Proteins and Water

PROTEINS

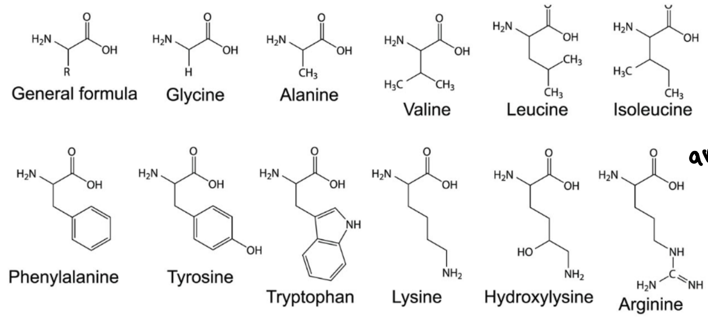
Proteins are an extremely important class of macromolecules in living organisms. More than 50% of the dry mass of most cells is protein. Proteins have many important functions, for instance:

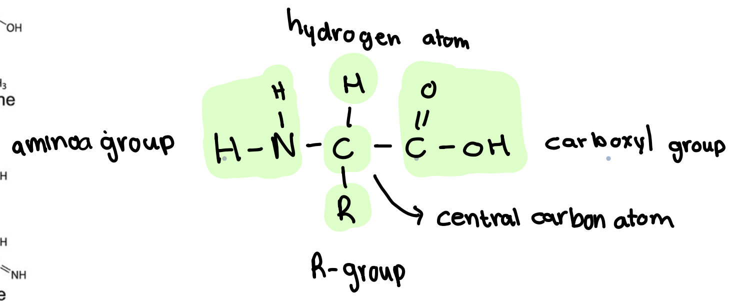
- all enzymes are proteins;
- proteins are essential components of cell membranes - their functions in membranes, such as receptor proteins and signalling proteins,
- some hormones are proteins - for example, insulin and glucagon;
- the oxygen-carrying pigments haemoglobin and myoglobin are proteins; o antibodies, which attack and destroy invading microorganisms, are proteins;
- collagen, another protein, adds strength to many animal tissues, such as bone and the walls of arteries;
- hair, nails and the surface layers of skin contain the protein keratin;
- actin and myosin are the proteins responsible for muscle contraction;
- proteins may be storage products - for example, casein in milk and ovalbumin in egg white.

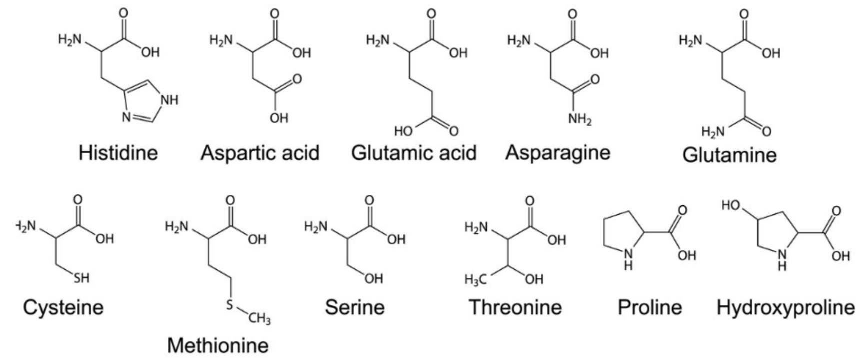
Despite their tremendous range of functions, all proteins are made from the same basic monomers. These are amino acids. Proteins are macromolecules made from one or more chains of amino acids known as polypeptides. Polypeptides are unbranched molecules composed of approximately ten or more amino acids linked together by peptide bonds. Haemoglobin is a protein consisting of 574 amino acids, and the insulin hormone consists of 51 amino acids.

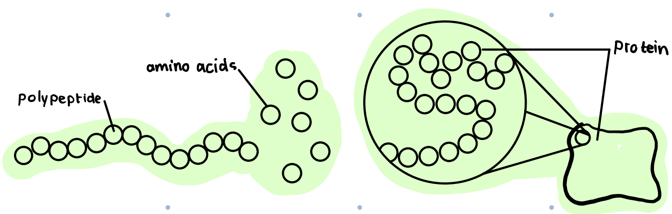
Structure of an amino acid

Amino acids are molecules used to build proteins. All amino acids have a central carbon atom surrounded by a hydrogen atom, a carboxyl group (COOH), an amino group (NH_2) and an R-group. It is the R-group or side chain that differs between the 20 amino acids.





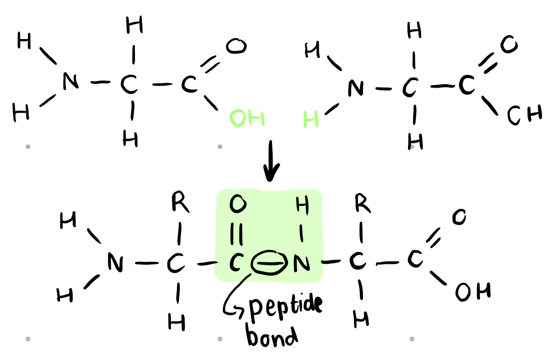




Cells use 20 different types of amino acids to make different proteins.

