ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN-POLONIA
VOL. LX, N1, 15 SECTIO D 2005

Department of Biochemistry and Molecular Biology, Medical University of Lublin

BOLESŁAW FLORIAŃCZYK

Sephadex G-75 gel filtration chromatography of metallothionein and zinc chloride

Metallothioneins (MTs) are the widespread proteins in the animal world. While isolated from the different organs of different animals they only slightly differ in the amino acid composition from one another. The number of amino acids is fixed in every animal group, that is 60 (or 61) amino acids, 20 of which are the cysteine radicals which makes over 30% of the amino acid composition. Such a large amount of cysteine, which include the sulphydryl groups -SH determines the metallothionein's functions (1,3,5,6,9,12).

Metallothioneins take part in the homeostasis of the ions of metals which are necessary for the proper metabolism of the organism (zinc, copper), regulation of the synthesis of the zinc proteins (for example the zinc-dependent transcription factors). They also take part in the removal of toxic metals from the tissue. Besides, they also protect the tissue from the free radicals, radiation, electrophilic pharmacological agents used in the cancer therapy and the mutagens (10,11,13).

The aim of this work was to determine the content of metallothioneins and zinc in fractions after gel filtration (Sephadex G-75). The research was supposed to answer the question whether the metallothioneins contain zinc ions after heat--treated cytosol.

MATERIAL AND METHODS

Tissues. The rabbit liver was weighted, washed with physiological saline and homogenized in fourfold volumes of 10 mM Tris-HCl buffer, pH 7.4 with a glass homogenizer. The homogenates were centrifuged at 10 000 x g for 10 min., and then supernatant was also centrifuged at 100,000. x g, 4° C for 1 hr, and the supernatant was heated in a boiling water bath for 2 min. Precipitated proteins were separated by centrifugation 10,000 x g, 4°C for 10 minutes, and then supernatant was collected and stored in a freezer.

Gel filtration. 10 ml samples were applied to a Sephadex G-75 column (2 x 50 cm. Uppsala) equilibrated with elution buffer (10 mmol, Tris-HCl pH 7.4). The samples was eluted a flow rate of 2 ml/min, and 3 ml were collected in each probe. In the same conditions zinc saline solution ($ZnCl_2$) was applied onto the Sephadex G-75 column at the concentration of 0.25 mol/l.

Determination of metallothioneins. The levels of the metallothionein were determined by cadmium-hemoglobin affinity assay using the cadmium isotope (^{109}Cd) (4).

Determination of zinc. The concentration of zinc was determined spectrophotometrically using Pye Unicam (SP-192) spectrophotometer (14).

RESULTS

The content of MTs in each tube after gel filtration of heat-denaturated tissue supernatant was shown in Figures 1. Metallothioneins concentration was shown in impuls on tribe cpm (Beckman counter type LS 6000TA). After column chromatography, the zinc content in each fraction was measured (zinc concentration was shown in mmol/l).

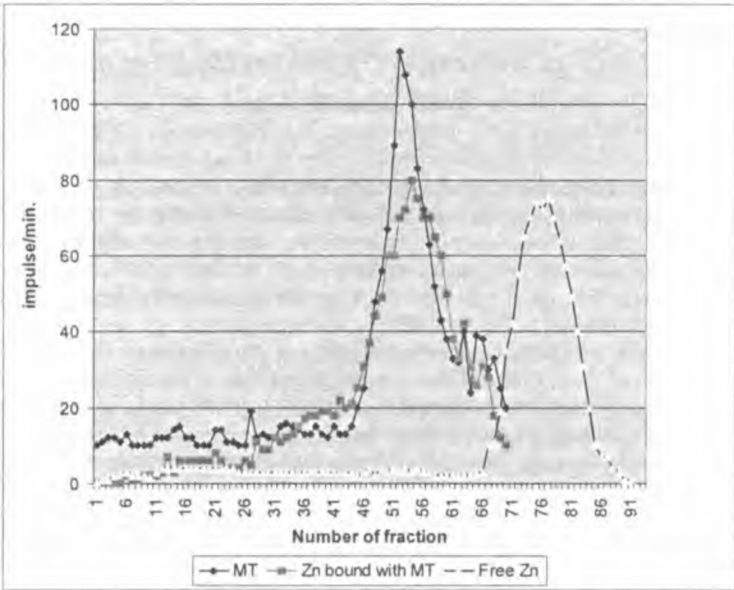

Fig. 1. Gel filtration of cytosol from rabbit liver. Rabbit liver cytosol subjected to heat treatment was applied to a Sephadex G-75 columns. Metallothioneins contents in the eluted fractions were determined by cadmium-hemoglobin affinity assay using the cadmium isotope. Zinc contents in the eluted fractions were determined by atomic absorption spectrophotometry

DISCUSSION

Metallothioneins have been isolated from a wide range of tissues, including liver, kidney, pancreas, and intestine. Indeed immunologic techniques for their detection have improved, metallothioneins have been found in most other tissues, including brain, thymus, bone marrow, and reproductive organs. Detection by subcellular fractionation indicates that metallothionein occurs principally in the cytosol, but immuhistochemical studies have consistently revealed its presence also in nuclei. Although metallothionein is mainly of intracellular origin, it also occurs in small amounts in extracellular fluids such as plasma, bile, and urine (1).

