

Enzymes: summary notes

These notes cover Topic 3 (Enzymes) of the Cambridge International AS & A Level Biology syllabus (9700). They follow the syllabus topics, with the practical skills collected in Section 7. The first part covers how enzymes work: globular protein catalysts, the active site, the lock-and-key and induced-fit hypotheses, activation energy, and intracellular and extracellular enzymes. The second part covers what affects enzyme activity: temperature, pH, enzyme concentration, substrate concentration and inhibitor concentration, $V_{\max}$ and the Michaelis–Menten constant K_m , competitive and non-competitive inhibition, and immobilised enzymes. The practical section shows how to measure rates, use a colorimeter and work out a rate from a tangent. Worked examples appear throughout, and the notes end with a glossary and quick-check questions with answers.

1. What enzymes are

Enzymes are **globular proteins** that act as **biological catalysts**. They speed up metabolic reactions but are not used up or permanently changed. Each enzyme molecule can therefore be used again and again.

Because they are globular proteins:

- they have a precise **tertiary structure**, held by hydrogen bonds, ionic bonds, disulfide bonds and hydrophobic interactions between R groups (some enzymes also have quaternary structure)
- they are roughly spherical, and hydrophilic R groups on the outside make them **soluble** in water
- a small region of the molecule, the **active site**, has a specific three-dimensional shape and charge pattern that is complementary to the substrate
- anything that changes the tertiary structure, such as high temperature or extreme pH, can change the shape of the active site

Learn these definitions

Enzyme: a globular protein that acts as a biological catalyst, speeding up a reaction by lowering its activation energy, without being permanently changed.

Active site: the region of an enzyme, made of a small number of amino acids, that binds the substrate and where the reaction is catalysed; its shape is complementary to that of the substrate.

Activation energy: the minimum energy that must be supplied for a reaction to start.

Intracellular and extracellular enzymes

Feature	Intracellular enzymes	Extracellular enzymes
Where they act	Inside the cell that made them (in the cytoplasm, or inside organelles such as mitochondria, chloroplasts or the nucleus)	Outside the cell that made them, after being secreted by exocytosis

Examples	Catalase (breaks down hydrogen peroxide, a toxic by-product of metabolism); DNA polymerase; ATP synthase; enzymes of glycolysis	Salivary and pancreatic amylase; trypsin; pepsin (these two are secreted as the inactive forms trypsinogen and pepsinogen, then activated outside the cell); enzymes secreted by fungi onto their food
Route out of the cell	Not secreted	Made on ribosomes on the rough ER, modified in the Golgi body, carried in vesicles to the cell surface membrane

Catalase catalyses this reaction: $2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$. It is found in many tissues (it is plentiful in liver, yeast, potato and celery) and is often used in practicals because the oxygen it releases is easy to measure.

2. How enzymes work

The enzyme–substrate complex

Enzyme and substrate molecules move randomly and collide. If a substrate molecule collides with the active site in the right orientation, it binds and forms an **enzyme–substrate complex (ES complex)**. It is held there by temporary bonds, such as hydrogen bonds and ionic attractions, between the substrate and R groups in the active site. The reaction happens, giving an **enzyme–product complex**. The products are then released and the active site is free to bind another substrate molecule.

In summary: $\text{E} + \text{S} \rightleftharpoons \text{ES} \rightarrow \text{EP} \rightarrow \text{E} + \text{P}$

Enzymes are **specific**: each one catalyses only one reaction, or one type of reaction, because only a substrate with a complementary shape can fit its active site.

Lock-and-key and induced fit

Feature	Lock-and-key hypothesis	Induced-fit hypothesis
Active site shape	Rigid; exactly complementary to the substrate before binding	Flexible; roughly complementary, and changes shape slightly as the substrate binds
What happens on binding	Substrate slots into the active site like a key into a lock	The active site closes around the substrate, forming a closer fit
How it explains catalysis	Holds substrate molecules close together in the right orientation	The change in shape puts strain on bonds in the substrate, or brings reacting groups together, so the reaction happens more easily
Status	Simple model; explains specificity well	Current model; supported by evidence that many enzymes change shape when the substrate binds; explains how binding can strain bonds in the substrate and lower the activation energy

Lowering the activation energy

Enzymes speed up reactions by providing a different reaction pathway with a **lower activation**

energy. More molecules then have enough energy to react at body temperature, so reactions happen quickly without the high temperatures that would damage cells. An enzyme does **not** change the overall energy change of a reaction or the final position of equilibrium. It only makes equilibrium reached faster.

