PHYSICO-CHEMICAL STUDY OF CADMIUM-METALLOTHIONEIN COMPLEXES ISOLATED FROM CADMIUM EXPOSED MUSSELS (*MYTILUS GALLOPROVINCIALIS*)

Marijana Erk*, Dusica Juric, Sonja Kozar, Jasenka Pavicic and Biserka Raspor

Rudjer Boskovic Institute, Center for Marine and Environmental Research, Zagreb, Croatia

Abstract

The amperometric titrations of the purified metallothionein (MT) chromatographic fractions with cadmium ions were performed in the buffered 0.59 M NaCl solution, pH 7.9 at 25°C. MTs were isolated from digestive glands of the mussels (*Mytilus galloprovincialis*) exposed to cadmium. Different purification procedures of the chromatographic fractions could be the reason for the differences in their metal and MT content. It is perhaps the reason for their different complexing properties for cadmium. The applied differential pulse anodic stripping voltammetry (DPASV) is suitable for physico-chemical characterization of MTs due to its species-selectivity and sensitivity.

Key words: cadmium, complexation, metallothionein, Mytilus galloprovincialis

Metallothioneins are rather small, metal-inducible and metal-binding proteins that are rich in cysteine residues, and are responsible for the metal detoxification and metal homeostasis in living organisms [1,2]. Since the knowledge of the biological functions of MTs, which is based on understanding their molecular and physico-chemical properties, is still not complete, our aim was to contribute to this knowledge by characterizing the metal-binding properties of MT isoforms, induced and isolated from the digestive gland of *M. galloprovincialis* exposed to cadmium, at a concentration level close to the cellular one at which metal interacts with the protein.

Experimental

Adult specimens of *M. galloprovincialis* (5-7 cm, from Lim channel, Northern Adriatic) were exposed for 14 days to 200 μg Cd dm^{-3} (1.8x10^{-6} M Cd^{2+} added as $CdCl_2$) in a continuous flow-through seawater system (S=38‰, 20°C). The biochemical procedure of the isolation and purification of MTs from the composite sample of the digestive glands of cadmium-exposed *M. galloprovincialis* is described elsewhere [3,4].

For further complexation studies the chromatographic fractions which contained MT were finally selected based on their highest cadmium content [4,5]. MT content was determined according to the Brdicka reaction [6,7]. Amperometric titrations of MTs with cadmium were performed at constant temperature (25.0±0.5 °C), ionic strength (0.59 M) and pH (7.9)[8]. Voltammetric measurements in a differential pulse anodic stripping mode (DPASV) were carried out with a μAutolab instrument (Eco Chemie, The Netherlands); the measurement parameters are defined elsewhere[8].

Results and Discussion

Before commencing the amperometric titration, the cadmium [4]. and metallothionein [7]. content was analysed in the selected chromatographic fractions (Table 1). It was found that dithiotreitol (DTT), added as a reducing agent during the MT isolation, competes with MT for Cd^{2+} ions and masks CdMT complex formation [6]. Therefore, DTT must be removed from the MT samples. Preferably, in the MT isolation procedure ß-mercaptoethanol should be used instead.

Table 1.Cadmium and MT content in the selected chromatographic fractions

Purified chromatographic fractions	*c*(Cd) / M	*c*(MT) / M
MT10(IV)	$7.54 \cdot 10^{-8}$	$1.5 \cdot 10^{-6}$
MT10(V)	$5.05 \cdot 10^{-8}$	$3.3 \cdot 10^{-6}$
MT10(27)	$4.34 \cdot 10^{-7}$	$2.1 \cdot 10^{-7}$
MT20(18)	$5.34 \cdot 10^{-7}$	$1.1 \cdot 10^{-7}$
MT10(12)(13)E.P.	$1.08 \cdot 10^{-6}$	not determined

CdMT complexes [9,10]. give the anodic signal (Fig. 1) with the peak potential at $E_p \approx$ -0.66 V. The most positive peak at -0.45 V is assigned to the oxidation of the mercury electrode in the presence of thiol groups complexed with cadmium [11]. (Fig. 1). The fact that during the amperometric titration of MT with $CdCl_2$ solution two distinct anodic signals, one of the CdMT complex and the other of Cd_{ionic} (E_p = -0.60 V; Fig. 1), are observed indicates that under the selected measuring conditions the CdMT complex is electrochemically inert and a reversible type of complex. From the peak heights of CdMT and Cd_{ionic} signals using the modified van den Berg-Ruzic-Lee method[6-8], the complexing capacity (C_L) (Table 2), and the apparent stability constants of CdMT were determined. Normalizing the available MT concentration for complexing cadmium (C_L) with the total (analytical) concentration of metallothionein (*c*(MT)) the number of cadmium ions additionally bound by the MT molecule could be determined. The variations of the Cd[4] and MT[7] concentrations (Table 1) and the stoichiometric ratio (Table 2) in the selected chromatographic fractions, suggest that different purification procedures caused differences in the metal and MT content of the chromatographic fractions and therefore resulted in different complexing properties for cadmium.

