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Role of Metallothioneins in Disease*

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ABSTRACT

Metallothioneins are small proteins (6,000 to 10,000 mw) with similar amino acid compositions, high content of sulfhydryl amino acids and no aromatic amino acids. E_{max} at 250 nm is due to cadmium mercaptide bond. Synthesis is induced by a number of metals including zinc, cadmium, mercury, copper, bismuth, gold and silver. These proteins function as a mechanism of intracellular storage of some essential metals such as zinc and copper. Binding of potentially toxic metals like cadmium may be protective; but with saturation of protein by metal, toxicity may occur. Excessive cadmium-thionein may have a role in the pathogenesis of cadmium induced kidney disease. A more sensitive method for detection and measurement of this protein will greatly enhance future studies, particularly the potential for clinical application.

This brief review concerns some current notions regarding the biochemistry and metabolism of the metallothioneins. The results of experimental studies in our laboratory investigating the possible role of these proteins in the pathogenesis of cadmium-induced renal disease are also presented.

The metallothioneins are a group of low molecular weight cytoplasmic proteins with a high affinity for a number of metals, particularly cadmium but also zinc, copper and probably mercury and silver as well. The protein moiety of the metalloproteins is similar in amino acid composition and, because of the high sulfhydryl content, the protein has been termed thionein, or metallothionein when coupled with a metal. It is not certain at present to what degree thionein associated with different metals is similar or different. For purposes of discussion, however, it is probably best at present to term a particular thionein with respect to the metal it is bound with, that is, thionein bound with cadmium or zinc might be referred to as cadmium-thionein or zinc-thionein, respectively.

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Properties of Metallothioneins

In table I are summarized some common properties of the metallothioneins. They contain about 6 to 11 percent metal and 30 percent cysteine and do not contain any aromatic amino acids.[5] The metal

TABLE I

Properties of Thionein

1. Induced synthesis by certain metals.
2. Low molecular weight (6,000 - 10,000).
3. High content of cystein (30 percent) and affinity for metals.
4. Absence of disulfide bonds and aromatic amino acids.
5. Absorption maximum at 250 nm. Cd-Cyst bond, minimum at 280 nm.
6. Heat stability.

ion is bound to the protein by mercaptide linkages, and three cysteinyl residues are involved in the binding of one metal ion. Also, these proteins do not have any disulfide bridges. This feature has been recently confirmed in the publication of the amino acid sequence and primary structure of cadmium-thionein isolated from horse kidney.[7] The maximum absorption at 250 nm is due to the cadmium-mercaptide bond. The absorption at 250 nm is decreased when cadmium is replaced by zinc, mercury or copper. The minimal absorption of the metallothioneins at 280 nm is due to the absence of aromatic amino acids. The thioneins are heat stable and are not destroyed by heating at 80° for 10 minutes.[1]

Synthesis of Thioneins

It is interesting that the synthesis of thioneins induced by different metals is somewhat organ specific. The mechanism whereby these metals, particularly a non-essential one like cadmium, induces the synthesis of thionein is not known. There are, however, two notions reported in recent literature. One study suggests that there is synthesis of new messenger ribonucleic acid (RNA) and that regulation of metallothionein synthesis takes place only at the transcriptional level without any effect on the translational step of protein biosynthesis.[10] A second study suggests that there is a control mechanism on the induction only at the translational step.[12] This can only occur if the messenger RNA for the thionein is already present in the cell and metals like cadmium can activate the messenger, that is, the metal must "unmask" the messenger RNA for the thioneins which are normally present in the cell. However, this is an area where more study is needed.

TABLE II

Increase in Thionein-like Proteins in Organs in Response to Various Metals

Metal	*Liver*	*Kidney*	*Spleen*
Cadmium	+	+	+
Zinc	+	-	-
Mercury	-	+	-
Copper	+	+	-
Bismuth	?	+	-
Gold	?	+	-
Silver	+	+	?
Lead	-	-	-

Synthesis or at least increase in amounts of thioneins that can be isolated have been observed in liver, kidney and/or spleen after administration of several metals table II).

Even trace amounts of cadmium, intraperitoneal injections or oral ingestion of as little as a few micrograms, will induce the synthesis of cadmium-thionein in liver and kidney. However, it requires considerably more zinc (30 μg per g) to induce thionein synthesis and it only seems to occur in liver and not kidney. Mercury, on the other hand, will only induce thioneins in kidney but not in other organs. Copper may also induce copper thionein in liver and kidney. There are reports of thionein synthesis following exposure to bismuth, gold and silver, but lead does not induce any response.

