



## Structure and Biological Functions of Proteins

By studying this Factsheet the student should gain knowledge and understanding of:

- The primary, secondary, tertiary and quaternary structure of proteins, including fibrous and globular types.
- The effect of pH on amino acids and proteins.
- Denaturation by extremes of pH or temperature.
- The biological functions of proteins, enzymes, hormones, carriers, membrane proteins, including structural, contraction, protection (antibodies), osmotic and buffering roles.

For a full description of the chemical bonds referred to in this Factsheet the student should refer to Factsheet No.78, September 2000, Chemical Bonding in Biological Molecules.

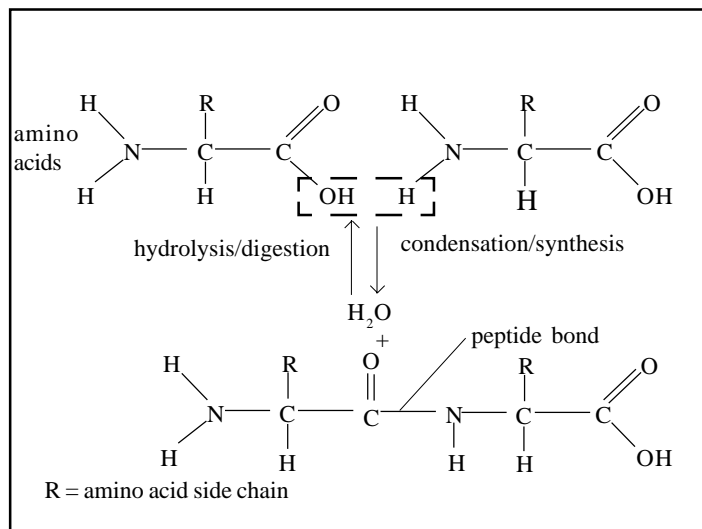
**Remember** - amino acids are made in autotrophic green plants as products of photosynthesis, and then are assembled into proteins. Heterotrophic organisms gain their amino acids and proteins from plants through food chains in the case of animals, or in decay processes in the case of bacteria and fungi.

### The structure of proteins

Twenty types of amino acid occur which form the 'building blocks' of proteins. Amino acids join together by peptide bonds, formed by condensation between the acid group of one amino acid and the amine group of the other amino acid (Fig 1). When two amino acids join in this way the product is a dipeptide. Many amino acids joined in this way make up a polypeptide.

**Remember** - condensation is the joining of molecules by the removal of water and is used in many synthetic processes. The reverse process is hydrolysis which is the splitting of molecules by the addition of water and is used in digestion.

Fig 1. Formation of a peptide bond between two amino acids



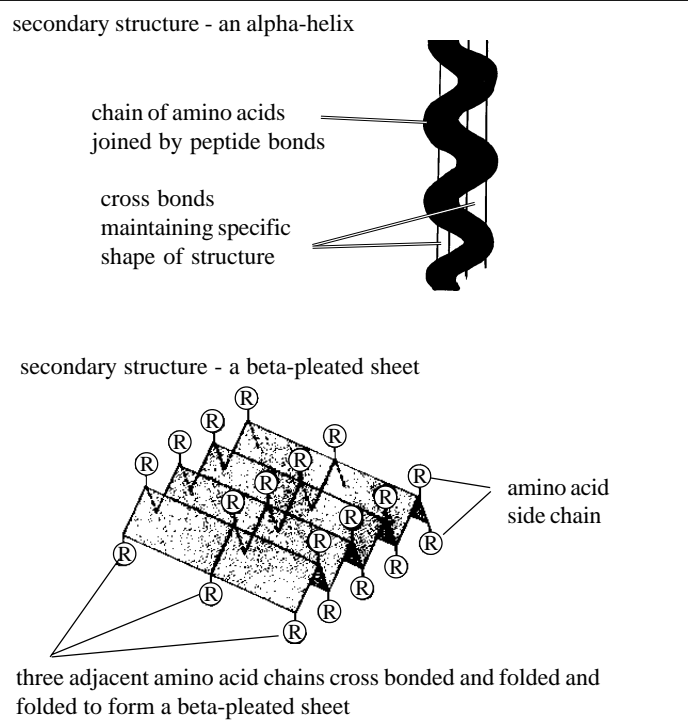
More amino acids can join by peptide bonds onto the ends of the dipeptide resulting in the formation of a polypeptide. The polypeptide with its specific sequence of amino acids is called the 'primary structure of the protein'.

