Chem Factsheet

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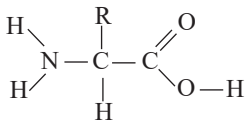
Proteins

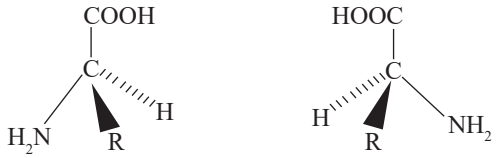
Proteins are vital biomolecules with a diverse range of roles such as providing structure, catalysis of metabolic reactions, cell signalling and genetic regulation to name a few. Without proteins, life would be very different, and certainly a lot slower than it is now.

Amino Acids

The basic building blocks of any protein are amino acids. These are organic molecules with a primary amine group (—NH_2) at one end and a carboxylic acid group (—COOH) at the other. In between these two groups is a carbon atom attached to a variable side chain (R) and a hydrogen atom. In nature, all proteins are made from a pool of 20 different amino acids resulting from 20 different R groups.



With the exception of the amino acid glycine (i.e. 2-aminoethanoic acid where R = H), all amino acids are chiral and exhibit optical isomerism, existing as a pair of enantiomers (non-superimposable mirror images).



d-isomer l-isomer

In nature, amino acids almost exclusively adopt the L-configuration which means that all proteins are also chiral which is vital for the specific functions they carry out.

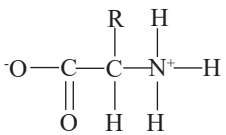
Variable Side Groups

The side group, R, may vary significantly in size and chemistry. Some side groups are made of non-polar hydrocarbons, e.g., alanine (R = CH_3) and leucine (R = $CH_2CH(CH_3)_2$). Some contain polar hydroxyl, amine or carboxyl groups, e.g., serine (R = CH_2OH), lysine (R = $(CH_2)_4NH_2$) and glutamic acid (R = CH_2CH_2COOH), while others contains sulphur atoms, e.g., cysteine (R = CH_2SH).

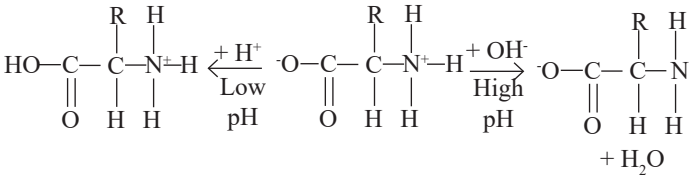
The side groups of the amino acids in the protein are pivotal for determining the three-dimensional shape it adopts as well as the nature of the molecules with which the protein can interact.

Zwitterions

At a physiological range of pH values, amino acids possess charged carboxylate and ammonium groups (some may also contain charged side groups) and exist as zwitterions which have no overall electrical charge.



If the pH drops (more acidic), the carboxylate group (COO^-) gains a proton to reform the carboxylic acid (COOH); should the pH rise (more alkaline) then the ammonium group (NH_3^+) loses a proton to reform the amine group (NH_2).

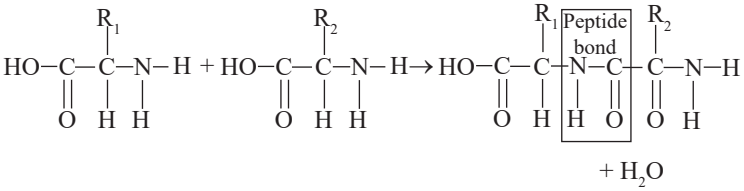


Isoelectric Point [pI or pH(I)]

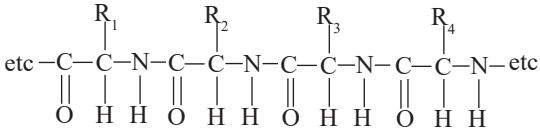
The isoelectric point of an amino acid or protein is the pH value at which the molecule has no overall electrical charge; in the case of amino acids this is when they exist as zwitterions. When the pH is below the pI value then the molecule will have an overall positive charge (NH_3^+ predominates); when the pH is above the pI value, the molecule will have an overall negative charge (COO^- predominates). The ability to modify the charge of a protein by altering the pH of the environment is important for the separation of proteins and amino acids by gel electrophoresis (see later).