Describing the energy diagram (no image). The y-axis is energy and the x-axis is progress of reaction. The substrate starts at a higher energy level and the products end at a lower level (for an exergonic reaction). Between them the uncatalysed reaction rises to a tall peak. The enzyme-catalysed reaction rises to a much lower peak. The activation energy is the height from the substrate level to the top of each peak. The difference between substrate and product levels is the same for both.

3. Factors that affect the rate of enzyme-catalysed reactions

The rate of an enzyme-catalysed reaction depends on how many successful collisions happen per unit time, and so on how many ES complexes form per unit time. Always explain your answer using these ideas: kinetic energy, collisions, active sites, ES complexes and the shape of the active site.

Temperature

Graph description: rate (y-axis) against temperature (x-axis). Rate rises gradually from low temperatures to a peak at the **optimum temperature**, then falls steeply. The curve is **not symmetrical**: the fall above the optimum is much steeper than the rise below it.

- **Below the optimum:** as temperature rises, enzyme and substrate molecules have more kinetic energy. They move faster and collide more often, and more collisions have at least the activation energy. More ES complexes form per unit time, so the rate increases.
- **At the optimum:** the rate is highest. For many human enzymes the optimum is about 37–40 °C. Enzymes from thermophilic bacteria in hot springs can have optima of 70 °C or more.
- **Above the optimum:** the extra vibration breaks hydrogen bonds and ionic bonds that hold the tertiary structure. The active site changes shape, so it is no longer complementary to the substrate and fewer ES complexes form. The enzyme is **denatured**. Denaturation is usually permanent.
- **At low temperatures** (for example 0–5 °C) the enzyme has **very low activity but is not denatured**. Molecules have little kinetic energy and collisions are rare, but the active site keeps its shape and activity returns when it is warmed.

Exam trap. Never write that enzymes are "killed" (they are not alive); write "denatured". Never write that the active site is the "same shape" as the substrate; write "complementary".

pH

Graph description: rate against pH gives a curve that peaks at the **optimum pH** and falls on either side. It is often roughly bell-shaped, but may be narrow for some enzymes.

- pH is a measure of hydrogen ion (H^+) concentration. Changes in H^+ concentration change the charges on R groups: for example, $-COO^-$ can gain H^+ to become $-COOH$, and $-NH_2$ can gain H^+ to become $-NH_3^+$.

- This disrupts **ionic bonds and hydrogen bonds** in the tertiary structure, so the active site changes shape.
- It can also change the charges on R groups **in the active site**, so the substrate can no longer bind even if the shape is almost unchanged.
- Small changes from the optimum are usually reversible. Extreme pH values cause permanent denaturation.
- Examples of optimum pH: pepsin in the stomach, about pH 2; salivary amylase, about pH 7; trypsin in the small intestine, about pH 8.

In practicals, pH is kept constant with **buffer solutions**, which resist changes in pH.

Enzyme concentration

Graph description: initial rate against enzyme concentration is a **straight line through the origin** (directly proportional), as long as substrate is in excess.

- More enzyme molecules mean more active sites available, so more ES complexes form per unit time.
- If the amount of substrate is limited, the line eventually levels off. Adding more enzyme then has no effect because not enough substrate molecules are left to fill the extra active sites. **Substrate concentration** has become the limiting factor.

Substrate concentration

Graph description: initial rate against substrate concentration (with enzyme concentration fixed) rises steeply at first, then curves and levels off to a plateau. The plateau is **V_{max}** .

- **At low substrate concentration** many active sites are empty. Adding more substrate increases collisions and ES complex formation, so the rate rises almost in proportion. Substrate concentration is the limiting factor.
- **As concentration increases**, more and more active sites are occupied at any moment, so each extra bit of substrate adds less to the rate.
- **At the plateau** all active sites are occupied (the enzyme is **saturated**). Adding more substrate cannot increase the rate. **Enzyme concentration** is now the limiting factor. The rate could only rise if more enzyme were added (or the temperature raised towards the optimum).

Inhibitor concentration

Graph description: initial rate (y-axis) against inhibitor concentration (x-axis), with substrate and enzyme concentration fixed. The rate is highest with no inhibitor. It falls as inhibitor concentration increases, steeply at first, then more gradually, levelling off towards zero.

