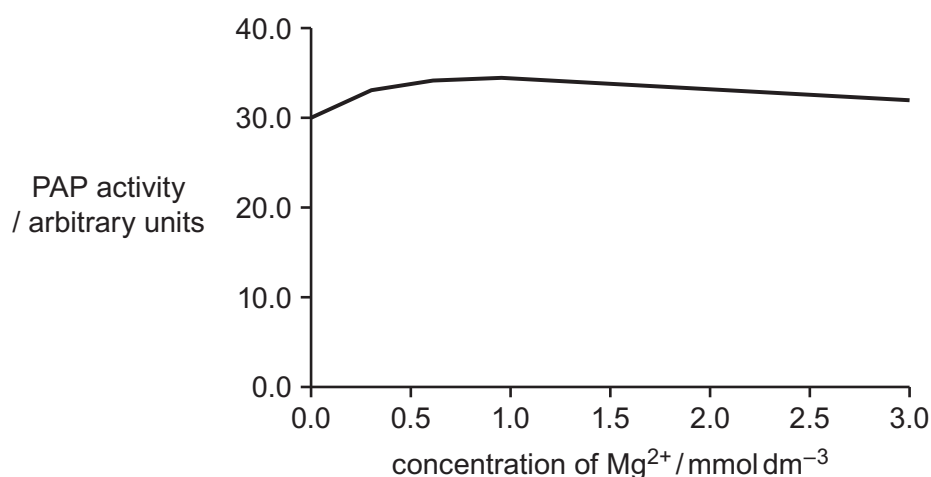


Fig. 2.2

Explain, with reference to Fig. 2.2, whether the PAP extracted from *M. charantia* is a PAP1 enzyme or a PAP2 enzyme.

.....

.....

.....

.....

.....

..... [2]



With reference to Fig. 2.3, describe **and** explain the effect of increasing phosphatidate concentration on the activity of PAP.

..... [4]

(c) The diglycerides formed as a result of the action of PAP can be used to synthesise triglycerides and membrane phospholipids.

(i) Explain how the structure of a triglyceride is suited to its function as an energy storage molecule.

.....

.....

.....

.....

.....

..... [2]

(ii) Explain why phospholipids are able to form a bilayer in cell membranes.

.....

.....

.....

.....

.....

..... [2]

[Total: 10]

21. Question part 1/2 | 9700 May-June 2020 P2 V1 Q3

- 3 The enzyme glucose 6-phosphate dehydrogenase (G6PD) is composed of two identical polypeptide chains.

- (a) Students investigated the activity of two forms of G6PD, **J** and **K**, at different concentrations of substrate. **K** is a form of the enzyme that results from a mutation that changes one amino acid in the polypeptide.

The results are shown in Fig. 3.1.

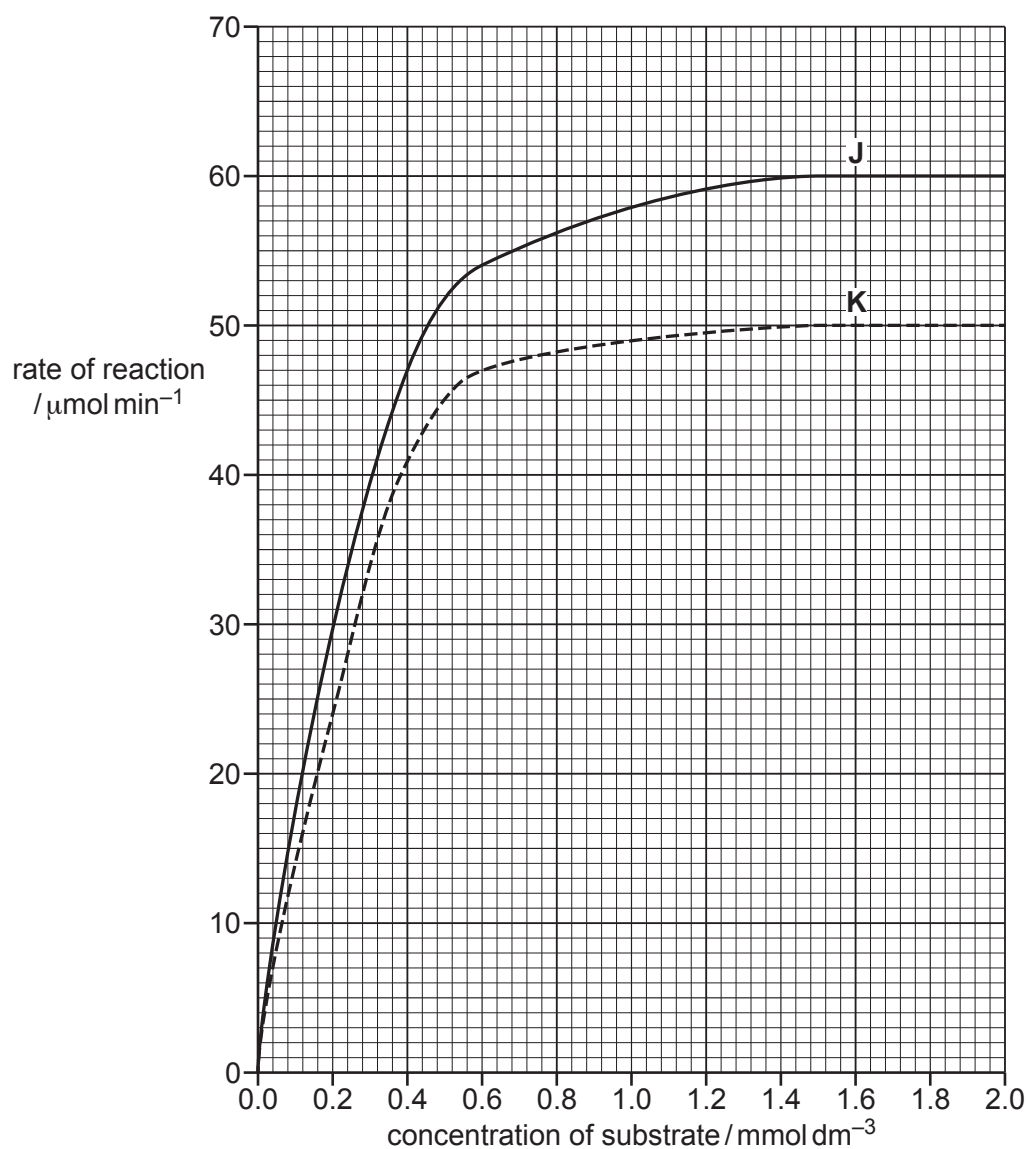


Fig. 3.1

21. Question part 2/2 | 9700 May-June 2020 P2 V1 Q3

- (i) Use Fig. 3.1 to complete Table 3.1 by stating:
- the V_{max} and the K_m for enzymes **J** and **K**
 - the units for V_{max} and K_m .

Table 3.1

	V_{max} /	K_m /
J		
K		

[3]

- (ii) With reference to Fig. 3.1 and Table 3.1, describe the effect of the mutation on the activity of G6PD **and** suggest an explanation for this effect.

.....

.....

.....

.....

.....

.....

.....

.....

.....

..... [4]

- (b) In certain conditions, G6PD may also exist as four identical polypeptide chains rather than two identical polypeptide chains.

Explain why both of these types of G6PD have all four levels of protein structure.

.....

.....

.....

.....

..... [2]

- 5** Sucrose phosphorylase is an enzyme found in some species of bacteria. One function of this enzyme is for the production of compounds that help to protect the cell from harmful osmotic changes in the external environment.

Fig. 5.1 shows the reversible reaction that takes place within the bacterial cell.

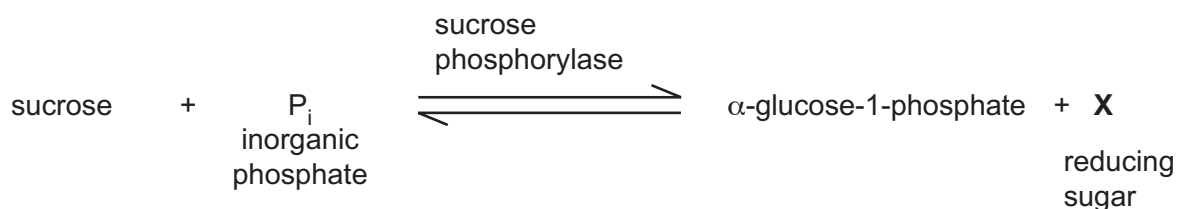


Fig. 5.1

- (a)** Name reducing sugar **X** in Fig. 5.1.

..... [1]

- (b)** In the absence of sucrose phosphorylase as a catalyst, the reaction shown in Fig. 5.1 would take too long to occur to allow the bacterial cell to function efficiently.

Explain why the reaction shown in Fig. 5.1 proceeds at a much faster rate in the presence of the enzyme.

.....
.....
.....
.....
..... [2]

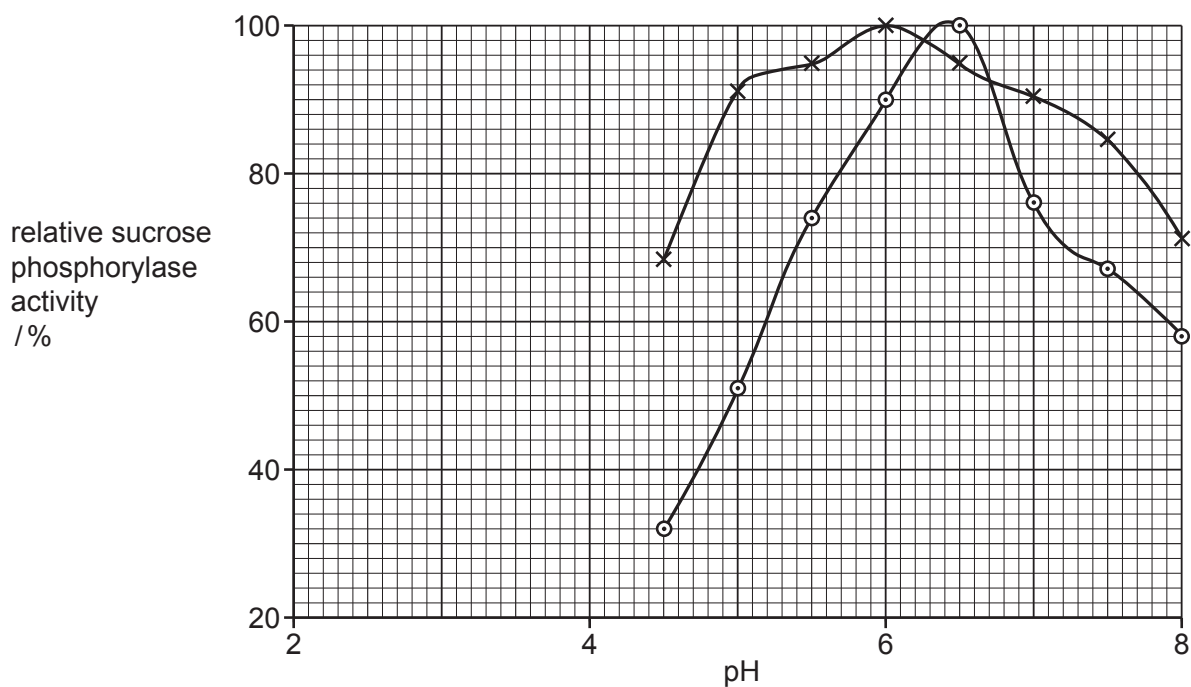
(c) An enzyme that catalyses a reaction of commercial interest needs to be investigated to see if it is suitable for use in industry.

For example:

- immobilised enzymes may be used as they have a longer shelf-life than the enzyme free in solution
- many industrial reactions are carried out at higher temperatures to minimise contamination of products by microorganisms.

Fig. 5.2 shows the results of an investigation to compare the activity of sucrose phosphorylase free in solution (free enzyme) with immobilised sucrose phosphorylase (immobilised enzyme) at different pHs.

