ANNALES
UNIVERSITATIS MARIE CURIE-SKŁODOWSKA
LUBLIN-POLONIA
VOL. LVIII, N 2, 103 SECTIO D 2003

Department of Biochemistry and Molecular Biology, Medical University of Lublin

BOLESŁAW FLORIAŃCZYK

Gel filtration chromatography of metallothionein obtained from rabbit liver

Metallothioneins (MT) are the widespread proteins in 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 amono acid composition. Such a large amount of cysteine, which includes the sulfhydryl groups – SH determines 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) and they also take part in the removal of toxic metals from the tissue. Apart from these they also protect the tissue from the free radicals, radiation, electrophilic pharmacological agents used in the cancer therapy and the mutagens (10,13).

The induction of metallothionein synthesis is influenced by many factors including heavy metals, inflammatory factors, free radicals, glucocorticoides and other hormones and pharmacologic agents (11).

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 wheter metallothioneins contein zinc ions after heat-treated cytosol.

MATERIAL AND METHODS

The rabbit liver was weighted, washed with physiological saline and homogenized in 4-times 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 minutes, and then supernatant was also centrifuged at 100 tys. x g, 4° C for 1 hr, and the supernatant was heated in a boiling water bath for 2 minutes. 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. Ten ml samples were applied to a Sephadex G-75 column (2 x 50 cm, Upsala) equilibrated with elution buffer (10 mmol, Tris-HCl pH 7,4). The samples was eluted at a flow rate of 2 ml/min, and 3 ml were collected in ich probe.

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

Zinc determination. The concentration of zinc was determined spectrophotometricaly using Pye Unicam (SP-192) spectrophotometer (14).

Statistical analysis. The results were analyzed statistically by means of the Cohran-Cox test accepting the differences as intrinsic at the intrinsicity level of $p < 0.05$. The results are presented in the table.

RESULTS

The content of MTs in each tube after gel filtration of heat-denaturated tissue supernatant is shown in Figure 1a. 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. 1b).

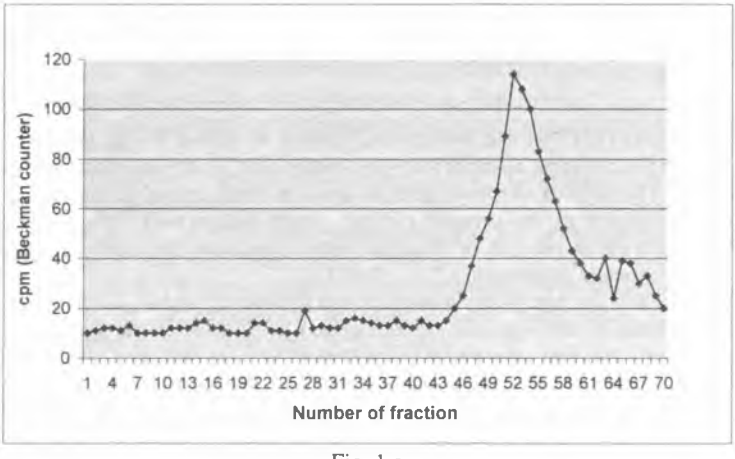

Fig. 1 a

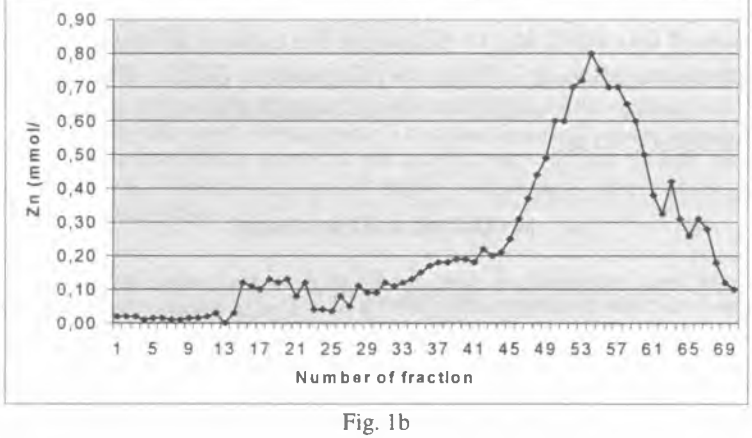

Fig. 1b

Fig. 1. Gel filtration of rabbit liver cytosol. 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 (Fig. 1a). Zinc contents in the eluted fractions were determined by atomic absorption spectrophotometry (Fig. 1b)

DISCUSSION

Metallothioneins have been isolated from a wide range of tissues, including liver, kidney, pancreas, and intestine. 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 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 protein in tissues is highly variable and is iduced 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 metallothioneins are its low molecular weight and its 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).

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 was measured and the result is shown in Figure 1a. Another experiment was conducted to further confirm whether the MT peak contains zinc. A metal peak appeared at low molecular weight fractions. The MT isolated from rabbit liver contains zinc (ryc. 1b).

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 funtions (7). The responsibility for the homeostasis of zinc and copper inside the cell is held by metallothioneins. Sulfhydryl groups frequently occurring in these proteins permit the binding of metallic ions. Although metallothioneins bind Zn and Cu with considerable affinity, microelements exchange is possible both between particular MT molecules as well as other proteins and micro-molecular ligands. This exchange helps to supply proteins with zinc and copper, which they need in order to function properly (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., 1991 205.
5. Ebadi M.: Metallothionein and other zinc-binding proteins in brain. Methods Enzymol., 205, 363, 1991.
6. Florianczyk B.: Funkcja metalotionein w ustroju. Post. Hig. Med. Dośw., 50, 375, 1996.
7. Florianczyk B.: Pompy ATP-azowe dla jonów miedzi. Nowiny Lek., 65, 561, 1996.
8. Florianczyk B.: Metalotioneiny - białka szoku chemicznego. Mag. Med., 9, 31, 1998.
9. Florianczyk B.: Metallothioneins in rat exposed to cadmium. Rocz. AM Białyst., 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. (Suppl.) 61, 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

Metallothioneins (MTs) are known as heat-stable proteins and are able to be precipitated at 100^0 C for 2 min. The aim of this work was to determine the content of metallothioneins and zinc in fraction after gel filtration (Sephadex G-75). The research was supposed to answer the question whether metallothioneins contain zinc ions after heat-treated cytosol. The rabbit liver cytosol was initially heat-treated to remove the heat-liable proteins and then cytosol was applied to a gel filtration column (Sephadex G-75). Metallothioneins content in each eluted fraction was measured by cadmium-hemoglobin affinity assay using the cadmium isotope (^{109}Cd). Another experiment was conducted to further confirm whether the MTs peak contains zinc. A metal peak appeared at low molecular weight fractions. The metallothioneins isolated from rabbit liver contain zinc.

Filtracja żelowa metalotionein otrzymanych z wątroby królika

Metalotioneiny są białkami odpornymi na ogrzewanie i są stabilne podczas stosowania temperatury 100^0 C przez 2 minuty. Celem pracy było oznaczenie zawartości metalotionein oraz cynku w cytozolu z wątroby królika po filtracji żelowej. Homogenat uzyskany z wątroby królika podddano wcześniej denaturacji termicznej. Zawartość metalotionein w każdej próbce oszacowano metodą kadmowo-hemoglobinową z zastosowaniem izotopu kadmu ^{109}Cd. Ponadto w każdej frakcji oznaczono zawartość cynku. Przeprowadzone badania pozwoliły na stwierdzenie, że metalotioneiny poddane wcześniej wysokiej temperaturze zachowały zdolność wiązania jonów cynku.