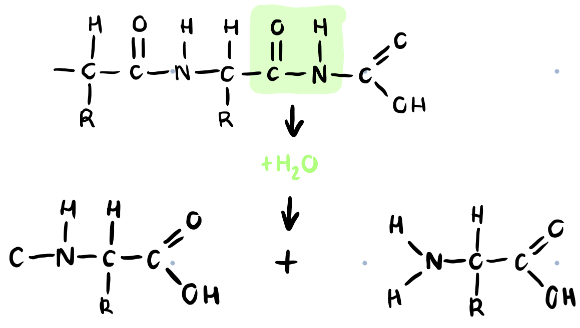
The formation of a peptide bond

A peptide bond is a chemical bond formed between two amino acid molecules when the carboxyl group of one molecule reacts with the amino group of the other molecule. An H from the amino group combines with an OH from the carboxyl group, forming and eliminating a molecule of water (H_2O) during a condensation reaction. Amino acids are joined by peptide bonds. These bonds link the C of the carboxyl group of one amino acid to the N of the amino group of the other.



A dipeptide (di = 2) is a short protein consisting of only two amino acids linked by one peptide bond.
Plece
A tripeptide (tri = 3) is a short protein consisting of three amino acids joined by two peptide bonds.
An oligopeptide (oligo = few) is a short protein consisting of less than ten amino acids.
A polypeptide (poly = many) is a protein chain of more than ten amino acids.
A protein is a long chain having fifty or more amino acids.

The breakage of a peptide bond



Proteins and polypeptides can be broken down into amino acids by breaking the peptide bonds. This is a hydrolysis reaction involving the addition of water and catalysation of enzymes.
This happens naturally in the stomach and small intestine during digestion where proteins are broken down into amino acids for absorption.

ORGANISATION OF PROTEIN STRUCTURE

There are four levels of protein structure: primary, secondary, tertiary and quaternary. It is helpful to understand the nature and function of each level of protein structure, to fully understand how a protein works.

Primary structure is the sequence of amino acids in a polypeptide or protein.

Secondary structure is the structure of a protein molecule resulting from the regular coiling or folding of the chain of amino acids, e.g. an α-helix or β-pleated sheet.

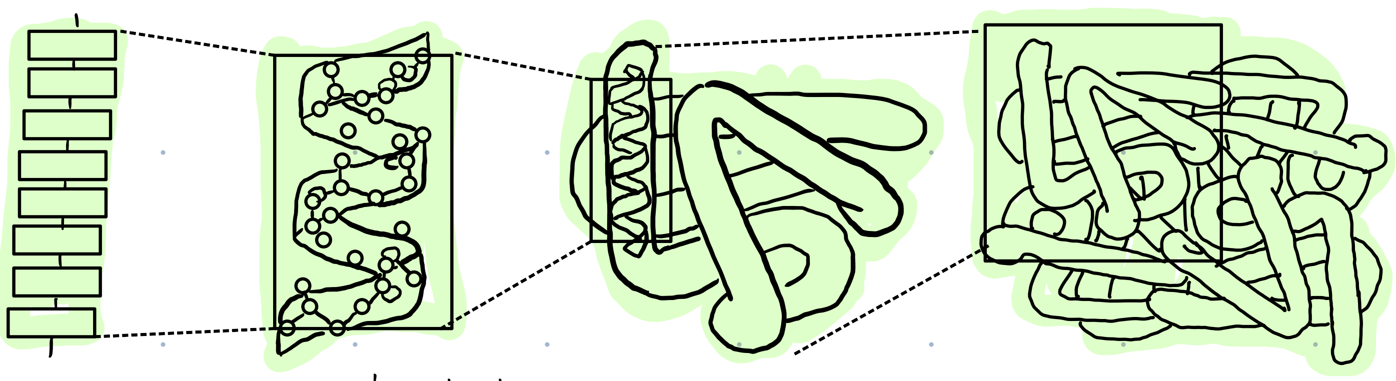
Tertiary structure is the compact structure of a protein molecule resulting from the three-dimensional coiling of the already-folded chain of amino acids.

Quaternary structure is the three-dimensional arrangement of two or more polypeptides, or of a polypeptide and a non-protein component such as haem, in a protein molecule.

- Each polypeptide chain shows the primary structure, which is a long linear chain of amino acids in specific sequence (order) and number (quantity). The number and sequence of amino acids are determined by the gene in the DNA that codes for a specific polypeptide. A polypeptide or protein molecule may contain several hundred amino acids linked into a long chain. The amino acids are held by peptide bonds that are made during the process of proteinsynthesis.

  There is an enormous number of different possible primary structures. Even a change in one amino acid in a chain made up of thousands may completely alter the properties of the polypeptide or protein.

