Review Article

Biochemistry of zinc

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Summary

Zinc forms part of the active site and/or stabilizes the conformation of several enzymes. The protein metallothionein constitutes a zinc deposit from which the metal is transferred to enzymes.

Zinc participates in the synthesis of nucleic acids and hence in the synthesis of proteins, principally through multi-enzyme pir 1-3 and DNA-dependent RNA polymerase, which are metallo-enzymes of zinc. Zinc is therefore necessary for normal growth, development, healing and ossification.

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Zinc belongs to group IIB of the periodic table of elements; its 3d level is complete and its 4s level has two electrons. As a result of these characteristics it always behaves as a cation with two positive charges (Zn^{2+}), and does not undergo redox reactions. Zinc combines with bivalent anions to form neutral compounds and may also bind neutral or charged atoms or molecules to form co-ordination complexes, generally involving four chemical groups. In the case of metalloproteins, these groups may be different amino-acid residues of the same protein. Theoretically, the zinc co-ordination complexes should have a tetrahydric form determined by the cation; however, zinc-enzyme complexes are unstable because the 3d level of the cation is complete, a fact that confers atypical co-ordination properties upon zinc.

More than 100 zinc metalloproteins have been identified, including enzymes belonging to all six groups listed in the International Union of Biochemistry classification of enzymes (oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases). In some cases zinc forms part of the active site of the enzyme, and in others it merely stabilizes the conformation of the enzyme molecule without participating in its catalytic function.

Zinc may also be of some importance in stabilizing the structure of nucleic acids, although its actual role *in vivo* has not been precisely elucidated.

Metallothionein

Zinc and copper form part of a group of proteins generically designated metallothioneins, which are abundant in tissues which undergo rapid growth or development (such as the liver, kidney, testes and gastro-intestinal tract). Metallothioneins are rich in cysteine, which forms two special groupings that may accumulate seven metallic ions per protein molecule. Zinc thionein transfers the ion to an apo-enzyme, which is a structurally complete enzyme except in its metallic component, and transforms it into an active holo-enzyme:

$$nZn^{2+} + \text{metallo-enzyme} = (Zn)_n \text{ thionein}$$

$$(Zn)_n \text{ thionein} + \text{apo-enzyme} = (Zn)_{n-1} \text{ thionein} + \text{holo-enzyme}.$$

In addition to this function in the synthesis of macromolecules, metallothionein plays a role in some detoxification processes, including those involving cadmium, mercury and silver.

Metallo-enzymes of zinc

Zinc forms an intrinsic part of numerous metallo-enzymes. These include several of fundamental importance, such as carboxypeptidases, nucleotide polymerases, superoxide dismutases and alcohol dehydrogenase.

Carboxypeptidases

The carboxypeptidases include digestive enzymes, angiotensin I-converting enzyme (kininase II), and pir 1-3, a multi-enzymatic complex involved in the synthesis of pyrimidine nucleotides and thus in the synthesis of nucleic acids. The characteristics of angiotensin I-converting enzyme are described separately.[1]

Digestive carboxypeptidases are enzymes of relatively low molecular weight (*c.* 35 000 dalton) which hydrolyse small peptides formed by the actions of pepsin, trypsin and chymotrypsin. Their active site is in the form of a cleft and they are secreted as inactive precursors called zymogens, which are activated by trypsin. Two groups of carboxypeptidases exist (A and B), and each group includes numerous iso-enzymes which need zinc for their activation.[2] The zinc forms a tetravalent complex binding a glutamic residue and two histidine residues of the enzyme. The fourth position is occupied by carbonyl oxygen of the first peptidic bond of the substrate; this bond is hydrolysed in the enzymatic process.