**Remember** - the sequence of amino acids in the polypeptide is governed by the sequence of codons in the gene that assembles that polypeptide by using the messenger RNA/transfer RNA/ribosome mechanism.

The polypeptide chain is folded to make particular three dimensional shapes known as the 'secondary structure of the protein'. These shapes may either be of the alpha-helix type or the beta-pleated-sheet type. They are characteristic of fibrous type structural proteins. The secondary structure may be further folded tightly to give the 'tertiary structure of the protein'. This is characteristic of globular type proteins such as enzymes and antibodies. Secondary and tertiary structures are still single polypeptides. The 'quaternary structure of a protein' is the way in which polypeptides (in secondary or tertiary form) join together to form proteins.

The secondary, tertiary and quaternary structures are not loosely, randomly folded structures but are precisely shaped and cross-bonded by ionic, hydrogen, sulphur and peptide bonds. These are formed between reactive groups in the amino acid side chains. (The core acid and amine groups of the amino acids are already involved in joining the amino acids by peptide links). Figs 2 and 3 show three dimensional forms of secondary, tertiary and quaternary polypeptides and protein molecules.

Fig 2. Three dimensional forms of polypeptide- Secondary structures



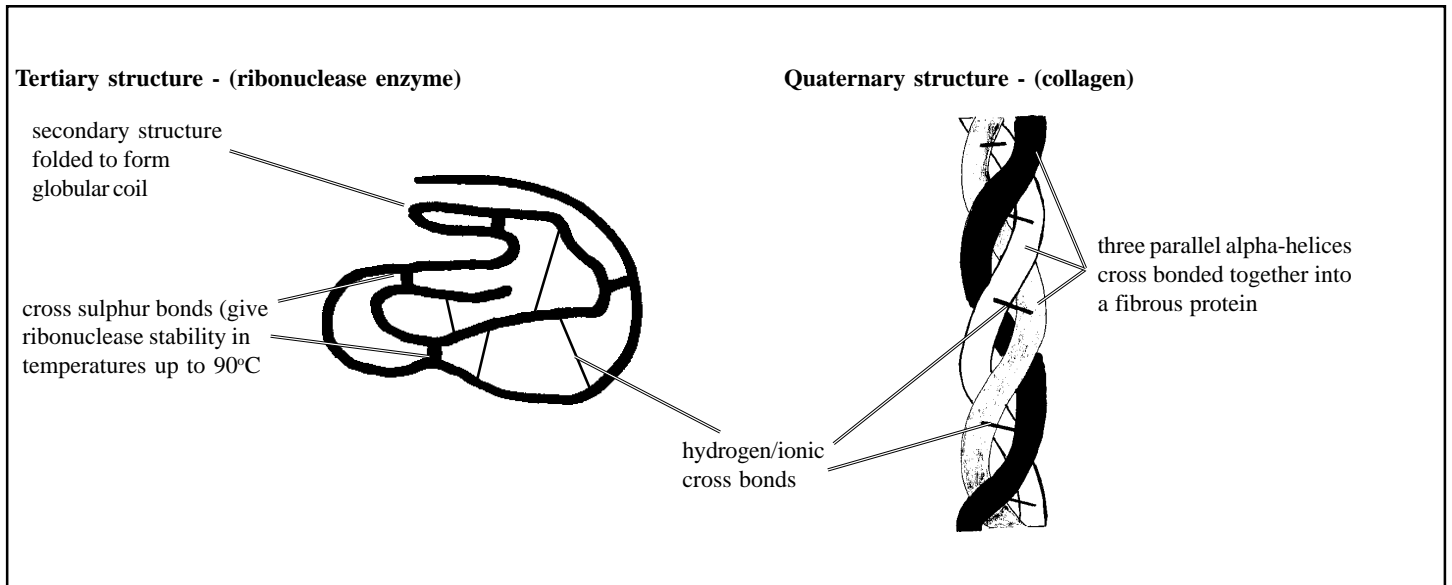
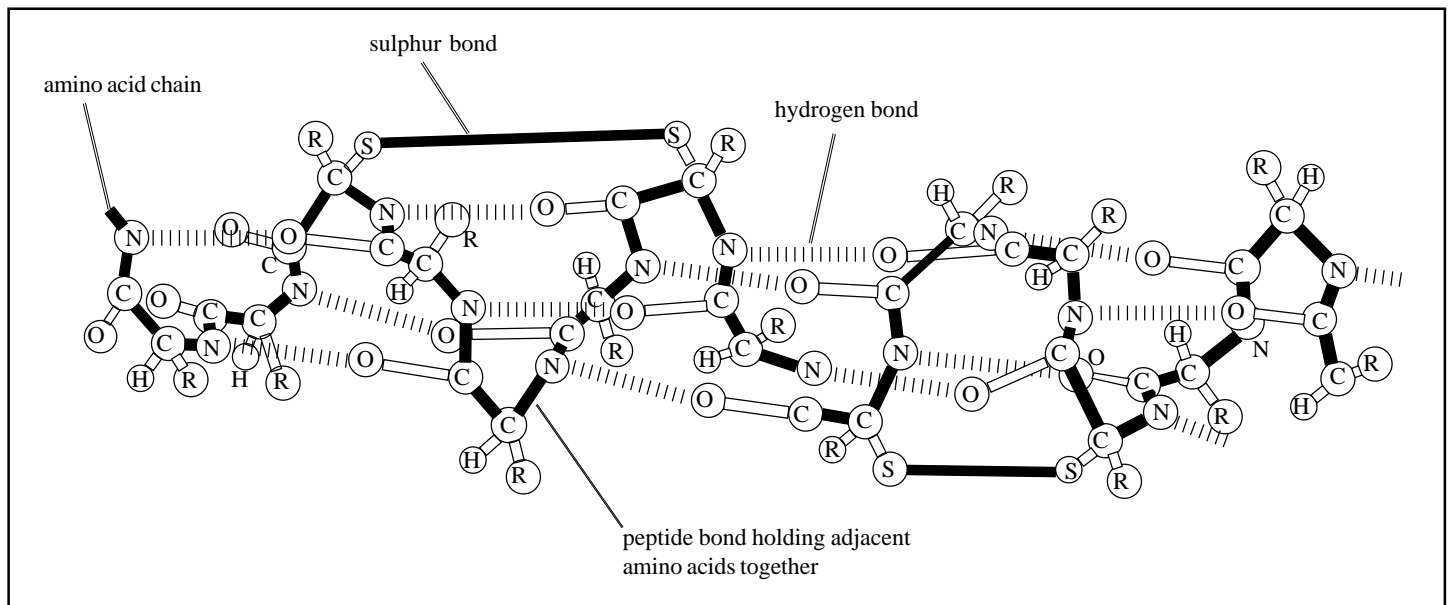
**Fig 3. Three dimensional forms of polypeptide and protein molecules - Tertiary and Quaternary**