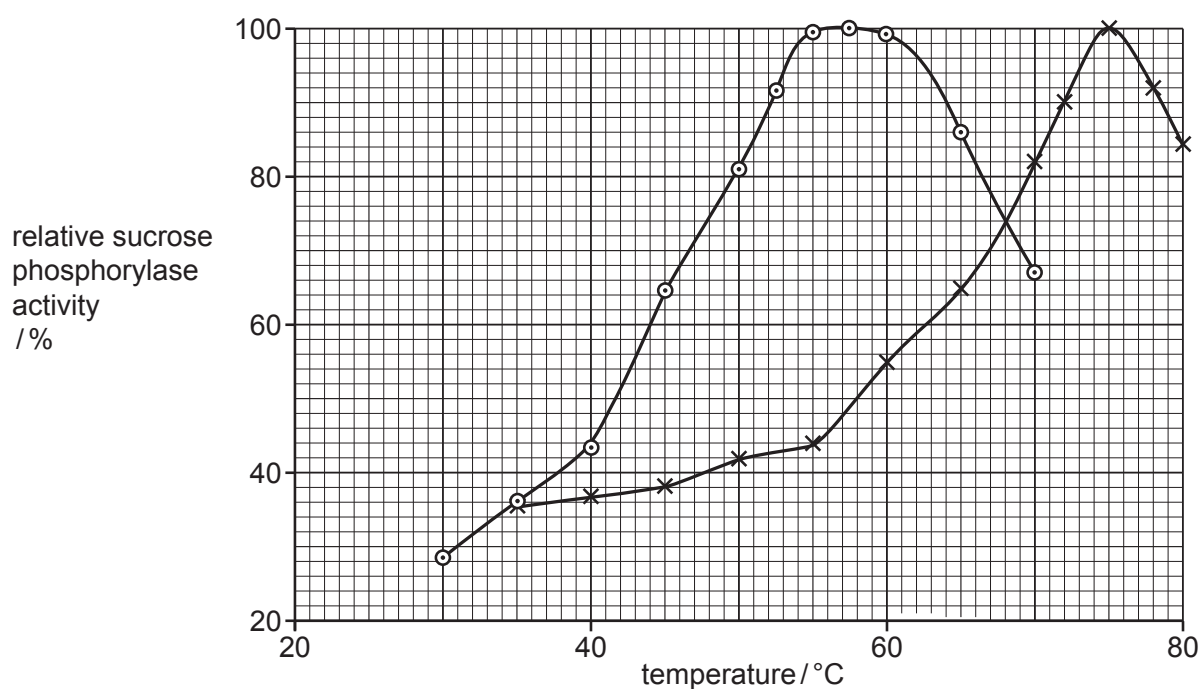
Fig. 5.3 shows the activity of the free enzyme and immobilised enzyme at different temperatures.



Key

- free enzyme
- × immobilised enzyme

Fig. 5.2

**Key**

- free enzyme
- × immobilised enzyme

Fig. 5.3

With reference to the results shown in Fig. 5.2 and Fig. 5.3, discuss which sucrose phosphorylase enzyme, free or immobilised, is better for use in industrial reactions.

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

..... [4]

[Total: 7]

- 4 (a) The induced-fit hypothesis and the lock-and-key hypothesis are used to describe the mode of action of enzymes.

Explain the induced-fit hypothesis.

.....

.....

.....

.....

.....

..... [3]

- (b) Radish plants contain the enzyme peroxidase that catalyses the breakdown of hydrogen peroxide.

Students investigated the effect of increasing the concentration of hydrogen peroxide on the activity of peroxidase extracted from radish.

The results of their investigation are shown in Fig. 4.1.

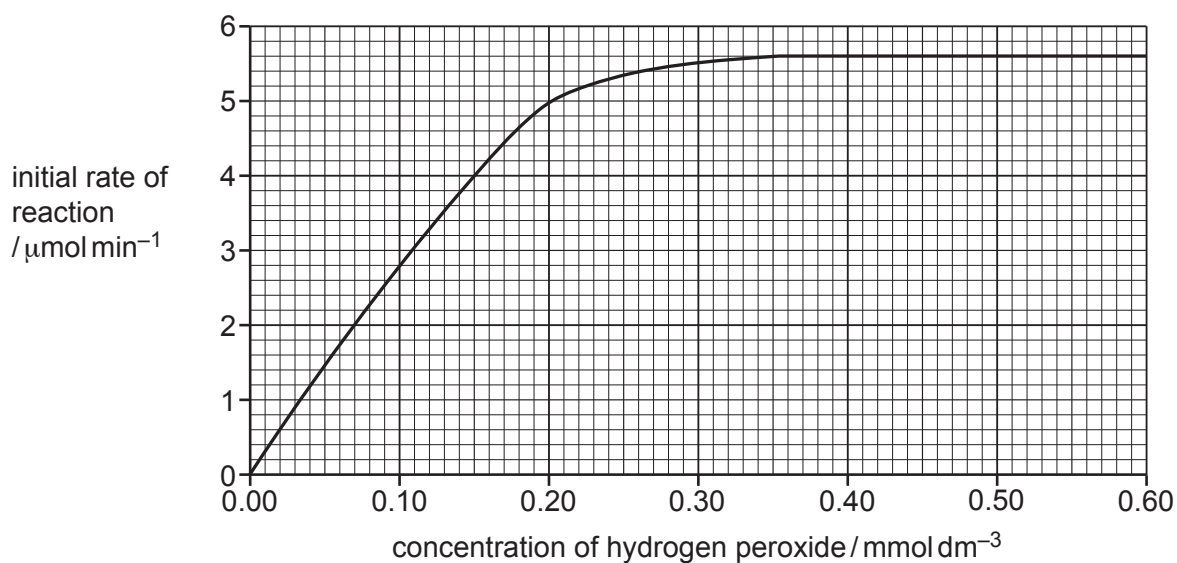


Fig. 4.1

(i) Explain the effect of increasing the concentration of hydrogen peroxide on the initial rate of reaction as shown in Fig. 4.1.

.....

.....

.....

.....

.....

..... [3]

(ii) The students determined the K_m for radish peroxidase as 0.10 mmol dm^{-3} .

With reference to Fig. 4.1, describe how they determined the K_m .

.....

.....

.....

..... [2]

(c) A further investigation found that the K_m for carbonic anhydrase is 12 mmol dm^{-3} .

Describe the role of carbonic anhydrase in the transport of carbon dioxide.

.....

.....

.....

.....

.....

..... [3]

- 6** Amylase is an enzyme that catalyses the hydrolysis of starch into reducing sugars.

A student carried out an experiment to investigate the hydrolysis of starch using immobilised amylase. Fig. 6.1 is a diagram of the apparatus that was used in the investigation.

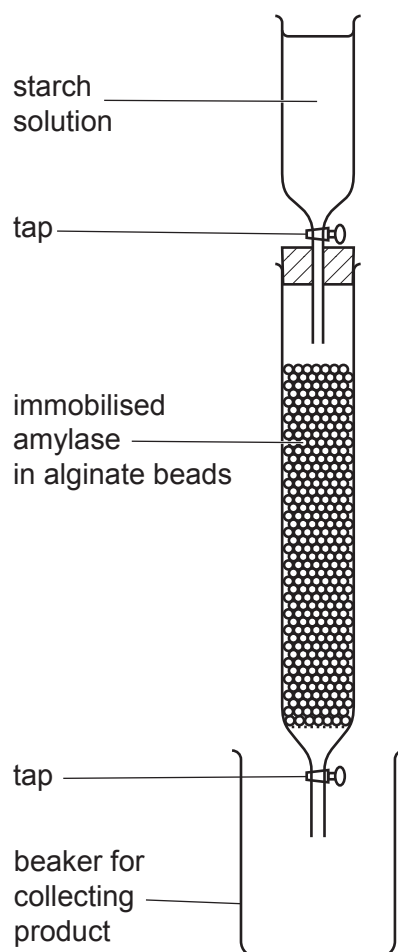


Fig. 6.1

The alginate beads were all the same size.

Both taps were opened to allow the starch solution to flow down the column and for the product to be collected.

The product was tested for the presence of reducing sugar and starch.

(a) The results of the investigation showed that the product collected in the beaker contained reducing sugar **and** starch.

With reference to Fig. 6.1, describe a method that would allow the student to use the immobilised amylase to collect a product that contains **only** reducing sugar.

.....

.....

.....

.....

.....

.....

..... [3]

(b) One standardised variable in the investigation is the size of the alginate beads.

Suggest **one** reason why using larger or smaller alginate beads in the column would affect the results obtained.

.....

.....

..... [1]

[Total: 4]

25. Question part 1/3 | 9700 Oct-Nov 2021 P2 V3 Q2

- 2** Pepsin is an enzyme that hydrolyses proteins (protease). Some students used pepsin from the stomach of a mammal.

The activity of the pepsin was investigated by placing a small quantity of the enzyme with a known concentration of the protein albumen.

Fig. 2.1 shows the progress of the enzyme-catalysed reaction that was carried out at 20 °C.

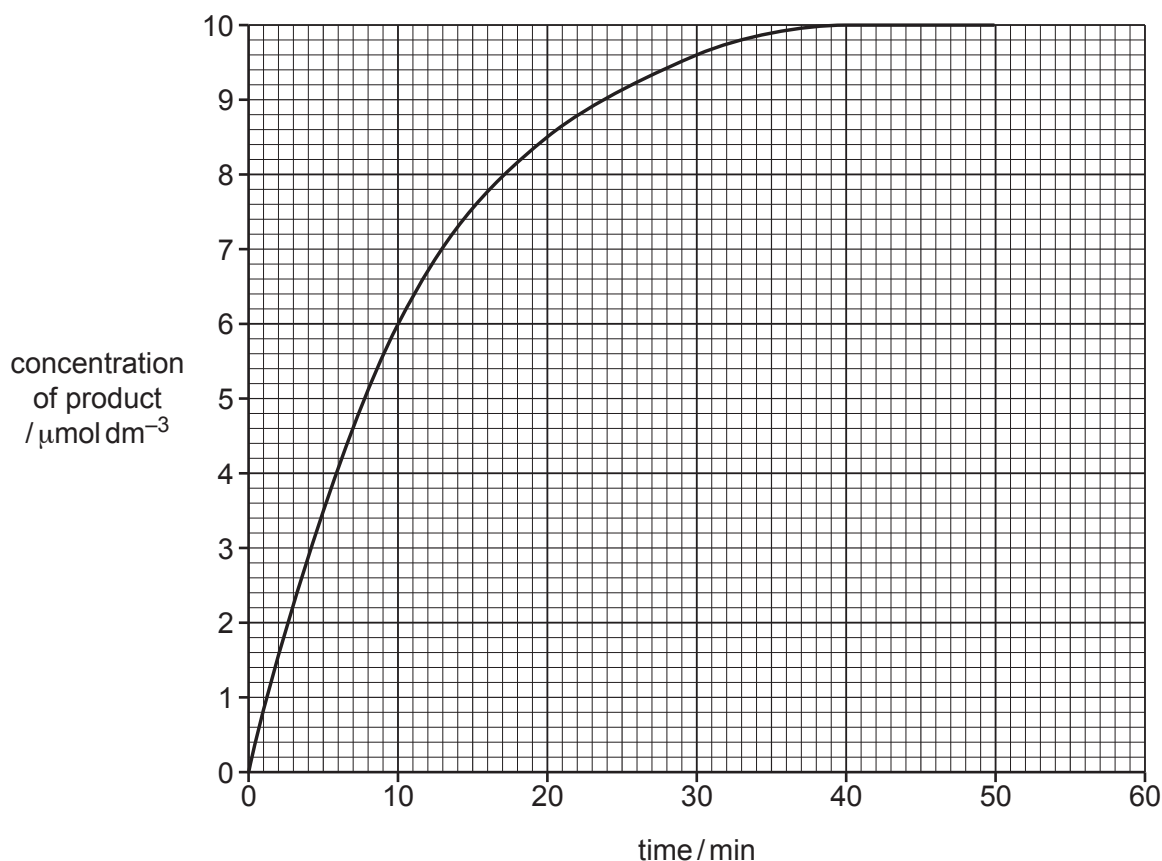


Fig. 2.1

- (a)** Calculate the initial rate of the reaction.

initial rate of reaction = ... [2]

- (b) (i)** The procedure was repeated to find the effects on the activity of the pepsin using a competitive inhibitor at the same temperature, 20 °C.

Predict the results that will be obtained using the competitive inhibitor.

.....
.....
.....
..... [2]

(ii) The procedure was repeated **without** the competitive inhibitor but at the higher temperature of 30 °C.

Predict the results that will be obtained at 30 °C.

.....

.....

.....

..... [2]

(c) The students extended their investigation by using pepsin from a different species of mammal. The experiments were carried out at 20 °C and **without** a competitive inhibitor.

With reference to Fig. 2.1, explain the advantage of calculating the initial rate of reaction in each experiment.

.....

.....

.....

.....

.....

..... [2]

[Total: 8]

9700 Biology Paper 2 - Enzymes

Topical questions | 39 questions

25. Question part 3/3 | 9700 Oct-Nov 2021 P2 V3 Q2

BLANK PAGE

- 3 (a) Enzymes are polymers of amino acids.

Complete Fig. 3.1 to show the general structure of an amino acid.

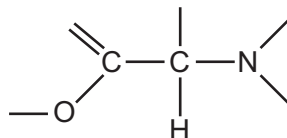


Fig. 3.1

[1]

- (b) When bananas are peeled, the exposed tissue gradually turns brown in the presence of oxygen in the air. This is due to an enzyme called catechol oxidase, which acts on the substrate catechol. Catechol and catechol oxidase are present in the banana tissue.

The overall reaction is shown in Fig. 3.2.

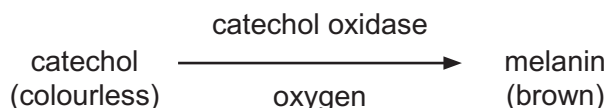


Fig. 3.2

A student investigated how the concentration of catechol oxidase affects the rate of this reaction. All other variables were kept constant throughout the investigation.

For each concentration of catechol oxidase used, the student mixed catechol oxidase solution with catechol and recorded the time taken for the mixture to reach a standard brown colour.

The rate of reaction, **R**, for each concentration of catechol oxidase used was then calculated using the formula:

$$R = \frac{1}{\text{time to reach standard brown colour in minutes}}$$

- (i) Calculate the rate of reaction when the standard brown colour was reached in 2 minutes 30 seconds.

rate of reaction = ... min⁻¹ [1]

(ii) Fig. 3.3 is a graph showing the results of the investigation.

