4. The metabolism and activity of protein

All proteins contain nitrogen in their basic structure in addition to the carbon and water (= hydrogen and oxygen) which are typically found in polysaccharides. Proteins are polymer forms of the nitrogen containing amino acids. We will characterize their specific area of activity by looking at their properties, and will compare this to similar processes in nature to give us a view of their role in nature.

4.1. Protein metabolism and nitrogen

4.1.1. The nitrogen balance

The catabolism of proteins in organisms is not an expedient way to provide energy to the organism since the catabolism of larger amounts of protein would result in a structural breakdown of chiefly muscle proteins. This becomes visible in the nitrogen balance of organisms. Under physiological conditions the amount of nitrogen excreted must equal the amount taken up in nutrition.

4.1.2. Nitrogen metabolism in nature

The nitrogen that is in amino acids and proteins ultimately comes from the air, which contains 80% N_2. Yet it cannot be taken up directly from the air by most living organisms. It first enters the soil through nitrogen fixation by special bacteria that form the root nodules of legumes. Through this process N_2 is first converted to ammonia and subsequently incorporated in α-ketoglutarate, a citric acid cycle intermediate, to form the amino acid glutamate. The amine group of glutamate is transferred in transamination processes to further citric acid cycle intermediates and other metabolites of carbohydrates and lipids (acetyl-CoA and acetoacetyl-CoA). Complicated conversion processes can ultimately lead to the formation of all 20 amino acids.

Plants take up the organic nitrogen-containing compounds of bacteria in the soil into their organism.

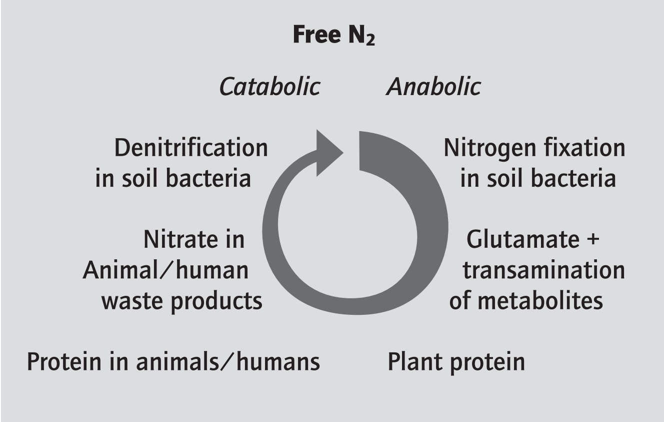


Fig. 4.1 The cycle of nitrogen metabolism in nature

Higher organisms get their nitrogen from the plants through their food. Their waste products contribute nitrogen to the soil again.

Denitrification reactions in bacteria in the soil render the nitrogen back into the atmosphere.

In humans, 10 of the 20 amino acids cannot be synthesized in sufficient quantity, especially in growing children, and must be taken up in the diet to prevent structural breakdown.

4.1.3. The urea cycle in organisms

Nitrogen metabolism in organisms is also cyclic. In the *urea cycle,* glutamate donates ammonia to ornithine through de-amination in the mitochondrion, which leads to the formation of citruline. This passes to the cytosol and via two more steps is converted to arginine, which gives off urea to form ornithine again. The urea cycle is linked to both anabolic and catabolic processes in nitrogen metabolism. The urea cycle has two links

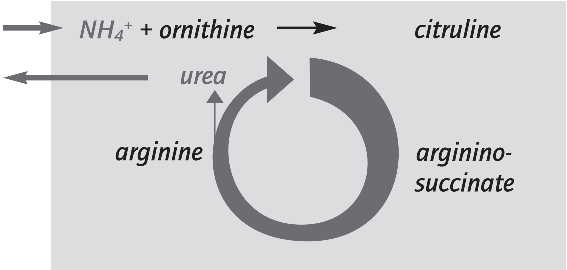


Fig.4.2 The urea cycle, the nitrogen cycle in organisms

with the citric acid cycle, via oxaloacetate and fumarate.

The secretion of urea requires the presence of water, therefore many mammals excrete it in their urine as a waste product. Sufficient quantities of water are needed to be able to excrete it properly.

Nitrogen metabolism is a controlled mechanism with limits on the supply and the excretion sides. It is controlled by *negative feedback* systems. When the amount of end product reaches a certain level it inhibits its own synthesis. Feedback systems function as control cycles. They are characteristic for nitrogen metabolism because of the latter's inherent tight supply and demand limits.

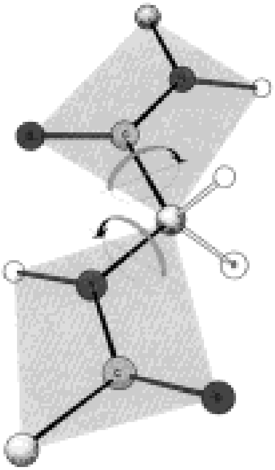


Fig. 4.3 Polypeptide chainformation (from Campbell, 1999)

4.2. Protein structure as the basis for protein function

4.2.1. The specificity of protein structure

Protein primary structure consists of varying combinations of 20 different amino acids in long polypeptide chains (section 2.1.2.). Protein conformation is more differentiated and more complicated than carbohydrate structure. Proteins have three possible levels of conformation beyond their primary structure of amino acid chains (polypeptide chains).