- Each primary structure forms a secondary structure immediately after formation at the ribosome. The different amino acids cause folding or twisting of the chain in various ways. it can coil into a corkscrew shape called an alpha helix (α-helix) where the polypeptide forms a right-handed helix. The beta-pleated sheet (β -pleated sheet) is where the polypeptide folds back and forth to fold into a flat sheet. These shapes are permanent and are held and stabilised by hydrogen bonds. These interactions are between the R-groups of amino acids. Hydrogen bonds, although strong enough to hold the α-helix and β -pleated sheet structures in shape, are easily broken by high temperatures and pH changes. As you will see, this has important consequences for living organisms.
- The secondary structure forms the tertiary structure, because the secondary structure folds further into a unique complex 3D shape. Some proteins take up a tertiary structure in the form of a long, much-coiled chain and are called fibrous proteins, e.g. collagen. Other proteins take up a tertiary structure that is more spherical and are called globular proteins, e.g. enzymes. This structure is stabilised by ionic and di bonds and hydrogen interactions.
- Many protein molecules are made up of two or more polypeptide chains. The quaternary structure of proteins thus arises when two or more polypeptides or proteins associate together to form a complex, biologically active protein molecule. These polypeptides can be identical or different. The chains are held together by the same four types of bonds as in the tertiary structure. Haemoglobin is an example of this, having four polypeptide chains in each molecule. More details of haemoglobin are discussed later.



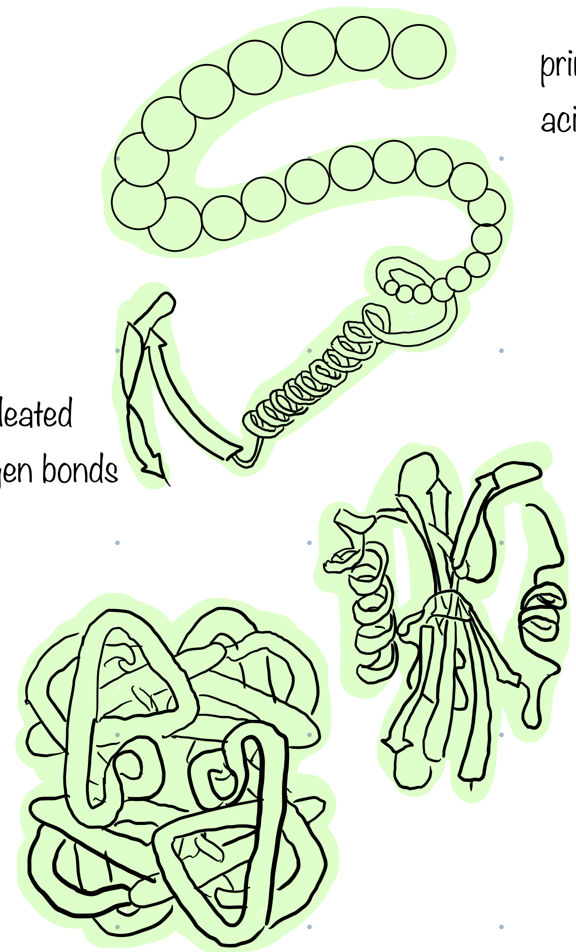
primary structure: long chain of amino acids held by peptide bonds

secondary structure: primary structure coils into alpha helix held by hydrogen bonds

tertiary structure: forms a unique complex 3D shape stabilised by disulfide and ionic bonds

quaternary structure: two or more polypeptides or proteins associated together primary structure: long chain of amino acids held by peptide bonds

secondary structure:
primary structure coils into beta-pleated sheet or alpha helix held by hydrogen bonds

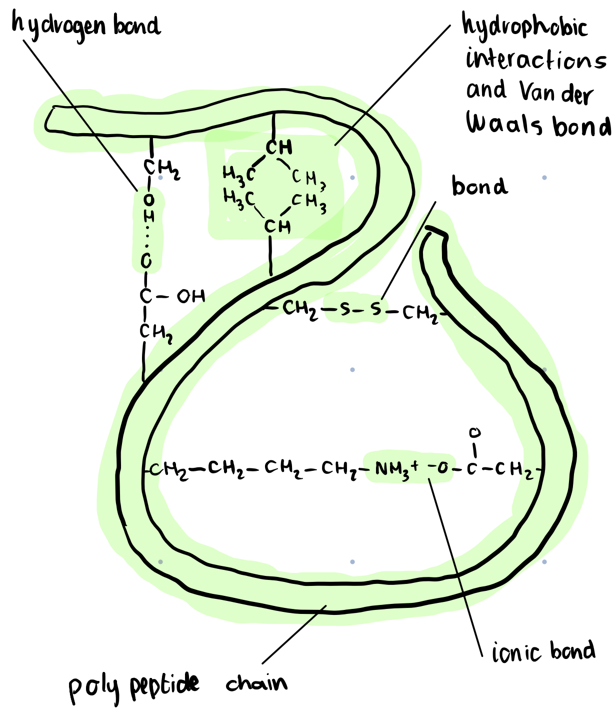


tertiary structure:
forms a unique complex 3D shape stabilised by and ionic bonds

quaternary structure: two or more polypeptides or proteins associated together

BONDS THAT STABILISE PROTEIN STRUCTURE (HYDROGEN, IONIC, DISULFIDE AND HYDROPHOBIC INTERACTIONS)