- More inhibitor molecules occupy or alter more enzyme molecules at any moment, so fewer active sites are available to the substrate and fewer ES complexes form per unit time.
- Each extra bit of inhibitor has a smaller effect, because there are fewer uninhibited enzyme molecules left for it to act on.
- With a **competitive** inhibitor, the size of the fall depends on the ratio of inhibitor to substrate: at a higher substrate concentration, more inhibitor is needed to cause the same fall in rate.
- With a **non-competitive** inhibitor, the size of the fall depends on inhibitor concentration and is

not reduced by adding more substrate.

Why we use initial rates

During a reaction the substrate is used up, so its concentration falls and the rate falls too. A product against time graph therefore starts steep and gradually levels off. When the substrate has all been used up, no more product forms. To compare conditions fairly, we measure the **initial rate**, the gradient at time zero. At that point the substrate concentration is known and has not yet fallen.

4. $V_{\max}$ and the Michaelis–Menten constant (K_m)

$V_{\max}$: the maximum initial rate of an enzyme-catalysed reaction, reached when all the active sites are occupied by substrate (the enzyme is saturated).

Michaelis–Menten constant, K_m : the substrate concentration at which the rate of reaction is half of $V_{\max}$.

What K_m tells you: K_m is a measure of an enzyme's **affinity** for its substrate.

- A **low K_m** means only a low substrate concentration is needed to reach half $V_{\max}$. The enzyme binds substrate readily, so it has a **high affinity** for the substrate. ES complexes form easily, even when little substrate is present.
- A **high K_m** means a lot of substrate is needed to reach half $V_{\max}$, so the enzyme has a **low affinity** for the substrate.

K_m has the **units of concentration** (for example mmol dm^{-3}). $V_{\max}$ has the **units of rate** (for example $\mu\text{mol min}^{-1}$). $V_{\max}$ depends on the amount of enzyme used, but K_m is a property of the enzyme and substrate pair under given conditions and does not depend on enzyme concentration.

Finding them from a graph: (1) draw a horizontal line at the plateau and read $V_{\max}$; (2) halve it; (3) draw across from $\frac{1}{2}V_{\max}$ to the curve, then down to the x-axis; (4) read K_m .

Worked example 1: finding $V_{\max}$ and K_m

A student measured the initial rate of an enzyme at different substrate concentrations, keeping the enzyme concentration, temperature and pH constant.

Substrate concentration / mmol dm^{-3}	Initial rate / $\mu\text{mol min}^{-1}$
1.0	0.23
2.0	0.36
5.0	0.53
10.0	0.64
20.0	0.71
40.0	0.75
80.0	0.78

Step 1: estimate $V_{\max}$. The rate rises by less and less, and is levelling off towards about 0.80

$\mu\text{mol min}^{-1}$. On a plotted graph the curve approaches this value, so $V_{\text{max}} \approx 0.80 \mu\text{mol min}^{-1}$.

Step 2: halve V_{max} . $\frac{1}{2}V_{\text{max}} = 0.80 \div 2 = 0.40 \mu\text{mol min}^{-1}$.

Step 3: find the substrate concentration that gives this rate. 0.40 lies between the rates at 2.0 mmol dm^{-3} (0.36) and 5.0 mmol dm^{-3} (0.53). Plot the data yourself (rate on the y-axis, substrate concentration on the x-axis) and draw a smooth curve through the points. Reading from the curve, the rate is $0.40 \mu\text{mol min}^{-1}$ at a substrate concentration of about 2.5 mmol dm^{-3} . Straight-line interpolation between the 2.0 and 5.0 points would give about 2.7, but the curve bends, so the true value is nearer 2.5.

Answer: $V_{\text{max}} \approx 0.80 \mu\text{mol min}^{-1}$; $K_{\text{m}} \approx 2.5 \text{ mmol dm}^{-3}$.

Step 4: compare. A second enzyme that acts on the same substrate has $K_{\text{m}} = 0.6 \text{ mmol dm}^{-3}$. It has a **higher affinity** for the substrate than the first enzyme, because it reaches half its maximum rate at a lower substrate concentration.

Notice: do not simply take V_{max} as the highest measured rate (0.78). V_{max} is the plateau the curve approaches, which may lie a little above the highest reading. Using 0.78 instead gives $\frac{1}{2}V_{\text{max}} = 0.39$ and $K_{\text{m}} \approx 2.4 \text{ mmol dm}^{-3}$. Because values are read from a hand-drawn graph, examiners usually accept a small range of answers.