Secondary structure involves the formation of α-helical forms and β-pleated sheets (see also section 2.1.2.). Helices and planar forms in separate compounds also exist in carbohydrates. In protein they exist in the same compound. Tertiary structure is the overall folding of the protein on itself, which results in the typical three-dimensional arrangement that allows its function. Quaternary structure exists when several polypeptide chains (subunits) together form an interacting molecule such as hemoglobin.

The variety of amino acids creates the potential for differentiated polypeptide chains, which allows for many different proteins. The proteins in an organism are very varied and they can perform a large number of functions. Proteins, in contradistinction to carbohydrates, are also highly specific to the function they fulfill. The alteration of one or more amino acids in the polypeptide chain can give them a different function (as for instance in myoglobin, in relation to the hemoglobin α and β chains) or render them dysfunctional (as in hemoglobin S in sickle-cell anemia).

Protein conformation can be *fibrous* or *globular*. *Membrane proteins* are situated in the membranes of cells. They can have different forms, varying with their function. *Glycoproteins* are proteins with carbohydrate residues.

Fig. 4.4 Collagen (from Campbell, 1999)

4.2.2. Fibrous proteins

The overall shape of fibrous proteins is a long rod. Fibrous proteins are *non-soluble in water*.

Examples of typical fibrous proteins:

• *Collagen*, is the prototype of fibrous proteins and the most frequently occurring protein in vertebrates. Collagen consists of three helical polypeptide chains wrapped around each other to form a superimposed triple helix. Hydrogen bonds hold the three strands together. Collagen is located extracellularly in bone and connective tissue. The polypeptide chains consist mainly of a relatively simple triad of three amino acids: glycine, proline and hydroxyproline. Every third amino acid is glycine, the second is proline or hydroxyproline, and the first amino acid is mostly also proline or hydroxyproline or can be another amino acid. Collagen has an important structural function. It is the main fiber in connective tissue: the tissue between organs and other tissues in which cells and organ parts are embedded.

• Another example of fibrous conformation in proteins is *keratin* in wool and hair. It is mostly α-helical. The fibers of *fibroin*, the main protein in silk, has largely β-sheets.

• The *muscle proteins,* actin and myosin, are also mainly fibrous proteins. They are the principal constituents of muscle fibers, but do also occur in other kinds of cells. These proteins are always located intracellularly. Their structure is relatively simple, albeit more complex than that of collagen. Myosin has a globular head that is instrumental in muscle contraction. It provides the power stroke for the contraction by interacting with actin. In action, the interlocking of actin and myosin components intensifies and the muscle contracts, thus moving the body in part or as a whole and altering the relation of the organ or organism to its surroundings. These proteins are structural proteins in the resting state as well as functional proteins when in action. Muscle fibers also affect movement within cells.

Fibrous proteins provide structural elements to the animal and human organism.

4.2.3. Globular proteins

Globular proteins are compact *functional* proteins. The overall shape of a globular protein is spherical, as the name indicates. Their tertiary and quaternary structures are complex. Globular proteins are *water-soluble*.

Examples of globular proteins

- Most *enzymes* are globular proteins and are principally only functional. They catalyze reaction processes between molecules. In the lock and key model of enzyme function they bind substrate and catalyze a specific reaction, then let go of the reaction products. Their configuration changes reversibly with their activity. Otherwise they are unchanged by the process they catalyze.

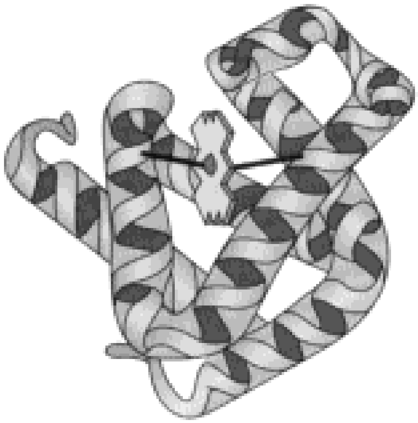


Fig. 4.5 Myoglobin, a globular protein (from Campbell, 1999)

Enzymes catalyze numerous metabolic reactions throughout the organism. Reactions catalyzed by enzymes will proceed up to 10^{14} times faster than non-catalyzed reactions. Each reaction process has a specific enzyme that catalyzes it. This diversity of form in proteins becomes possible through the configuration of their primary structure with 20 different amino acids, which subsequently enables the formation of their specific secondary, tertiary, and possibly quaternary structures. The amino acid sequence has to be exact for the protein to be biologically active.

- Other examples of globular proteins include myoglobin and hemoglobin.

