Proteins and enzymes

Amino acids and the levels of protein structure, globular and fibrous proteins, how enzymes work and what affects them, and the core practical on enzyme rates.

Amino acids and proteins

An **amino acid** has a central carbon bonded to an **amino group** ($-NH_2$), a **carboxyl group** ($-COOH$), a **hydrogen** and an **R group**. The R group differs between the 20 amino acids.

Amino acids join by **condensation**, forming a **peptide bond** and releasing a molecule of water. A chain is a **polypeptide**, and one or more polypeptides folded into a functional shape make a **protein**.

Levels of structure

Level	Description	Bonds
Primary	The **sequence** of amino acids	Peptide bonds
Secondary	The chain folds into an **α-helix** or **β-pleated sheet**	**Hydrogen bonds** between the C=O and N–H of the backbone
Tertiary	The 3D folding of the whole chain	**Hydrogen bonds**, **ionic bonds**, **disulfide bridges** (between cysteines) and **hydrophobic interactions** between R groups
Quaternary	Two or more polypeptide chains (and sometimes non-protein groups)	The same bonds as the tertiary structure

The primary structure determines the secondary and tertiary structures, and so the shape and properties of the protein. A change in one amino acid can change the whole shape (as in sickle cell haemoglobin).

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Globular and fibrous proteins

	Globular (e.g. haemoglobin, enzymes)	**Fibrous (e.g. collagen)**
Shape	Compact, rounded	Long, rope-like
Solubility	Usually **soluble**, with hydrophilic R groups on the outside	**Insoluble**
Function	Metabolic: transport, enzymes, hormones	**Structural**: strength

Haemoglobin has four polypeptide chains, each with a haem group (quaternary structure). It is soluble, so it can be carried in the red blood cell cytoplasm, and its shape changes as it binds oxygen.

Collagen has three polypeptide chains in a **triple helix**, with **hydrogen bonds** between them and **cross-links** between molecules. It is strong and flexible and is found in tendons, skin and artery walls. Every third amino acid is glycine, which is small, so the chains can pack tightly.

Enzymes

Enzymes are **biological catalysts** (globular proteins) that **lower the activation energy** of a reaction by providing an alternative reaction pathway, so the reaction goes faster without the enzyme being used up.

- **Intracellular** enzymes act inside cells (e.g. catalase, the respiratory enzymes).
- **Extracellular** enzymes act outside the cell that made them (e.g. digestive amylase).

Mechanism and specificity

The **active site** is a region of the enzyme with a specific shape, formed by the tertiary structure. The **substrate** binds, forming an **enzyme–substrate complex**.

- **Lock and key model**: the active site is a fixed shape that exactly matches the substrate.
- **Induced fit model**: the active site is **flexible** and changes shape slightly as the substrate binds, which puts a strain on the bonds in the substrate. This better explains the evidence.

The enzyme is **specific** because only a substrate with a **complementary shape** fits the active site.

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Factors affecting the rate

Factor	Effect
Temperature	Rate increases as particles gain kinetic energy and collide more often with more energy, up to an **optimum**. Above it, hydrogen bonds break, the **active site changes shape** and the enzyme is **denatured**. Cold does not denature; it slows the rate
pH	Each enzyme has an **optimum pH**. A change alters the ionic and hydrogen bonds, changing the shape of the active site, and denatures the enzyme at extremes
Enzyme concentration	The rate increases in proportion while substrate is in excess, as there are more active sites
Substrate concentration	The rate increases until all active sites are occupied (**saturation**), where the rate levels off and is limited by the enzyme concentration
Inhibitors	**Competitive** inhibitors are similar to the substrate and bind to the active site, so the effect is reduced by raising substrate concentration. **Non-competitive** inhibitors bind elsewhere and change the shape of the active site, so more substrate does not overcome the effect

Core Practical 4: initial rates

To investigate the effect of a factor on the **initial rate** of an enzyme-catalysed reaction:

1. Use a measurable product or substrate (e.g. volume of oxygen from catalase and hydrogen peroxide; the time for starch to disappear with amylase and iodine).
2. Measure **at the start** (the initial rate), when the substrate concentration is highest and the product has not yet built up. The rate is the **gradient of the tangent at** $t = 0$ of a graph of product against time, or $1/t$.
3. Vary one factor and **control** the others: use a **water bath** for temperature, **buffers** for pH, and the same volumes.
4. Pre-incubate the enzyme and substrate at the test temperature before mixing.
5. Repeat and calculate means.

> **TRY IT**
>
> Explain why the rate of an enzyme-catalysed reaction decreases after some time.

The substrate is used up (its concentration falls), so fewer enzyme–substrate collisions occur. Product may build up and the pH may change. For this reason the **initial rate** is used for comparisons.

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