

Enzymes

A-Level Biology ■ Topic 3

A-Level Biology



What we will cover

- 1 How enzymes work
- 2 Activation energy
- 3 Measuring the rate
- 4 Temperature & pH
- 5 $V_{\max}$ & K_m
- 6 Inhibitors & immobilisation



enzymes catalyse reactions



How enzymes work

Enzymes are **globular proteins** that **catalyse** reactions – **intracellular** ones work inside the cell, **extracellular** ones are **secreted** to work outside it, like amylase in the gut. The **active site** is complementary to the **substrate** .

lock and key

the site is a rigid match
for one substrate

induced fit

the site moulds around
the substrate as it binds

Both form an enzyme–substrate complex that lowers activation energy. Induced fit is the better model.

Two models of the active site

Lock-and-key

The **lock-and-key hypothesis** : the active site is a fixed shape, and only a substrate with the matching shape fits.

Induced fit

The **induced-fit hypothesis** : the site is not quite the right shape at first, and moulds itself around the substrate as it binds.

The complex

Either way the substrate forms an **enzyme-substrate complex** ; the reaction happens, the **products** leave, and the site is free again.

Specificity

An example is **amylase** , released into the gut to **digest starch** – and nothing else.

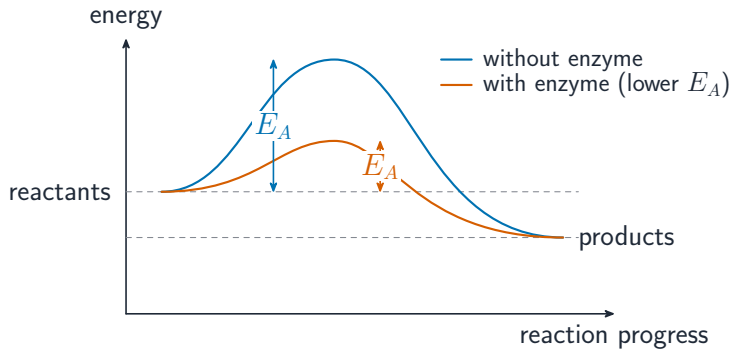
Induced fit explains what lock-and-key cannot: why binding itself strains the substrate's bonds and lowers the activation energy.

Lowering activation energy

Every reaction needs an **activation energy** to start.

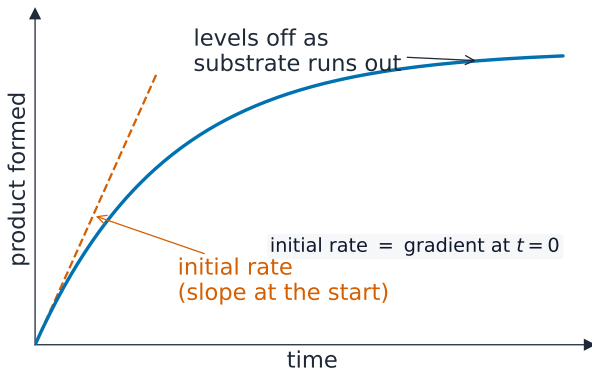
An enzyme **lowers** it, so the reaction runs fast at the cell's normal temperature – no high heat needed.

Lowering activation energy, in detail



Every reaction needs an **activation energy** to start.

Measuring the rate



Follow the product made, or the substrate used up – with amylase, the iodine test's blue-black fades. A **colorimeter** measures the absorbance, turning a colour change into a number you can plot.

Product builds fastest at the start, so the **initial rate** (slope at time zero) is the fairest value to compare.

Measuring an enzyme rate fairly

the initial rate

the reaction is **fastest at the start**, so measure over a **short early interval**

why

averaging over the whole reaction underestimates it, because the substrate runs low

controlling pH

use a **buffer solution**, so pH is held while another variable is changed

controlling temperature

a water bath, and let the reactants reach temperature **before** mixing

kinetic energy

raising temperature makes molecules move faster, so they **collide** more often and with more energy

beyond the optimum

the enzyme **denatures** – the active site changes shape and no **enzyme-substrate complex** – can form

Optimum pH and optimum temperature are both about the **shape** of the active site, which is why both curves rise and then fall sharply.

Why the initial rate is the fair comparison

It falls with time

The **rate of reaction** is steepest at the start, when the most substrate is present, and flattens as substrate runs out.

So use time zero

The **initial rate** – the slope of the tangent at $t = 0$ – is the only value that compares two conditions fairly.