Fig 4 shows the structural formula of an alpha-helix.

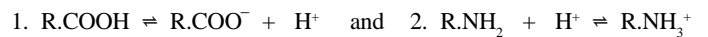
**Fig 4. Structural forms of an alpha-helix**

### Types of protein

In addition to being classed as fibrous or globular forms according to their 3D structure, proteins may be classed as **simple** or **conjugated**. Simple proteins only contain amino acids in their structure and exist as several different types, such as albumins, globulins and scleroproteins. Examples of these types will be named later. Conjugated proteins contain amino acids plus some other type of chemical molecule, such as nucleic acids in nucleoproteins, phosphoric acid in phosphoproteins and lipids in lipoproteins. Haemoglobin is a conjugated protein consisting of four globular polypeptides each of which contains a porphyrin ring which also contains iron.

### The effect of pH on amino acids and proteins

The pH measures the hydrogen ion concentration of the medium in which the amino acid or protein is, whether, for example, in blood, tissue fluid, cell, animal or plant or soil. The hydrogen ion concentration will affect how the amino acids and proteins ionise. The acid and amine groups of amino acids ionise as shown in the equilibrium reactions:



Thus, in a high hydrogen ion concentration (acid pH) reaction 1 will tend to be pushed to the left and reaction 2 will tend to be pushed to the right. The amino acids will therefore be predominately positively charged **cations**.

In a lower hydrogen ion concentration (less acid or alkaline pH) reaction 1 will tend to proceed to the right and reaction 2 will tend to proceed to the left. The amino acids will therefore be predominately negatively charged **anions**.

