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DISTRIBUTION OF CADMIUM, ZINC AND COPPER IN THE MUSSEL *Mytilus edulis*. EXISTENCE OF CADMIUM-BINDING PROTEINS SIMILAR TO METALLOTHIONEINS.

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Introduction.

The existence of metallothioneins, specific Zn-, Cd-, Hg- and Cu- binding proteins is now quite established in various organs of mammals, birds and recently of fishes [1,2]. These proteins, present in the cytoplasmic soluble fraction, are characterized by their extraordinary metal binding capacity, their high content in cystein (approximately one third of the total amino acid residues), their low molecular weight (about 10.000) and their inducible nature on administration of heavy metals [2,3,4,5]. The role of metallothioneins in the detoxification of Cd and Hg has been frequently implied[6,7,8]. The very important accumulation of these two metals in the organisms as well as their very slow rate of elimination is likely to be explained by their binding to metallothioneins.

To our knowledge, there is no report on the finding of such proteins in invertebrates. Owing to the enormous cadmium accumulation observed in molluscs[9], the possible existence of metallothioneins in one species of the phylum has been investigated.

Methods.

For each essay, the soft parts of two mussels are pooled and homogenized in three volumes of 0.5M sucrose by means of a Polytron Homogenizer. The supernatants obtained by centrifugation at 100.000g are chromatographied on Sephadex G-75 or LKB Ultrogel AcA 54 columns equilibrated in NH_4HCO_3 0.05M. The fractions are analysed for Cd, Zn and Cu by atomic absorptions spectrophotometry.

Results and discussion.

The results presented in fig.1 have been confirmed by many other chromatographies. Graphs A and B show that the Cd accumulated by the mussels during the chronic intoxications is associated with three fractions. Fraction I is situated at the level of high molecular weight (mol.wt.) proteins. Fraction II, when large columns are used, corresponds to two unresolved peaks, the second one (IIb) having an elution volume corresponding exactly to that observed for metallothionein in different eel tissues[2] ; the position of this peak is characteristic of substances having a mol.wt. close to 10.000. Fraction III has the same elution volume as free Cd. It must be pointed out that a number of authors have already observed heterogeneity at the level of the Cd-binding peak of low mol.wt.[1,7,10,11]. Note that fraction II does not contain high amounts of Zn and Cu contrary to metallothionein from eel liver[12].

In the control mussels (graph C) Cd is almost exclusively bound to high mol.wt. proteins. This result and the fact that in the case of a short intoxication (fig.2) the fraction II only contains very little Cd, seems well to indicate that the metalloproteins contained in fraction II on chromatograms of animals submitted to a long Cd exposure does not exist in detectable amount in control mussels. It thus appears that Cd, even at very low doses, induces the synthesis of low mol.wt. Cd-binding proteins (Cd-BP) in mussel tissues.

According to preliminary results, these Cd-BP possesses a high percentage of cysteine and an ultraviolet absorption spectrum typical of metallothioneins. However further isolation steps are now necessary for a better characterization.

Thus like various vertebrates, mussels are able to react in presence of Cd by producing Cd-BP of low mol. wt. that seem to belong to the group of metallothioneins. In mammals, the induction of metallothioneins is considered to be a protective mechanism against the toxic Cd^{++} ion. The existence of such proteins in molluscs would allow them to accumulate large amounts of Cd and to resist to it, becoming a potential danger to man as food.

As OLAFSON and THOMPSON[1] have recently pointed out referring to the finding of a Cd-BP of low mol.wt. in blue green alga[13] : "it thus appears that metallothioneins may be ubiquitous in the living world".

Summary.

The study of the distribution of Cd, Zn and Cu in homogenates of mussels has shown that Zn and Cu are principally associated with high mol.wt. proteins. The same distribution is observed for Cd in untreated mussels but in Cd chronically intoxicated animals, the metal is principally bound to low mol.wt. proteins synthesized in response to the uptake of the cation and similar to metallothioneins of vertebrates.

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References.