There are four bonds that stabilise polypeptides and proteins.
- Hydrogen bonds form between polar groups, such as the dipolar -OH and -CO groups on either side of the -C-N- bond. A hydrogen atom is shared by two other atoms. Hydrogen bonds are weak, but are common to help stabilise protein structure.
- Disulfide bonds form between the sulfur-containing R-groups (of cysteine residues). It is a strong covalent bond formed by oxidation of -SH groups of two cysteine side-chains.
- Ionic bonds form between ionised amino ($-NH_3^+$) and carboxyl (-COO-) groups of R-groups. This may often be broken by changing the pH.
- Hydrophobic interactions and Van der Waals forces (bonds) form between non-polar R-groups. It is the clustering of hydrophobic groups away from water. These come into play when two or more atoms are very close (0.3 - 0.4m apart).



GLOBULAR AND FIBROUS PROTEINS

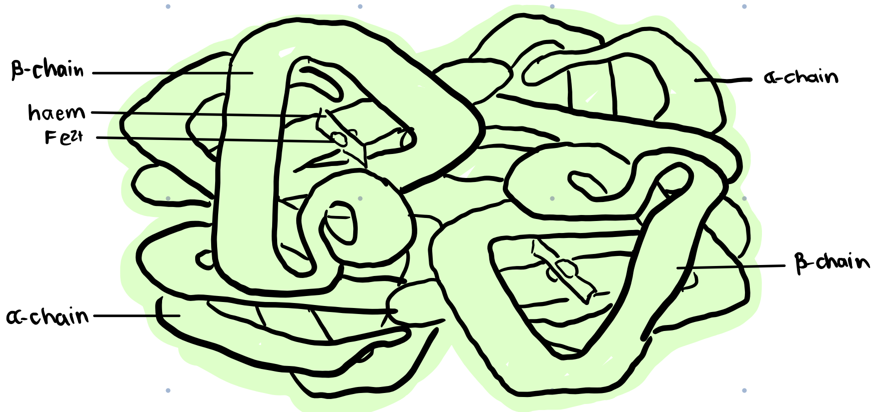
Some proteins take up a tertiary structure in the form of a long, much-coiled chain and are called fibrous proteins, e.g. collagen. Fibrous proteins are insoluble in water and have simple shapes, such as a helix. They are structural proteins. Other proteins take up a tertiary structure that is more spherical and are called globular proteins, e.g. haemoglobin and enzymes. Globular proteins are soluble in water and are folded into complex 3D shapes. They are metabolic proteins because they carry out a range of functions that contribute to the metabolism of organisms.

Globular protein:
It is a metabolic protein that is soluble in water and has a spherical or near-spherical shape, e.g. haemoglobin and enzymes.

Fibrous protein:
It is a structural protein that is insoluble in water and has a chain-like shape, e.g. collagen and keratin.

The molecular structure and function of haemoglobin (globular protein)

Haemoglobin is a conjugated protein with a quaternary structure.
Each haemoglobin molecule is composed of four polypeptide chains - two a-globins (α-chains / alpha chains) and two -globins (β-chains / beta chains). Each polypeptide has a tertiary structure consisting of α-helices only. There is a ham group in the centre of each polypeptide. Each ham group has a central atom of ferrous iron (Fe^{2+}).