5. Enzyme inhibitors

An **inhibitor** is a substance that reduces the rate of an enzyme-catalysed reaction. The syllabus focuses on **reversible** inhibitors, which bind temporarily (non-covalently) and can leave the enzyme again.

Competitive inhibition

- The inhibitor has a shape **similar to the substrate**, so it can fit into the **active site**.
- While the inhibitor occupies the active site, the substrate cannot bind, so fewer ES complexes form.
- Inhibitor and substrate **compete** for the active sites. The effect depends on the **relative concentrations** of substrate and inhibitor.
- If substrate concentration is increased, a substrate molecule is more likely than an inhibitor molecule to collide with any free active site. At very high substrate concentration the inhibition is overcome and the rate reaches the **same V_{max}** .
- More substrate is needed to reach half V_{max} , so **K_{m} increases** (the enzyme appears to have a lower affinity for the substrate).
- Example: malonate competitively inhibits succinate dehydrogenase in the Krebs cycle, because its shape is similar to succinate.

Non-competitive inhibition

- The inhibitor binds to the enzyme at a site **other than the active site** (a site elsewhere on the enzyme, sometimes called an **allosteric site**).
- This changes the tertiary structure, so the **shape of the active site changes**. The substrate can no longer bind, or the enzyme cannot catalyse the reaction.
- The inhibitor does not compete with the substrate, so **increasing substrate concentration does not overcome** the inhibition.

- In effect some enzyme molecules are taken out of action, so **$V_{\max}$ is lower**. The enzyme molecules that are unaffected work normally, so **K_m stays the same**.
- Example: many metabolic pathways are controlled by **end-product inhibition**. The final product of the pathway often acts as a non-competitive inhibitor, binding to a site other than the active site of an enzyme near the start of the pathway. When plenty of product has built up, the pathway slows down.

Feature	No inhibitor	Competitive inhibitor	Non-competitive inhibitor
Where the inhibitor binds	—	Active site	Site other than the active site
Shape of inhibitor	—	Similar to substrate	Need not be similar to substrate (does not bind the active site)
Effect of raising substrate concentration	—	Reduces the inhibition; can overcome it	Does not overcome the inhibition
$V_{\max}$	Normal	Unchanged (reached at a higher substrate concentration)	Decreased
K_m	Normal	Increased	Unchanged
Rate against substrate concentration curve	Rises and plateaus at $V_{\max}$	Rises more slowly, lies below the normal curve, but reaches the same plateau at high substrate concentration	Same general shape, but plateaus at a lower $V_{\max}$

6. Immobilised enzymes

An **immobilised enzyme** is held in place so that it cannot move freely in solution. A common school method traps enzyme in **alginate beads**:

1. Mix the enzyme solution with **sodium alginate** solution.
2. Drip the mixture from a syringe into **calcium chloride** solution.
3. Calcium ions cross-link the alginate chains and form insoluble **calcium alginate** beads, with enzyme molecules trapped inside.
4. Leave the beads to harden, then rinse them with distilled water to remove any enzyme on the surface.
5. Pack the beads into a column (for example a syringe barrel with a tap or clip) and run substrate solution through it. Product flows out of the bottom.

A familiar example: **lactase** immobilised in alginate beads can be used to make lactose-free milk. Milk is run through a column of beads and the lactose is hydrolysed to glucose and galactose. The presence of glucose in the milk that comes out can be shown with glucose test strips.

Feature	Enzyme free in solution	Immobilised enzyme
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Separating enzyme from product	Difficult; product is contaminated with enzyme, so it may need purifying	Easy; product flows out and is not contaminated
Reuse	Lost with the product; usually not reused	Can be reused many times, which saves cost
Type of process	Usually batch	Suits a continuous process
Stability	Denatured more easily by temperature and pH changes	More stable across a wider range of temperature and pH, because the surrounding matrix helps hold the molecule's shape and shields it
Rate at the start	Can be faster; all active sites are freely reached by substrate	Can be slower; substrate must diffuse into the beads, and some active sites are hard to reach
Set-up cost	Lower	Higher (making beads and columns)

To compare immobilised and free enzyme fairly, use the same mass of enzyme, the same volume and concentration of substrate, the same temperature and pH, and the same method of measuring product. Blank beads (alginate with no enzyme) make a useful control.