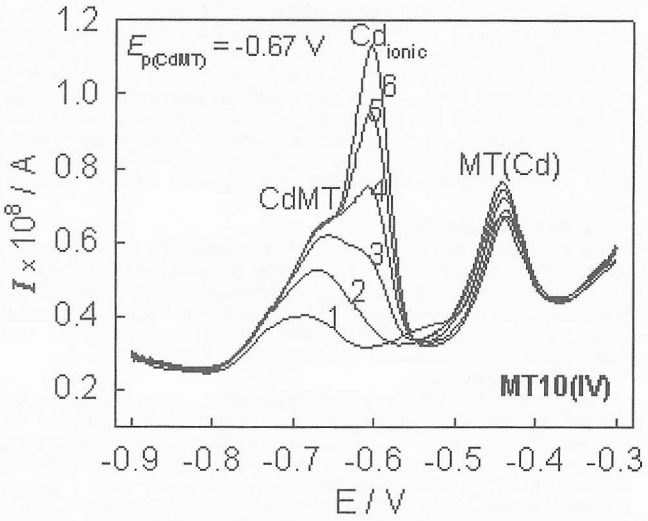

Figure 1 Amperometric titration of MT in 0.59 M NaCl, pH 7.9 with cadmium: to 50 µl MT10(IV)(or $4 \cdot 10^{-9}$ M) Cd^{2+} solution is added in the concentration range from $1.42 \cdot 10^{-9}$ M to $8.52 \cdot 10^{-9}$ M[10].

Table 2.Cadmium complexing capacity (CL) of MT from differently purified chromatographic fractions. The results refer to 0.59 M NaCl, pH 7.9 at (25.0±0.5) °C

Purified chromatographic fractions	*c*(MT)* / M	C_L / M	C_L / *c*(MT)
MT10(IV)	$4.0 \cdot 10^{-9}$	$4.0 \cdot 10^{-9}$	1.0
MT10(V)	$8.0 \cdot 10^{-9}$	$1.0 \cdot 10^{-8}$	1.3
MT10(27)	$5.8 \cdot 10^{-10}$	$1.0 \cdot 10^{-9}$	1.7
MT20(18)	$4.3 \cdot 10^{-10}$	$5.7 \cdot 10^{-10}$	1.3
MT10(12)(13)E.P.	$1.3 \cdot 10^{-8}$	$7.0 \cdot 10^{-9}$	0.5
MT20(17)(18)E.P.	$4.2 \cdot 10^{-9}$	$1.4 \cdot 10^{-8}$	3.3

** aliquot of c(MT) from Table 1 diluted in 20 ml supporting electrolyte*

Acknowledgments. The financial support of the Ministry of Science and Technology of the Republic of Croatia, projects No. 00981511 and No.098431, is gratefully acknowledged.

References

1.Binz P.A and Kägi J.H.R., 1999. Metallotionein: Molecular evolution and classification. In: Klaasen, C.D. (editor) Metallothionein IV. Birkhäuser Verlag, Basel:7-13.

2.Roesijadi G., 1996. Metallotionein and its role in toxic metal regulation. *Comp. Biochem. Physiol.*, 113C: 117-123.

3.Pavicic J., Skreblin M., Raspor B., Branica M., Tusek-Znidaric M., Kregar I. and Stegnar P., 1987. Metal pollution assessment of the marine environment by determination of metal-binding proteins in *Mytilus galloprovincialis*. *Mar. Chem.*, 22: 235-248.

4.Juric D., 1998. Cadmium-induced synthesis of metallothionein isoforms in the digestive gland of the mussel *Mytilus galloprovinsialis*. M.Sc. Thesis, University of Zagreb, pp. 88 (in Croatian).

5.Raspor B., Kozar S., Pavicic J. and Juric D., 1998. Determination of the cadmium and copper content inherent to metallothionein. *Fresenius J. Anal. Chem.*, 361:197-200.

6.Erk M. and Raspor B., 2000. Advantages and disadvantages of voltammetric method in studying cadmium-metallothionein interactions. *Cell. Mol. Biol.*, 46: 269-281.

7.Erk M., 2000. Study of chemical reactivity of metallothioneins using electrochemical techniques. Ph.D.Thesis, University of Zagreb, pp. 107 (in Croatian).

8.Erk M. and Raspor B., 1998. Evaluation of cadmium-metallothionein stability constants based on voltammetric measurements. *Anal. Chim. Acta*, 360: 189-194.

9.Raspor B and Pavicic J., 1996. Electrochemical methods for quantification and characterization of metallotioneins induced in *Mytilus galloprovincialis*. *Fresenius J. Anal. Chem.*, 354: 529-534.

10.Erk M. and Raspor B., 1999. Electrochemical study on Cd binding to metallotioneins isolated from the mussel, *Mytilus galloprovincialis*. *J. Electroanal. Chem.*, 466: 75-81.

11.Mendieta J., Chivot J., Muñoz A. and RodrÍguez A.R., 1995. Electrochemical behavior of metallothioneins and related molecules. Part I: Lys-Cys-Thr-Cys-Cys-Ala thionein fragment [56-61] MT I. *Electroanalysis*, 7: 663-669.