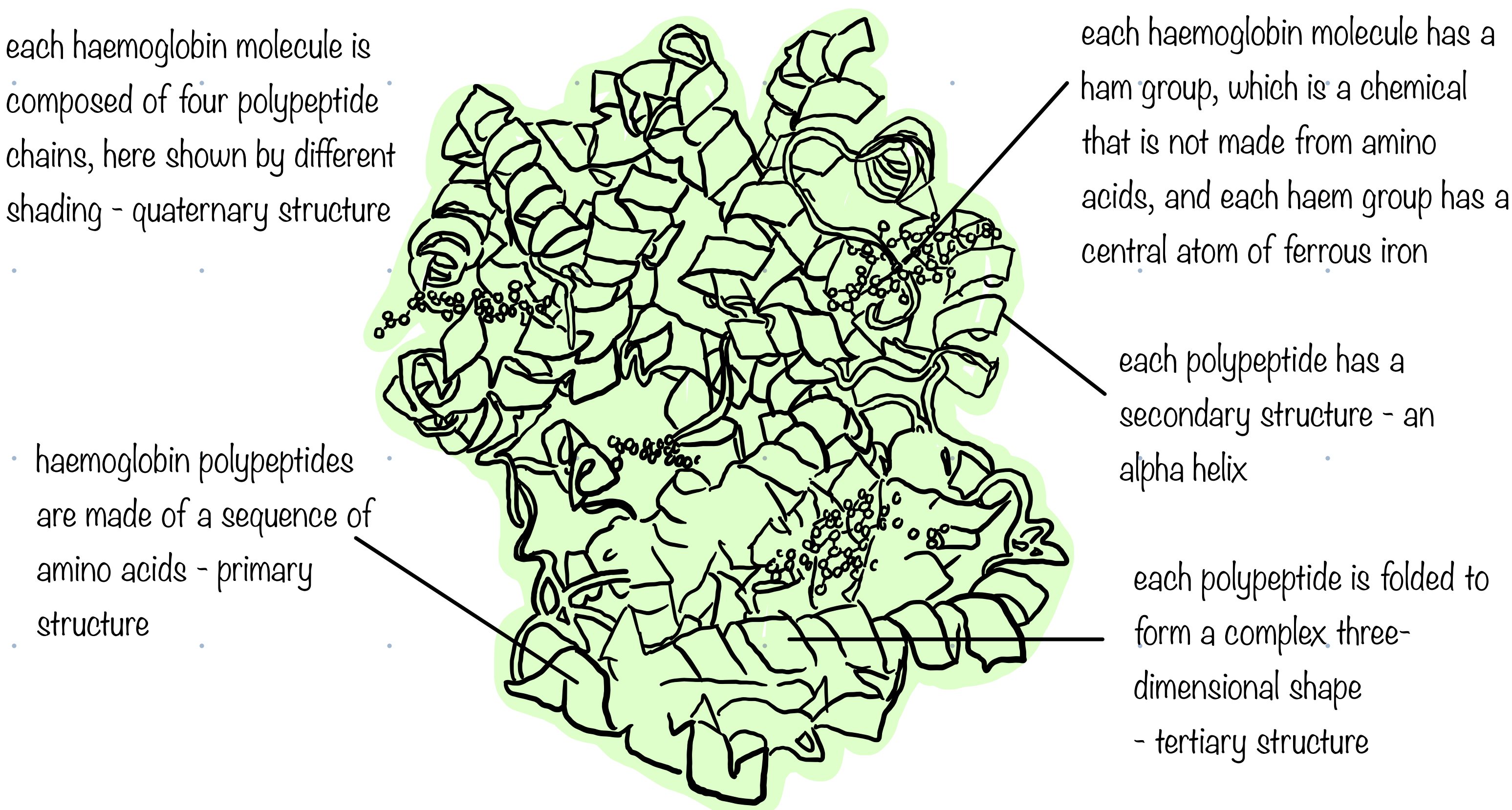


Haemoglobin is a protein in your red blood cells that carries oxygen to your body's organs and tissues and transports carbon dioxide from your organs and tissues back to your lungs.

Each haem group can form a temporary bond with an oxygen molecule, which loosely combines with the iron. The addition of each molecule of oxygen causes a change in the shape of haemoglobin, which makes it easier to accept the next oxygen molecule. This changing of the shape causes the molecule to expose the ham groups so they can accept oxygen.

Haem is an example of a prosthetic group - a part of a protein molecule that is not made of amino acids. A protein with a prosthetic group is called a conjugated protein.

Diagrams of haemoglobin in red blood cells can make students think that each RBC contains only one haemoglobin molecule, but it is estimated that each RBC has 280 million of them! One haemoglobin carries 4 oxygen molecules ($Hb + 4O_2 = HbO_4$). One red blood cell can thus carry 1 120 000 000 molecules of oxygen!