7. Practical investigations

Two ways of following a reaction

Approach	Example	How it is measured
Rate of appearance of product	Catalase breaking down hydrogen peroxide	Collect oxygen in an upturned measuring cylinder or gas syringe and record the volume at regular time intervals; or count bubbles; or time how long an enzyme-soaked filter-paper disc takes to rise through hydrogen peroxide solution
Rate of disappearance of substrate	Amylase breaking down starch	Take samples at intervals and add iodine solution. Blue-black shows starch is present; when the sample stays orange-brown, the starch has gone. Starch concentration can be measured more precisely with a colorimeter

Controlling variables

- **Temperature:** use a thermostatically controlled water bath. Equilibrate the enzyme and substrate separately to the test temperature before mixing.
- **pH:** use a buffer solution of known pH.
- **Concentrations and volumes:** keep the enzyme and substrate concentrations and volumes the same, except for the independent variable. Make dilutions accurately with graduated pipettes or syringes.
- **Controls:** use boiled (denatured) enzyme, or water instead of enzyme, to show that the change is caused by the enzyme's activity.
- **Reliability:** repeat each condition (at least three times), calculate a mean and look for anomalous results.

Rate from an end-point time

If you record the time taken to reach an end point (for example the time until no starch is detected), then **rate** = $1 \div \text{time}$, with units s^{-1} when time is in seconds. Because the numbers are small, you can calculate $1000 \div \text{time}$ instead. Record this in a column headed "rate / 1000 s^{-1} " (meaning the value shown is $1000 \times$ the rate in s^{-1}), or give it in arbitrary units. Do not simply label $1000 \div \text{time}$ as " s^{-1} ". A longer time means a lower rate.

Using a colorimeter

A colorimeter shines light of a chosen colour through a sample in a cuvette and measures how much is **absorbed** (absorbance) or **transmitted** (percentage transmission). For the starch–iodine method:

1. Choose a filter giving light of the colour that the blue-black starch–iodine complex absorbs strongly (a red or orange filter).
2. Zero (calibrate) the colorimeter with a cuvette of distilled water. Iodine solution is itself slightly coloured, so a sample of iodine solution with no starch gives a small absorbance reading.
3. Make a **calibration curve**: measure the absorbance of a series of known starch concentrations mixed with a fixed volume of iodine solution, and plot absorbance against concentration.
4. During the reaction, remove samples at set times and stop the reaction in each one: for example, add it to an equal volume of dilute acid (the calibration standards must be treated with the same acid), or transfer it immediately to a boiling water bath for a set time and then cool it. Then add the fixed volume of iodine solution and measure absorbance.
5. Use the calibration curve to convert each absorbance into a starch concentration.

As amylase digests starch, the blue-black colour fades, so **absorbance falls** (and transmission rises). A colorimeter gives **quantitative** results and avoids the subjective judgement of colour by eye.

Worked example 2: using a calibration curve

A calibration curve for starch (with a fixed volume of iodine solution, colorimeter zeroed with distilled water) gave these results. They lie on a straight line: absorbance = $0.83 \times \text{concentration} + 0.02$. The reading of 0.02 at 0.0% starch comes from the slight colour of the iodine solution.

Starch concentration / %	0.0	0.2	0.4	0.6
Absorbance	0.02	0.19	0.35	0.52

Starch concentration / %	0.8	1.0
Absorbance	0.68	0.85

A reaction started with 1.0% starch. After 5.0 minutes a sample had an absorbance of 0.43. Calculate the mean rate of starch breakdown over the 5.0 minutes.

Step 1: convert absorbance to concentration. Reading from the graph (or rearranging the equation): concentration = $(0.43 - 0.02) \div 0.83 = 0.41 \div 0.83 = 0.494 \approx 0.49\%$.

Step 2: find the change in concentration. $1.0 - 0.49 = 0.51\%$ starch broken down.

Step 3: divide by time. Rate = $0.51 \div 5.0 = 0.102 \approx 0.10\% \text{ min}^{-1}$ (percentage starch per minute).

This is a *mean* rate over 5 minutes. The initial rate would be higher, because the rate falls as starch is used up.

Calculating a rate from a tangent

For a curved graph of product against time, the rate at any instant is the **gradient of the tangent** at that time. To find the **initial rate**, draw the tangent at $t = 0$.

1. Place a ruler so that it touches the curve at the chosen time without crossing it. At $t = 0$ it should follow the steepest, early part of the curve.
2. Draw a long straight line so that the triangle you use is large. This reduces reading errors.
3. Pick two points on the **tangent line** (not on the data points) that are far apart and easy to read.
4. Gradient = change in $y \div$ change in $x = \Delta y \div \Delta x$.
5. Give units: y -axis unit per x -axis unit, for example $\text{cm}^3 \text{s}^{-1}$.