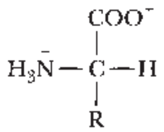
4.2.4. Membrane proteins

Membrane proteins are an important component of membranes. 20 - 80 % of the membrane weight of animal and human cells consists of protein. These proteins have a structural function, but their major role is functional. They form channels through the membrane that allow the passage of specific compounds under certain conditions, they effect active transport of certain compounds across the membrane, and they function as receptors on the membrane's inner and outer surface for compounds such as neurotransmitters. They can also be enzymes themselves.

4.2.5. Glycoproteins

Glycoproteins play a role as antibodies in immune recognition and as antigenic determinants in human cell membranes. The use of glycoproteins for the typing and matching of blood groups and grafts is exemplary of the specificity of proteins in organisms. Even though close matches can be found, for instance in twins, the perfect match is only found in proteins within an individual organism. *Proteins emphasize the singularity of organisms* and play an important role in recognizing the distinction between self and non-self, which is the function of the immune system.

4.3. Amino acids



General amino acid structure

Protein breakdown in the metabolism does not specifically yield energy-carrying substances, even though amino acids yield energy when they are broken down. Amino acids are important as metabolites that can be used by the organism in anabolic processes to build up its own proteins. The energy for this process comes from the catabolism of carbohydrates.

4.3.1. Amino acid activity

Many amino acids are themselves biologically active in the organism (such as glutamate or glycine) or with small structural changes (such as the monoamine serotonin, which is formed from tryptophan, and the catecholamines, which are derived from tyrosine) or as peptides (small amino acid chains). They are active as neurotransmitters in nervous tissue, where they connect nerve cells functionally by transmitting the electrical impulse chemically from the axon to the receptors on the axon or cell body of the next cell. Some have an important function as bile salt (glycine in glycocholic acid), in methylation reactions (methionine), or in inflammation (histamine from histidine). Thyroxine is a derivative of tyrosine, and functions as a hormonal substance important for the rate of metabolism. Oxytocin, vasopressin and insulin are peptide hormones, which effect contraction of smooth muscles in the uterus, contraction of smooth muscles in the blood vessels, and carbohydrate metabolism respectively.

Many of these compounds are also known to influence consciousness. Serotonin and histamine released from a bee sting provoke a strong sensation of pain as well as local inflammation. In schizophrenia we find increased levels of serotonin and catecholamines, including dopamine. In endogenous depressions, a lack of serotonin and catecholamine metabolism is found. The stimulating effect of coffee on consciousness is due to its stimulating influence on monoamine metabolism. Stimulants of consciousness such as cocaine and LSD mimic the central nervous system action of catecholamines and serotonin respectively. The rate of monoamine metabolism varies with the rhythm of sleeping and waking in the healthy organism. Serotonin and dopamine are secreted in the area of the brainstem that is active during waking (see Elsas, 1994).

4.3.2. Amino acid metabolism

Most amino acids are *glucogenic*. Their degradation after de-amination yields pyruvate or oxaloacetate. This gives the possibility for them to be converted to glucose through gluconeogenesis and enter carbohydrate metabolism, or they may be used up in the citric acid cycle. Amino acids can therefore provide energy for the organism like the carbohydrates, but they do so less efficiently. When body energy requirements need protein degradation in larger quantity, as in hunger states or malnutrition, this happens at the expense of other tasks of proteins and amino acids in the organism, both structural as well as functional (section 4.1.1.).

The amino acid leucine is only *ketogenic*, meaning it can only be broken down to acetyl-CoA or acetoacetyl-CoA and its breakdown may lead to the formation of ketone bodies or fatty acids. It can be used in the citric acid cycle but cannot be converted to glucose. Isoleucine, lysine, phenolalanine, tryptophan and tyrosine are both ketogenic and glucogenic.

Amino acids also contribute to the synthesis of nucleic acids and the pyrrole ring of hemoglobin.

4.4. Summary and conclusion

Nitrogen

Nitrogen is fixed from the atmosphere by soil bacteria and taken up by leguminous plants. When animals and humans eat the plants, the nitrogen-containing substances enter their organism. The nitrogen becomes part of the urea cycle in organisms, which has links to both anabolic and catabolic processes as well as to the citric acid cycle. Once the nitrogen is excreted as urea it enters the larger cycle in nature again and can be either taken up by plants again or denitrified by soil bacteria to become part of the nitrogen in the air in an even larger cycle. Nitrogen metabolism in organisms is controlled by extensive negative feedback mechanisms to support the tight nitrogen balance. Nitrogen intake has to match excretion to prevent structural breakdown.

The breakdown of protein in the digestive tract yields amino acids, which are important as metabolites for the organism to build up its own proteins in anabolic processes. The energy for these processes comes from the catabolism of carbohydrates. When proteins are broken down for energy in the organism as happens in hunger states, this means structural as well as functional breakdown of the organism. Proteins have a structural function in animals and humans, and they cannot be used as the regular energy source. We found that carbohydrates take on the structural function in plants, and also have a major role in the structural components of bacteria and invertebrates. In the latter two the