1. R.W. OLAFSON and J.A.J. THOMPSON, Marine Biology *28*, 83 (1974).
2. J.M. BOUQUEGNEAU, Ch. GERDAY and A. DISTECHE, FEBS Letters *55*, 173 (1975).
3. Z.A. SHAIKH and O.J. LUCIS, Proc. Can. Fedn. Biol. Socs *13*, 158 (1970).
4. M. WEBB, Biochem. Pharmacol. *21*, 2767 (1972).
5. M. WEBB and M. DANIEL, Chem. Biol. Interactions *10*, 269 (1975).
6. H.E. RUGSTAD and T. NORSETH, Nature *257*, 136 (1975).
7. P. PULIDO, J.W.R. KÄGI and B.L. VALLEE, Biochemistry *5*, 1768 (1966).
8. T. SUDA, N. HORIUCHI, E. OGATA, I. EZAWA, N. OTAKI and M. KIMURA, FEBS Letters *42*, 23 (1974).
9. J.D. PEDEN, J.H. CROTHERS, C.E. WATERFALL and J. BEASLEY, Mar.Pollution Bull. *4*, 7 (1973).
10. G.F. NORDBERG, M. NORDBERG, M. PISCATOR and O. VESTERBERG, Biochem.J. *126*, 491 (1972).
11. Z.A. SHAIKH and O.J. LUCIS, Arch. Environ. Health *24*, 419 (1972).
12. F. NOËL-LAMBOT, unpublished work.
13. F.I. MACLEAN, O.J. LUCIS, Z.A. SHAIKH and E.R. JANSZ, Fedn Proc. Fedn Am. Socs exp. Biol. *31*, 699 (1972).