Multi-enzyme pir 1-3. Triphosphate pyrimidine nucleotides are required as substrate for polymerization reactions in the synthesis of nucleic acids. Desoxycytidine triphosphate and desoxythymidine triphosphate are required for the synthesis of DNA, and cytidine triphosphate and uridine triphosphate are required for the synthesis of the various types of RNA. Uridine monophosphate (UMP) is the basic precursor required in the synthesis of these four pyrimidines.[3] UMP synthesis is a process in which six enzymes participate: carbamylphosphate synthetase II, aspartate transcarbamylase, dehydro-orotase, dehydro-orotase dehydrogenase, orotate-phosphoribosyl transferase and orotidilate descarbolzylase. These enzymes possess only one active site per molecule and the first three form a unique enzymatic complex which is active in the synthesis of pyrimidines and known as multi-enzyme pir 1-3; the last two enzymes form another enzymatic complex called multi-enzyme pir 5-6, and the fourth enzyme acts on its own. The first complex possesses three

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different enzymatic activities on the same polypeptide chain and requires zinc for the activation of dehydro-orotase.

Nucleotide polymerases

Zinc is indispensable for growth and development. All rapidly growing tissues are rich in zinc thionein, a protein that serves as a zinc deposit and which transfers it to the metallo-enzymes of zinc during their synthesis. In cell cultures of rat liver[4] and rabbit kidney[5] the process of growth may be inhibited by adding the chelating agent ethylenediamine tetra-acetate (EDTA), which sequesters the metallic ions in the culture medium. This effect can be reversed by incorporating zinc in the culture medium.[4,5]

When zinc deficiency is provoked in the last trimester of pregnancy in rats, the offspring have a reduced total content of encephalic DNA and behave abnormally; these manifestations are irreversible.

Many experiments have led to the conclusion that zinc participates in the mechanism of action of DNA-dependent RNA polymerase and in those of the enzymes that take part in the synthesis of pyrimidines. It is not yet clear whether zinc has the same functions with respect to the same enzyme in all species.

RNA polymerase III is a metallo-enzyme which has 2 atoms of zinc in every molecule.[6] The union between metal and enzyme is strong and is only lost when the protein is denatured; however, the precise function of zinc in the protein is not well understood, although it probably forms part of the active site, because adequate concentrations of chelating agents, such as 1,10-phenanthroline, inhibit the enzyme completely.

Superoxide dismutases

In the mitochondria, oxygen is reduced by a flow of electrons, and water is formed in the presence of protons. This process, called the respiratory chain, produces large amounts of energy, which is stored as adenosine triphosphate.

When oxygen is reduced within the intracellular organelles, it receives only 1 electron at a time and a series of highly toxic intermediate compounds are produced, among which are the superoxide ion (O_2-), the free radical hydroperoxide (HO_2) and hydrogen peroxide (H_2O_2).[7,8] The cell has a defence mechanism which prevents the transfer of these toxic substances to the *milieu intérieur*; superoxide dismutases and catalases intervene in the elimination of the superoxides and the peroxides respectively. These dismutases are metallo-enzymes of zinc and copper.[9]

Superoxides alter the stability of several structural molecules of great importance, including polyunsaturated lipids of the cell membranes. *In vitro* work indicates that the superoxides destroy polyunsaturated fatty acids such as linoleic and arachidonic acids, an effect that may be inhibited by superoxide dismutase and catalase.[10]

Alcohol dehydrogenase

Less than 10% of absorbed alcohol is eliminated through the kidney and the lung, and the remainder is oxidized within the body, principally in the liver; alcohol is transformed into acetaldehyde, which in turn forms acetyl co-enzyme A. The enzyme responsible for the oxidation of alcohol, alcohol dehydrogenase, is formed by two subunits, each of which has 2 atoms of zinc.[11] One of these zinc atoms forms part of the active site and satisfies its co-ordination index by binding to amino acid residues of cysteine in positions 47 and 174, histidine in position 67, and the oxygen of alcohol. The apo-enzyme is inactive.

Connective tissue

Fibroblasts synthesize the two fundamental macromolecules which constitute and characterize connective tissue, namely collagen, a fibrillar component, and the proteoglycans, which form the interstitium. The synthesis of all these compounds requires the presence of zinc, as shown by studies of wound healing and the normal development of bone, although the exact biochemical role of the metal in these processes remains unknown. In the absence of an adequate amount of zinc the composition of collagen is altered with a marked diminution in the percentage of recently formed soluble collagen, and modification of the quantity and type of bonds between collagen units.[12] The composition of the interstitial substance is also changed, resulting in an unfavourable environment for both fibroblasts and the specialized cellular species derived from them. As a result, healing and ossification are retarded.[13]

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