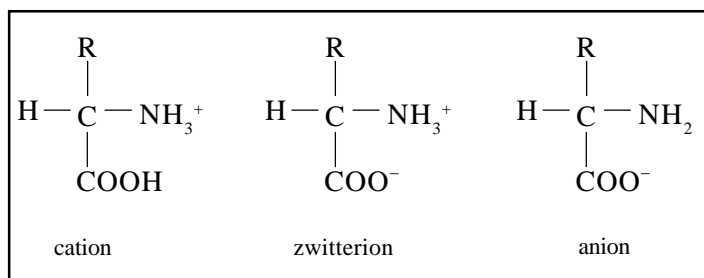
There is an intermediate hydrogen ion concentration where the forward and backward rates of reactions 1 and 2 are equal (50:50). The amino acids will then carry 50% of amine groups charged and 50% of amine groups uncharged, 50% of acid groups charged and 50% of acid groups uncharged. Such ions are called **zwitterions** (German for 'ions of two types'). The pH at which this occurs is a physical constant for each specific amino acid or protein and is called the **iso-electric point (IEP)**.

**Remember** – the iso-electric point is the pH at which the amino acids or protein carry no net charge/carry equal amounts of negative and positive charges.

**Exam hint** – a common omission when defining 'iso-electric point' is to fail to refer to pH. Candidates often just say 'the iso-electric point is the point at which the protein carries no net charge'. Candidates also often incorrectly say that the IEP must be pH 7.

Proteins behave in a similar way to amino acids but the charges are on acid, amine and hydroxide groups in the amino acid side chains – the core amine and acid groups are bound up in the peptide bonding. The ionic state of amino acids is shown in Fig 5.

**Fig 5. Ionic states of an amino acid**



The charges on a protein resulting from the pH effect may have an influence on its behaviour:

- the charges on the active sites of an enzyme may affect the capability of the enzyme to join with its specific substrate. This is why enzymes tend to work best at specific pHs.
- at the IEP the protein carries equal numbers of opposite charges. Opposite charges attract which may make the protein molecules clump together and precipitate. At other pHs the protein only carries like charges. These repel molecules from each other and thus may increase the solubility.
- at extremes of pH the protein molecules may carry huge numbers of like charges as reactions 1 and 2 go almost to completion. These charges may exert a large repulsive force which breaks apart the hydrogen and ionic bonds holding the 3D structure together. The 3D structure therefore breaks apart and the protein is denatured since its structure and functional ability is lost

**Remember** – denaturation is the loss of function of a protein caused by a loss of structure. Another agent of denaturation may be heat. This can disrupt the hydrogen and ionic bonds thus causing the 3D structure to unravel. Most proteins denature around 45°C. Sulphur bonds are more stable to heat and thus proteins with many such bonds can withstand higher temperatures. e.g. enzymes in bacteria which live in hot springs and ribonuclease in saliva.

### The range of biological functions of proteins

- **structural proteins:** Many structural proteins belong to the class of scleroproteins. Examples are;
  - **collagen** – found as strong non-elastic white fibres in tendons, cartilage and bone.
  - **elastin** – found as yellow elastic fibres in ligaments and joint capsules.
  - **keratin** – found as a horny impermeable protein in skin, hair, feathers, nails and hooves.

Other structural proteins are the **lipoproteins** of cell membranes, **viral coat proteins**, **fibroin** found as spider silk and cocoon silk, **sclerotin** found in insect exoskeletons, and **mucoproteins** found in lubricating joint (synovial) fluid.

- **enzymes:** syllabus examples are **hydrolases** such as amylases, proteases and lipases used in digestion, **oxido-reductases** such as the dehydrogenases used in the metabolic cycles and **ligases** which enable molecules to be bonded together using the energy from ATP.
- **hormones:** some hormones are protein in nature, such as **somatotropin** – pituitary growth hormone and **insulin** which regulates blood glucose concentrations.
- **contractile proteins:** some proteins can contract and lengthen and thus enable movement. Examples are **actin** and **myosin** found in muscles and **dynein** making up the structure of cilia and flagella.
- **storage proteins:** because of their toxic amine groups, amino acids cannot be stored, unless they are bound within protein structure. Examples are **ovalbumin** or egg white protein, **casein** and **lactalbumins** which are milk proteins, **glutelins** and **gliadins** which are cereal seed proteins and **ferritin** which binds up iron and stores it in the spleen, liver and red bone marrow.
- **transport proteins:** bind on to and release insoluble or inadequately soluble substances so that they can be transported through the body. Examples are **haemoglobin** for oxygen transport in vertebrate blood, **myoglobin** for oxygen transport in muscles, **plasma albumin** which transports fatty acids in blood, **transferritin** which transports iron through blood to the iron storage sites and **binding globulins** which transport insoluble thyroid hormones through blood.
- **protective proteins;** examples are the blood clotting factors such as **thrombin** and **fibrinogen** which reduce bleeding during injury, **antibodies** (gamma globulins) which can react with foreign proteins (antigens) to neutralise them, thus giving protection against disease, and **complement** which can form complexes with antigen-antibody systems enhancing their activity.
- **buffers:** many amino acids and proteins have buffering ability and thus reduce pH change within the organism. A classic example is **haemoglobin** which can react with hydrogen ions forming **reduced haemoglobin**. This buffers the blood between pH 7.2 and 7.6.
- **osmotic proteins:** **plasma albumin** in blood is responsible for much of the osmotic pressure or water potential of blood, which tends to hold water in the blood plasma thus maintaining the blood volume. Proteins in most biological fluids, such as cell sap in plant cells and in invertebrate bloods, have a similar role.
- **toxins;** some proteins act as toxins or poisons. Examples are the **phospholipase** enzymes found in many snake venoms – these destroy cell membranes. Many bacteria such as **Clostridium tetani**, **Clostridium botulinum** and **Diphtheria**, release toxic chemicals that are very dangerous to humans. **Ricin** is a toxic chemical that is found in castor oil beans which if taken, in contaminated castor oil, causes jaundice, gastrointestinal problems and heart failure.