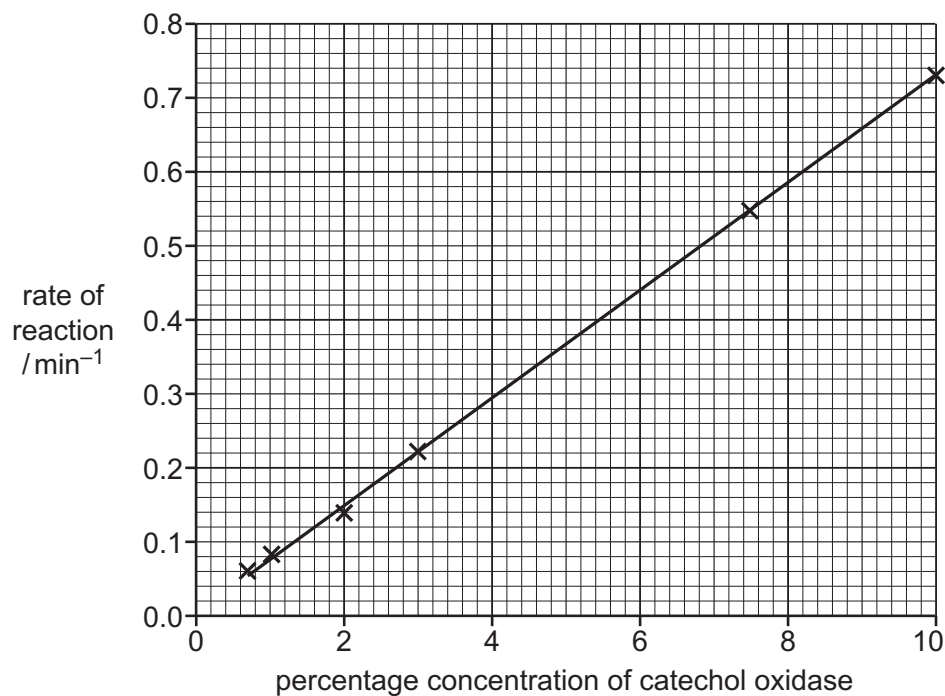


Fig. 3.3

State how the results shown in Fig. 3.3 show that substrate was in excess at all concentrations of catechol oxidase tested.

.....
.....
..... [1]

- (c) The student carried out a further experiment to investigate how the concentration of catechol affects the initial rate of reaction. All other variables were kept constant throughout this investigation.

Fig. 3.4 is a graph showing the effect of varying the concentration of catechol on the initial rate of reaction.

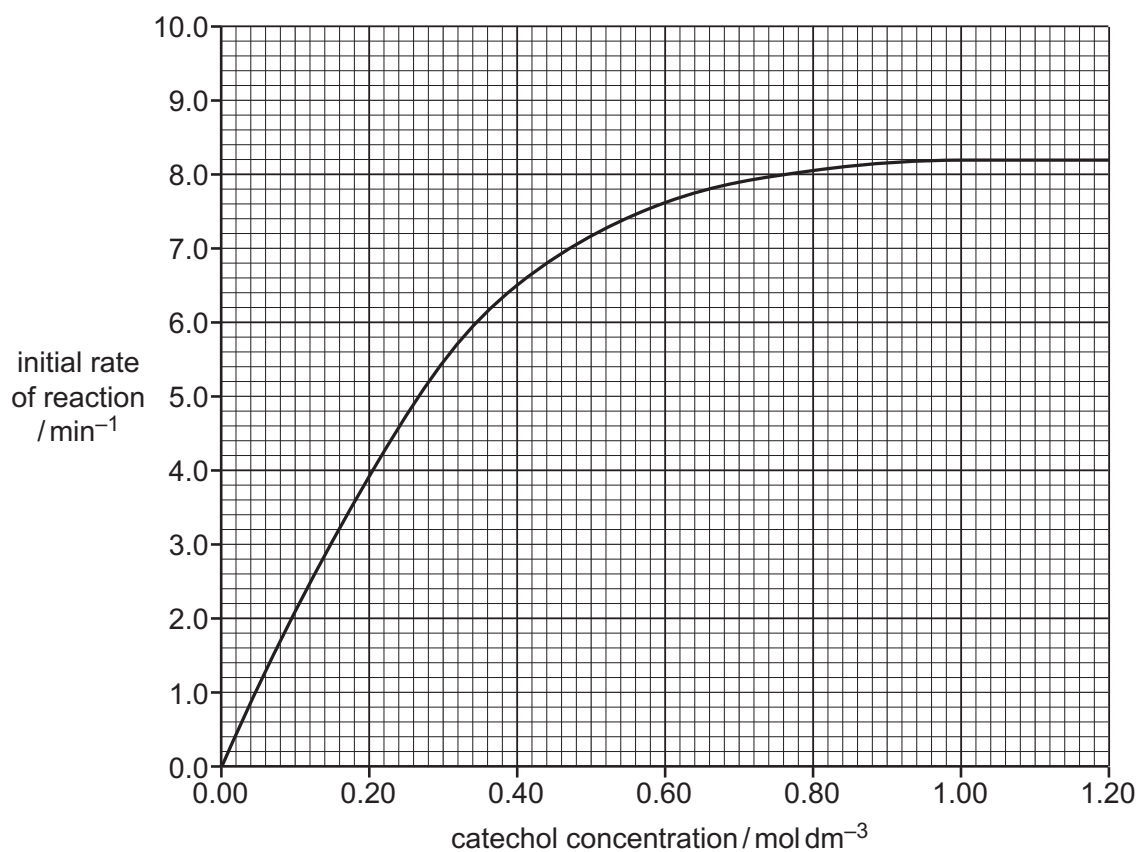


Fig. 3.4

- (i) Explain the shape of the curve shown in Fig. 3.4.

.....

.....

.....

.....

.....

.....

..... [3]

- (ii) Use Fig. 3.4 to calculate the value of the Michaelis–Menten constant (K_m) for the reaction between catechol oxidase and catechol.

$K_m = \dots\dots\dots \text{mol dm}^{-3}$ [1]

- (iii) Methylcatechol has a similar shape to catechol. Catechol oxidase can also use methylcatechol as a substrate.

The K_m value for the reaction using methylcatechol as the substrate was found to be much lower than the K_m value for the reaction using catechol as the substrate, when the reactions were carried out under the same conditions.

State what these K_m values indicate about the relationship between the enzyme and the two substrates.

.....
.....
..... [1]

[Total: 8]

27. Question part 1/3 | 9700 May-June 2022 P2 V1 Q3

- 3 A tyrosine kinase receptor (TKR) is a protein complex found in the cell surface membrane of mammalian cells.

TKR has two components involved in the process of cell signalling:

- a receptor for the signalling molecule (ligand)
- an enzyme that catalyses the transfer of a phosphate group from ATP to an intracellular protein.

Fig. 3.1 is a diagram to show how TKR is involved in cell signalling.

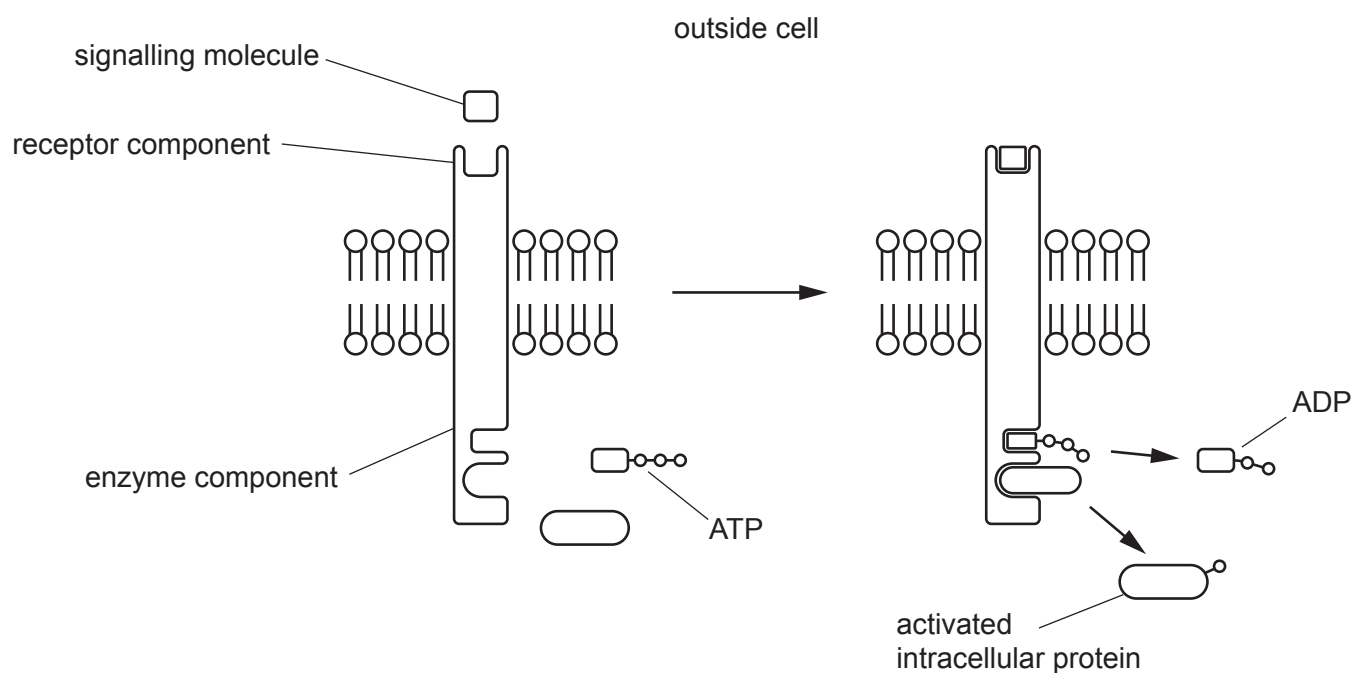


Fig. 3.1

- (a) Most enzymes are specific to one reaction.

With reference to Fig. 3.1, explain how the structure of an enzyme provides its specificity.

.....

.....

.....

.....

.....

.....

..... [2]

The drug GNF-5 is used in the treatment of some cancers. GNF-5 affects the activity of TKR by binding to the enzyme component of the complex.

Researchers investigated the effect of GNF-5 on the activity of TKR using different concentrations of ATP solution. In an experiment the activity of TKR was measured with no GNF-5 **and** with GNF-5.

The results are shown in Fig. 3.2.

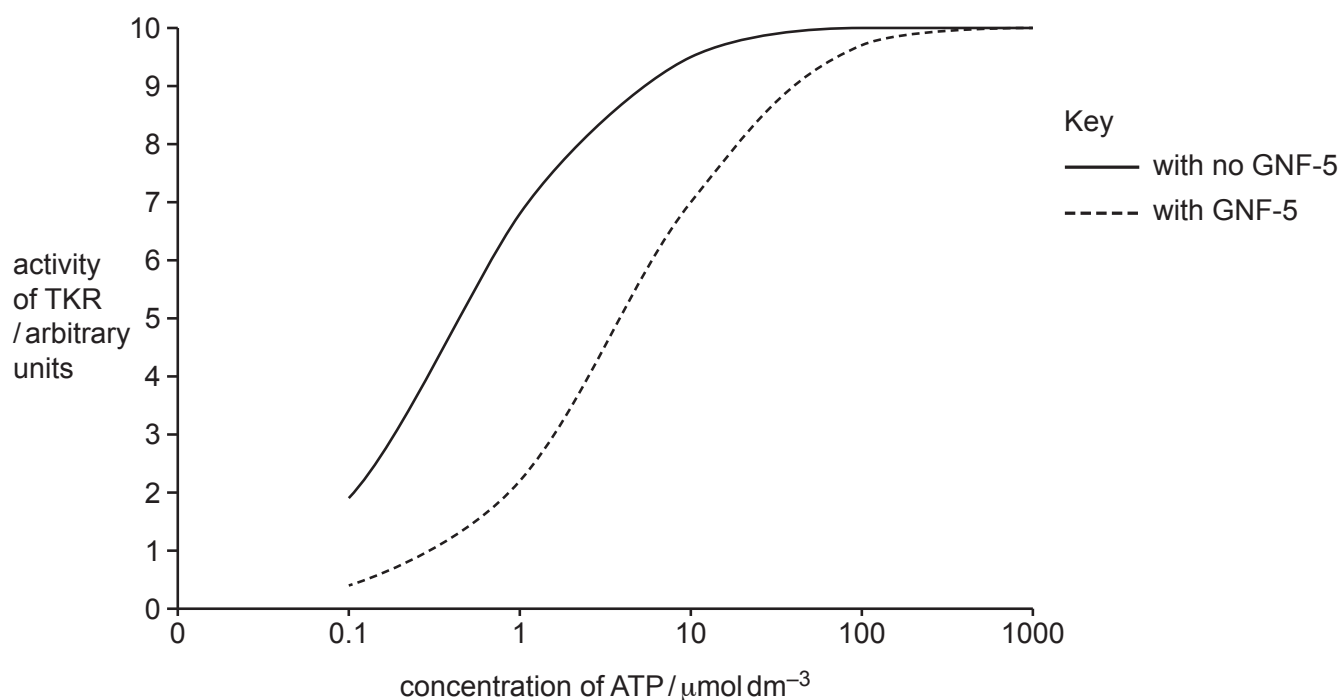


Fig. 3.2

- (b) The researchers concluded that GNF-5 acts as an inhibitor of the enzyme component of TKR **and** that it is a competitive inhibitor.