Worked example 3: initial rate from a tangent

A yeast suspension (a source of catalase) was added to hydrogen peroxide solution buffered at pH 7, at 25 °C. The oxygen released was collected in a gas syringe.

Time / s	0	30	60	90
Volume of O_2 / cm^3	0.0	12.0	21.0	27.5

Time / s	120	150	180
Volume of O_2 / cm^3	32.0	34.5	35.5

(a) Calculate the initial rate of reaction. A tangent drawn to the curve at $t = 0$ passes through (0 s, 0.0 cm^3) and (50 s, 22.0 cm^3).

Step 1: $\Delta y = 22.0 - 0.0 = 22.0 \text{ cm}^3$

Step 2: $\Delta x = 50 - 0 = 50 \text{ s}$

Step 3: gradient = $22.0 \div 50 = 0.44 \text{ cm}^3 \text{s}^{-1}$ (which is $0.44 \times 60 = 26.4 \text{ cm}^3 \text{min}^{-1}$)

(b) Calculate the mean rate over the first 60 s and explain why it is lower than the initial rate.

Step 1: mean rate = total volume $\div$ time = $21.0 \div 60 = 0.35 \text{ cm}^3 \text{s}^{-1}$

Step 2 (explain): as hydrogen peroxide is broken down, its concentration falls. There are fewer collisions between substrate molecules and active sites, and fewer ES complexes form per second, so the rate falls during the 60 s. The mean includes these slower later rates, so it is lower than the rate at $t = 0$.

(c) Suggest why the volume levels off at about 36 cm^3 .

The hydrogen peroxide is almost all used up (substrate is now limiting), so almost no more oxygen is produced. The final volume depends on the amount of substrate, not the amount of enzyme.

Worked example 4: interpreting inhibitor data

An enzyme was tested with and without inhibitor X. Without X: $V_{\text{max}} = 1.20 \mu\text{mol min}^{-1}$ and $K_m = 4.0 \text{ mmol dm}^{-3}$. With X: $V_{\text{max}} = 1.20 \mu\text{mol min}^{-1}$ and $K_m = 9.0 \text{ mmol dm}^{-3}$. Identify the type of inhibition and explain.

Step 1: compare V_{max} . It is unchanged, so at high substrate concentration the inhibition is

overcome.

Step 2: compare K_m . It rises from 4.0 to 9.0 mmol dm⁻³, so more substrate is needed to reach half V_{max} ($\frac{1}{2} \times 1.20 = 0.60 \mu\text{mol min}^{-1}$).

Conclusion: X is a **competitive** inhibitor. It binds to the active site and competes with the substrate. At high substrate concentration, substrate molecules occupy almost all active sites, so the same V_{max} is reached.

Key terms

Term	Definition
Enzyme	A globular protein that acts as a biological catalyst, lowering the activation energy of a reaction without being permanently changed
Active site	The region of an enzyme with a shape complementary to the substrate, where the substrate binds and the reaction is catalysed
Substrate	The molecule that an enzyme acts on
Enzyme–substrate complex	The temporary structure formed when a substrate is bound in an enzyme's active site
Specificity	An enzyme catalyses only one reaction or type of reaction, because only a substrate of complementary shape fits its active site
Activation energy	The minimum energy needed for a reaction to start
Lock-and-key hypothesis	A model in which the active site has a fixed shape exactly complementary to the substrate
Induced-fit hypothesis	A model in which the active site changes shape slightly as the substrate binds, forming a closer fit and helping the reaction happen
Intracellular enzyme	An enzyme that acts inside the cell that made it
Extracellular enzyme	An enzyme secreted by a cell that acts outside that cell
Denaturation	A change to the tertiary structure of a protein, caused by bonds breaking, that changes the shape of the active site so it no longer binds the substrate; usually permanent
Optimum	The temperature or pH at which the rate of an enzyme-catalysed reaction is greatest
Initial rate	The rate at the very start of a reaction, found from the gradient of the tangent at time zero
Limiting factor	The factor that is in shortest supply and therefore controls the rate
V_{max}	The maximum initial rate of reaction, reached when all active sites are occupied
K_m (Michaelis–Menten constant)	The substrate concentration at which the rate is half V_{max} ; a low value shows high affinity for the substrate
Affinity	How readily an enzyme binds its substrate and forms enzyme–substrate complexes

