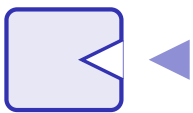
Enzymes

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

1 How enzymes work

How enzymes work

Enzymes 酶 are **globular proteins** 球状蛋白质 that **catalyse** 催化 reactions – **intracellular** 细胞内 ones work inside the cell, **extracellular** 细胞外 ones are **secreted** 分泌 to work outside it, like amylase in the gut. The **active site** 活性位点 is complementary to the **substrate** 底物.

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** 锁钥假说: the active site is a fixed shape, and only a substrate with the matching shape fits.
Induced fit	The **induced-fit hypothesis** 诱导契合假说: 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** 酶底物复合物; the reaction happens, the **products** 产物 leave, and the site is free again.
Specificity	An example is **amylase** 淀粉酶, released into the gut to **digest** 消化 **starch** 淀粉 – and nothing else.

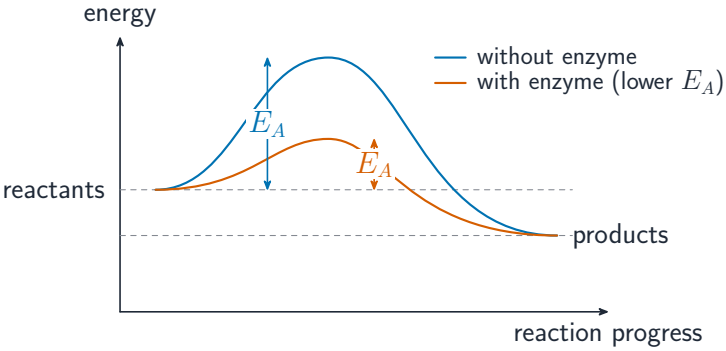
Induced fit explains what lock-and-key cannot: why binding itself strains the substrate's bonds and lowers the activation energy.

Lowering activation energy

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

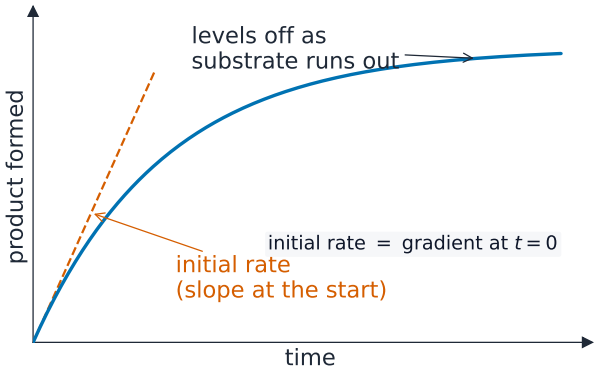
> An enzyme lowers it, so the reaction runs fast at the cell's normal temperature – no high heat needed.

Lowering activation energy, in detail



Every reaction needs an **activation energy** 活化能 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** 比色计 measures the absorbance, turning a colour change into a number you can plot.

> Product builds fastest at the start, so the **initial rate** 初速率 (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** 缓冲溶液, so pH is held while another variable is changed
controlling temperature	a water bath, and let the reactants reach temperature before mixing
kinetic energy 动能	raising temperature makes molecules move faster, so they **collide** 碰撞 more often and with more energy
beyond the optimum	the enzyme denatures – the active site changes shape and no **enzyme–substrate complex** 酶-底物复合物 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.

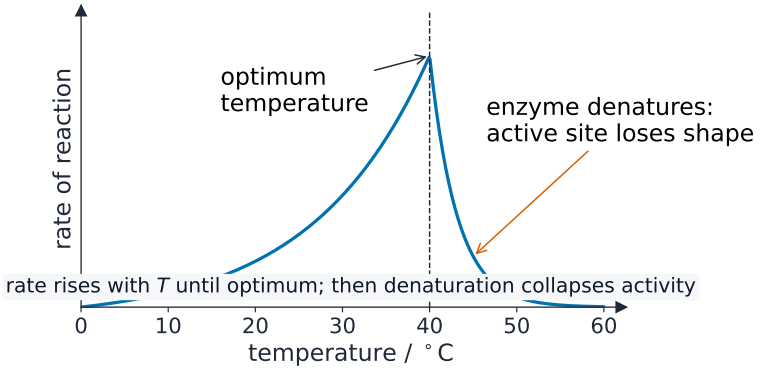
Why the initial rate is the fair comparison

It falls with time	The **rate of reaction** 反应速率 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.