Polypeptides

Amino acids are able to polymerise to form long chains called **polypeptides**, which are the basis of protein structure. The amine group (NH_2) of one amino acid reacts with the carboxyl group (COOH) of another in a **condensation** reaction; a molecule of **water** is released in the process. The chemical bond that forms between them (NH-CO) is termed an **amide linkage** or **peptide bond**.



This condensation process can be repeated at both ends of this **dipeptide** to eventually form the polypeptide which is therefore a **condensation polymer**.

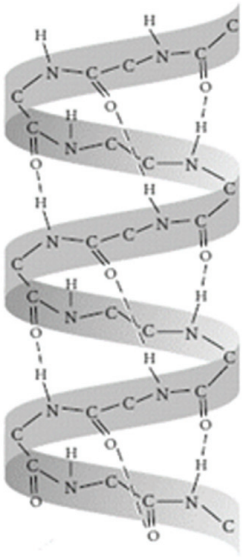


Structure of Proteins

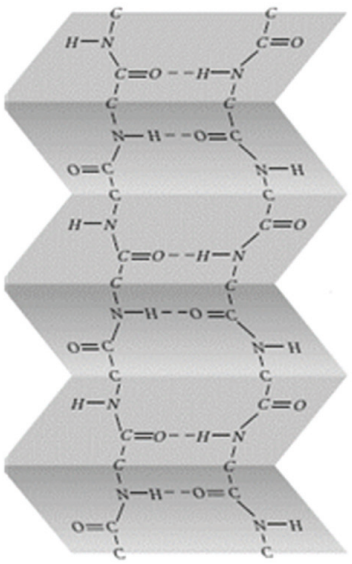
Protein structure can be split into four distinct categories.

Primary structure – this is the linear arrangement of contiguous amino acids as indicated in the previous general diagram. It is characterised by (a) which amino acids are present (b) how many of each of those amino acids are present and (c) the sequence in which those amino acids are linked. Complete hydrolysis followed by chromatography (see later) can elucidate (a) and (b). Partial hydrolysis followed by chromatography (see later) can elucidate a lot about (c). Proteins do not adopt primary structures in nature.

Secondary structure – the primary structure is able to coil up to form an **alpha-helix** or form **beta-pleated sheets** of parallel amino acid chains. These structures are stabilised by **hydrogen bonding** between the N—H and C=O groups in the amide (peptide) linkages between amino acids. Keratin (found in hair and nails) is an example of a protein with only secondary structure.

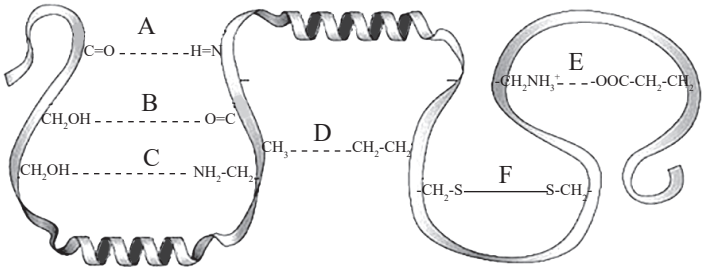


α - helix



β - pleated sheet

Tertiary structure – linear amino acid chains and secondary structures are able to fold up into a three-dimensional shape via the interactions of the variable R side groups (see later) : or hydrogen bonds between CO and NH groups within the primary chain (type A) : or between a side group and a primary chain group (type B). Many proteins adopt tertiary structures and are called globular proteins. The three-dimensional nature of these proteins creates specifically shaped active sites through which they can catalyse reactions and interact with signalling molecules.