**Exam hint** – questions on functions of protein may often require continuous prose or essay type answers. Make sure that you can illustrate your answers by reference to specific examples for each function.

**Specimen Questions**

1. Read through the following account of protein structure and then complete the passage by writing in the most suitable word or words in the spaces.

Amino acids join together into polypeptide chains by ..... bonds which are formed by ..... reactions. The linking bonds are formed between the ..... and ..... groups of the amino acids when ..... is released from the reaction.

The polypeptide chains may be folded into secondary structures, such as the ..... and ..... . Secondary structures may be further folded into tertiary structures, such as the ..... . Polypeptides are combined together to give the ..... structure of the protein. These three dimensional shapes of the protein are held firmly in place by ionic bonds, ..... bonds and covalent ..... bonds. Disruption of these bonds, by extremes of pH or exposure to ..... causes ..... of the protein.

**Total 13**

2. Suggest reasons for the following observations:
  - (a) Proteins may precipitate at certain pHs but be soluble at other pHs. 4
  - (b) Casein precipitates at pH 4.7 but lysozyme precipitates at pH 11.0. 3
  - (c) Most enzymes denature around 45°C but ribonuclease does not denature until 90°C. 3

**Total 10**

3. Complete the following table which concerns proteins and their functions. Give one example only in each case.

Function	Example
Forms a waterproof hard layer on skin surface.	
Forms a foodstore in cereal grains.	
Enables joint capsules to stretch.	
Transports iron through blood	
Can regulate growth in mammals.	
Forms strong fibres in muscle tendons.	
Holds oxygen in muscles.	
Can join molecules using energy from ATP.	
Combines with antigen-antibody debris.	
Makes cilia mobile.	

**Total 10**

**Answers**

1. peptide; condensation; acid/amine;; water; alpha helix/beta pleated sheet;; globular/coil/type/shape; quaternary; hydrogen; sulphur; high temperatures; denaturation;

**Total 13**

2. (a) different proteins have different iso-electric points; at the IEP equal numbers of opposite charges attract; thus protein molecules clump together and precipitate; at other pHs all charges are similar and so repel, preventing clumping and precipitation; 4
- (b) the IEP of casein is probably pH 4.7 and that of lysozyme is probably pH 11.0; proteins are most likely to precipitate at their IEPs; due to carrying equal numbers of attracting opposite charges; 3
- (c) most enzymes are bonded together by mainly ionic and hydrogen bonds; these are very susceptible to disruption by heat/are not heat stable; ribonuclease contains many sulphur bonds which are heat stable; 3

**Total 10**

- 3.

Function	Example
Forms a waterproof hard layer on skin surface.	<b>keratin;</b>
Forms a foodstore in cereal grains.	<b>glutelins/gliadins;</b>
Enables joint capsules to stretch.	<b>elastin;</b>
Transports iron through blood	<b>transferritin;</b>
Can regulate growth in mammals.	<b>somatotropin;</b>
Forms strong fibres in muscle tendons.	<b>collagen;</b>
Holds oxygen in muscles.	<b>myoglobin;</b>
Can join molecules using energy from ATP.	<b>ligase (enzyme);</b>
Combines with antigen-antibody debris.	<b>complement;</b>
Makes cilia mobile.	<b>dynein;</b>

**Total 10**

**Acknowledgements;**

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