Competitive inhibitor	A molecule with a shape similar to the substrate that binds reversibly to the active site; raises K_m but does not change V_{max}
Non-competitive inhibitor	A molecule that binds to a site other than the active site and changes the active site's shape; lowers V_{max} but does not change K_m
Allosteric site	A site on an enzyme other than the active site, where a molecule such as a non-competitive inhibitor can bind
End-product inhibition	Control of a metabolic pathway in which the final product inhibits an enzyme earlier in the pathway, often non-competitively
Immobilised enzyme	An enzyme held in or on an inert matrix, such as alginate beads, so it cannot move freely in solution
Buffer	A solution that resists changes in pH
Calibration curve	A graph of a measured value (such as absorbance) against known concentrations, used to find unknown concentrations

Quick check

1. State what is meant by the term *activation energy*. [1]
2. State **one** example of an intracellular enzyme and **one** example of an extracellular enzyme. [2]
3. Outline the induced-fit hypothesis of enzyme action. [3]
4. Explain why an enzyme can catalyse only one type of reaction. [2]
5. Describe and explain the effect of increasing temperature from 20 °C to 60 °C on the rate of a reaction catalysed by a human enzyme. [5]
6. Explain how a pH far below the optimum reduces the activity of an enzyme. [3]
7. A student timed how long amylase took to break down all the starch in a sample. At 30 °C it took 125 s. Calculate the rate as $1000 \div \text{time}$ and state how the value should be recorded, with units. [2]
8. In an experiment with a fixed amount of enzyme, the rate stopped increasing when substrate concentration was raised above 15 mmol dm⁻³. Explain why. [2]
9. Enzyme P has $K_m = 0.2 \text{ mmol dm}^{-3}$ and enzyme Q has $K_m = 5.0 \text{ mmol dm}^{-3}$ for the same substrate. Compare the affinities of P and Q for the substrate and explain your answer. [2]
10. A tangent is drawn at $t = 0$ on a graph of product concentration (y-axis, mmol dm⁻³) against time (x-axis, min). It passes through (0 min, 0.0 mmol dm⁻³) and (4.0 min, 3.0 mmol dm⁻³). Calculate the initial rate of reaction. [2]
11. The initial rate of an enzyme was measured at different substrate concentrations, with enzyme concentration, temperature and pH kept constant.

Substrate concentration / mmol dm ⁻³	1.0	2.0	4.0	8.0
Initial rate / $\mu\text{mol min}^{-1}$	0.40	0.67	1.00	1.33

Substrate concentration / mmol dm ⁻³	16.0	32.0	64.0
Initial rate / $\mu\text{mol min}^{-1}$			

Initial rate / $\mu\text{mol min}^{-1}$	1.60	1.78	1.88
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Use the data to estimate V_{max} and K_m . Show your working. [3]

- An inhibitor lowered V_{max} from 2.0 to 1.2 $\mu\text{mol min}^{-1}$ but left K_m at 3.0 mmol dm^{-3} . Identify the type of inhibitor and explain how it reduces the rate. [4]
- Explain why increasing substrate concentration reduces the effect of a competitive inhibitor but not the effect of a non-competitive inhibitor. [3]
- Describe and explain how increasing the concentration of a competitive inhibitor affects the rate of reaction when substrate concentration is kept constant. [3]
- Suggest **two** reasons why an immobilised enzyme in alginate beads may give a lower initial rate than the same mass of free enzyme, and **one** advantage of using the immobilised enzyme in industry. [3]
- Describe how you would compare the activity of lactase free in solution with lactase immobilised in alginate beads. Include the variables you would control. [5]

Answers

For multi-mark questions, each numbered point is one mark point. Award up to the maximum shown.

