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The adaptation of mussels *Crenomytilus grayanus* to cadmium accumulation result in alterations in organization of microsomal enzyme–membrane complex (non-specific phosphatase)

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Abstract

The kinetic parameters (V_m, K_m and slope) of membrane-bound microsomal non-specific phosphatase (NPase, with G6P as the substrate) from the digestive gland of unexposed and cadmium adapted (45 days for 100 μg Cd^{2+}/l) mussels were investigated. In vivo and in vitro approaches were used. Adaptation of mussels (*Crenomytilus grayanus*) to cadmium resulted in a 1.6-fold increase in NPase activity. V_m was increased by 1.6-fold, but K_m was the same in terms of enzyme kinetics. This indicates that the total concentration of the enzymes in the digestive gland increased. Cd^{2+} (1 mM) did not significantly alter the activity of the membrane-bound enzyme in vitro both for unexposed and for cadmium adapted mussels, meaning that cadmium ions are not a direct inhibitor of the membrane-bound enzyme in this concentration. The microsomal NPase activity in both unexposed and cadmium adapted mussels was inhibited by in vitro solubilization of microsomes with non-ionic detergent (Triton X100, 0.01%). This inhibition was uncompetitive for microsomes of unexposed mussels (K_m decreased 3.1-fold). The most drastic events were observed in cadmium adapted mussels, where inhibition was mixed (K_m decreased 7.2-fold). The simultaneous actions of detergent and cadmium ions did not alter NPase activity significantly in comparison with action of the detergent alone. The differences in the types and the extents of inhibition of the enzymes activity by membrane disordering agent (Triton X100) indicated that the enzyme–membrane complex (NPase) has been altered as a result of adaptation of mussels to cadmium accumulation. We conclude that the mussels produced a new enzyme–membrane complex, with the same K_m as the previous complex, but with other detergent sensitivity and greater amounts. Thus, the adaptation capacity of this enzyme is reduced as result of adaptation of mussels to cadmium accumulation.

Keywords: In vivo; In vitro; Non-specific phosphatase; Membrane-bound enzyme; Cadmium accumulation; Bivalve mollusc; Mechanism of adaptation

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1. Introduction

Membrane-bound enzymes are suitable models to study some mechanisms of cadmium adaptation, because, changes in their kinetics indicate fine changes in membrane–enzyme relationships. On the other hand, general cell processes (energy turnover, biosynthesis etc.) are based on membrane-bound enzymes as well. In order to study the mechanism of adaptation to cadmium accumulation in detail according to this model, scientists have to use two experimental approaches (in vivo and in vitro) simultaneously to distinguish non-specific compensatory reactions of the energy metabolism to chronic toxicity. For example, Ros et al. (1992) has revealed that Mg^{2+}-ATPase from rice shoots and roots showed the increase of activity during cadmium accumulation in vivo, but in the same time an in vitro experiment resulted in the inhibition of enzyme by cadmium directly. Other general drawbacks of some articles is that authors discuss changes in common enzyme activity, but not in enzyme kinetics, although analysis of kinetic parameters can give a lot of additional information.

There are two possibilities for Cd^{2+} to alter the enzyme activity for membrane-bound enzymes: directly and by means of disturbing the membrane surrounding.

