GradeMax

Cambridge International A Level

Cambridge International Examinations
Cambridge International Advanced Level

CANDIDATE NAME

CENTRE NUMBER

CANDIDATE NUMBER

5726098057

BIOLOGY **9700/52**

Paper 5 Planning, Analysis and Evaluation **October/November 2015**

1 hour 15 minutes

Candidates answer on the Question Paper.

No Additional Materials are required.

READ THESE INSTRUCTIONS FIRST

Write your Centre number, candidate number and name on all the work you hand in.
Write in dark blue or black pen.
You may use an HB pencil for any diagrams or graphs.
Do not use staples, paper clips, glue or correction fluid.
DO **NOT** WRITE IN ANY BARCODES.

Answer **all** questions.

Electronic calculators may be used.

You may lose marks if you do not show your working or if you do not use appropriate units.

At the end of the examination, fasten all your work securely together.
The number of marks is given in brackets [ ] at the end of each question or part question.

This document consists of **8** printed pages.

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1 Protease enzymes catalyse the hydrolysis of protein. There are a number of different types of protease enzyme.

A student was provided with two proteases that hydrolyse the amino acid chains of protein in different ways, producing mixtures of single amino acids and peptides of varying lengths.

1. An endoprotease that hydrolyses peptide bonds between specific amino acids within the protein molecule. The enzyme only functions if there is a minimum of two amino acids on each side of the hydrolysis site.

2. An exoprotease that hydrolyses the terminal peptide bond of a molecule. The enzyme only functions if the substrate molecule has a minimum of three amino acids.

The student used these enzymes to hydrolyse a protein formed by the linking of two polypeptides.

Fig. 1.1 shows the possible hydrolysis sites of these two enzymes on this protein.