- The minimum energy that must be supplied for a reaction to start. (1)
- Intracellular: catalase, or DNA polymerase, or ATP synthase (any one) (1). Extracellular: amylase, or trypsin, or pepsin (any one) (1).
- (1) the active site is flexible / not exactly complementary to the substrate at first; (2) as the substrate binds, the active site changes shape slightly / moulds around the substrate to give a close fit (ES complex); (3) this puts strain on bonds in the substrate, or brings reacting groups together; (4) so the activation energy is lowered. **Max 3.**
- (1) the active site has a specific shape (determined by the tertiary structure); (2) only a substrate with a complementary shape can bind and form an ES complex. **Max 2.**
- (1) rate increases up to the optimum, about 37–40 °C; (2) molecules gain kinetic energy / move faster; (3) more frequent (successful) collisions / more collisions exceed the activation energy; (4) more ES complexes form per unit time; (5) above the optimum the rate decreases (steeply); (6) hydrogen bonds / ionic bonds in the tertiary structure break; (7) the active site changes shape / is no longer complementary to the substrate; (8) the enzyme is denatured, so by 60 °C the rate is very low or zero. **Max 5.**
- (1) high H^+ concentration changes the charges on R groups (for example $-\text{COO}^-$ gains H^+); (2) ionic bonds / hydrogen bonds in the tertiary structure are disrupted; (3) the active site changes shape / is no longer complementary; (4) charges in the active site change, so the substrate cannot bind; (5) fewer ES complexes form. **Max 3.**
- (1) $1000 \div 125 = 8.0$; (2) correct recording: rate = $8.0 \times 10^{-3} \text{ s}^{-1}$, or 8.0 in a column headed "rate / 1000 s^{-1} ", or 8.0 arbitrary units. Do not credit " 8.0 s^{-1} ".
- (1) all the active sites are occupied / the enzyme is saturated, so V_{max} has been reached; (2) enzyme concentration is now the limiting factor, so extra substrate cannot increase the rate. **Max 2.**
- (1) P has the higher affinity; (2) its K_m is lower, so it reaches half V_{max} at a much lower

substrate concentration / forms ES complexes more readily. **Max 2.**

10. (1) gradient = $(3.0 - 0.0) \div (4.0 - 0) = 0.75$; (2) units $\text{mmol dm}^{-3} \text{ min}^{-1}$. Answer: **0.75 mmol $\text{dm}^{-3} \text{ min}^{-1}$.**
11. (1) $V_{\text{max}} \approx 2.0 \mu\text{mol min}^{-1}$ (the rate is levelling off towards a plateau a little above the highest reading of 1.88); (2) $\frac{1}{2}V_{\text{max}} = 2.0 \div 2 = 1.0 \mu\text{mol min}^{-1}$; (3) $K_m \approx 4.0 \text{ mmol dm}^{-3}$ (the substrate concentration giving a rate of 1.0). Allow a V_{max} of 1.9–2.0 with K_m read consistently from it (about 3.6–4.0 mmol dm^{-3}); units needed. **Max 3.**
12. (1) non-competitive inhibitor, because V_{max} is lower and K_m is unchanged; (2) it binds to a site other than the active site; (3) this changes the tertiary structure / shape of the active site; (4) the substrate can no longer bind or react, so fewer ES complexes form; (5) adding more substrate does not overcome this, because the inhibitor is not competing for the active site. **Max 4.**
13. (1) a competitive inhibitor competes with the substrate for the active site; (2) at higher substrate concentration a substrate molecule is more likely than an inhibitor molecule to collide with / occupy a free active site, so more ES complexes form (the same V_{max} can be reached); (3) a non-competitive inhibitor binds to a site other than the active site / does not compete with the substrate; (4) enzyme molecules with inhibitor bound have an altered active site and cannot work whatever the substrate concentration, so V_{max} stays lower. **Max 3.**
14. (1) the rate decreases as inhibitor concentration increases; (2) the fall is steep at first, then levels off, with the rate approaching zero; (3) more inhibitor molecules, so an inhibitor molecule is more likely than a substrate molecule to occupy a free active site; (4) fewer active sites are available to the substrate, so fewer ES complexes form per unit time. **Max 3.**
15. Reasons (any two, 1 mark each): (1) substrate must diffuse into the bead before it can reach the enzyme; (2) some active sites are buried or blocked by the alginate matrix; (3) the enzyme molecules cannot move freely, so there are fewer collisions with substrate.
Advantage (any one, 1 mark): the enzyme can be reused; the product is not contaminated with enzyme; it is more stable over a range of temperature and pH; a continuous process can be used. **Max 3.**
16. (1) make beads from sodium alginate containing a known mass/volume of lactase, dripped into calcium chloride, and use the same mass/volume of lactase free in solution for the comparison; (2) add the same volume and concentration of milk or lactose solution to each (for example running it through a column of beads, and mixing it with the free enzyme); (3) measure glucose in the product at set time intervals, for example with glucose test strips, and compare the time taken to reach a set glucose concentration, or the concentration reached at a set time; (4) control temperature with a water bath; (5) control pH with a buffer; (6) keep the flow rate / contact time the same, and use beads of the same size and number; (7) control: blank beads with no enzyme, or boiled enzyme, to show glucose comes only from lactase activity; (8) repeat at least three times and calculate a mean. **Max 5.**