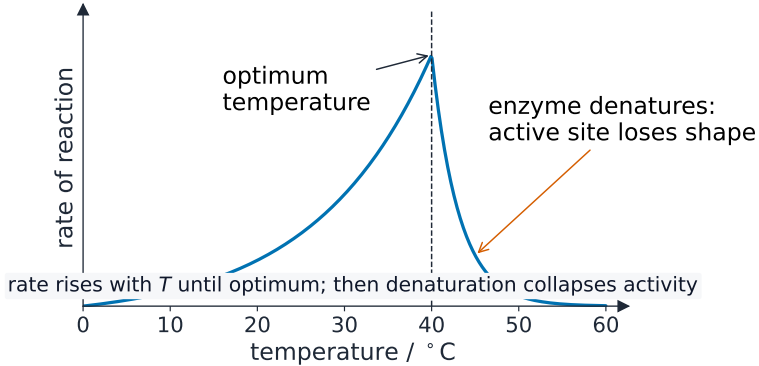
How to get it

Plot product against time, draw the tangent at the origin, and read its gradient.

Comparing total product after five minutes compares how much substrate there was, not how good the enzyme is.



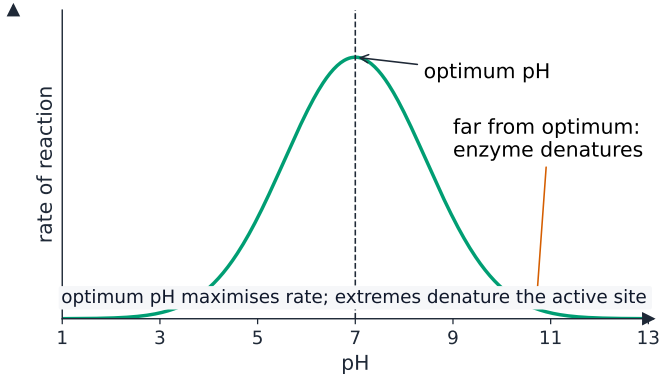
Optimum, then denatures



optimum, then denatures

optimum, then denatures

A bell-shaped optimum pH



a bell-shaped optimum pH

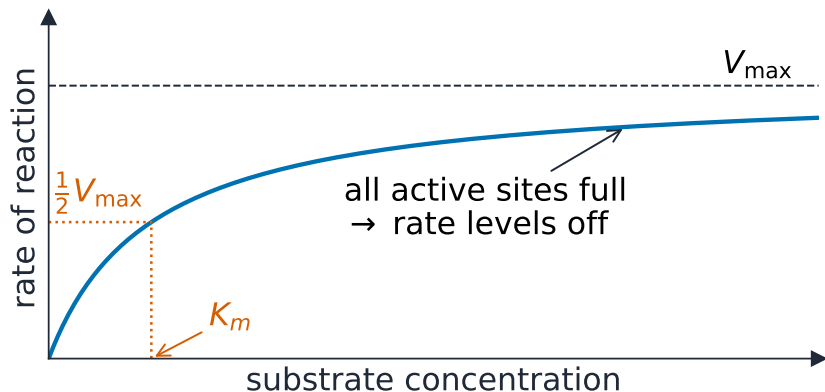
a bell-shaped optimum pH

$V_{\max}$ and the Michaelis–Menten constant

Rate climbs to $V_{\max}$ once all active sites are full.

K_m (**Michaelis–Menten constant**) is the substrate concentration giving half $V_{\max}$ – a **low** K_m means high **affinity** .

$V_{\max}$ and the Michaelis–Menten constant, in detail



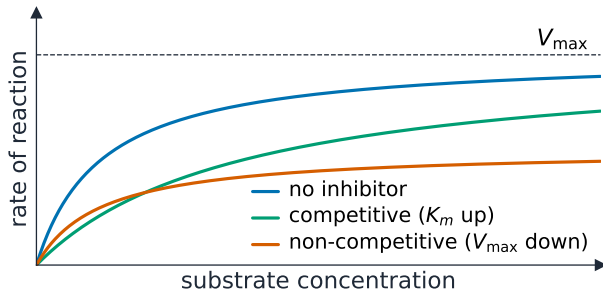
$$v = \frac{V_{\max}[S]}{K_m + [S]} \quad \cdot \quad K_m = [S] \text{ at half } V_{\max}$$

Rate climbs to $V_{\max}$ once all active sites are full.

Reversible inhibitors

- **competitive** – binds the active site; more substrate overcomes it ($K_m \uparrow$, $V_{\max}$ same)
- **non-competitive** – binds elsewhere; substrate can't help ($V_{\max} \downarrow$, K_m same)

Reversible inhibitors, in detail



competitive: $\uparrow K_m$, same $V_{\max}$ · non-competitive:
 $\downarrow V_{\max}$

A competitive inhibitor raises K_m (more substrate overcomes it); a non-competitive one lowers $V_{\max}$

The two reversible inhibitors, compared

competitive

binds at the active site, competing with the substrate

add more substrate

the inhibitor is outcompeted, so the effect is overcome

its effect

$V_{\max}$ unchanged, K_m increased

non-competitive

binds elsewhere, changing the active site's shape

add more substrate

no help – the sites are still distorted

its effect

$V_{\max}$ reduced, K_m unchanged

K_m measures how strongly an enzyme holds its substrate, so it lets you compare enzymes. A low K_m means a high affinity.