Use Fig. 3.2 to provide evidence for these conclusions.

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

- (c) A mutation of the gene coding for TKR results in changes to the enzyme component of TKR. This altered form of TKR is known as T315L.

The effect of GNF-5 on the activity of T315L was also investigated.

The results of this investigation are shown in Fig. 3.3.

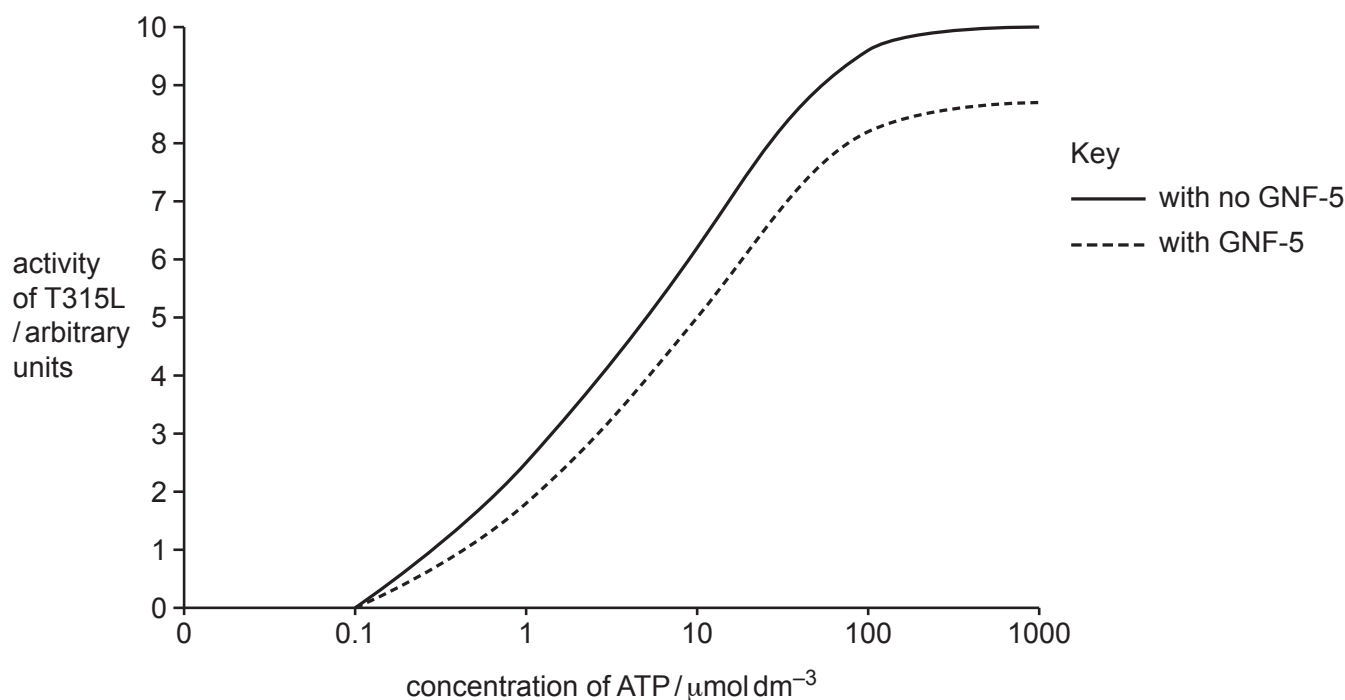


Fig. 3.3

Use Fig. 3.2 **and** Fig. 3.3 to:

- (i) State how the activity of T315L differs from TKR when **no** GNF-5 was present.

.....
.....
..... [1]

- (ii) State how the effect of GNF-5 on T315L differs from the effect of GNF-5 on TKR.

.....
.....
.....
.....
.....
..... [2]

[Total: 8]

- All carbonic anhydrase enzymes catalyse the same reversible reaction, shown in Fig. 4.1.

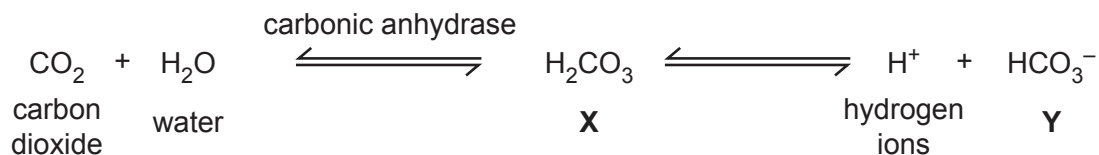


Fig. 4.1

[2]

- Suggest how all carbonic anhydrase enzymes can catalyse the same reaction, even though they have different primary structures.

.....

.....

..... [1]

- Outline the similarities and differences between the introns and the exons of genes coding for proteins such as carbonic anhydrase.

.....

..... [3]

All mammals have the same type of carbonic anhydrase, known as α -carbonic anhydrase. Many different forms, or isoforms, of α -carbonic anhydrase have been identified in mammals.

There are 15 isoforms of α -carbonic anhydrase (CA) in humans. Cells of different tissues have one or more isoforms. Within cells the isoforms may be in different locations.

(d) Red blood cells contain two isoforms, CA1 and CA2.

Suggest the location of CA1 and CA2 in red blood cells **and** give a reason for your answer.

.....

.....

.....

.....

..... [2]

(e) Isoform CA6 forms part of human breast milk. Mammary gland cells package CA6 in Golgi vesicles for release from the cells.

Name the transport mechanism associated with CA6 secretion.

..... [1]

(f) Human CA isoforms in some epithelial cells in the eye have a role in the formation of the clear fluid of the eye known as aqueous humour. Overactivity of the enzyme may lead to a harmful increase of pressure within the eye and cause a condition known as glaucoma.

Acetazolamide is a therapeutic drug that can be used in the treatment of glaucoma. It acts as a reversible non-competitive inhibitor.

Describe the mechanism of action of acetazolamide as a reversible non-competitive inhibitor of carbonic anhydrase.

.....

.....

.....

.....

.....

.....

.....

..... [3]

- 5 Arachidonic acid is a fatty acid that is a common component of phospholipids.

Phospholipids can be used as a source of arachidonic acid when it is metabolised within cells in an enzyme-catalysed pathway known as the cyclooxygenase (COX) pathway.

The final products of the COX pathway can be different in different cell types, causing a range of responses. In some cells, the products are involved in the inflammatory response, which is a response by the body to infection. In other cells, cell division is stimulated.

Fig. 5.1 shows the **first reaction** in the COX pathway. This reaction is catalysed by an enzyme known as COX-2.

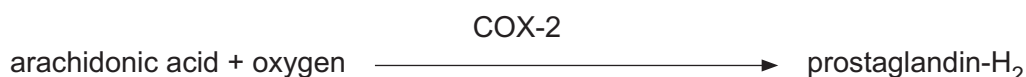


Fig. 5.1

- (a) The enzymes involved in the COX pathway are located in the membrane of rough endoplasmic reticulum.

Suggest the advantages to the cell of enzyme pathways being located in cell membranes, rather than in the cytosol of the cell (fluid portion of cytoplasm).

.....
.....
.....
..... [2]

- (b) During an inflammatory response, compounds produced by the COX pathway cause an increased sensitivity to pain.

Some anti-inflammatory drugs are reversible competitive inhibitors of COX-2.

Fig. 5.2 shows how increasing arachidonic acid concentration affects COX-2 activity.

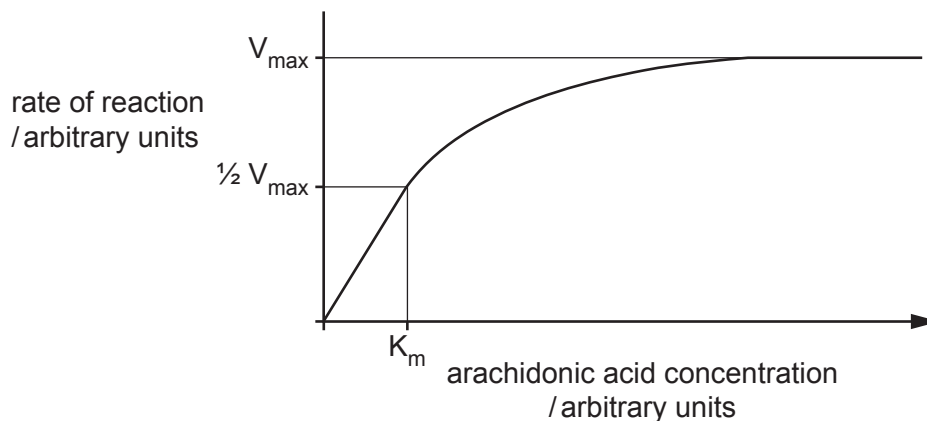


Fig. 5.2

9700 Biology Paper 2 - Enzymes

Topical questions | 39 questions

29. Question part 2/3 | 9700 May-June 2022 P2 V3 Q5

- (i) Sketch on Fig. 5.2 the curve obtained if an anti-inflammatory drug, which is a **competitive** inhibitor, is present with arachidonic acid. [1]

- (ii) Complete the statements to show whether the maximum rate of reaction ($V_{\max}$) and the Michaelis-Menten constant (K_m) of COX-2 **increases**, **decreases**, or **stays the same** in the presence of a competitive inhibitor.

In the presence of a competitive inhibitor:

$V_{\max}$ of COX-2

K_m of COX-2 [2]

- (c) COX-2 is composed of two identical polypeptides. The enzyme is produced when a gene, *PTGS2*, located on chromosome 1, is switched on and transcription begins.

- (i) Using gene *PTGS2* and enzyme COX-2 as examples, explain what is meant by a gene.

.....
.....
.....
.....
..... [2]

- (ii) Some mutations in *PTGS2* lead to an increased rate of transcription. These mutations have been linked to an increased risk of certain types of cancer.

Suggest why mutations in *PTGS2* may increase the risk of cancer.

.....
.....
.....
.....
..... [2]

(d) Fig. 5.3 shows the molecular structure of arachidonic acid. Not all hydrogen atoms are shown.

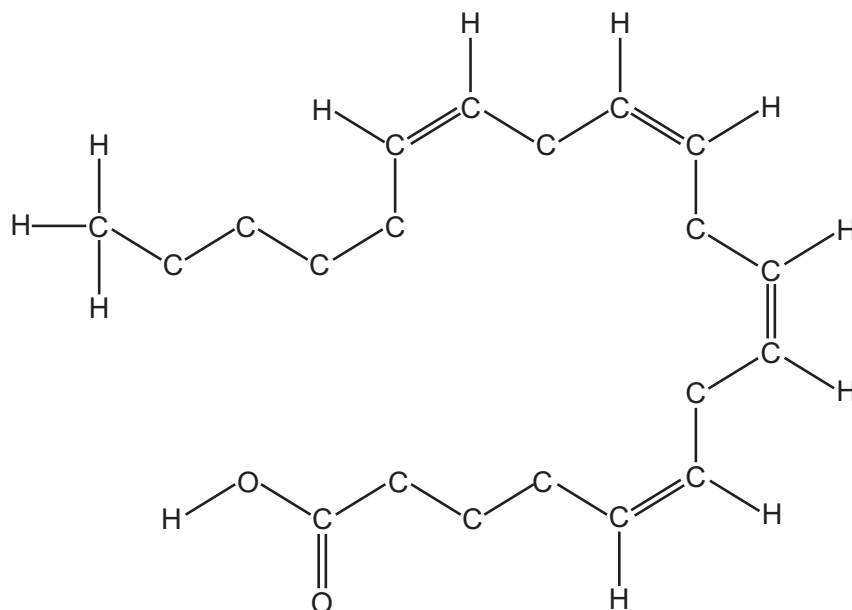


Fig. 5.3

With reference to Fig. 5.3, explain why increasing the proportion of phospholipids with arachidonic acid in a cell will increase the fluidity of the cell surface membrane of the cell.

.....

.....

.....

.....

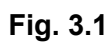
.....

.....

..... [3]

[Total: 12]

The reaction catalysed by lactase is summarised in Fig. 3.1.



In your answer, identify **Y** and product **Z**.

[4]

[4]

(b) On a commercial scale, immobilised lactase can be used to produce lactose-free milk.

One of the products of the reaction shown in Fig. 3.1 acts as an inhibitor of lactase. This is an example of product inhibition.

(i) Explain why product inhibition is useful in *K. lactis* when lactase is acting as an intracellular enzyme, but can be a disadvantage when extracted lactase is used free in solution for the production of lactose-free milk.

.....

.....

.....

.....

..... [2]

(ii) Suggest how using immobilised lactase for the production of lactose-free milk helps to reduce the problem of product inhibition.

.....

..... [1]

(iii) The first large-scale production of lactose-free milk with an immobilised enzyme used lactase trapped in cellulose triacetate fibres.

