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Copper in the organism – transport and storage in the cells

The essential role of copper (Cu) in the animal world ought to be seen in the context of its appearance in numerous oxidation-reduction enzymes. Copper is an important component of cytochrome-c oxidase (which is one of the elements of the respiratory chain); lysine oxidase (an enzyme taking part in the processes of collagen fibre maturing); Cu/Zn superoxide dismutase (an enzyme assisting in free radicals elimination); dopamine β-hydroxylase (the enzyme of the biosynthesis path of the catechol amines – adrenaline and noradrenaline); ceruloplasmin (which is a double-function protein fulfilling an enzymatic and transporting role); tyrosinase – catechol oxidase (which is an enzyme protein participating in melanin synthesis); and a protein that probably does not perform any enzymatic function, called metallothionein (8, 9).

Copper, one of the essential metals, is required for the activity of enzymes associated with ferrum (Fe) metabolism, elastin and collagen formation, melanin production and integrity of the central nervous system. On the other hand, Cu ions are known to be a toxicant, to both eukaryotic and prokaryotic cells. Copper ions can bind to proteins and nucleic acids and can cause the oxidation of lipids and proteins. The formation of deleterious free radicals is also enhanced by copper ions (14,15).

For cell viability, regulation of intracellular copper activity is thus crucially important and mechanisms must exist for the homeostasis of copper (13).

IONIC PUMPS FOR COPPER

In the process of evolution living creatures have developed techniques allowing the resorption of copper, its transport and storage in the organism as well as systems protecting them against its toxic activity. These systems contain proteins of strictly determined functions (5,13).

Recent studies of copper resistance in the Gram-positive bacterium *Enterococcus hirae* has led to the discovery of two enzymes exhibiting extensive sequence identity to two human ATPases that are defective in the copper-related Menkes and Wilson disease. The inherited Menkes and Wilson disease both cause a disturbance of copper metabolism. In the Menkes disease, copper is normal in the liver, but accumulates in intestinal mucosa, kidney, and connective tissue due to a defect in export. This results in a deficiency in copper-dependent enzymes. The candidate Menkes disease gene has been cloned, it encodes a P-type ATPase of 1500 amino acids that was proposed to be a copper-transporting ATPase. It was shown to be expressed in heart, brain, placenta, lung, muscle, kidney and pancreas, but not in the liver.

In the autosomal Wilson disease, copper secretion into the bile is reduced, with a concomitant toxic accumulation of copper in the liver and eventually also other tissues. The Wilson disease gene encodes a P-type ATPase of 1411 amino acids (5). In contrast to the Menkes gene produkt, this ATPase is most strongly expressed in liver and kidney.

The two human enzymes are approximately twice as large as the bacterial ones. The Wilson sequence shares 59% identity with the Menkes sequence (Fig. 1). The ATPases exhibit the typical features that are conserved in all known P-type ATPases. The Menkes and Wilson proteins contain, in their N-terminal region, conserved domains containing the invariant motif -CXXC-. This motif is repeated six times in the Menkes and Wilson gene products. This conserved -CXXC- is a copper binding site. The putative ion transduction regions of the ATPases here contains a proline that is located in a hydrophobic domains (CPC region) (2).

