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Isolation of mouse isometallothioneins : A comparison of isometallothioneins in growing cells and post-mitotic cells.

Shizuko KOBAYASHI and Junko SUZUKI

小林静子，鈴木純子

As we were interested in the relationship between cell growth and MT synthesis, we carried out an analysis of isoMTs in neonatal-mouse liver, and in Zn-, Cd- or glucocorticoid-treated mouse tumour cells, using an anion-exchange h.p.l.c. column and the results were compared with those for metal-treated adult liver. In the present paper, we describe the findings obtained, which suggest that MT-2 has a relationship with glucocorticoid in growing cells.

The h.p.l.c. instrument consisted of a chromatograph (Shimadzu HPLC CL-6A Gradient System; Shimadzu Co., Kyoto, Japan) and an anion-exchange chromatography column (Asahipak ES-502N, 13±0.5 μm particle size; 7.6 mm×100 mm column; Asahi Chemical Industries Co., Kawasaki, Japan). A 10 μl portion of the concentrated MT fraction obtained from gel filtration, containing 0.1—1.0 μg of metal, was applied to the column and eluted with 4 mM-potassium phosphate buffer, pH 7.5, at a flow rate of 0.5 ml/min. Subsequently, the sample was eluted with the same buffer for 10 min and then with a linear gradient of 4-52 mM potassium phosphate buffer, pH 7.5, for 30 min at 29 C, and the A_{220} was determined with a Shimadzu SPD-6A u.v. detector. The peak fractions detected by the A_{220} were collected with a Frac-100 (Pharmacia Fine Chemicals, Uppsala, Sweden) by switching them from the u.v. monitor, and heavy-metal concentrations were determined by atomic-absorption spectrometry.

Mouse metallothioneins (MTs) were separated into three isoforms by an anion-exchange h.p.l.c. column; conventionally isolated MT-1 and MT-2 showed a single peak (MT-1-1) and two peaks (MT-2-1 and MT-2-2), respectively. In growing cells, developing hepatocytes and growing tumour cells, MT-1/MT-2 ratios were less than 0.6, irrespective of the type of MT inducer, whereas adult liver post-mitotic cells had a ratio of more than 1.0. A large amount of the MT-2-2 subfraction was found in dexamethasone-treated FM3A cells; 90% of MTs was MT-2-2, suggesting that glucocorticoid hormone mainly induces MT-2-2 in tumour cells.

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