Suggest **one** feature of cellulose triacetate that makes it useful as an immobilising material.

.....

..... [1]

(c) When developing an enzyme-catalysed reaction for use in industry, the progress of the reaction is studied.

Outline how the progress of an enzyme-catalysed reaction can be investigated experimentally.

.....

.....

.....

.....

.....

.....

..... [3]

31. Question part 1/2 | 9700 Oct-Nov 2023 P2 V3 Q2

- 2 Glucose is used in the synthesis of amylose. Glucose is first converted to glucose 1-phosphate (G 1-P).

Starch phosphorylase is an intracellular enzyme that can catalyse the synthesis of amylose from G 1-P, which is the substrate for the reaction:



n = a large number

Students used a colorimeter to investigate the progress of the reaction.

The students made a reaction mixture containing 0.01 mol dm^{-3} G 1-P in a buffer solution at pH6.0. A very small quantity of amylose was added to initiate the reaction.

A solution of starch phosphorylase was added to the reaction mixture and samples were taken at 1-minute intervals. Each sample was added to a dilute iodine solution, stirred and then poured into a cuvette. The absorbance of each solution was recorded.

The results of the investigation are shown in Fig. 2.1.

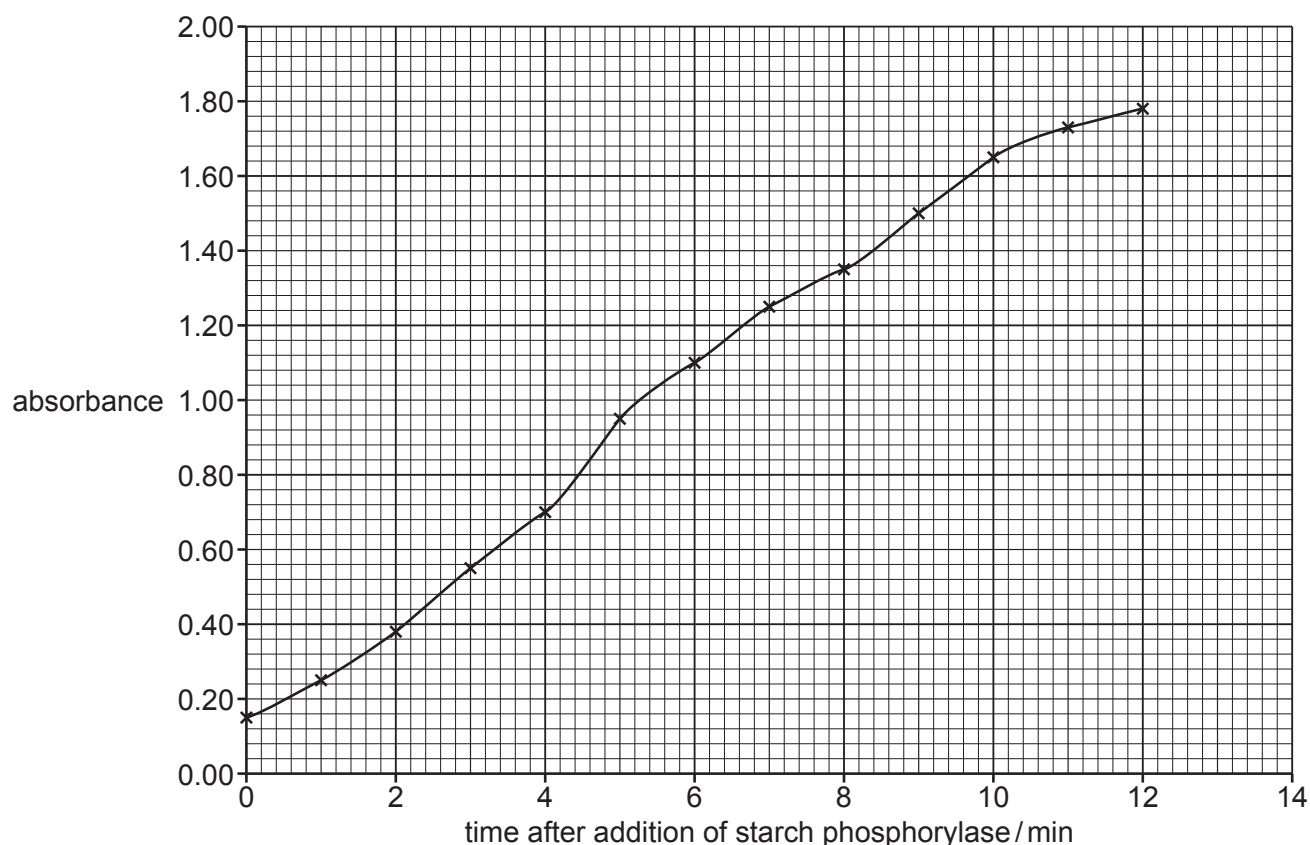


Fig. 2.1

- (a) (i) Explain why the absorbance increases, as shown in Fig. 2.1.

.....

.....

..... [1]

(ii) The students took their final sample at 12 minutes.

Predict the results for absorbance if the students had continued to take samples for a further 10 minutes. Explain your answer.

prediction

.....

.....

explanation

.....

.....

[2]

(iii) State an advantage of using a colorimeter in determining the progress of the reaction.

.....

.....

.....

..... [1]

(b) The reaction catalysed by starch phosphorylase occurs at the ends of amylose molecules.

Describe the sequence of events that occurs when starch phosphorylase catalyses the addition of a molecule of glucose to the end of an amylose molecule.

.....

.....

.....

.....

.....

.....

.....

.....

.....

..... [4]

32. Question | 9700 May-June 2024 P2 V2 Q3

3 Lysozyme is an antibacterial enzyme that was discovered in 1921 by Alexander Fleming, the scientist who discovered penicillin.

Lysozyme catalyses the hydrolysis of glycosidic bonds present in peptidoglycan molecules to form smaller products, NAG (N-acetylglucosamine) and NAM (N-acetylmuramic acid).

(a) Before the induced fit hypothesis was proposed in 1958, scientists believed that the lock and key hypothesis explained how lysozyme catalyses the hydrolysis of peptidoglycan to its products.

Draw labelled **and** annotated diagrams in the space provided to show how the **lock and key hypothesis** was used to explain the mechanism of action of lysozyme on peptidoglycan.

[3]

(b) Lysozyme and penicillin can be described as antibacterial agents.

Compare lysozyme **and** penicillin to show the similarities **and** differences between these two antibacterial agents.

.....

.....

.....

.....

.....

.....

.....

.....

..... [3]

[Total: 6]

Milk that contains casein has a cloudy, white appearance. As the casein is hydrolysed by trypsin, the milk changes in appearance to a clear (transparent), colourless solution.

A student carried out an experiment to investigate the effect of enzyme concentration on the rate at which trypsin hydrolyses casein.

The student added a solution of trypsin to a sample of milk and recorded the time taken for the milk to become transparent. The student repeated the experiment with different concentrations of trypsin. All other variables were kept constant.

Fig. 5.1 shows the results from the experiment.

- (a) (i) When the concentration of trypsin increases from 2.0% to 4.0%, the time taken for the milk to become transparent decreases by 48%.

Calculate the percentage decrease in the time taken for milk to become transparent when the concentration of trypsin increases from 0.25% to 0.5%.

Write your answer to the nearest whole number.

percentage decrease [1]

- (ii) Explain the results shown in Fig. 5.1.

..... [5]

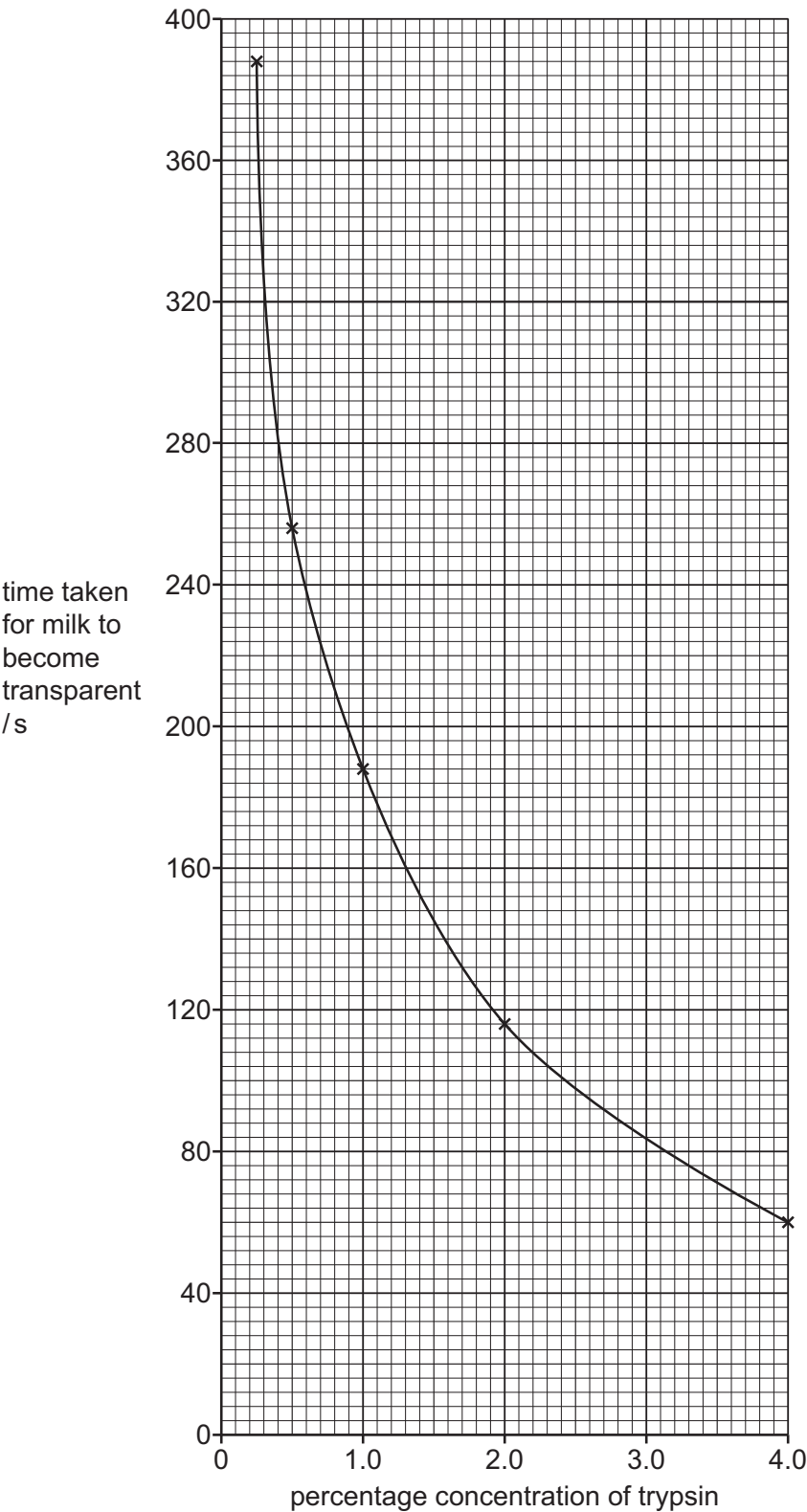


Fig. 5.1

33. Question part 3/3 | 9700 Oct-Nov 2024 P2 V1 Q5



(b) Trypsin has the potential to be used in a wide range of industrial processes.

The use of immobilised enzymes in industrial processes has many advantages.

Scientists investigated the effect of temperature on the activity of trypsin immobilised on the surface of a material and trypsin free in solution.

Table 5.1 shows the results of the investigation.

Table 5.1

temperature / °C	percentage of maximum activity of immobilised trypsin	percentage of maximum activity of trypsin free in solution
25	60	100
35	85	100
45	98	80
55	95	20
65	100	5

(i) State a reason for the difference in percentage of maximum activity of immobilised trypsin and trypsin free in solution at 25 °C.

.....

.....

..... [1]

(ii) Suggest **and** explain why the percentage of maximum activity of immobilised trypsin at 55 °C is higher than the percentage of maximum activity of trypsin free in solution at 55 °C.

.....

.....

.....

.....

.....

..... [2]

- Scientists investigated the production of sugars from crop waste for biofuel production. The scientists discovered that a strain of the fungus *Penicillium citrinum*, isolated from soil, was a good source of three different extracellular enzymes, **M**, **N** and **O**. These enzymes break down polysaccharides in cell walls.

The results are shown in Table 4.1.

enzyme	maximum activity /arbitrary units
M	292.83
N	111.72
O	6.54

The scientists carried out further research so that a solution containing the three enzymes (enzyme mixture) could be used for the most efficient production of sugars from crop waste.

..... [4]

34. Question part 2/2 | 9700 Oct-Nov 2024 P2 V3 Q4



(b) Students investigated the composition of the cell wall of leaf cells of thale cress, *Arabidopsis thaliana*. The students began by isolating the cell wall components from the rest of the cell material.

The students used enzymes extracted from a fungal pathogen of *A. thaliana* to hydrolyse the cell wall components to smaller molecules.

The students prepared a reaction mixture containing the cell wall components and the enzymes.

After 24 hours, they separated and identified the smaller molecules found in the reaction mixture.