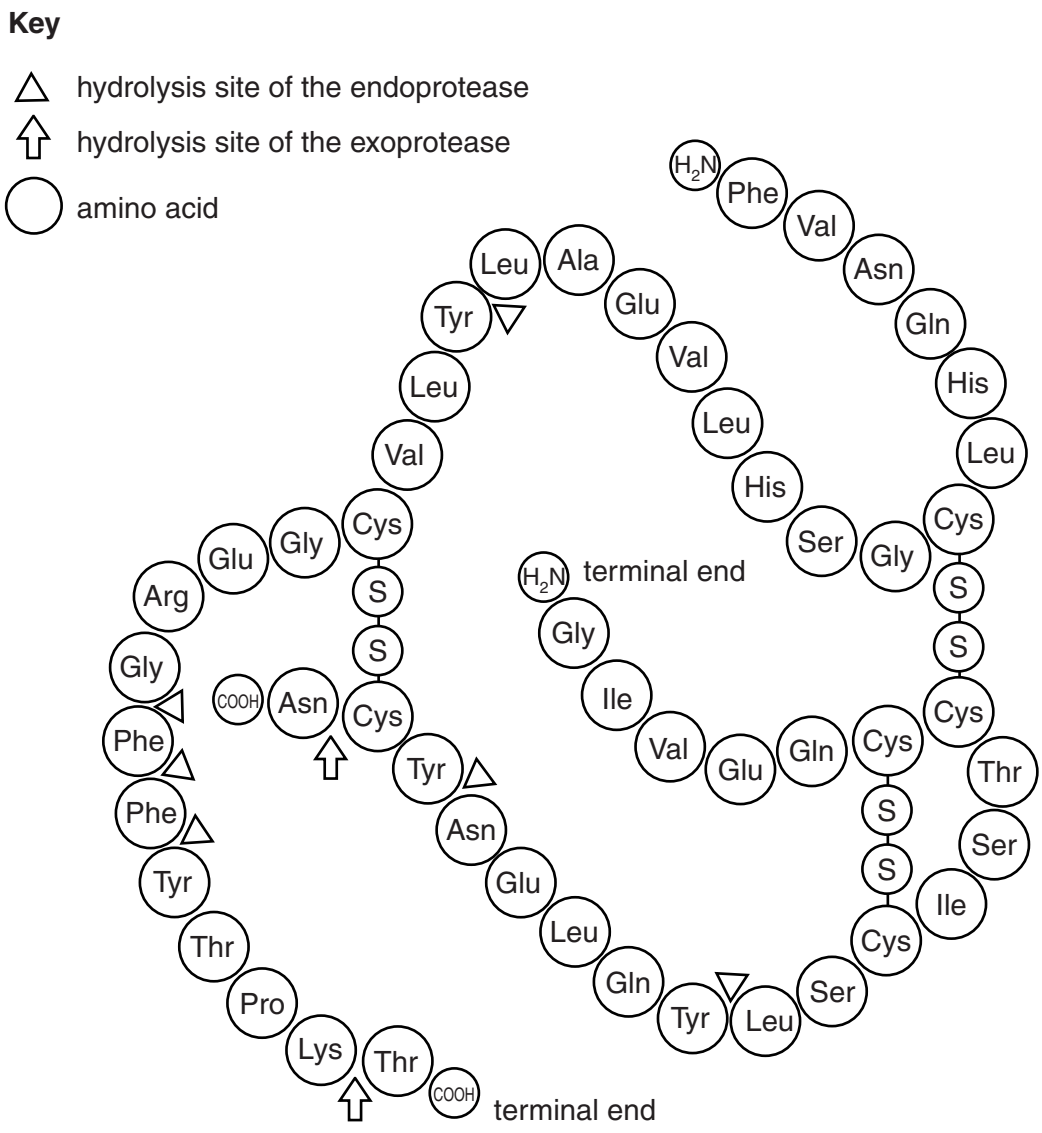

Fig. 1.1

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(a) The student investigated the effect of these two enzymes on the hydrolysis of this protein by incubating the protein separately with each of the enzymes.

- Each mixture of enzyme and protein was incubated at 35 °C and at a pH of 7.6.
- At intervals of 5 minutes, samples of each mixture were removed using a capillary tube.
- The products of hydrolysis of each mixture were separated by chromatography using the same solvent.
- The products of hydrolysis were located by spraying the chromatogram with the same specific dye.
- The student continued sampling every 5 minutes and running chromatograms for each mixture until hydrolysis of the protein was completed by each enzyme.

(i) Identify the **independent** variable in this investigation.

.....[1]

(ii) Identify **three** variables, **other than** the chromatography solvent and the specific dye, that the student has standardised in this investigation.

Describe how the student might have standardised **two** of these variables.

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.....[3]

(iii) Suggest how the student determined when the hydrolysis was complete.

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.....[1]

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(b) Describe a method that the student could use to prepare and use chromatograms to compare the changes in the products of hydrolysis of the protein by the two different proteases over time.
Your method should be detailed enough for another person to follow.

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(c) Fig. 1.2 shows the appearance of the chromatograms at the times when hydrolysis by the different enzymes was complete.

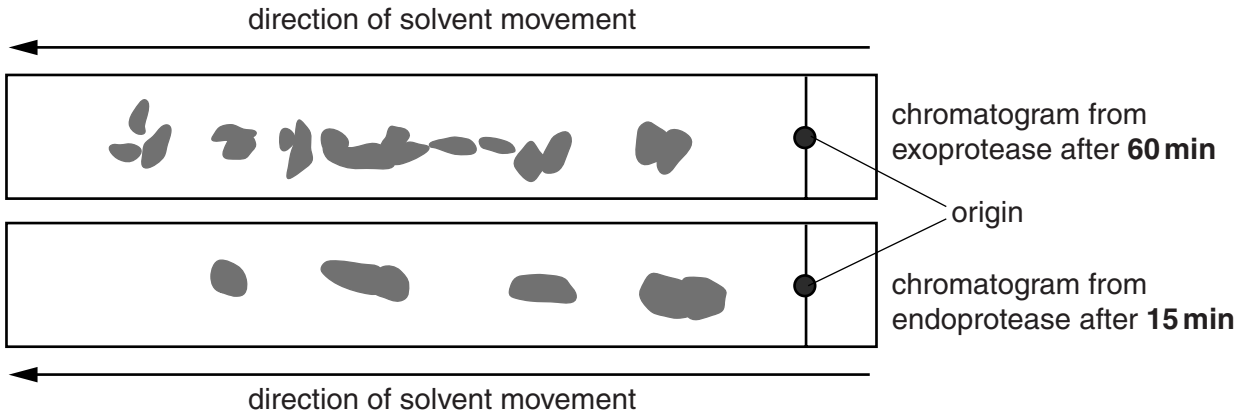

Fig. 1.2

The student concluded that:

1. The endoprotease worked faster than the exoprotease because fewer bonds were hydrolysed.
2. The products of hydrolysis of the exoprotease were all single amino acids giving more spots on the chromatogram.
3. The larger peptides released by the endoprotease have fewer charges and moved more slowly than single amino acids and small peptides.
4. Hydrolysing the protein with a mixture of endoprotease and exoprotease would give the same results as for the exoprotease more quickly.

State **and** explain whether each of these conclusions about the hydrolysis of protein is supported **or** not supported by the evidence in Fig. 1.2 and the information in the question about these two enzymes.

conclusion 1 .....

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conclusion 2 .....

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conclusion 3 .....

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conclusion 4 .....

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.....[4]

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(d) Using an internet search the student found that:

- electrophoresis can also be used to separate the products of enzyme hydrolysis of proteins
- a combination of electrophoresis and chromatography was used to study haemoglobin from people with sickle cell anaemia (SCA).

In an investigation, the haemoglobin from people with SCA and people without the disease was hydrolysed by an endoprotease. The resulting mixture was placed centrally on one side of chromatography paper and separated by electrophoresis.

Negatively charged peptides moved to the anode (+) and positively charged peptides moved to the cathode (–). The paper was turned through 90° and chromatography used to separate the mixture.

Fig. 1.3 shows the main stages of this experiment.