Membrane structures participate in the process of cadmium accumulation and detoxication (at least as the border of cell compartments). Primarily Cd^{2+} accumulates in the cytosol (Belcheva, 1988), as metal–ligand complexes (as a rule, as glutathione or metallothionein) which are then dislocated into membrane structures (Kunimoto and Miura, 1988) for depositing Cd^{2+} accumulation in vivo results in the intensification of lipid peroxidation (LP) (Bompart et al., 1991; Pal et al., 1993). Although cadmium is not a direct inductor of LP (Chelomin and Belcheva, 1992), it displays its action by suppression of the antioxidant system. The amount of reduced glutathione and tocopherol decreases in cells (Hussain et al., 1985; Bompart et al., 1991; Chelomin and Belcheva, 1992; Strubelt et al., 1996), and the activity of some enzymes of antioxidant system (superoxide dismutase, catalase, glutathione peroxidase) and reductase activity are decreased by the influence of cadmium as well (Bompart et al., 1991; Strubelt et al., 1996). Cadmium accumulation results in a decrease of the content of important membranous component such as cholesterol (Katti and Sathyaneasan, 1984). Cadmium accumulation also disrupts membranogenes: the rate of phospholipids exchange decreases (Belcheva and Chelomin, 1988), the total amount of phospholipids decreases (Hussain et al., 1985), and the lipid composition and ratio of phospholipid classes changes (Gulati et al., 1986). Fatty acid composition of membranous phospholipids (Donaldson, 1985) and speed of turnover of many fatty acids (Chelomin and Belcheva, 1991) are changed due to inhibition of fatty acid biosynthesis as a result of cadmium accumulation (Steibert and Kokot, 1980). Protein/phospholipid ratio in the membrane fraction increases in erythrocytes incubated with Cd^{2+} (Kunimoto and Miura, 1988). Experiments on artificial phospholipid bilayer membranes have shown that the heavy metal ions binds to phospholipids of the membrane (Omelchenko, 1991), and it can cause a lateral phase division directly in a membrane (Sasaki et al., 1995; Maloney et al., 1996). Cd^{2+} has an impact on microviscosity of membranes and makes them rigid (Mayfield and Munawar, 1983; Boadi et al., 1992; Chelomin and Tyurin, 1992). All these preconditions indicate that cadmium accumulation have influences on membrane functioning.

The presented research was carried out on the hepatopancreas (digestive gland), which is the main organ that deposits cadmium in mussel body (Chelomin et al., 1995). For our study we have chosen non-specific phosphatase (NPase), which locates in the endoplasmic reticulum and displays activity in the microsomal fraction of cell (Viarengo et al., 1986). This enzyme functions as glucose-6-phosphatase (G6Pase) in this cell compartment, because G6Pase is absent in mussel digestive glands (Viarengo et al., 1986), and it is membrane-bound enzyme as well as

G6Pase (Viarengo et al., 1986). Thus, we determined common non-specific phosphatase activity with glucose-6-phosphate as substrate.

The aim of this research was to study some features of adaptation of the mussel's microsomal enzyme–membrane complex to cadmium accumulation.

2. Materials and methods

2.1. Animals

The experimental research was carried out on adult mussels *Crenomytilus grayanus* (Dunker) on adult mussels, of 8–12 cm total length, collected from Russkii and Popov islands (Peter the Great Bay, Sea of Japan, Russia) in March. After a 2-week acclimation to aquarium conditions, mussels were divided into two groups (each of 15 animals). The first group was used as a control and it was called 'unexposed mussels'. The second group was exposed during 1.5 months (45 days, March–April) to a cadmium concentration 100 μg Cd^{2+}/l, added as $CdCl_2$ salt. We called this group 'cadmium adapted mussels'. Water in the 140-l aquaria was well-aerated (90–100% oxygen saturation) and had a constant salinity (34‰) during the experiment. The photoperiod and temperature regime (4–12°C) was in accordance with the time of the season. Water was changed every 2 days with fresh unfiltered seawater; therefore the mussels were not fed specially. Cadmium was added every time after the water was changed.

2.2. Isolation of microsomes

Mussels were dissected on the ice and pieces of digestive gland (1–2 g) were homogenized with ice-cold Tris Buffer Solution with sucrose (TBS-sucrose: 0.05 M Tris–HCl, 0.25 M sucrose, 0.5 M NaCl, pH 7.5). Differential centrifugation (at + 4°C) with the Ca^{2+}-method was used for the isolation of a microsomal fraction (Kamath and Narayan, 1972). After precipitation of the cell/nuclear fraction (5×10^3 g for 15 min) and the mitochondrial fraction (1×10^4 g for 20 min), the supernatant was diluted 5 times with ice-cold TBS-Ca^{2+} (0.05 M Tris–HCl, 0.5 M NaCl, pH 7.5, 40 mM $CaCl_2$). The diluted supernatant was kept in a refrigerator for 15 min and then the microsomes were precipitated (1×10^4 g for 15 min). The pellets were suspended in ice-cold TBS-EDTA (0.05 M Tris–HCl, 5 mM EDTA, 0.5 M NaCl, pH 7.5). The surplus of Ca^{2+} was released by double precipitation (1×10^4 g for 15 min) of the microsomes in TBS-EDTA. A final pellet of microsomes was suspended in 3 ml of ice-cold TBS (0.05 M Tris–HCl, 0.5 M NaCl, pH 6.5) and stored in a refrigerator on ice (+4°C) not more than a day.