Four types of molecule were identified:

- short chains of β -glucose
- β -glucose
- peptides
- amino acids.

Explain the presence of these molecules in the reaction mixture after 24 hours of hydrolysis.

.....

.....

.....

.....

.....

.....

.....

.....

..... [4]

[Total: 8]

35. Question part 1/5 | 9700 May-June 2025 P2 V1 Q3

- 3 (a) Scientists investigated the progress of reactions catalysed by two enzymes: dopa oxidase and neutrase. The reactions catalysed by these enzymes result in changes to the appearance of the reaction mixtures.

The reactions are shown in Fig. 3.1.

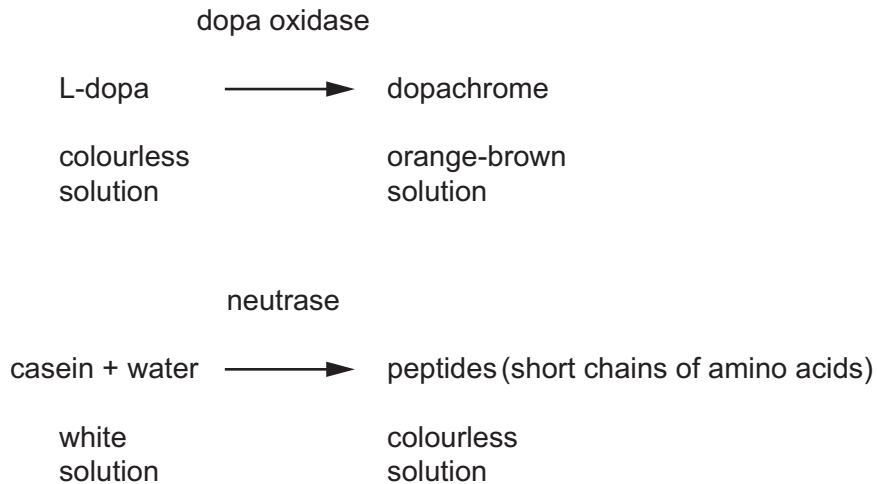


Fig. 3.1

The changes in appearance of the reaction mixtures make it possible to follow the reactions using a colorimeter.

Fig. 3.2 shows the progress of the reaction catalysed by dopa oxidase as recorded from a colorimeter.

Fig. 3.3 shows the progress of the reaction catalysed by neutrase as recorded from a colorimeter.

35. Question part 2/5 | 9700 May-June 2025 P2 V1 Q3

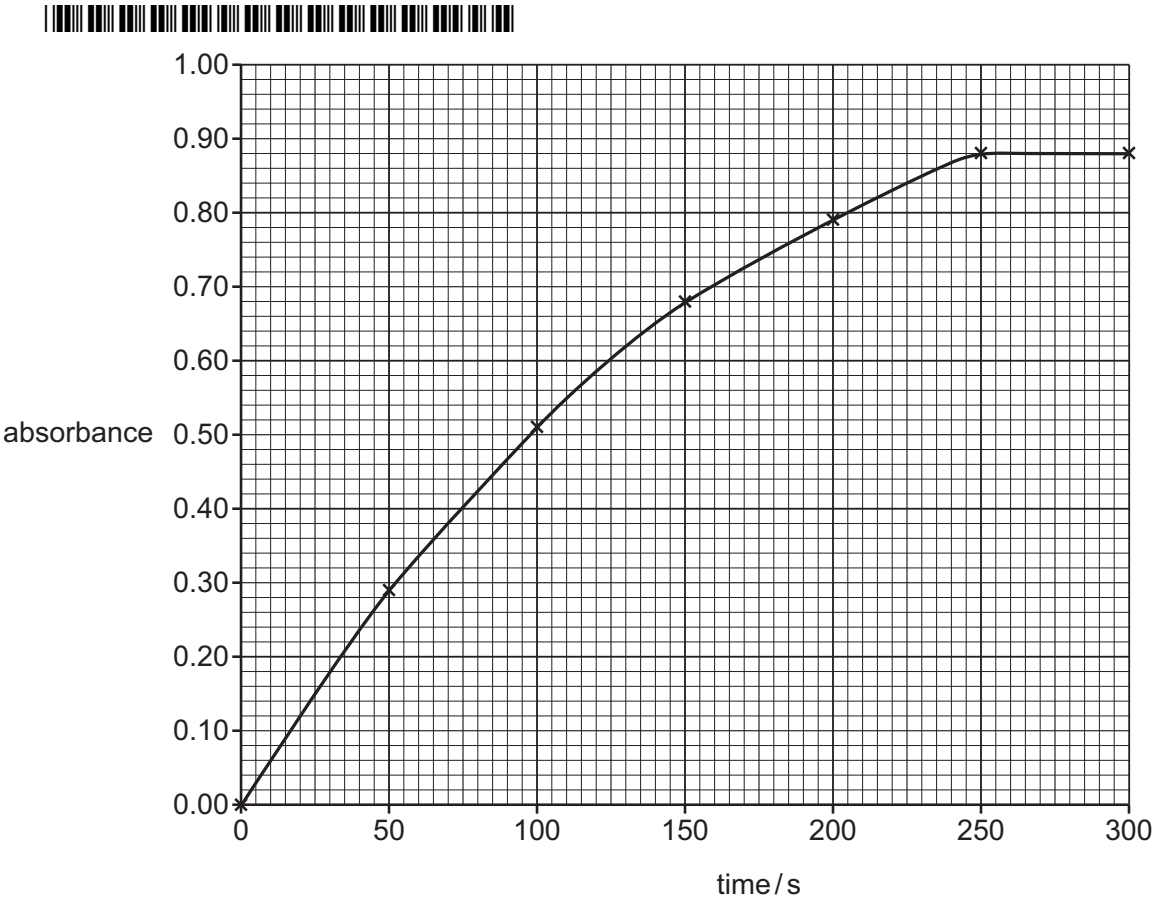


Fig. 3.2



- [3]

35. Question part 4/5 | 9700 May-June 2025 P2 V1 Q3



(ii) Suggest **two** advantages of using a colorimeter to investigate the progress of reactions such as those shown in Fig. 3.1.

.....

.....

.....

.....

..... [2]

(b) Scientists searching for a suitable enzyme to use in an industrial process isolated the bacterium *Vibrio parahaemolyticus* from the mouth of the Mediterranean eel, *Muraena helena*.

The scientists discovered an enzyme in the bacterium that was suitable for the industrial process. The scientists named the enzyme VpSP37.

The scientists investigated how the rate of reaction catalysed by VpSP37 is affected by the concentration of its substrate. The results of the investigation are shown in Fig. 3.4.

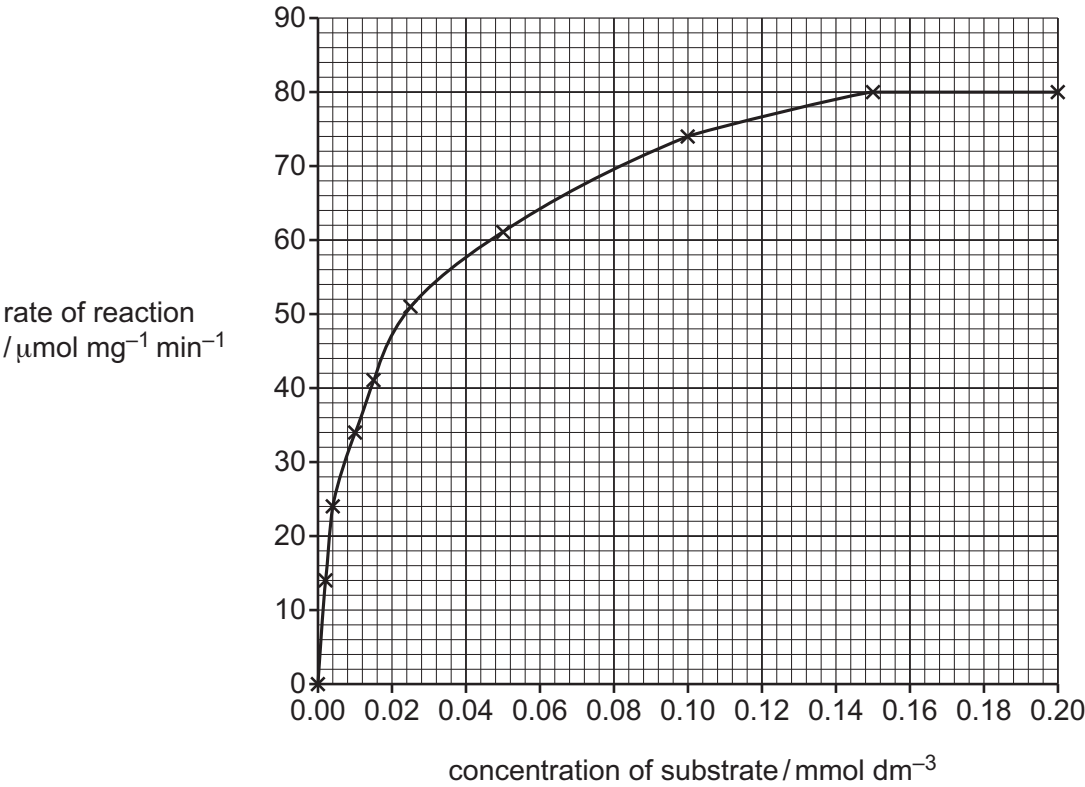


Fig. 3.4

35. Question part 5/5 | 9700 May-June 2025 P2 V1 Q3



- (i) Calculate the Michaelis–Menten constant, K_m , for the enzyme VpSP37 using the information in Fig. 3.4.

Show your working.

$K_m =$ [2]

- (ii) The scientists discovered other enzymes that were suitable for the industrial process. These enzymes had higher K_m values than VpSP37.

Explain the advantage of using the enzyme VpSP37 in the industrial process rather than one of these other enzymes with higher K_m values.

.....

.....

.....

.....

..... [2]

[Total: 9]

36. Question part 1/3 | 9700 May-June 2025 P2 V2 Q6

6 Horseradish peroxidase (HRP) is an enzyme that can be extracted from the roots of the horseradish plant, *Armoracia rusticana*. HRP is used extensively in industry and technology.

In the reaction catalysed by HRP, hydrogen peroxide (H_2O_2) is used to oxidise an organic substrate. This is summarised in Fig. 6.1.

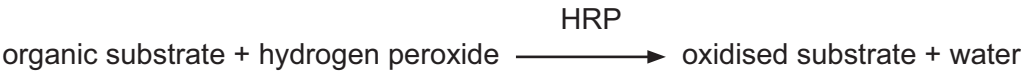


Fig. 6.1

(a) Inhibitors can have an effect on V_{max} , the maximum rate of reaction, and K_m , the Michaelis–Menten constant, of HRP.

Fig. 6.2 shows the effect of substrate concentration on the rate of reaction of HRP.

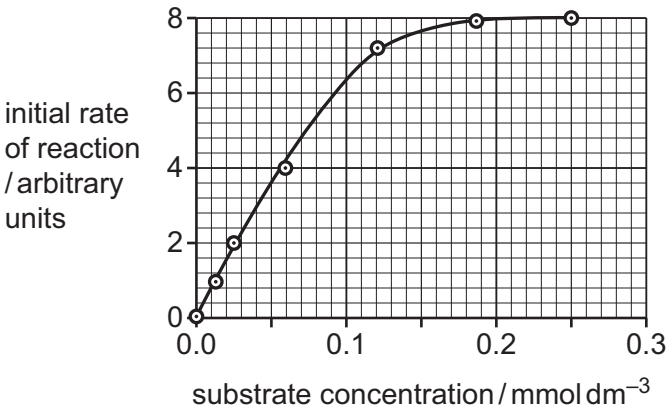


Fig. 6.2

Complete Fig. 6.2 by drawing a curve to show how the presence of a non-competitive inhibitor will affect the rate of reaction of HRP.

Use the curve **you have drawn** to obtain an estimate of K_m .

$K_m = \dots\dots\dots$ [2]

(b) Scientists can design synthetic DNA nucleotide sequences to produce a synthetic HRP gene. These sequences will include a start codon and a stop codon so that translation of messenger RNA (mRNA) can occur.

Explain what is meant by a start codon **and** a stop codon.

.....

.....

.....

..... [2]

36. Question part 2/3 | 9700 May-June 2025 P2 V2 Q6



- (c) HRP is used in an immunological test known as a sandwich ELISA. One use of the test is to diagnose disease.

Fig. 6.3 outlines the main steps in a sandwich ELISA in which a toxin released by a pathogen is detected in a sample of body fluid taken from a person who is ill.

The test involves two types of monoclonal antibody that can bind to the toxin, a capture antibody and a detection antibody.

