Bio Factsheet

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Answering Questions: enzyme activity

It is certain that there will be a question about enzyme activity in your exams! Every session – Jan and Jun of each exam board – features a question about active sites or the factors affecting enzyme activity. Some exam Boards –AQA especially –like to "dress up" the question by asking about an enzyme or application you've probably never heard of. It doesn't matter, the basic biology is always the same.

This Factsheet summarises the most common questions about enzyme activity that have appeared over the 5 years on all the Boards.

The Basics: 12 facts you MUST know before entering the exam hall

- enzymes are globular proteins which act as biological catalysts
- they speed up the rate of reaction by lowering the activation energy.
- enzymes are a complex tertiary and sometimes quaternary shape and catalyse reactions by forming a complex (known as the enzyme substrate complex) at a specific region of the enzyme called the active site.
- the active site has a specific shape and is formed from just a few amino acids
- the substrate – the substance that the enzyme acts upon – has a complementary structure to this active site
- this means that only this substrate can fit into the enzyme's active site
- thus, enzymes are specific -any individual enzyme can usually only catalyse one particular reaction
- the mechanism by which enzyme and substrate come together is called the induced-fit hypothesis
- substrate approaches the active site of the enzyme
- the shape of the active site then changes to fit precisely around the substrate – in other words, the substrate **induces** the active site to change shape
- the reaction is catalysed and products form
- the products are a different shape from the substrate and therefore diffuse away from the active site. As they do, the active site reverts to its original shape.

Once you have learned these 12 facts, some of the exam questions become a doddle:

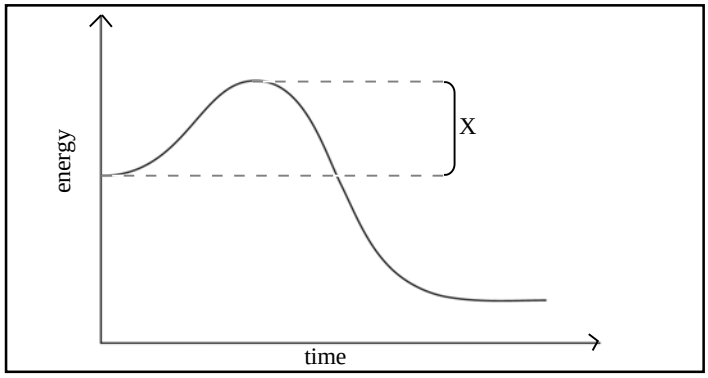
Activation energy

Before a chemical reaction can proceed, bonds need to be broken. For example, before sucrose can be converted into glucose and fructose, the bonds inside sucrose must be broken. The energy needed to do this is called the activation energy.

Enzymes work by lowering the activation energy. Fig 1 shows the energy changes which take place when heated hydrogen peroxide breaks down to water and oxygen. $2H_2O_2 \rightarrow 2H_2O + O_2$

X represents the activation energy. Inside cells, this reaction is catalysed by the enzyme **catalase**. Catalase is an extremely efficient enzyme. The **turnover number** of an enzyme is the number of substrate molecules converted to product per second. The maximum turnover number of catalase is 200 000 molecules per second.

Fig1



Sometimes you may be asked to explain why the turnover number varies with changing conditions. Why, for example, would the turnover number increase if the temperature at which the reaction took place was increased? This is exactly the same as asking why the rate increases with temperature (molecules have more energy/ move faster/more collisions / more enzyme-substrate complexes form etc)

Question

Explain how the shape of an enzyme molecule is related to its function. 4 marks

Examiners markschemes are brief; they contain the key terms that get the marks. Thus, you need to practice writing just the key terms – in this case –

active site *3D shape* *tertiary structure* *substrate* *complementary shape* *induced fit*

And then join them up with a few words in between:

Enzymes have an active site. Its complex 3D shape is determined by the tertiary structure of the protein. The substrate has a complementary shape and this means that and only that substrate can be acted upon by the enzyme.
The mechanism by which enzyme and substrate bind is called induced fit

This would get maximum marks. Don't waffle and don't say the same thing twice.

Denaturation

Questions about denaturation are common.

- All enzymes have an optimum temperature and pH, at which they work most efficiently.
- extremes of temperature or pH cause denaturation
- the tertiary structure/shape changes
- because H^+/ionic bonds break
- the shape of the active site is changed and the substrate is no longer a complementary shape/ is unable to bind to the active site.

