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METALLOTHIONEIN AND OTHER LOW MOLECULAR WEIGHT METAL-BINDING PROTEINS – A REPORT FROM AN INTERNATIONAL MEETING

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ABSTRACT

In this paper the results of discussions and conclusions reached at an international meeting on metallothionein are summarized. The definition of this important low molecular weight metal binding protein and its role in toxicity and metabolism of metals are pointed out.

In the field of metal toxicology meetings have been arranged by the Scientific Committee on the Toxicology of Metals under the Permanent Commission and International Association on Occupational Health[1,2,3,5,6,8]. The increasing interest in the field of research on metallothionein and other low molecular weight metal-binding proteins, as part of metal toxicology, has created a need for evaluating present knowledge on this subject.

A meeting was arranged in Zürich, July 17–22, 1978, by the Scientific Committee on the Toxicology of Metals under the Permanent Commission and International Association on Occupational Health in collaboration with the Department of Biochemistry, University of Zürich and the Department of Environmental Hygiene, Karolinska Institute, a WHO collaborating center in Environmental Health. Drs Jeremias Kägi and Magnus Piscator were elected chairmen and Dr Monica Nordberg was elected secretary of the meeting. A report from the meeting was prepared and was published in full length together with working papers by the individual participants[7].

The meeting was focused on metallothionein, an important low molecular weight metal binding protein involved in the toxicity of cadmium, zinc, mercury and copper. The participants, totally 38 persons, from different parts of the world and with special interest in biochemistry, toxicology, physiology, nutrition and medicine, and actively working in this field of research discussed important questions concerning the low molecular weight metal-binding proteins. The main topics were: 1) history of metallothionein research, 2) definition, nomenclature, and occurrence in vertebrates, invertebrates and microorganisms, 3) biochemical properties such as isolation and quantification, chemical composition: contents of metal, cysteine and other amino acids, amino acid

sequence, multiple forms, and evolutionary aspects, 4) physico-chemical properties such as molecular weight, electrochemical and optical properties, metal-binding site and spatial structure, 5) metabolism e.g. biosynthesis, induction and degradation, 6) role in metal metabolism and toxicity, aspects on metal metabolism for cadmium and mercury, studies on zinc and copper metabolism, toxicological aspects, involvement in short-term toxicity of cadmium, distribution and effects of metallothionein administered to experimental animals, involvement in chronic cadmium toxicity, aspects related to other metals, e.g. copper, mercury, 7) special attention was focused on other low molecular weight metal-binding proteins such as copper-binding proteins, zinc--binding ligands in milk and intestine, renal bismuth-, gold- and mercury--binding proteins, lead-binding protein of low molecular weight in human erythrocytes.

The history of metallothionein research started primarily in 1957 by the publication by Margoshes and Vallee[4]. Due to the presence of metallothionein in animal tissues, such as liver, kidney, erythrocytes, plasma, spleen, testis and fetus, and in fungi, metallothionein is accepted as a widespread and ancient protein. The amount of metallothionein varies a great deal among species and tissues.

DEFINITION OF METALLOTHIONEIN

The nomenclature of the protein was accepted as follows: "Metallothionein" used to designate the cadmium-, zinc- and copper-containing sulfur-rich protein from the liver and kidney and fulfills the following criteria:

- molecular weight 6000–7000 (on gel filtration molecular weight of 10000)
- high metal content of Cd, Zn, Hg, Cu
- characteristic amino acid composition (high cysteinyl content, no aromatic amino acid nor histidine)
- optical features characteristic of metal-mercaptides
- unique amino acid sequence (fixed distribution of cysteinyl residues).

The presence in microorganisms e.g. *Neurospora crassa* of proteins with the same characteristics as metallothionein, but with lower molecular weight indicates that the molecular weight may vary between species.

Proteins resembling equine renal metallothionein in several of these features can be designated as "metallothionein". The definition of metallothionein and guide-lines for its nomenclature were in accordance with the above, and extended to more specific terms such as "cadmium-metallothionein" and "cadmium--thionein", which can be employed when metallothionein contains only cadmium. For metallothionein containing another metal an analogous nomenclature should be adopted. The metal prefixed to the word "thionein" should not be used to designate the metal employed for metallothionein induction.

The term "multiple forms of metallothionein" or "isometallothioneins" should be used as a broad term covering all metallothioneins occurring naturally in a single species. These terms should apply only to forms of metallothioneins

arising from genetically determined differences in primary structure and not to forms derived by modification of the same primary sequence nor to forms differing only in metal composition. Metallothionein contains the following amino acids: lysin, asparagin, cystein, threonine, serine, glutamic acid, prolin, glycin, alanin, methionein, isoleucine, valine, arginine. Cystein predominates, approximately 30%.

Complete amino acid sequences are known from 10 different metallothioneins. That the primary structure has been conserved from neurospora to man suggests its involvement in and its indispensability to some fundamental biological processes in the cell. Biochemical and physico-chemical data show that metallothionein is a compact molecule containing characteristic metal-mercaptide complexes.

Concentrations of metallothionein primarily in the liver have been shown to increase after cadmium and zinc exposure. The turnover of metallothionein is rapid and increased formation of metallothionein upon administration of cadmium or zinc is correlated with an increase of metallothionein mRNA in the cell.

ROLE IN METAL METABOLISM AND TOXICITY

Acute toxicity of injected cadmium-metallothionein was found to be five times higher than of cadmium as cadmium chloride. Injected cadmium--metallothionein is taken up by the kidney to a greater extent than cadmium received as cadmium chloride. On the contrary, for cadmium given as cadmium chloride the target organ is the liver.

The involvement of metallothionein in cadmium metabolism has been proposed and partly shown as follows: Exposure to cadmium induces the synthesis of metallothionein in the liver. Via blood, both in plasma and erythrocytes, cadmium is transported partly bound to metallothionein. Metallothionein is cleared from the plasma and taken up in the kidney – filtration through the glomeruli and reabsorption in the tubules. Released cadmium from catabolized metallothionein stimulates the synthesis of metallothionein in the kidney. This might explain the long biological half-time for cadmium. After renal tubular damage has occurred in the kidney, cadmium is excreted in the urine partly bound to metallothionein. However, 20 years after the discovery of the protein, there is still a lack of knowledge concerning its biochemical function. Important involvement in the metabolism of zinc, cadmium and copper has been shown. At elevated exposure of an organism to cadmium metallothionein might modulate the toxic effects of the metal.

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