2.3. Protein assay

Protein content in the microsomal fraction was determined by the bromphenol dye (Greenberg and Gaddok, 1982) with Triton X100 as detergent. Calibration was carried out with bovine serum albumin.

2.4. Enzyme assay

The activity of non-specific phosphatase (NPase) was determined by measuring of liberated inorganic orthophosphate (P_i) (Schulze et al., 1986), formed as a result of the enzymatic cleavage of the substrate glucose-6-phosphate disodium salt (5 mM ÷ 20 mM). The final volume of the incubation medium was 2 ml in TBS (pH 6.5). The enzymatic reaction was started by adding the microsomal suspension (100 μg of microsomal proteins) and was developed under 37°C. Adding cooled $HClO_4$ up to a final concentration of 8%, stopped the enzymatic reaction after 90 min. The incubation medium was centrifuged for 5 min at 1×10^4 g for precipitation of the denatured protein. The amount of liberated inorganic orthophosphate was measured in the obtained supernatant by Murphy's method (Murphy and Riley, 1962). Enzyme activity was expressed as μg P_i produced per h by 1 mg of microsomal proteins (μg P_i/h per mg microsomal proteins).

2.5. Determination of the K_m and V_m

The main kinetic parameters (V_m, maximum speed of enzyme reaction and K_m, apparent Michaelis constant; slope, ratio K_m/V_m) of enzyme activity were calculated from the enzyme activity under different concentrations of the substrate (G6P, 5 ÷ 20 mM) by the method of Lineweaver–Burk.

2.6. In vitro effects of detergent and Cd^{2+} on NPase activity

The in vitro effects of non-ionic detergent (0.01% Triton X100) and cadmium ions (1 mM Cd^{2+}) on the activity of NPase were evaluated by means of changing of K_m, V_m, and slope of the equations.

2.7. Statistics

Measurements of kinetic parameters were carried out for each individual mussel, but afterwards all data were merged in one database. A test of data distribution was done by Kolmogorov–Smirnov procedure. K_m and V_m were calculated as constants for mentioned data set. The significance of differences of two regression lines $y_1 = a_1 + b_1x$ and $y_2 = a_2 + b_2x$) was evaluated by following method:

$$S_g^2 = \frac{\left(\sum(y_{1i} - Y_{1i})^2\right) + \left(\sum(y_{2i} - Y_{2i})^2\right)}{m_1 + m_2 - 4}$$

$$S_d^2 = S_g^2\left[\frac{1}{\sum(x_{1i} - \bar{x}_1)^2} + \frac{1}{\sum(x_{2i} - \bar{x}_2)^2}\right]$$

$$t = \frac{|b_1 - b_2|}{S_d}$$

The differences are significant if $t \geq t_{st}$ ($f = m_1 + m_2 - 4$; $P = 0.95$) (Doerffeld, 1990). Where: m, number of items in a group; f, degree of freedom; t, calculated t-Student's coefficient; t_{st}, standard t-Student's coefficient; Y_{ji}, predicted Y_i for j-group; $\bar{x}_j$, average x for j-group; S_d^2, dispersion of $|b_1 - b_2|$; S_g^2, general dispersion of y_1 and y_2.

3. Results

As a result of the mussels' adaptation to cadmium accumulation, the activity of their NPase increased 1.6-fold. In terms of enzyme kinetics, V_m was increased 1.6-fold, from 49.5 μg P_i/h per mg microsomal proteins in unexposed mussels up to 80.64 μg P_i/h per mg microsomal proteins in cadmium adapted mussels. But K_m remained approximately the same, 32.39 mM in unexposed mussels and 35.95 mM in cadmium adapted mussels (Table 1).

Cd^{2+} (1 mM) did not significantly alter the activity of the membrane-bound enzyme in vitro for both unexposed and cadmium adapted mussels (Table 1).

The microsomal NPase activity of both unexposed and cadmium adapted mussels was altered significantly by in vitro solubilization of microsomes with a non-ionic detergent (Triton X100, 0.01%) (Table 1, Figs. 1 and 2). From the analysis of the Lineweaver–Burk plot we can conclude that uncompetitive inhibition occurred in the microsomes of unexposed mussels (Fig. 1), and mixed inhibition in the microsomes of cadmium adapted mussels (Fig. 2). The most drastic events were observed in cadmium adapted mussels, the sensitivity of the enzyme to the membrane-disordering agent (Triton X100) was very high: K_m decreased 7.1-fold, in comparison with 3.1-fold for unexposed mussels (Table 1).

