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THE EFFECT OF ZINC AND RARE EARTH ELEMENTS ON METALLOTHIONEIN IN BIVALVE MOLLUSC *IN VITRO*

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Rare earth elements (REE) are attracting increasing attention as emerging environmental pollutants, especially in aquatic ecosystems, due to their rapid use in various industrial sectors. Studies show that REEs have harmful effects on aquatic organisms [1, 4].

Metallothioneins (MT) are low-molecular weight proteins (6–8 kDa), rich in cysteine residues (~30%), which have a high capacity to bind certain physiological metals (Zn^{2+} and Cu^{+}) and toxic metals (Cd^{2+} and Hg^{2+}), forming metal-thiolate clusters and thereby regulating their homeostasis [3]. Although there are some doubts regarding the ability of REEs to interact with MT via the sulphur of cysteines or to displace physiological metals such as Zn^{2+} and Cu^{+} from MT, their ability to interact with other amino acid residues of the protein, including carboxyl groups, should not be ruled out [2].

The aim of this study was to investigate the effect of zinc (Zn), gadolinium (Gd) and yttrium (Y) on partially metalated MT (pMT) *in vitro* by determining their spectral parameters, as well as to assess the stability of the clusters upon the addition of H_2O_2 at two concentrations (0.5 mM and 5 mM). MT were isolated from the digestive gland of the bivalve mollusc *Unio tumidus* using gel permeation chromatography of a thermostable protein fraction. To obtain apo-MT, the isolated MT-containing fraction was repeatedly eluted on a Sepadex G-50 column using 0.05 M HCl solution, resulting in the isolation of pMT, indicating its stability to HCl. The collected protein was used to reconstruct MT by incubation with freshly prepared 0.6% solutions of Zn, Gd and Y.

After 1 hour of incubation of Zn, Gd and Y with pMT, the spectral intensity at 220 nm and 255 nm decreased in all groups. During the 24 hour incubation, the spectra for all groups were practically identical; however, during this time, Zn significantly altered its spectral

absorption in the 220–230 nm range, which is characteristic of it [2]. No such significant changes were observed for Gd and Y. Following the addition of 0.5 mM H_2O_2 over a period of 1 hour, an increase in spectral absorption at 220 nm and 255 nm was observed in the Zn group. In the Gd group, an increase in spectral absorption at 255 nm and slight fluctuations at 220 nm were also observed. Y, in turn, was characterised by a decrease in absorption at 255 nm. The addition of 5 mM H_2O_2 over a period of 1 hour to the Zn and Gd groups led to an increase in spectral absorption at 255 nm, whereas Y exhibited a significant decrease in absorbance in these regions, indicating Y ability to influence the stability of MT clusters.

Overall, the REEs demonstrated varying effects on the structures of pMT clusters *in vitro*, which may indicate their ability to influence the stability of these complexes. However, whether they can bind directly to MT remains unknown and requires further investigation.

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