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Gel filtration of proteins as a result of heat-treated denaturation of cancer cells homogenate

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Abstract: The study covered material from patients with brain cancer (astrocytoma G-2). Cancer cells were homogenized, and heat-treated denaturation of cancer cells homogenate was performed. The solution containing heat-stable proteins was applied to a column filled with Sephadex. The results of the study showed that metallothioneins – heat-stable proteins – appear in the eluate in the same fractions as proteins obtained from a rabbit's liver in the same experimental conditions. The presence of zinc ions was observed in the same fractions as metallothioneins, which indicates that zinc is a stable element in the structure of proteins.

Key words: metallothioneins, zinc, gel filtratiom, astrocytoma

INTRODUCTION

Metallothioneins (MT) are widespread proteins in the animal world. While isolated from the different organs of different animals they only differ slightly from one another in the amino acid composition. In every animal the number of amino acids in MT are in a fixed group, i.e. 60 or 61 amino acids, 20 of which are the cysteine radicals which constitutes more than 30% of the amino acid composition. Such a large ammount of cysteine, which include the sulfhydryl groups –SH, determines the functions of metallothionein [1-4].

Metallothioneins take part in the homeostasis of the ions of the metals necessary for the proper metabolism of the organism (zinc, copper), regulation of the synthesis of the zinc proteins (e.g. zinc-dependent transcription factors), and they also participate in the removed of toxic metals from the tissue. In addition, they also protect the tissue from free radicals, radiation, electrophilic pharmacological agents used in cancer therapy, and mutagens [5-9].

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

MATERIALS AND METHODS

The experimental materials were the brain neoplastic tissues resected during neurosurgical procedures. The brain tumours were benign gliomas (*astrocytoma* G-2), established by histopathological studies.

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Tissues. The experimental material 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 × 4,000 g for 10 min.; the supernatant was then also centrifuged at × 10,000 g, 4°C for 1 hr, and the supernatant heated in a boiling water bath for 2 min. Precipitated proteins were separated by centrifugation × 10,000 g, 4°C for 10 min.

Gel filtration. 10 ml samples were applied to a Sephadex G-75 column (2 × 50 cm, Upsala, SWden) equilibrated with elution buffer (10 mmol, Tris-HCl pH 7.4). The samples was eluted at a flow rate of 2 ml/min; 3 ml were collected in each probe.

Determination of metallothioneins. The levels of metallothioneins were determined by cadmium-hemoglobin affinity assay using a cadmium isotope (^{109}Cd) [10].

Determination of zinc. The concentration of zinc was determined spectrophotometrically using a Pye Unicam (SP-192) spectrophotometer [11].

RESULTS

The content of total protein in each tube after gel filtration of heat-denarurated tissue supernatant is shown in Fig. 1a. The content of MT in each tube after gel filtration of heat-denaturated tissue supernatant is shown in Fig. 1c. Metallothioneins concentration was shown in impulse on tribe cpm (Beckman counter type LS 6000TA) (Fig. 1c).

Another experiment was conducted to further confirm whether the MT peak contained zinc.

After column chromatography, the zinc content in each fraction was measured (zinc concentration shown in mmol/l) (Fig. 1b). A metal peak appeared at low molecular weight fractions.

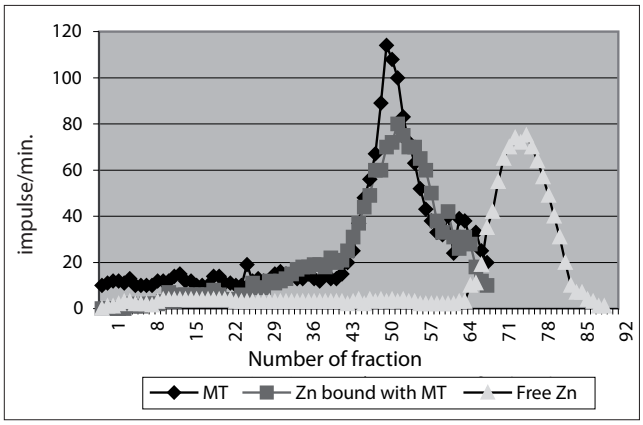

Figure 1 Gel filtration (Sephadex G-75) of proteins as a result of heat-treated denaturation of cancer cells homogenate.