The simultaneous action of detergent and cadmium ions did not alter NPase activity significantly in comparison with the action of detergent alone for both groups of mussels (Table 1).

4. Discussion

This study was carried out on hepatopancreas (digestive gland), which is the main organ that deposits cadmium in mussel *C. grayanus*. The conditions of treatment was the same as in a study of Chelomin et al. (1995), therefore we did not determine the level of cadmium accumulation in this study.

Cadmium accumulation in vivo results in its different effects on enzyme activities. For in-

Table 1
The results of kinetic analysis of NPase from microsomes of digestive gland of unexposed and cadmium adapted mussels (45 days, 100 μg Cd^{2+}/l) *C. grayanus*

[G6P] mM	Velocity of reaction (μg P_i/h per mg microsomal proteins)				Equation	V_m[e] (μg P_i/h per mg microsomal proteins)	K_m[f] (mM G6P)	Slope[g]
	5	10	15	20				
Unexposed mussels ($n = 15$)								
Control[a]	6.568 ± 0.211	12.344 ± 2.175	14.017 ± 1.625	19.936 ± 3.300	$y = 0.0202 + 0.6543x$	49.50	32.39	0.65
+Cd[b]	7.667 ± 1.027	11.793 ± 2.024	17.713 ± 1.489	20.861 ± 2.487	$y = 0.0199 + 0.6227x$	50.25	31.29	0.62
+D[c]	5.554 ± 1.241	7.389 ± 4.975	11.358 ± 1.874	14.457 ± 4.975	$y = 0.0716 + 0.7433x$*	13.96	10.38	0.74
+D+Cd[d]	6.383 ± 1.318	7.336 ± 5.086	13.246 ± 2.383	12.875 ± 3.302	$y = 0.0978 + 0,6047x$*	10.22	6.18	0.60
Cadmium adapted mussels ($n = 15$)								
Control	11.053 ± 2.030	15.466 ± 2.415	27.036 ± 1.254	42.375 ± 1.751	$y = 0.0124 + 0.4459x$	80.64	35.95	0.45
+Cd	12.224 ± 2.834	17.347 ± 2.807	26.418 ± 1.157	41.804 ± 4.450	$y = 0.0107 + 0.4147x$	93.45	38.75	0.41
+D	15.682 ± 0.662	16.455 ± 3.508	19.316 ± 1.694	33.420 ± 2.732	$y = 0.0349 + 0.1741x$*	28.65	4.98	0.17
+D+Cd	14.090 ± 0.570	16.488 ± 3.095	21.482 ± 2.131	31.588 ± 2.727	$y = 0.0291 + 0.2396x$*	34.36	8.23	0.24

[a] Control, control activity of NPase in vitro.
[b] +Cd, NPase activity in vitro presence 1 mM Cd^{2+};
[c] +D, NPase activity in vitro presence detergent 0.01% Triton X100.
[d] +D+Cd, NPase activity in vitro presence both cadmium and detergent;
[e] V_m, maximum speed of enzymatic reaction (μg P_i/h per mg microsomal proteins).
[f] K_m, apparent Michaelis constant (mM G6P);
[g] Slope, the ratio of K_m to V_m; value are reported as a mean ± standard deviation.
* The differences are significant ($P = 0.05$); n, number of animals.

stance, benz(a)perene hydroxilase activity from the liver of industrial water fish was higher as consequence of the Cd^{2+} content in these waters (Ueng et al., 1996). Block et al. (1992) has registered increasing proteinkinase C activity in response to influence of Cd^{2+} in vivo. Cd^{2+} activated in vivo *p*-nitroanisole *O*-demethylase (*p*-NAOD), the enzyme of monooxygenase system (Iscan et al., 1993). However, cadmium exposure in vivo inhibits the activity of a considerably larger number of membrane-bound enzymes. For example, Cd^{2+} induct decreases in cytochrom P_{450} activity in experiments in vivo (Bompart et al., 1991; Rosenberg and Kappas, 1991; Iscan et al., 1992; Stagg et al., 1992; Iscan, et al., 1993), cytochrom b_5 (Iscan et al., 1992, 1993), and cytochrom *c* oxydase (Rao et al., 1993), all of which are parts oxygenase system. This implies that