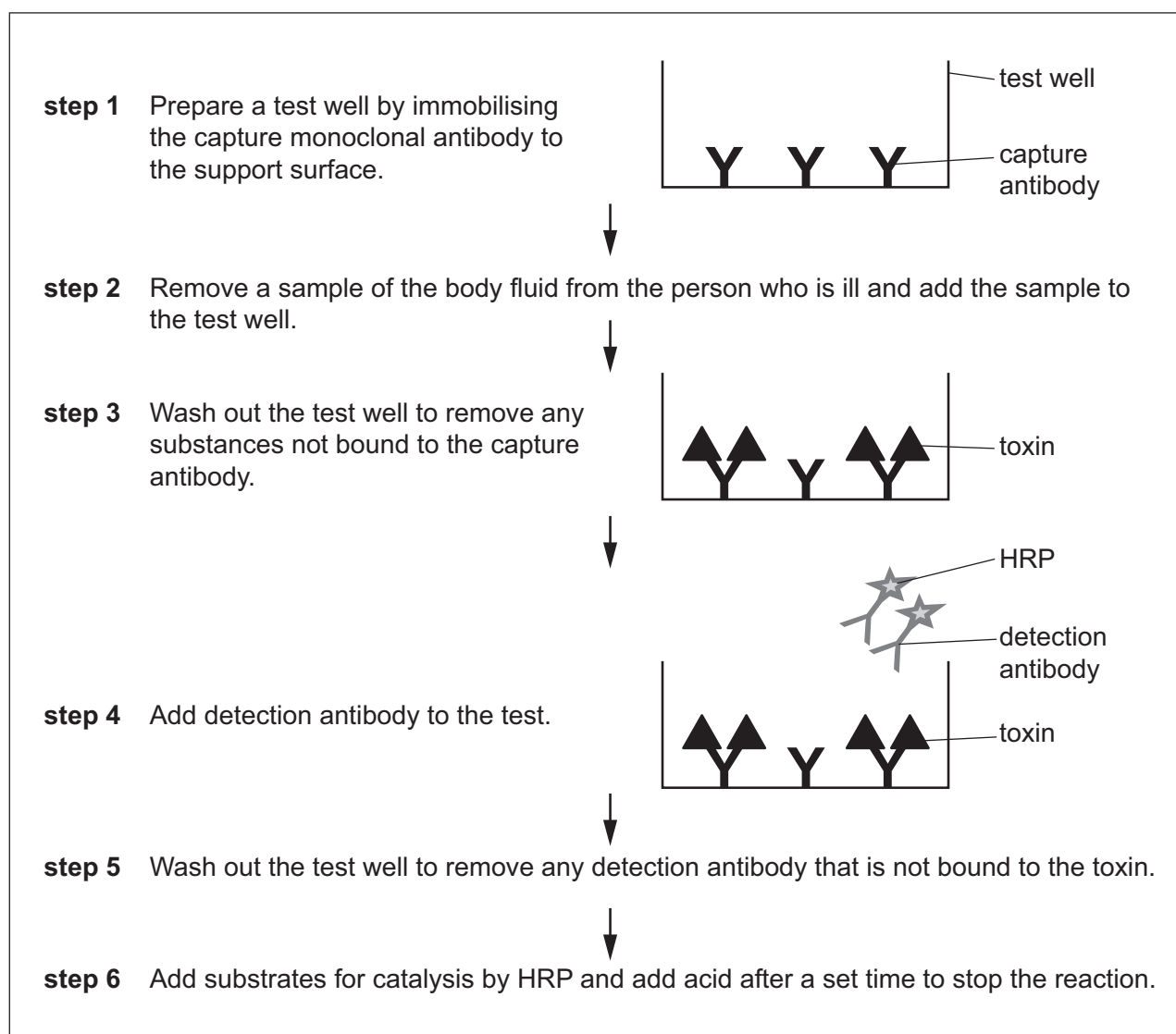


Fig. 6.3

- (i) Complete Fig. 6.4 to show the situation in the test well after **step 5**.



Fig. 6.4

36. Question part 3/3 | 9700 May-June 2025 P2 V2 Q6



(ii) With reference to Fig. 6.3, describe **one** difference between the capture antibody and the detection antibody.

.....

.....

.....

..... [1]

(iii) Fig. 6.5 outlines the reaction catalysed by HRP in **step 6**. The organic substrate, TMB, is in excess and changes colour when it is oxidised. This indicates a positive test result.



Fig. 6.5

A student suggested that:

- a low concentration of toxin may be diagnosed as a negative result instead of a positive result
- using a colorimeter after **step 6** in the sandwich ELISA test would provide a quantitative result and help to avoid this error.

With reference to Fig. 6.5, explain why using a colorimeter would provide a quantitative measurement for detection of a low concentration of the toxin and help to confirm a positive result.

.....

.....

.....

.....

.....

.....

.....

.....

.....

..... [4]

37. Question part 1/3 | 9700 May-June 2025 P2 V4 Q2

- 2 The human pancreas synthesises and secretes a number of different digestive enzymes. These enzymes have their effect in the small intestine.

Pancreatic lipase acts by breaking the bond between glycerol and fatty acids in triglycerides.

- (a) State the term used to describe any enzyme that is secreted to the outside of the cell where it has its effect.

..... [1]

- (b) One of the triglycerides found in olive oil is shown in Fig. 2.1.

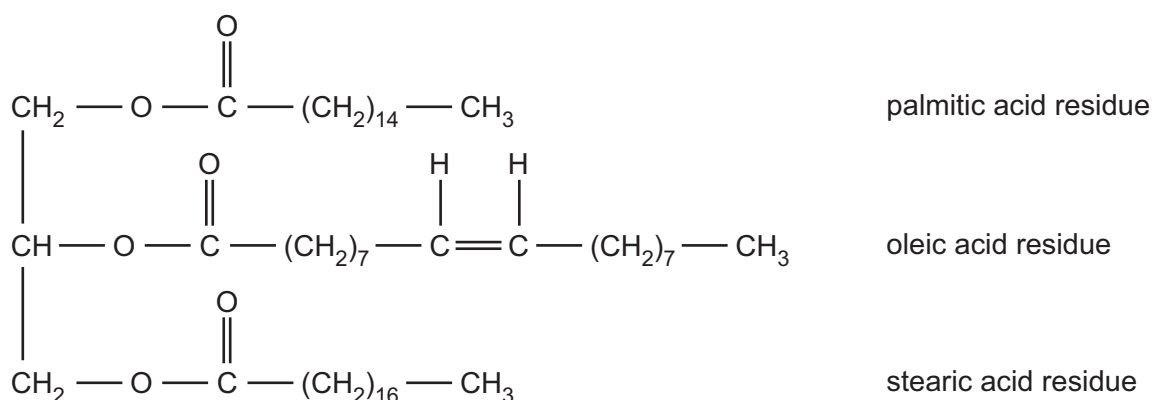


Fig. 2.1

- (i) Draw an arrow on Fig. 2.1 to show where the bond between the glycerol residue and the stearic acid residue is broken by lipase digestion. [1]
- (ii) State the type of reaction that occurs to produce stearic acid from this triglyceride **and** name the type of bond that is broken.

type of reaction

type of bond

[2]

37. Question part 2/3 | 9700 May-June 2025 P2 V4 Q2



Investigations were carried out into the activity of a lipase extracted from a strain of bacterium that lives in hot springs.

- (c) The activity of the bacterial lipase was measured at 37 °C using different concentrations of olive oil as the substrate.

The results were used to derive a Michaelis–Menten constant (K_m) of 91.76 mmol dm⁻³.

Explain what is meant by a K_m of 91.76 mmol dm⁻³.

.....

.....

.....

.....

..... [2]

- (d) Fig. 2.2 shows the results of an investigation into the effect of temperature on the activity of the bacterial lipase.

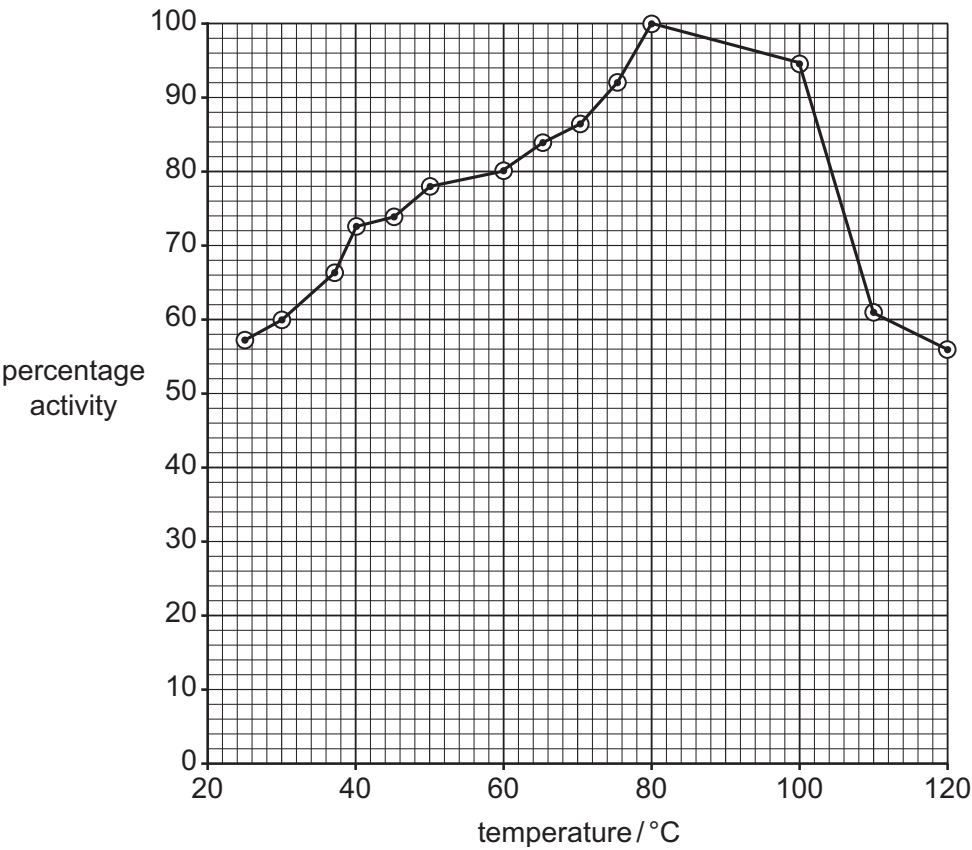


Fig. 2.2

9700 Biology Paper 2 - Enzymes

Topical questions | 39 questions

37. Question part 3/3 | 9700 May-June 2025 P2 V4 Q2



(i) Describe **and** explain the results shown in Fig. 2.2.

..... [4]

(ii) An investigation using human pancreatic lipase at the same range of temperature will produce different results from those shown in Fig. 2.2.

Predict how the trend will be different from that shown in Fig. 2.2.

..... [2]

[Total: 12]

38. Question part 1/4 | 9700 Oct-Nov 2025 P2 V1 Q3

- 3 (a) The lock-and-key hypothesis and the induced-fit hypothesis are used to describe the interaction of enzymes and their substrates.

Describe **one** similarity and **one** difference between the lock-and-key hypothesis and the induced-fit hypothesis.

similarity

.....

.....

difference

.....

.....

[2]

- (b) Phosphorylase enzymes can catalyse the synthesis of starch **and** the breakdown of starch in some plant tissues. A reaction catalysed by starch phosphorylase is shown in Fig. 3.1.

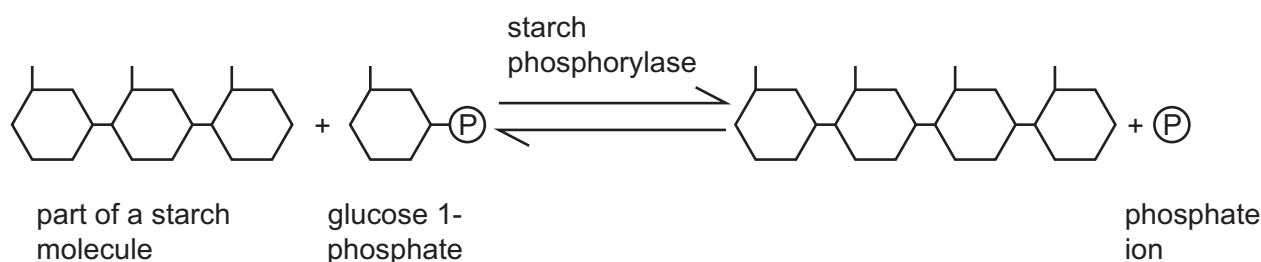


Fig. 3.1

A student carried out an experiment to study the synthesis of starch by phosphorylase found in potato tissue.

The student was provided with a solution, **E**, extracted from potato tissue. The extract was filtered to remove all the starch grains.

Extract **E** contained biological molecules from the potato tissue including phosphorylase but **no** starch.

- (i) Iodine solution was used to confirm that starch was **not** present in extract **E**.

State the colour observed when iodine solution was added to a sample of extract **E**.

..... [1]

38. Question part 2/4 | 9700 Oct-Nov 2025 P2 V1 Q3



The student added a small drop of a dilute starch solution and a solution of glucose 1-phosphate to a test-tube containing extract **E**.

Samples of the reaction mixture in the test-tube were removed every minute and a drop of iodine solution was added to each.

The student used a colorimeter to measure the absorbance of the solution in each sample.

The results are shown in Fig. 3.2.