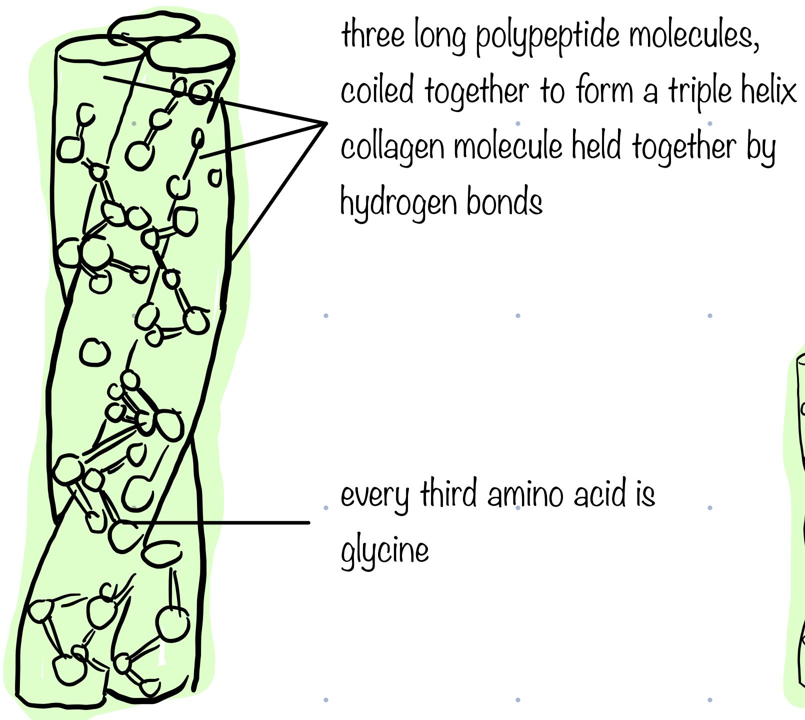


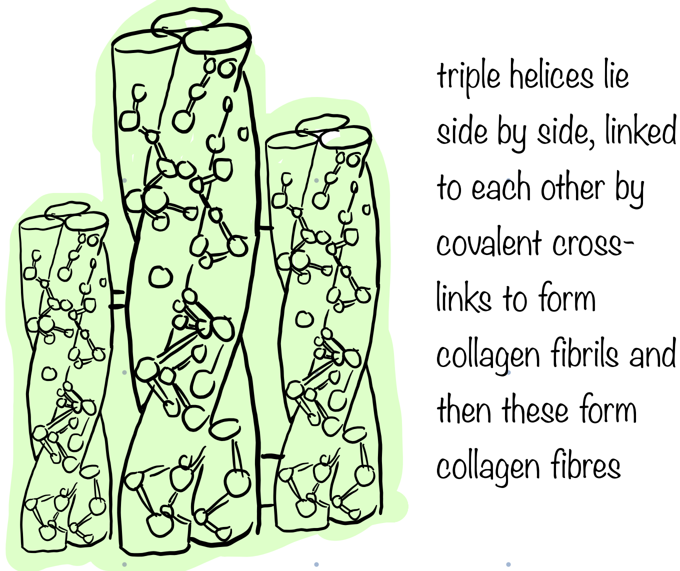
The molecular structure of collagen (fibrous protein)

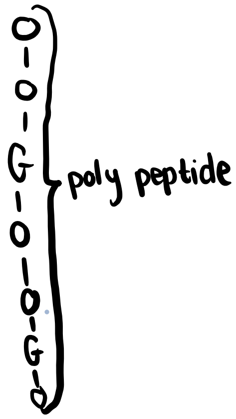
Collagen is a fibrous protein, the most abundant structural protein found in animals, making up 25% of the total protein in mammals. It is an insoluble fibrous protein found in skin (leather is preserved collagen), tendons, cartilage, bones, teeth and the walls of blood vessels. Skin is largely composed of collagen.

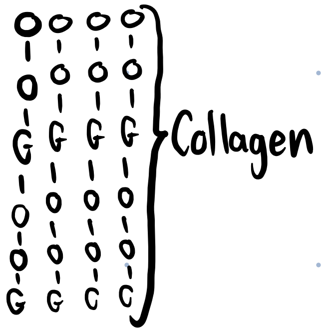
Chemically, a collagen molecule consists of three identical polypeptide chains, each about 1 000 amino acid residues long, wound together as a triple left-handed helix. (Under a microscope a collagen fibril looks like a twisted rope. Each polypeptide chain has a secondary structure in the form of an alpha helix. Every third amino acid is glycine. Glycine has the smallest R-group (-H) so it does not take up much space and allows close packing. This means the helices can be wound tightly together and form many hydrogen bonds between them.

Many triple helices (collagen molecules) are joined together by covalent bonds to form collagen fibrils, which group together to form collagen fires. The ends of triple helices do not coincide (do not occupy the same place) within each fibril so there are no lines of weakness where the fibre may break. Collagen then binds with other components to enormously increase its strength, e.g. in healthy bone, it combines with a mineral to form calcium phosphate. This makes collagen suitable for structures such as tendons, which connect muscles to bone, because it has high tensile strength and resists pulling forces.









x3 poly petides twist; hydrogen bonds = Collagen molecules

Collagen molecules twist = collagen fibil -> Twist fibers

THE IMPORTANCE OF IRON IN THE HAEMOGLOBIN MOLECULE

Iron is an essential element for blood production. About 70 percent of your body's iron is found in the red blood cells of your blood, called haemoglobin, and in muscle cells, called myoglobin. Haemoglobin is essential for transferring oxygen in your blood from the lungs to the tissues. Myoglobin in muscle cells accepts, stores, transports and releases oxygen.

Each polypeptide chain of haemoglobin contains a ham group. Each haem group contains an iron atom. One oxygen molecule, O_2 can bind loosely with each iron atom. So, a complete haemoglobin molecule, with four ham groups, can carry four oxygen molecules (eight oxygen atoms) at a time. The addition of each molecule of oxygen with each iron atom causes a change in the shape of haemoglobin which makes it easier to accept the next oxygen molecule. This changing of the shape causes the molecule to expose the ham groups so they can accept oxygen. The binding of one molecule of oxygen makes it easier to bind to another, and once the second oxygen molecule binds, it makes it easier to bind the third and so on.

It is the ham group which is responsible for the colour of haemoglobin. This colour changes depending on whether or not the iron atoms are combined with oxygen. If they are, the molecule is known as oxyhemoglobin and is bright red. If not, the colour is purplish.

BIURET TEST: TEST FOR PROTEINS

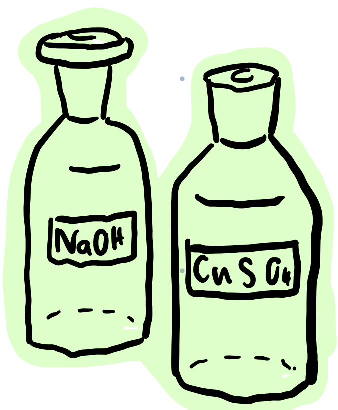
Background information

The biuret test is a simple test that can detect protein in food and biological specimens. You can do it easily in the classroom. The biuret reagent, which is blue, consists of a solution of potassium or sodium hydroxide and copper sulfate. It detects peptide bonds in proteins. All proteins have peptide bonds, containing nitrogen atoms. These form a purple complex with copper (II) ions and this forms the basis of the biuret test.