Biologic Role

The principle biologic role of the metallothioneins is not certain; but a number of functions have been suggested.[6] Since these proteins can bind with various metals, both essential and non-essential, they may have an important function in regulating the metabolism and toxicity of these metals. It has been known for some time

that metallothionein is an intracellular protein which can act as a storage protein. The long biological half life of cadmium in humans could be due to the specific binding of cadmium to this intracellular protein. Though the half life of the protein moiety of metallothionein is only four to five days, similar to other tissue proteins, cadmium stays bound to the protein in the cell once it is synthesized. Recent study[11] shows a half life of 20 hours for zinc-thionein in rat liver. Increased accumulation of cadmium and zinc in liver rats repeatedly treated with cadmium is also suggestive of a storage function for these proteins.

Cadmium-thionein complex also accumulates in the kidney particularly in the cortex. Persons in the general population have about 10 to 15 μg Cd per g of renal cortex and may have as much as 50 μg per g. In both experimental animals and persons with excessive exposure to cadmium, it has been observed that renal tubular dysfunction occurs when the cadmium concentration exceeds 100 μg per g and an irreversible nephropathy occurs when the cadmium concentration in renal cortex approaches 200 μg per g.[3] The pathogenesis of the cadmium nephropathy and the possible role of cadmium-thionein is under study in many laboratories.[2,8,13]

Cadmium-thionein Complex in Relation to Renal Disease

A few years ago, Nordberg at the Karolinska Institute in Sweden found that the injection of cadmium-thionein complex protected mice from the testicular necrosis that usually follows exposure to cadmium chloride.[8] This experiment, in a way, confirmed the protective role of cadmium-thionein complex. However, it was also noted that cadmium-thionein injected mice develop necrosis of renal tubular lining cells, a pathologic process similar to that expected when cadmium content of renal cortex exceeds 200 μg per g. This observation has led to considerable study attempting to define the role of cadmium-thionein in cadmium induced nephropathy.[2]

When workmen are exposed to excessive amounts of cadmium or mice are injected daily with cadmium, there is virtually no increase in urinary excretion of cadmium until renal cortex concentration is over 100 μg per g.[4] With increased cadmium in urine, there is usually evidence of renal tubular dysfunction, that is, glycosuria and aminoaciduria. An increase in excretion of beta-2-microglobulin is noted but cadmium-thionein is not detectable in urine.

However, in mice injected with cadmium chloride, cadmium-thionein becomes detectable in serum about the same time as renal tubular dysfunction and increase in cadmium in urine occurs. This observation suggests that cadmium-thionein does have a role in the pathogenesis of cadmium induced renal disease but the mechanism is unclear. Two hypotheses have been proposed. It has been suggested that the cadmium-thionein within the renal tubular lining cells becomes saturated and the renal cell injury is the result of unbound cadmium[9,13] or secondly, that cadmium-thionein somehow becomes extracellular and has a direct toxic effect on cell membranes.[2] This second hypothesis is favored in our laboratory at the present, principally because the intraperitoneal injection of relatively small amounts of cadmium-thionein complex has a necrotizing effect on renal tubular cells.[2]

A final comment concerns the detection of cadmium-thionein in plasma as an early indication of cadmium-induced renal disease. In rats given large doses of cadmium chloride intraperitoneally, the appearance of cadmium-thionein in plasma correlates well with the onset of the renal tubular dysfunction. However, in the past few months the present authors have been attempting to detect cadmium-thionein complex in plasma of workmen with very

early renal tubular dysfunction with no success. There may be several reasons for the failure to date. For one, the sephadex chromatography method is not ideally suited for this purpose. Chromatographic methods are most useful for isolation of proteins but lack sensitivity and precision for quantitation. Secondly, the renal clearance of the cadmium-thionein complex in persons with occupational exposure to cadmium may be much more efficient than in the rat model so that plasma levels of cadmium-thionein will, at least, be only modestly elevated. Certainly studies concerning the relationships between plasma cadmium-thionein levels and cadmium nephropathy will be greatly enhanced by the availability of a more specific and sensitive method for the measurement of this metal-protein complex.

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