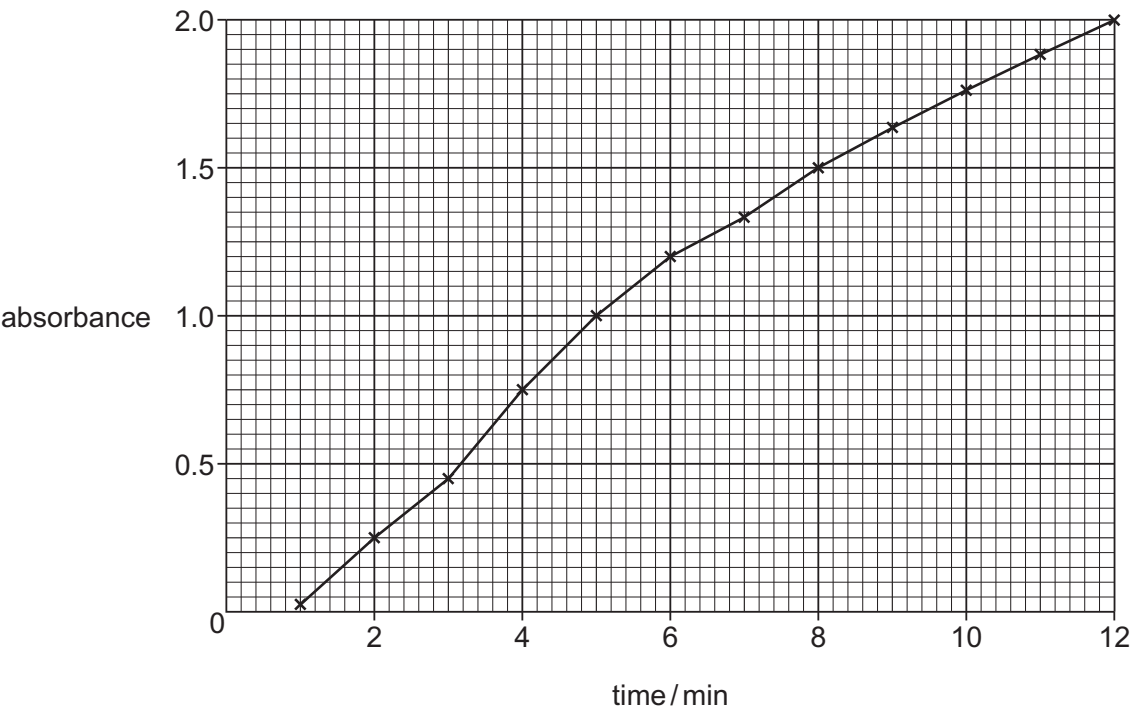


Fig. 3.2

(ii) Explain the results shown in Fig. 3.2.

.....

.....

.....

.....

.....

.....

..... [3]

38. Question part 3/4 | 9700 Oct-Nov 2025 P2 V1 Q3



(iii) After 12 minutes, the student added a solution containing phosphate ions to the reaction mixture. The student continued taking samples every minute, adding iodine solution to each sample.

The absorbance of the solution in each of these samples was measured. The results showed that the absorbance decreased over time.

Suggest why the absorbance decreased.

.....

.....

..... [1]

38. Question part 4/4 | 9700 Oct-Nov 2025 P2 V1 Q3



(c) Muscle cells contain glycogen phosphorylase.

Fig. 3.3 shows the effect of caffeine on the activity of glycogen phosphorylase at different concentrations of substrate.

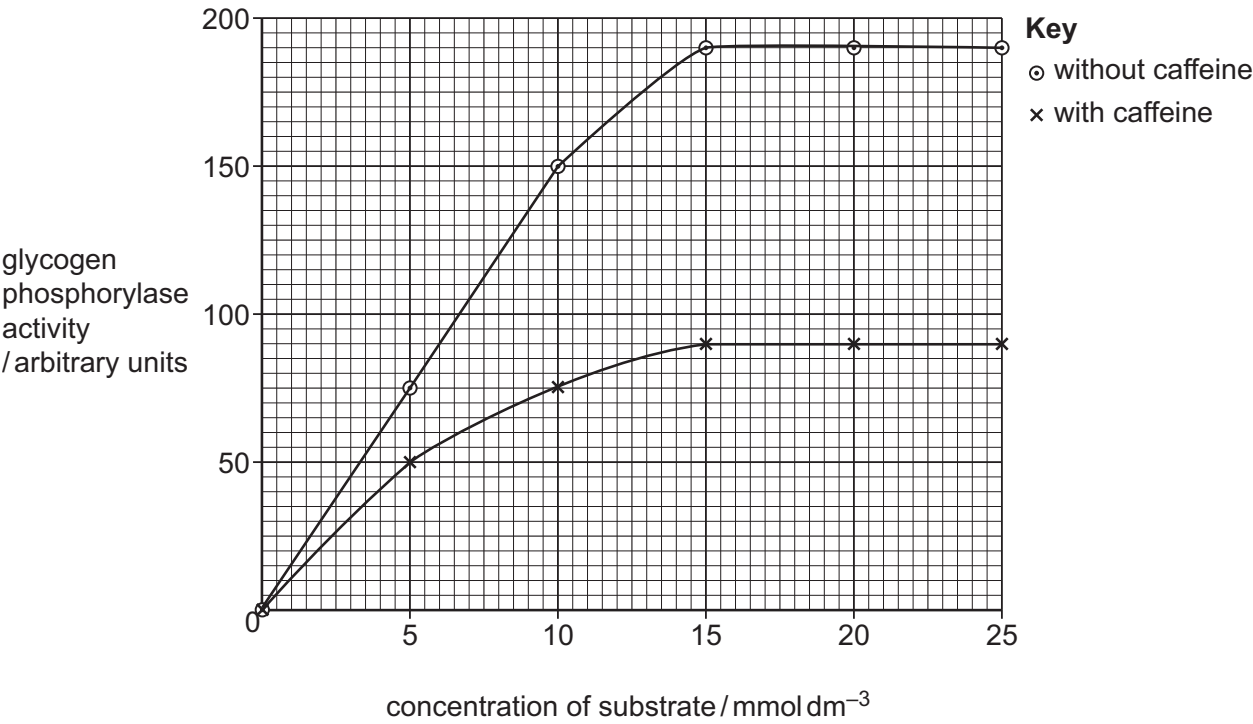


Fig. 3.3

A student concluded that caffeine acts as a non-competitive inhibitor of glycogen phosphorylase.

Explain how the results in Fig. 3.3 support this conclusion.

.....

.....

.....

.....

.....

.....

.....

..... [3]

[Total: 10]

39. Question part 1/4 | 9700 Oct-Nov 2025 P2 V3 Q3

- 3 (a) Describe the induced-fit hypothesis of enzyme action.

.....

.....

.....

.....

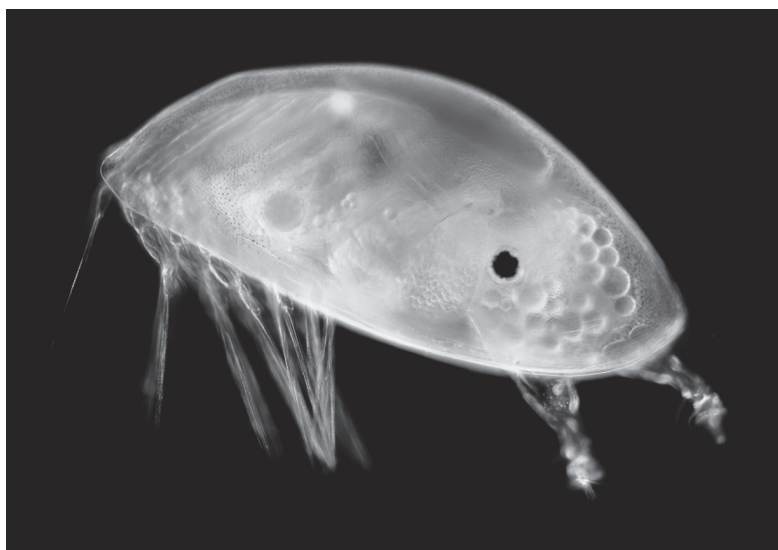
.....

.....

..... [3]

- (b) Many marine organisms can become attached to hard surfaces such as rocks or the surfaces of ships. These organisms are known as fouling organisms.

The larva of the acorn barnacle, *Amphibalanus amphitrite*, is an example of a fouling organism. One of these barnacle larvae is shown in Fig. 3.1.



magnification $\times 160$

Fig. 3.1

The larvae of *A. amphitrite* use a protein to attach themselves to the surfaces of ships.

It is expensive to remove fouling organisms from ships. Scientists have developed substances to prevent the attachment of larvae. However, some of these substances are toxic and have been responsible for a decrease in marine biodiversity.

Scientists investigated the effect of using an immobilised protease, subtilisin A, to prevent the attachment of the larvae of *A. amphitrite* to surfaces.

The scientists used 4 different concentrations of subtilisin A which had been immobilised onto the surface of a polymer film. As a control they used denatured subtilisin A immobilised onto the surface of the same polymer. Glass slides were also used as a control.



The larvae were released into 6 tanks of artificial sea water:

- 4 tanks, each with a polymer surface and a different concentration of immobilised subtilisin A
- 1 tank with a polymer surface and denatured immobilised subtilisin A
- 1 tank with glass slides instead of a polymer surface.

The number of larvae that attached to the different surfaces in the tanks was counted after 24 hours and again after 48 hours.

The number of larvae attached in each tank was expressed as the percentage of the total number of larvae released in each tank. The results are shown in Fig. 3.2.

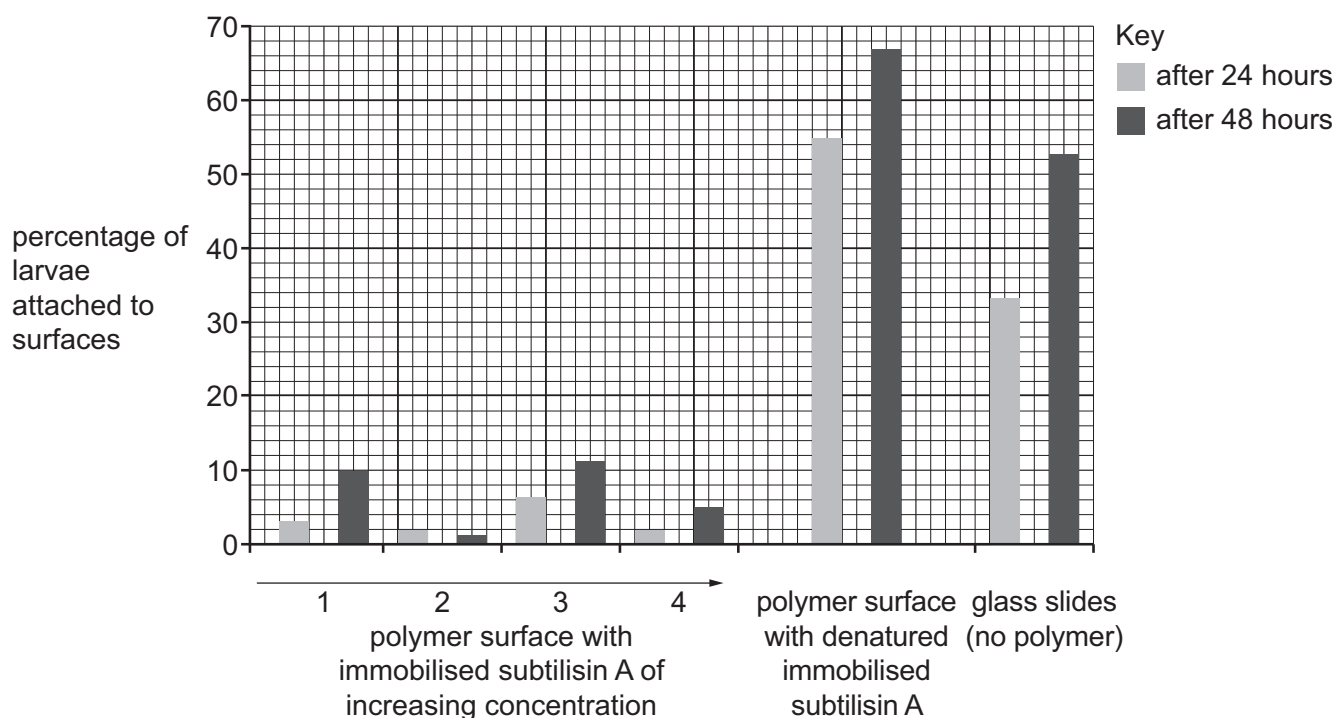


Fig. 3.2

- (i) With reference to the data in Fig. 3.2, discuss whether subtilisin A is effective in preventing the attachment of the larvae.

..... [4]

39. Question part 3/4 | 9700 Oct-Nov 2025 P2 V3 Q3



- (ii) The scientists extended their investigation by applying the polymer with immobilised subtilisin A to the outside of the bottom of small ships.

Two factors that need to be taken into consideration in this type of investigation are the temperature and pH of the sea water.

Outline **two other** factors that need to be taken into consideration when investigating the suitability of immobilised subtilisin A as an anti-fouling agent for ships.

.....

.....

.....

.....

.....

..... [2]

[Total: 9]

9700 Biology Paper 2 - Enzymes

Topical questions | 39 questions

39. Question part 4/4 | 9700 Oct-Nov 2025 P2 V3 Q3



Question 4 starts on page 12.