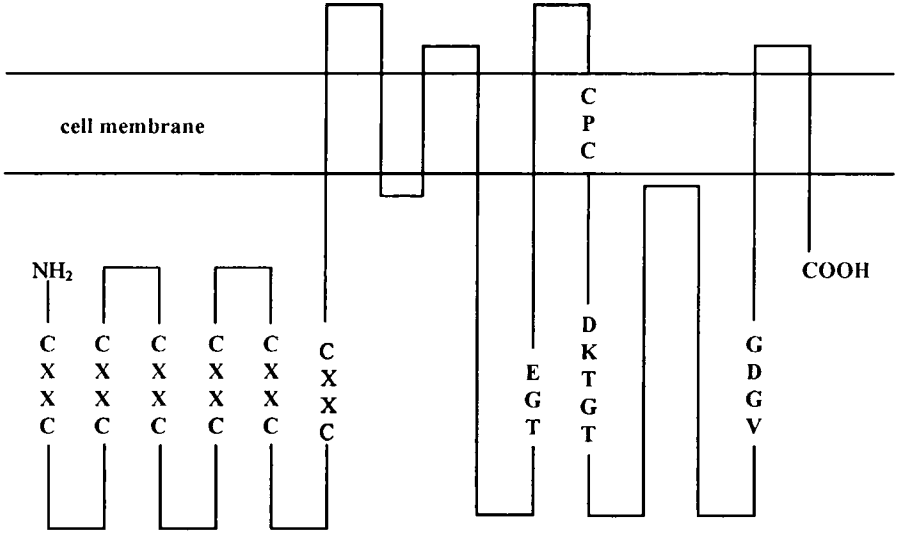

Fig. 1. Folding model for Menkes ATPase; -CXXC- heavy metal associated sequences, TGE - these residues and several others situated throughout the molecule are involved in transduction of the energy of ATP hydrolysis to cation transport, region -DKTGT- contains aspartate (D) residue - the aspartate residue forms a phosphorylated intermediate during the cation transport cycle, CPC motif - characteristic of the heavy-metal-transporting ATPases and probably located within the cation channel, region -VGDG- situated at the carboxy-terminal end of a large cytoplasmic ATP-binding domain

COPPER AND METALLOTHIONEINS

Metallothioneins (MT) are a class of low molecular mass (6-7 kDa), cysteine-rich proteins which bind with high affinity metal ions such as Zn(II), Cu(II), and Cu(I). One suggested role for MT lies in the handling of heavy metal ions in the maintenance of cellular homeostasis of essential trace metals, such as zinc and copper, and in the detoxification of physiologically harmful metals, such as Hg(II) and Cd(II) (6, 7).

Metallothioneins were isolated from a wide range of tissues, including liver, kidney, pancreas, and intestine. The an immunologic techniques for its detection have improved, metallothionein has been found in most other tissues, including brain, thymus, bone marrow, and reproductive organs. Detection by subcellula fractionation indicates that metallothioneins occur principally in the cytosol, but immuhistochemical studies have consistently revealed its

presence also in nuclei. Although metallothioneins are mainly of intracellular origin, they also occur in small amounts in extracellular fluids such as plasma, bile, and urine (1, 3).

The concentration of the protein in tissues is highly variable and is induced by many nutritional, physiologic, and developmental factors (4). For example, concentrations are greatly decreased in tissues of zinc-deficient animals and are increased after imposition of many types of stress or metal administration. They are generally elevated during fetal development and vary dramatically among species.

The characteristic features of metallothioneins are their low molecular weight and their unusual amino acid composition: cysteine accounts for 30% of the residues and aromatic acids absent. Sequence studies showed that the distribution of the cysteine residues along the polypeptide chain is fixed, regardless of the source or isoform of the protein (1,10). Another main feature of metallothioneins is high metal content, with 7 gram atoms of cadmium or zinc per mole or up to 12 gram atoms of copper per mole. This content is equivalent to one metal atom per three or two cysteine residues, respectivly. The cadmium- and copper-induced metallothioneins usually contain also zinc as a secondary metal (10).

The responsibility for the homeostasis of copper inside the cell is held by metallothioneins. SH sulphydryl groups frequently occuring in these proteins permit the binding of metallic ions. Although metallothioneins bind Cu with considerable affinity, copper exchange is possible both between particular MT molecules as well as other proteins and micro-molecular ligands. This exchange helps to supply proteins with copper, which they need in order to function properly.

In the case of Wilson's disease, inherited defects in the synthesis of a copper-binding protein result in abnormally high concentration of copper, especially in the liver and kidney (11). It was shown that copper accumulates in these organs largely as the Cu-MT complex. Indeed, the induction of increased MT biosynthesis through the administration of zinc is used as an alternative treatment for mild copper overload in instances when conventional chelation therapy is inappropriate.

Mammalian Cu(I)-MT isolated from the hepatic cytosol of acutely copper-overloaded individuals appears to contain about 12 Cu atoms (12).

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SUMMARY

Copper functions as cofactor in various redox enzymes. At the same time, copper is very toxic to both eukaryotic and prokaryotic cells. Copper ions can bind to proteins and nucleic acids and cause the oxidation of lipids and proteins. The formation of deleterious free radicals is also enhanced by copper ions. For cell viability, regulation of intracellular copper activity is thus crucially important and mechanisms must exist for the homeostasis of copper.

Miedź w organizmie – transport oraz magazynowanie w komórkach

Miedź w ustroju pełni funkcję katalizatora wielu enzymów z klasy oksydoreduktaz. Niemniej nadmiar tego pierwiastka jest toksyczny zarówno dla komórek organizmów eukariotycznych, jak i prokariotycznych. Miedź może wiązać się z białkami oraz kwasami nukleinowymi oraz powodować utlenienie lipidów i białek. Wytwarzanie wolnych rodników tlenowych jest również katalizowane za pośrednictwem miedzi. Zatem w organizmach muszą występować mechanizmy regulujące ilość miedzi oraz jej aktywność.