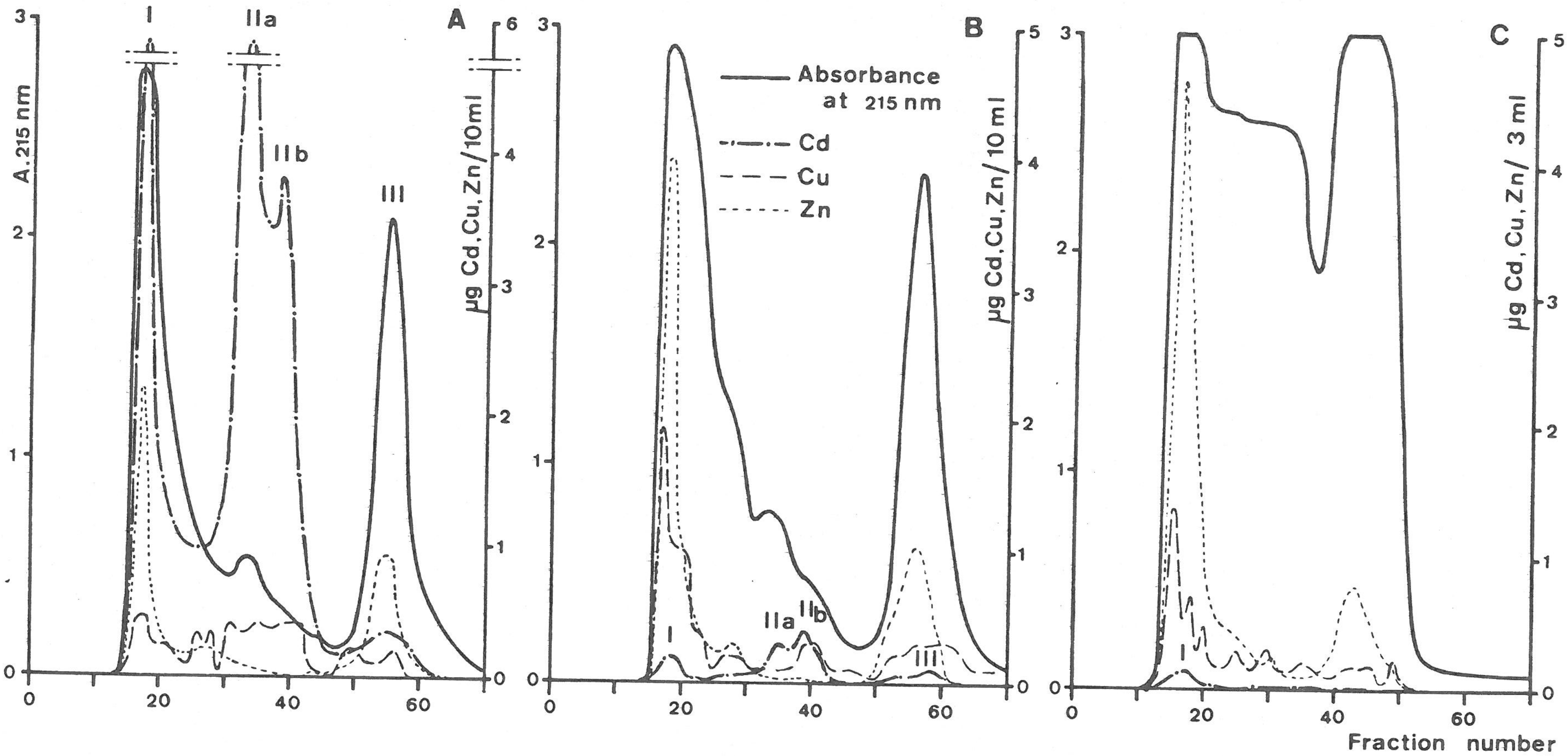

Fig.1 : Elution profiles on Sephadex G-75 column 76x3 cm (graphs A and B) or on Ultrogel column 40x2 cm (graph C) of supernatants of mussels.

A. Intoxication of 36 days in sea water containing 0.13 ppm Cd (as $CdCl_2$)
Concentration of Cd in animals : 80 ppm wet wt.

B. Intoxication of 90 days in sea water containing 0.005 ppm Cd.
Concentration of Cd in animals : 2.8 ppm wet wt.

C. Untreated mussels from the Belgian coast of the North Sea (concentration of Cd in water : 0.0001 à 0.001 ppm).
Concentration of Cd in animals : about 1 ppm wet wt.

In the three cases, the supernatant accounted for a little more than 50% of Cd.

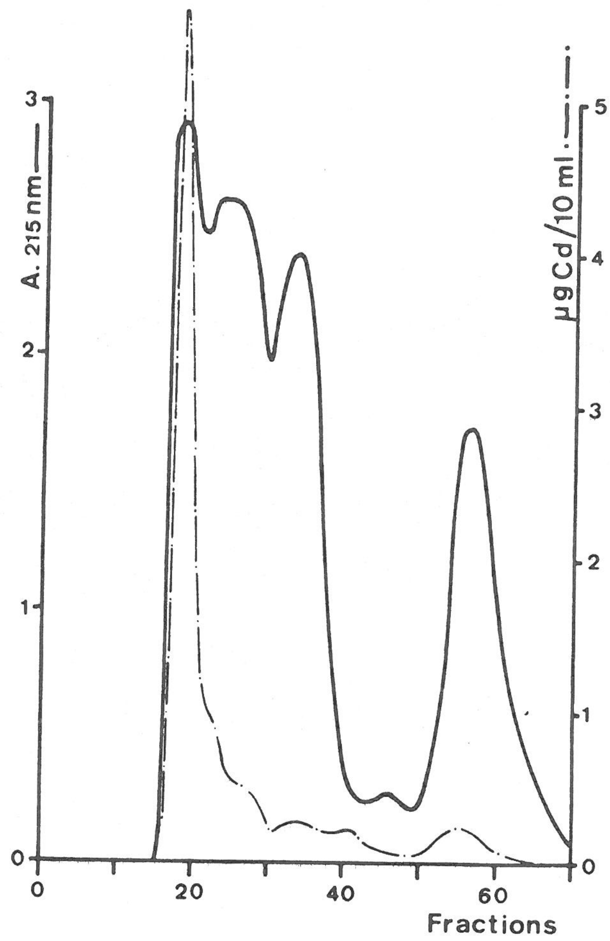

Fig.2. : Elution profile on Sephadex G-75column (76x3 cm) of supernatant from mussels intoxicated during 3 days in sea water containing 13 ppm Cd (acute intoxication).

Concentration of Cd in animals : 26 ppm wet wt.