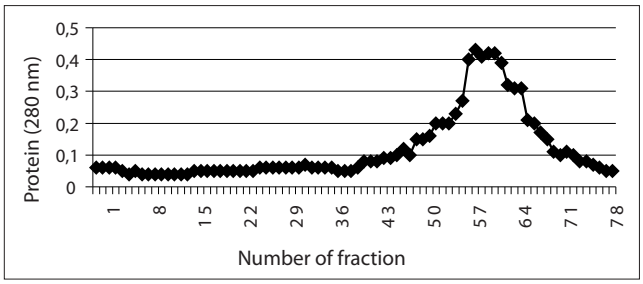

Figure 1a Total protein.

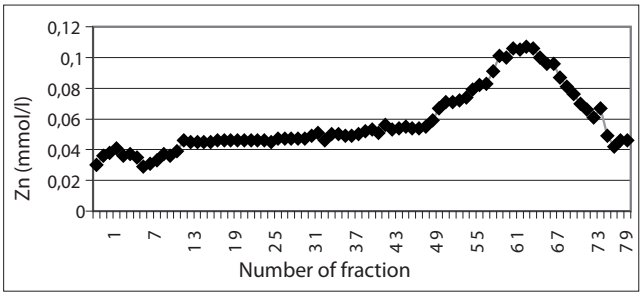

Figure 1b Zinc contents in the eluted fractions determined by atomic absorption spectrophotometry.

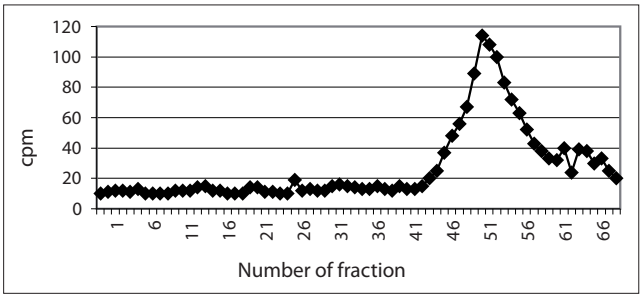

Figure 1c Metallothioneins contents in the eluted fractions determined by cadmium-hemoglobin affinity assay using a cadmium isotope.

DISCUSSION

Metallothioneins has been isolated from a wide range of tissues, including liver, kidney, pancreas, and intestine. Immunologic techniques for its detection have improved and metallothionein has been found in most other tissues, including brain, thymus, bone marrow, and reproductive organs. Although metallothioneins are mainly of intracellular origin, they also occur in small amounts in extracellular fluids such as plasma, bile, and urine [12].

The concentration of the protein in tissues is highly variable and is induced by many nutritional, physiologic, and developmental factors [13]. For example, concentrations are greatly decreased in the tissues of zinc-deficient animals, and increase after imposition of many types of stress or metal administration. They are generally elevated during foetal development and vary dramatically among species.

The characteristic features of metallothioneins are their low molecular weight and unusual amino acid composition: cysteine accounts for 30% of the residues and aromatic acids are absent. Sequence studies have shown that the distribution of cysteine residues along the polypeptide chain is fixed, regardless of the source or isoform of the protein [12].

MTs are known as heat-stable proteins which can be precipitated at 100° C for 2 min. The homogenate was initially heat-treated to remove the heat-liable proteins, after which cytosol was applied to a gel filtration column. Metallothioneins content in each eluted fraction was measured (result shown in Fig. 1).

Another experiment was conducted to further confirm whether the MT peak contained zinc. A metal peak appeared at low molecular weight fractions which, however, could not be free-zinc ions, because – according to results from previous studies – free-zinc flows out from the column in further fractions. In fractions flowing out of the column, free zinc appeared second to zinc and matallothioneins from the cytosole subjected to fractioning [14, 15]. The experiment suggests that zinc, being tightly bound with metallothionein, is a stable element of the structure,

During the process of evolution, living organisms have developed techniques for allowing the resorption of zinc and copper, their transport and storage in the organism, as well as systems protecting them against their toxic activity [1, 16]. These systems contain proteins with strictly determined functions. The responsibility for the homeostasis of zinc and copper within the cell is held by metallothioneins [17-19].

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