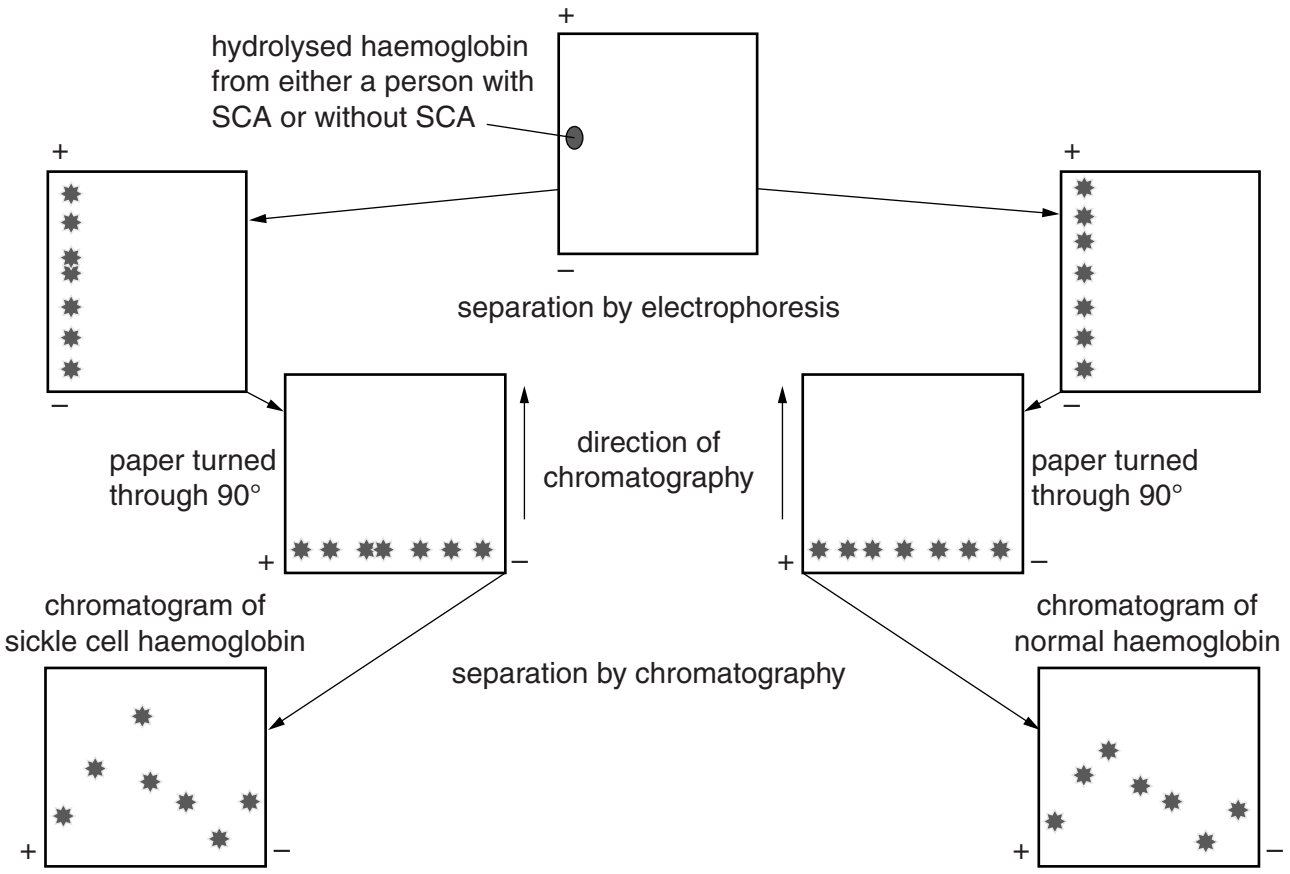

Fig. 1.3

(i) Sickle cell haemoglobin and normal haemoglobin have a difference in amino acid sequence.

On Fig. 1.3, draw a circle around the spot in each chromatogram that shows that the two types of haemoglobin have different amino acid sequences. [1]

(ii) Explain your answer to **(i)**.

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[Total: 19]

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2 Aphids are serious pests of many crop plants, causing wilting and spreading virus infections. A newly developed hybrid of a crop plant was found to be very susceptible to aphid infestation. Aphid infestations are usually controlled by spraying with insecticide.

An investigation into the effect of an insecticide was carried out by counting the number of aphids on leaves of the hybrid crop plant before and after treatment.

The insecticide was dissolved in an oily liquid.

- Ten hybrid plants were divided into two groups of five, **A** and **B**.
- Five leaves on each plant were used for the investigation.
- The total number of aphids on **both** surfaces of the five test leaves of each sample plant was counted before treatment.
- The upper surfaces of 5 leaves on the plants in group **A** were sprayed with insecticide.
- The lower surfaces of 5 leaves on the plants in group **B** were sprayed with the same insecticide.
- The same procedure was used to spray the leaves of control plants with a liquid.
- The total number of aphids on **both** surfaces of the treated leaves of all the plants was counted 24 hours, 48 hours and 72 hours after treatment.

(a) (i) State the **dependent** variable in this investigation.

.....[1]

(ii) Suggest what liquid should be used as the control.

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.....[1]

Table 2.1 shows the results of the investigation.

Table 2.1

time/h	mean total number of aphids on **both** surfaces of leaves ± standard error (S_M)			
	Group **A** upper surfaces of leaves sprayed		Group **B** lower surfaces of leaves sprayed	
	control	treated	control	treated
0	75 ± 5	75 ± 8	80 ± 20	85 ± 15
24	80 ± 6	85 ± 15	85 ± 25	3 ± 1
48	85 ± 5	90 ± 10	98 ± 20	2 ± 1
72	110 ± 4	110 ± 8	115 ± 25	2 ± 1

(b) (i) State what is meant by standard error.

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.....[1]

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(ii) Identify the results from Table 2.1 which suggest that the difference in the numbers of aphids as a result of the insecticide treatment may be significant.

Give a reason for your answer.

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.....[2]

(c) Identify **three other** features of the results shown in Table 2.1.

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(d) A *t*-test was used to find out if the number of aphids in Group **A** and in Group **B**, 72 hours after treatment with insecticide, differed significantly.

(i) State **one** reason why a *t*-test was used.

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(ii) State a null hypothesis for this test.

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.....[1]

(iii) The number of degrees of freedom for this statistical test is 48.

Describe how this is calculated.

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[Total: 11]

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