The concentration of the protein in tissues is highly variable and is induced by many nutritional, physiologic, and developmental factors (11). 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 metallothionein is its low molecular weight and its unusual amino acid composition: cysteine accounts for 30% of the residues and aromatic acids absent. Sequence studies have shown that the distribution of the cysteine residues along the polypeptide chain is fixed, regardless of the source or isoform of the protein (1). MTs are known as heat-stable proteins and are able to be precipitated at 100^0 C for 2 min (8). The liver cytosol was initially heat-treated to remove the heat-liable proteins and then cytosol was applied to a gel filtration column. Metallothioneins content in each eluted fraction were measured and the result is shown in Figure 1. Another experiment was conducted to further confirm whether the MT peak contains zinc. A metal peak appeared at low molecular weight fractions. The MTs isolated from rabbit liver contain zinc.

As part of the present paper zinc saline solution ($ZnCl_2$, so-called free zinc) was applied onto the Sephadex G-75 column and the gel filtration was performed in the same conditions as the filtration of the cytosol obtained from a rabbit liver. In fractions flowing out of the column free zinc appeared second to zinc and metallothioneins from the cytosole subjected to fractioning. The experiment suggests that zinc is a stable element of the structure, being tightly bound with metallothioneins obtained from a rabbit liver through thermal precipitation.

In the process of evolution living organisms have developed techniques allowing the resorption of zinc and copper, their transport and storage in the organism as well as systems protecting them against their toxic activity (3,15). These systems contain proteins of strictly determined functions (7). The responsibility for the homeostasis of zinc and copper inside the cell is held by metallothioneins (2).

REFERENCES

1. Bremner I.: Interaction between metallothionein and trace elements. Progr. Food Nutr. Sci., 11, 1, 1987.
2. Choudhuri S., Kramer K.K. et al.: Constitutive expression of metallothionein. Toxicol. Appl. Pharmacol., 131, 144, 1995.
3. Coleman J.E.: Zinc protein enzymes, storage protein, transcription factor, and regulation protein. Ann. Rev. Biochem., 61, 897, 1992.
4. Eaton D.L., Cherian M.G.: Determination of metallothionein in tissue by cadmium-hemoglobin affinity assay. Methods Enzymol., 205, 1991.
5. Ebadi M.: Metallothionein and other zinc-binding proteins in brain. Methods Enzymol., 205, 363, 1991.
6. Floriańczyk B.: Hamujący efekt cynku i metalotionein na toksyczne i kancerogenne działanie kadmu. Wiad. Lek., 48, 439, 1995.
7. Floriańczyk B.: Detoksykacyjna funkcja metalotionein. Nowiny Lek., 66, 310, 1996.
8. Floriańczyk B.: Wiązanie metali za pomocą metalotioneiny. Regulacja syntezy metalotionein. Praca zbiorowa: Biopierwiastki w Naszym Życiu. Poli ART. Studio s.c., Lublin 243, 1998.
9. Floriańczyk B.: Metallothioneins in rat exposed to cadmium. Rocz. AM Białystok, 43, 309, 1998.
10. Floriańczyk B.: Metallothionein and copper level in breast cancer. Adv. Clin. Exp. Med., 9, 29, 2000.
11. Floriańczyk B.: Czynniki indukujące syntezę metalotionein. Post. Hig. Med. Dośw., 5, 687, 2000.
12. Goyer R.A: Nutrition and metal toxicity. Am. J. Nutr., 61 (Suppl.), 646S, 1995.
13. Johnston S.W., Ozols R. F. et al.: Mechanisms of drug resistance in ovarian cancer. Cancer, 71, 644, 1993.
14. Pinta M.: Absorpcyjna spektrometria atomowa. PWN, Warszawa 1977.
15. Prasad A.: Zinc: An overview. Nutrition, 11, 93, 1995.

SUMMARY

The aim of this work was to determine the content of metallothioneins and zinc in fractions after gel filtration (Sephadex G-75) of cytosol obtained from rabbit liver. As part of the present paper zinc saline solution (so-called $ZnCl_2$ free zinc) was applied onto the Sephadex G-75 column and the gel filtration was performed in the same conditions as the filtration of the cytosol obtained from rabbit liver. In fractions flowing out of the column free zinc appeared second to zinc and metallothioneins from the cytosole subjected to fractioning. The experiment suggests that zinc is a stable element of the structure, being tightly bound with metallothioneins obtained from rabbit liver through thermal precipitation.

Filtracja żelowa (Sephadex G-75) metalotionein oraz roztworu chlorku cynku

Celem pracy było oznaczenie zawartości metalotionein oraz cynku we frakcjach uzyskanych z filtracji żelowej (sephadeks G-75) cytozolu z wątroby królika. Ponadto w pracy na kolumnę Sephadex G-75 nanoszono roztwór soli cynku (tzw. wolny cynk, $ZnCl_2$) i filtrację żelową prowadzono w tych samych warunkach, co filtrację cytozolu z wątroby królika. „Wolny cynk" pojawiał się w dalszych frakcjach wypływających z kolumny niż cynk i metalotioneiny z poddanego frakcjonowaniu cytozolu. Wykonane doświadczenie sugeruje, że cynk jest stałym elementem struktury metalotionein i mocno związany jest z tymi białkami, uzyskanymi z wątroby królika na drodze precypitacji termicznej.