The two inhibitors, and what each does to the constants

Competitive

Binds the **active site** itself, so it competes with the substrate. Adding more substrate outcompetes it: $V_{\max}$ unchanged, K_m rises.

Non-competitive

A **non-competitive inhibitor** binds elsewhere and changes the active site's shape. More substrate does **not** help: $V_{\max}$ falls, K_m unchanged.

Michaelis–Menten

The **Michaelis–Menten constant** K_m is the substrate concentration giving half of $V_{\max}$ – so a **low** K_m means a high affinity for the substrate.

Read the graph, not the story: which constant moved tells you which inhibitor it was.

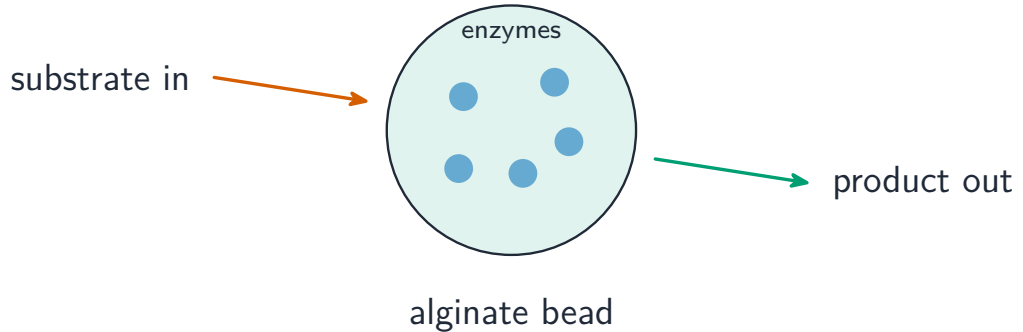
Immobilised enzymes

An **immobilised enzyme** is fixed in beads while substrate flows past.

- not washed away – reused
- pure product; more stable; runs continuously

Used in **biological washing powders** .

Immobilised enzymes, in detail



An **immobilised enzyme** is fixed in beads while substrate flows past.

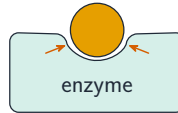
Specificity

lock-and-key



active site is a
fixed shape

induced fit



active site moulds
to fit the substrate

Lock-and-key: a fixed active site. Induced fit: the active site changes shape to grip the substrate

Immobilised enzymes, continued



Biological washing powders contain enzymes that digest food and blood stains at low temperatures

Immobilised enzymes: slower, and worth it

how

trapped in **alginate** beads, or bound to a surface

the cost

a free enzyme usually works a **little faster**, because the substrate reaches it more easily

reusable

the enzyme is recovered and used again – the largest saving

pure product

the product is not contaminated with enzyme, so no separation step is needed

more stable

immobilised enzymes tolerate higher temperature and a wider pH range

continuous flow

substrate can be run past the beads indefinitely

The rate loss is small and the practical gains are large – which is why nearly every industrial enzyme process immobilises.

Exam tips

- Explain enzyme action with the **induced-fit** model (the active site moulds around the substrate) – now preferred to lock-and-key.
- Above the optimum the enzyme **denatures** (hydrogen bonds and tertiary structure break) – write “denatures”, never “dies” or “is killed”.
- Distinguish inhibitors: **competitive** binds the active site (overcome by more substrate, same V_{max}); **non-competitive** binds elsewhere (lower V_{max}).
- Compare rates using the **initial rate** (tangent at time zero) – the fairest measure, before substrate becomes limiting.



Worked example – estimating a rate

In the first 20 s of a catalase reaction, 16 cm³ of oxygen is collected. Estimate the rate.

- Rate
$$= \frac{\text{volume of product}}{\text{time}} = \frac{16}{20} = 0.8 \text{ cm}^3 \text{ s}^{-1}$$
- This is the **average** over the first 20 s.
- The true **initial** rate is higher: the curve is steepest at $t = 0$ and flattens as substrate runs out.
- For an initial rate, draw a **tangent at the origin** and take its gradient.

Use the **initial** rate to compare conditions – at $t = 0$ substrate is not yet limiting, so the difference you see is the variable you changed.

Topic 3 – key takeaways

1

Active site

substrate fits; induced fit gives specificity

2

Activation energy

enzymes lower it to speed reactions

3

Factors

optimum temp & pH; denatures past them

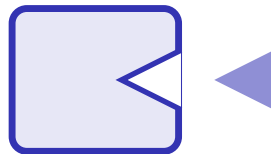
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Inhibitors

competitive raises K_m ; non-comp. lowers V_{max}

Check yourself

- 1 Which hypothesis says the active site moulds round the substrate?
- 2 What does an enzyme do to activation energy?
- 3 Above the optimum temperature, what happens to the enzyme?
- 4 A competitive inhibitor changes which constant?



Answers 1. induced fit 2. lowers it 3. it denatures 4. raises K_m ($V_{\max}$ unchanged)