Quaternary structure – multiple tertiary proteins come together as a series of subunits to form a much larger quaternary complex. Haemoglobin, made of four globular subunits, is good example of a quaternary structure.

Side Group Interaction

The variable side groups may interact in a number of ways to give individual globular proteins their unique three-dimensional shape:

TYPE C. Hydrogen bonding: between uncharged amine (NH_2), hydroxyl (OH), carboxyl (COOH) groups.

TYPE D. Non-polar interactions (London forces): between non-polar or aromatic groups, e.g., CH_3 or $CH_2C_6H_5$.

TYPE E. Electrostatic interactions: between electrically-charged ammonium (NH_3^+) and carboxylate (COO^-) groups.

TYPE F. Disulfide bridges: a covalent bonds forms between –SH groups on juxtaposed cysteine residues (–S—S—).

Factors affecting protein structure

1. **Heating** a globular protein to temperatures typically in excess of 40 °C, disrupts many of these interactions (A-E) and the specific three-dimensional shape of the protein is changed and functionality is lost. The protein is said to have been **denatured**.
2. **Changes to the pH** of the environment may also have the same effect. Low pH values with turn uncharged NH_2 groups into NH_3^+ and charged COO^- into uncharged COOH. High pH values turn NH_3^+ to NH_2 and COOH to COO^-. This disrupts both hydrogen bonding and electrostatic interactions.
3. **Heavy metal ions** such as Hg^{2+} (mercury) form covalent bonds to the sulphur atoms of cysteine, preventing the formation of disulphide bridges (this accounts for a part of their toxic nature).

Separating proteins and amino acids

Amino acids are normally separated via paper or thin layer **chromatography**.

Briefly,

1. the protein is hydrolysed by reflux in 6 mol dm^{-3} hydrochloric acid for a few hours.
2. the resulting mixture of amino acids is spotted on to the chromatography paper or plate.
3. The bottom of the plate is dipped in an appropriate solvent and the apparatus is sealed.
4. After the solvent and sample have had time to rise up the plate, the plate is removed and the position of the solvent front marked.
5. The solvent is evaporated from the plate and a locating agent such as **ninhydrin** is sprayed on to the plate. Ninhydrin turns purple in the presence of amino acids.
6. The positions of the amino acids are marked and either: (1) R_f values are calculated and compared to literature values or (2) the positions are compared with pure, known standards run at the same time.

Proteins are normally separated by gel **electrophoresis**. Briefly,

1. The mixture of proteins is injected into the receiving wells on a gel matrix immersed in a buffer solution.
2. A potential difference is applied across the gel using a power pack.
3. Positively-charged proteins migrate towards the negative electrode and negatively-charged proteins move towards the positive electrode. Neutrally-charged proteins do not migrate in either direction.
4. The distance travelled by any protein is dependent on its overall charge and its molecular mass.
5. The proteins can be visualised using a protein stain such as Coomassie Brilliant Blue.

Questions

1. Explain why the activity of an enzyme begins to decrease if it is heated above its optimum working temperature.
2. The isoelectric point of alanine ($H_2NCH(CH_3)COOH$) is 6.1. State the formula of the species present at a pH of: (a) 4.0 (b) 9.0
3. Suggest what types of interaction might occur between the side groups of:
   a. phenylalanine ($R = CH_2C_6H_5$) and valine ($R = CH(CH_3)_2$)
   b. aspartic acid ($R = CH_2COO^-$) and lysine ($R = (CH_2)_4NH_3^+$)
   c. two cysteine ($R = CH_2SH$) residues.

Answers

1. High temperature disrupts interactions between side groups, three-dimensional structure altered, protein denatured, active site of enzyme no longer recognises correct substrate.
2. a. $^+H_3NCH(CH_3)COOH$ b. $H_2NCH(CH_3)COO^-$
3. a. London forces/dispersion forces/hydrophobic/non-polar interactions/van der Waals' forces.
   b. electrostatic interaction.
   c. disulphide bridge.

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