The reagent used for this test is called biuret reagent. You can use it as two separate solutions: a dilute solution of potassium hydroxide or sodium hydroxide, and a dilute solution of copper(II) sulfate. Alternatively, you can use a ready-made buret reagent that contains both the copper(II) sulfate solution and the hydroxide already mixed.

Procedure to test for proteins

- If necessary, crush the food to be tested (like cheese liquidised with distilled water), or use liquid foods like milk or egg white and place it in a test tube.
- Mix equal amounts of biuret agent A (1% copper sulfate - Cus_4) and buret agent B (1% potassium hydroxide / sodium hydroxide - $NaOH$) by shaking the tube gently from side to side, or simply use a ready-made biuret reagent.
- Drop 5cm^3 blue biuret solution in the test tube with the food to be tested.
- Mix with a little distilled water.
- Shake gently and allow the mixture to stand for a few minutes. (There is no need for heating).
- If a purple (mauve, lilac or violet) colour appears, protein is present. This purple colour forms because there are peptide bonds. The colour develops slowly over several minutes.



WATER

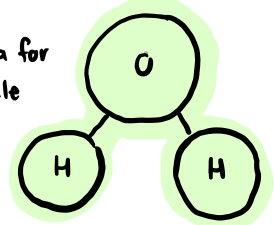
About 80% of the body of an organism is water. Water has unusual properties compared with other substances, because of the structure of its molecules.

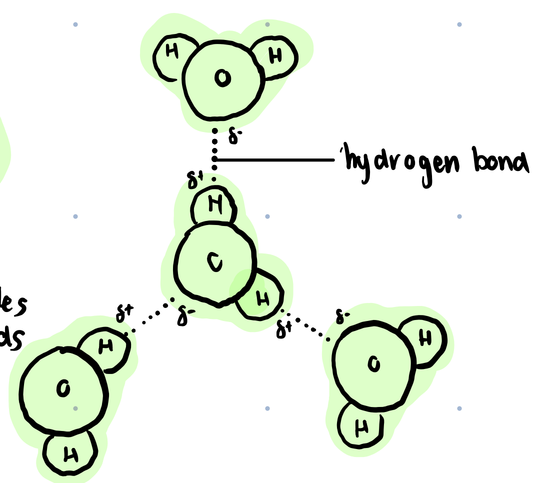
There are hydrogen bonds between water molecules. The oxygen atom of water can be described as slightly negative, while the hydrogen atom is slightly positive. A water molecule is thus dipolar.

The partial charges are indicated by δ (delta): δ^- on the oxygen and δ^+ on the hydrogen.

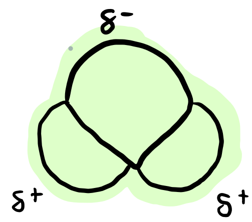
There is an attraction between the δ^- and δ^+ parts of neighbouring water molecules. A hydrogen bond forms between the slightly positive and slightly negative charge. Each hydrogen bond can easily be broken because it is very weak. Each water molecule can form hydrogen bonds with up to four other water molecules. In bodies of water, hydrogen bonds break and reform all the time.

Structural formula for a water molecule





four water molecules with hydrogen bonds



three dimensional model of a water molecule

Ion:
An ion is an atom or molecule or chemical group that has lost or gained one or more electrons and is either negatively or positively charged.

As water molecules are polar, they interact and are a good solvent for other polar molecules and ions. The hydrogen bonds between water molecules give them cohesion, which results in the properties that are important within organisms and cells, e.g. solvent action, specific heat capacity and latent heat of vapourisation.

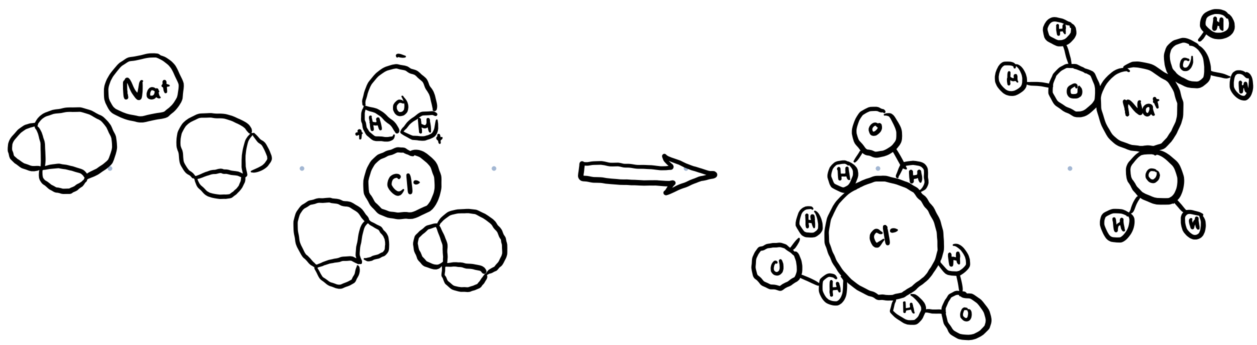
Properties of water relating to its roles in living organisms

Solvent action of water

Water is an excellent solvent for ions and polar molecules (molecules with an uneven charge distribution, such as sugars and glycerol), because the water molecules are attracted to the ions and polar molecules and therefore collect around and separate them. This is what happens when a chemical dissolves in water. Once a chemical is in solution, it is free to move about and react with other chemicals. Most processes in living organisms take place in solution in this way.