2 Factors

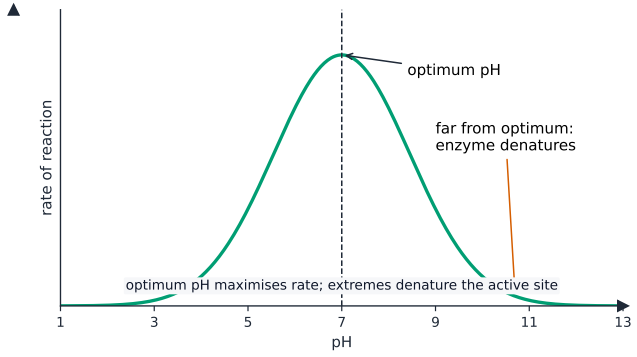
Optimum, then denatures



optimum, then denatures

optimum, then denatures

A bell-shaped optimum pH



a bell-shaped optimum pH

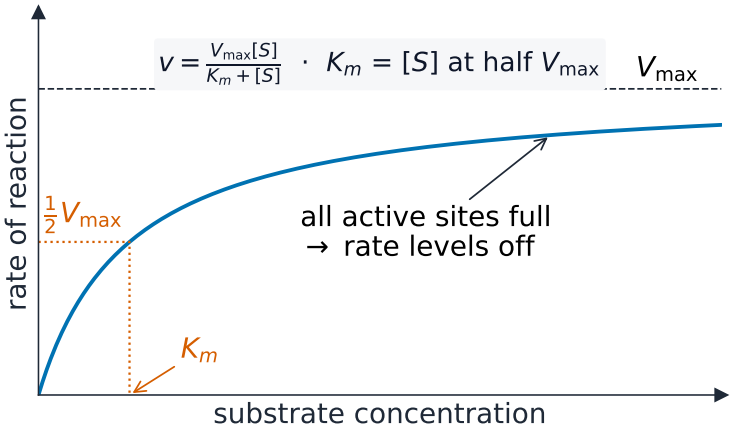
a bell-shaped optimum pH

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

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

> K_{m} (**Michaelis–Menten constant** 米氏常数) is the substrate concentration giving half $V_{\max}$ – a low K_{m} means high **affinity** 亲和力.

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

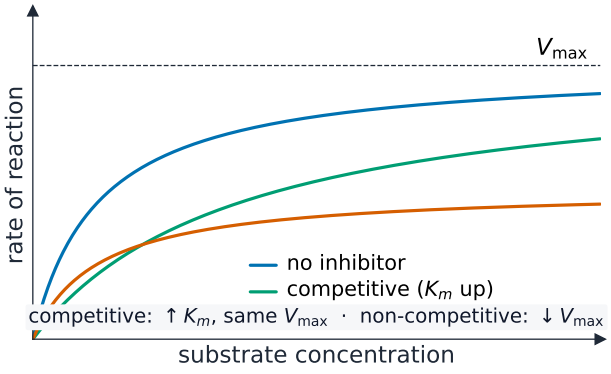


Rate climbs to $V_{\max}$ once all active sites are full.

Reversible inhibitors

- **competitive** 竞争性抑制剂 – binds the active site; more substrate overcomes it ($K_\mathrm{m}\uparrow$, V_max same)
- **non-competitive** 非竞争性抑制剂 – binds elsewhere; substrate can't help ($V_\mathrm{max}\downarrow$, K_m same)

Reversible inhibitors, in detail



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** 非竞争性抑制剂 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** 米氏常数 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.

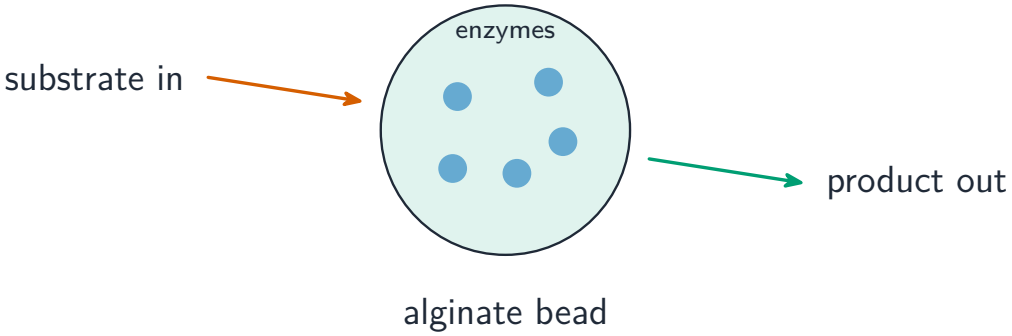
Immobilised enzymes

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

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

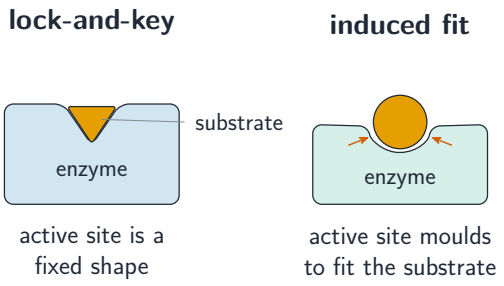
Used in **biological washing powders** 生物洗衣粉.

Immobilised enzymes, in detail



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

Specificity



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** 海藻酸盐 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.

3 Recap

Worked example – estimating a rate

In the first $20\,\mathrm{s}$ of a catalase reaction, $16\,\mathrm{cm}^3$ of oxygen is collected. Estimate the rate.

- Rate $= \dfrac{\text{volume of product}}{\text{time}} = \dfrac{16}{20} = 0.8\,\mathrm{cm}^3\,\mathrm{s}^{-1}$
- This is the average over the first $20\,\mathrm{s}$.
- 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

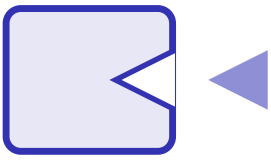
optimum temp & pH; denatures past them

4 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)