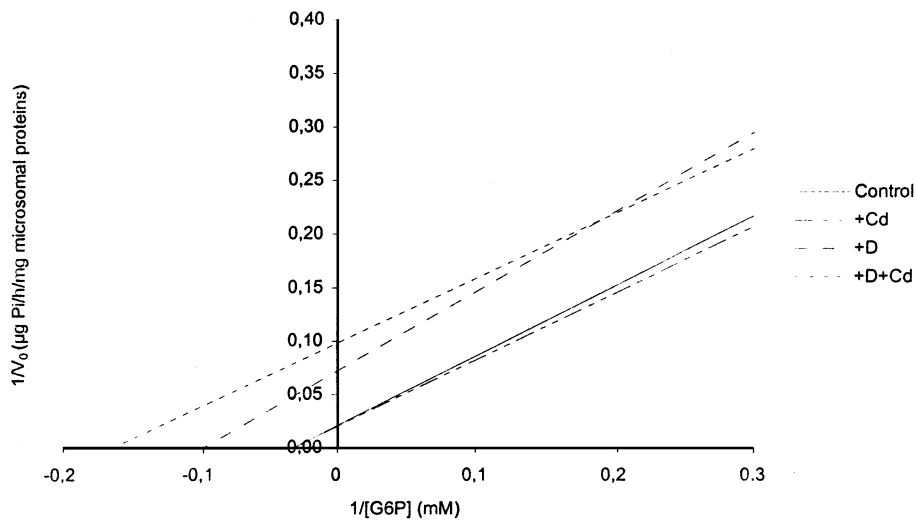

Fig. 1. The Lineweaver–Burk plot for NPase from microsomes of the digestive gland of unexposed mussels *C. grayanus*. V_o, velocity of enzymatic reaction, the rest conventional sign as in Table 1.

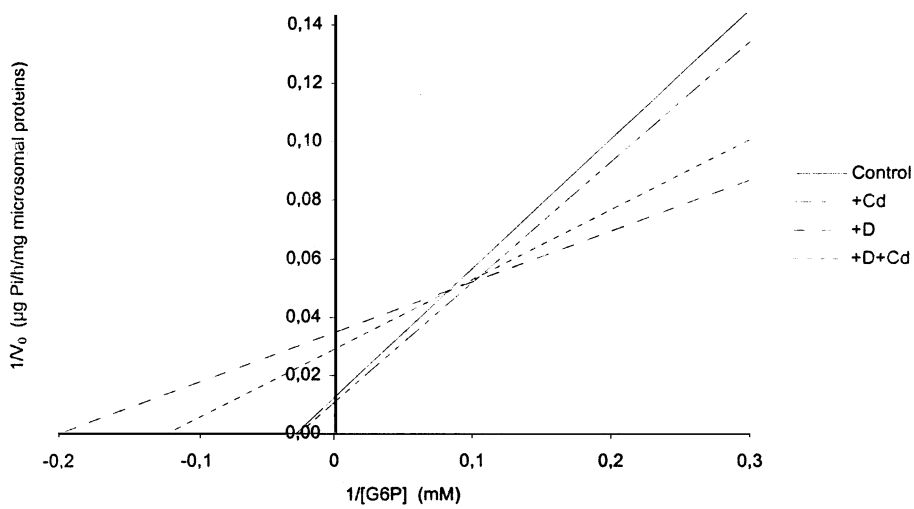

Fig. 2. The Lineweaver–Burk plot for NPase from microsomes of the digestive gland of cadmium adapted mussels (45 days for 100 µg Cd^{2+}/l) *C. grayanus*. V_o, velocity of enzymatic reaction, the rest conventional sign as in Table 1.

administration of Cd^{2+} in vivo reduces the biotransformation ability of the organism. There is work (Ueng et al., 1996) which shows that the influence of Cd^{2+} in vivo in model conditions has no effect to enzyme activity of the momooxygenase system (cytochrom P_{450}, NADPH-cytochrom P_{450}-reductase, benz(a)perene hydroxylase, 7-ethoxyresorufin *O*-deethylase; 7-ethoxycoumarine *O*-deehylase), unless the protective metallothionein response is significantly developed in the system. Also in vivo Cd^{2+} inhibits Na^+, K^+-ATPase activity (Gupta and Chandra, 1991; Pal et al., 1993), and reduces Na^+-dependent transport of D-galactose (Mesonero et al., 1993). It shows that Cd^{2+} is capable to reduce active transport through a membrane. In experiments in vivo it was established that influence of Cd^{2+} results in inhibition of acetylcholinesterase, 5′-nucleotidase, CNPase and succinate dehydrogenase (Gupta and Chandra, 1991; Pal et al., 1993), causing its neurotoxic effect.