By contrast, non-polar molecules such as lipids are insoluble in water and, if surrounded by water, tend to be pushed together by the water, since the water molecules are attracted to each other.

This is important, for example, in hydrophobic interactions in protein structure and in membrane structure and it increases the stability of these structures.



Water acts as a solvent within cells. It also acts as a solvent in transport media, e.g. blood plasma and lymph in animals, and in phloem and xylem in plants. Water is also a solvent for excretory wastes in urine.

The dipoles on water molecules make water an excellent solvent. Any substance that has fairly small molecules with charges on them, or that can separate into ions, can dissolve in water.

Because water is a good solvent, it helps to transport substances around the bodies of organisms.

For example, the blood plasma of mammals is mostly water and carries many substances in solution, including glucose, oxygen and ions such as sodium. Water also acts as a medium in which metabolic reactions can take place, as the reactants are able to dissolve in it. Water is the transport medium in the blood, in the lymphatic, excretory and digestive systems of animals, and in the vascular tissues of plants. Here, again, its solvent properties are essential.

Water has a high specific heat capacity

High specific heat capacity:
The heat capacity of a substance is the amount of heat required to raise its temperature by a given amount. The specific heat capacity of water is the amount of heat energy required to raise the temperature of 1 kg of water by 1°C.

Specific heat capacity is the amount of heat energy that has to be added to a given mass of a substance to raise its temperature by 1°C. Temperature is related to the kinetic energy of the molecules - the higher their kinetic energy, the higher the temperature. A lot of heat energy has to be added to water to raise its temperature, because much of the heat energy is used to break the hydrogen bonds between water molecules, not just to increase their speed of movement. Water has a relatively high heat capacity. In order for the temperature of a liquid to be raised, the molecules must gain energy and consequently move about more rapidly.

The hydrogen bonds that tend to make water molecules stick to each other make it more difficult for the molecules to move about freely; the bonds must be broken to allow free movement. This explains why more energy is needed to raise the temperature of water than would be the case if there were no hydrogen bonds. Water has the highest specific heat capacity of any liquid. The high heat capacity of water has important biological implications, because it makes water more resistant to changes in temperature.

- This means that large bodies of water, such as oceans, lakes, or large dams do not change their temperature as easily as air does. It means that large bodies of water are slow to change temperature as environmental temperature changes. As a result, they provide more stable habitats for aquatic organisms, because the temperature of the water will relatively stay the same day and night.
- It also means that the bodies of organisms, which contain large amounts of water, do not change temperature easily. Because of this property of water, it limits the fluctuations in the temperature of organisms. This means that the temperature within cells and within the bodies of organisms (which have a high proportion of water) tends to be more constant than that of the air around them. Biochemical reactions therefore operate at relatively constant rates and are less likely to be adversely affected by extremes of temperature.

Water has a high latent heat of vapourisation (evaporation)

Latent heat of vapourisation:
It is a measure of the heat energy needed to vaporise a liquid (cause it to evaporate), changing it from a liquid to a gas. In the case of water, it involves the change from liquid water to water vapour.

When a liquid is heated, its molecules gain kinetic energy, moving faster. Those molecules with the most energy are able to escape from the surface and fly off into the air. A great deal of heat energy has to be added to water molecules before they can do this, because the hydrogen bonds between them have to be broken. When water evaporates, it therefore absorbs a lot of heat from its surroundings.
Water has a relatively high latent heat of vapourisation. This is a consequence of its high heat capacity. The fact that water molecules tend to stick to each other by hydrogen bonds means that relatively large amounts of energy are needed for vapourisation to occur, because hydrogen bonds have to be broken before molecules can escape as a gas. The energy transferred to water molecules during vapourisation results in a corresponding loss of energy from their surroundings, which therefore cool down. A large amount of heat energy can be lost for relatively little loss of water, reducing the risk of dehydration.

This is biologically important, because it means that living organisms can use evaporation as a cooling mechanism, as in sweating or panting in mammals. Water loss from organisms means cooling - animals sweat and evaporation of water in sweat from the skin cause cooling. Plants lose water during evaporation from mesophyll cell walls and during transpiration out of stomata, which cause cooling of leaves. Transpiration from plant leaves is important in keeping them cool in hot climates. The reverse is true when water changes from liquid to solid ice. This time the water molecules must lose a relatively large amount of energy, making it less likely that the water will freeze. This is an advantage for aquatic organisms and makes it less likely that their bodies will freeze.