Typical exam questions just ask for the basic facts
Question
Salivary amylase breaks down starch. It works best at a pH of 8. Explain why it will not function in the stomach where the pH is much lower. *(4 marks)*

The last 4 bullet points get full marks

Enzyme experiments

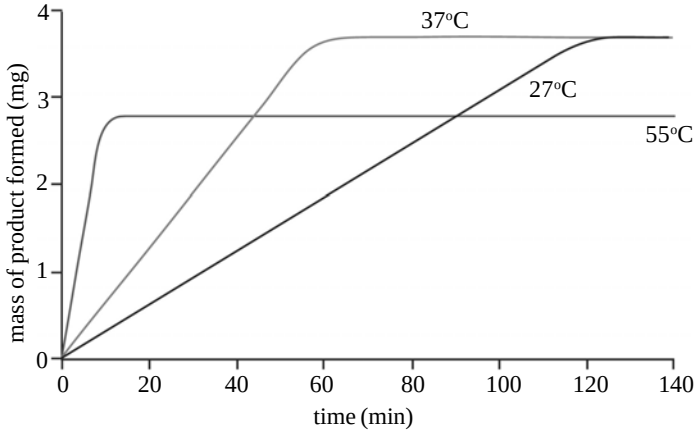
You may also be asked to draw, label or interpret graphs about enzyme experiments.

You can calculate the rate of an enzyme –catalysed reaction in two ways:

- By measuring the rate at which the substrate disappears
- By measuring the rate at which products form

Both of these approaches can come up in questions. Usually, the experiments are looking at the effect of temperature or pH on the rate of activity.

Here is a graph produced by a student investigating the effect of temperature on the amount of product formed.



Examiners like this type of graph because it enables them to identify students who are just regurgitating instead of thinking. Most students – upon seeing that the question is about temperature and enzymes – will want to write " at high temperatures enzymes are denatured ". This is true enough but look carefully at the graph. Over the first 10 minutes, the temperature that produced most product was 55°C –denaturation isn't instantaneous. Often, you will be asked to explain why the rate of reaction increases with temperature.

Exam Hint:- *"Many candidates have an extremely simplistic idea of denaturation. They believe that all enzymes denature instantly once the temperature rises above 37 °C."*

Here's part of a markscheme:

molecules have more (kinetic) energy/ are moving faster;
R: vibrate more collisions / more enzyme substrate complexes formed;

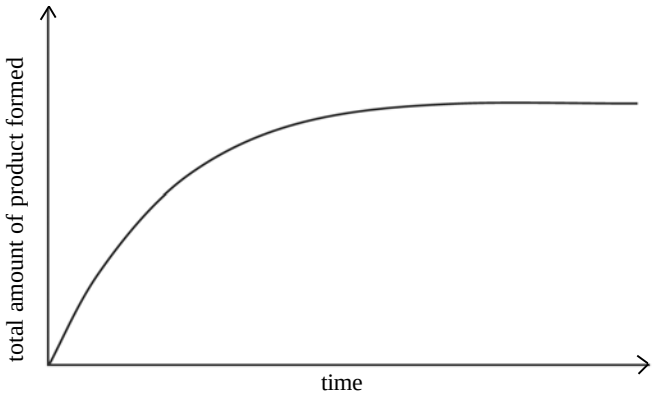
Note that if you write " the molecules vibrate faster" you do not get the mark. You must be precise at this level.

Look at the graph again. What evidence is there that denaturation occurred at 55 °C?

No more product was formed after 10 mins but it did at the other two temperatures.

Note that the mass of product formed was the same at the lower two temperatures. This is because the initial substrate concentration was the same and it has all now been converted into product.

The graph below shows the total amount of product formed during an enzyme-controlled reaction.



Make sure that you can explain all parts of this curve.
Why is it steep initially?
Why does the rate slow down?
Why does it plateau?

- initially, there was plenty of both enzyme active sites and substrate. Hence, product formed rapidly
- the rate slowed as the substrate was used up/ broken down/ became limiting
- maximum possible number of enzyme-substrate complexes have formed/ maximum turnover rate achieved
- eventually, all the substrate has been used up

Occasionally, you may be asked to explain why particular procedures were followed. Mostly, this is common sense.

For example, if you are investigating the effect of temperature on the rate of a reaction, you need to make sure that **both** the enzyme and the substrate are at the desired temperature before you mix them. Often, enzyme solutions are made by dissolving a known mass of enzyme in a buffer solution. A buffer solution is one which maintains a constant pH when hydrogen or hydroxyl ions are added to it. Buffers occur naturally and play an important role in keeping conditions inside living organisms constant. If you are investigating the effect of temperature on enzyme activity, then you do not want pH to be changing too; hence a buffer is essential.

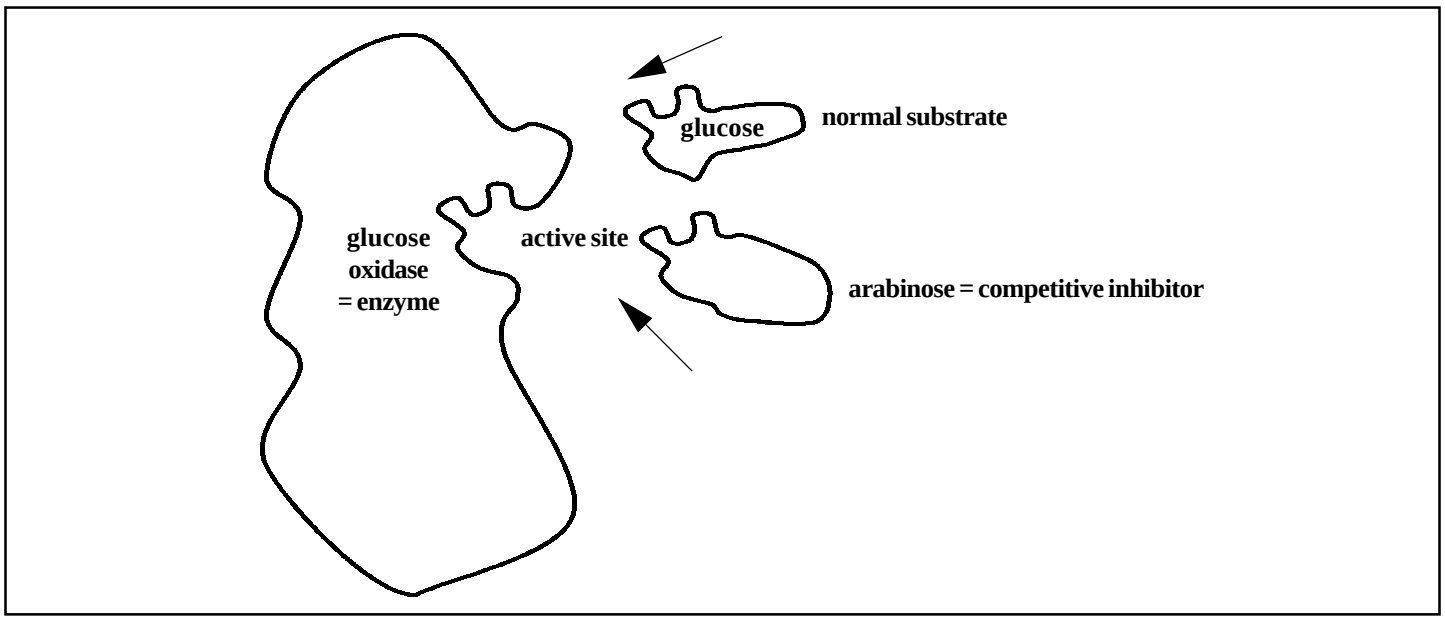
Exam Hint : *Be precise. State what buffers do. "Some candidates offered generalised answers in which they merely stated that buffers allowed enzymes to work better"*

Inhibitors

Inhibitors slow down the rate of reaction, giving the cell greater control of a reaction.

Competitive inhibitors are structurally similar **(but not identical)** to the normal substrate and compete with the substrate for the active sites

Competitive inhibition



Non-competitive inhibitors bind to the enzyme but not at the active site. This distorts the shape of the active site, so stopping the substrate from binding to it. Thus, there are fewer/no enzyme-substrate complexes formed and the reaction slows/stops

Exam Hint - *"Weaker candidates implied that competitive inhibitors and substrates were identical rather than chemically similar".*

Immobilised enzymes

In immobilisation the enzyme is held in a column of insoluble agent through which the substrate flows. Whole cells or extracted enzymes can be immobilised. The binding agent can be agar gel, cellulose, porous alumina or porous glass).

A very common question simply asks for the advantages and disadvantages of using immobilized enzymes

Advantages:

- stable at high temperature and across a large pH range
- can make more product/make product faster at high temperature;
- no contamination of product
- easy to re-use/recover the enzyme;
- can be used in continuous process;
- high temperature would denature other unwanted enzymes/would kill contaminating microorganisms;

Disadvantages:

- substrate needs to diffuse through the gel/membrane
- smaller surface area of enzyme
- enzyme at lower concentration when immobilised
- immobilised enzymes have little kinetic energy
- Immobilisation can affect shape of active site so fewer enzyme-substrate complexes form

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