Experiments that were only conducted in vivo do not clarify the physiological mechanism of adaptation to cadmium accumulation. As we have seen above, cadmium exposure in vivo can either induce or depress some enzyme systems. The influence will depend on many circumstances, which have to be examined in each individual case. It means that cadmium does not have obvious depressing influence on any enzyme system. Sometimes, cadmium toxicity or adaptation to cadmium accumulation induces a compensatory response rather then a direct effect of cadmium ions to the enzyme activities. Thus, there is the necessity to emphasize that compensatory reactions of cell can play a certain role in the detoxification answer under long-term exposure in vivo.

There are few studies in which the effect of cadmium on membrane-bound enzymes was evaluated in vivo and in vitro simultaneously. For example, Ros et al. (1992) has revealed that during cadmium accumulation Mg^{2+}-ATPase from rice shoots and roots showed the increasing of activity in vivo, but in the same time an in vitro experiment resulted in the inhibition of enzyme by cadmium directly. Another study (Canesi et al., 1998) on non-membrane-bound enzyme hexokinase shows that heavy metals can inactivate this enzyme both in vivo and in vitro. Such studies can reveal more information about the nature of cadmium effect on enzymes, but they still drawback the kinetic characteristics of enzyme activity.

In our experiment we have found out that cadmium exposure in vivo increases V_m of microsomal NPase in mussels digestive gland (Table 1). Earlier, such a phenomenon was found (Chapatwala et al., 1980) in liver and kidneys of rats exposed to cadmium. In another study (Soengas et al., 1996) it has been established that the exposure to Cd^{2+} in vivo resulted in increased activity of glycogen phosphorylase, and 6-phosphofructo-1-kinase, increased levels of glucose and lactate in blood serum, and a decrease of glycogen synthetase activity. It means that glycogenolysis and glycolysis processes are activated, which may be explained by the increase of cortisol levels in blood serum, or as a secondary stress answer on a disrupted energy exchange in the liver. Since these studies did not make a kinetic analysis of enzyme activity, there is some problem to make an exact conclusion about the nature of activation. Our data presented in terms of enzyme kinetics indicate that V_m was higher in 1.6-fold, but K_m was the same. This shows that cadmium accumulation induces an increase of the total concentration of this enzyme in the hepatopancreas.

A lot of literature data shows that cadmium ions in vitro alter the activity of membrane-bound enzymes. For instance, Cd^{2+} inhibits the work of cytochrom P_{450} (Chetty et al., 1992), Na^+, K^+-ATPase (Lijnen et al., 1991; Chetty et al., 1992; Kinnesaffran et al., 1993; Carfagna et al., 1996), (Na^+, K^+)-Mg^{2+}-ATPase (Pedrenho et al., 1996), Ca^{2+}-ATPase (Hechtenberg and Beyersmann, 1991; Jeanne et al., 1993; Viarengo et al., 1993; Visser et al., 1993), Mg^{2+}-ATPase (Carfagna et al., 1996) and basal ATPase activity (Hypponen et al., 1993). These data show that in vitro Cd^{2+} can disrupt the activity of transport ATPases and the activity of the oxygenase system. In vitro cadmium inhibits the transport of citrate through a membrane (Sato et al., 1995), Na^+-dependent transport of D-glucose (Bevan and Foulkes, 1989), L-alanine (Kim et al., 1990) and Na^+-dependent L-glutamate uptake (Lee et al., 1990; Kinne et al., 1995). Mesonero et al. (1994)

shows that Cd^{2+} in vitro impairs Na^+-dependent active transport of L-threonine through the epithelium membrane, by means of inhibition of the Na^+, K^+-ATPase, but it does not interfere with simple diffusion of this amino acid. In other studies Mesonero and coworkers (Mesonero et al., 1995, 1996) show that Cd^{2+} in vitro considerably reduces absorption of D-galactose on membranes and reduces transport of L-threonine. It was also shown that Cd^{2+} and Ca^{2+} have the same affinity to identical chemical groups, which are participating in the absorption of D-galactose and transportation of L-threonine. Thus, Cd^{2+} in vitro is capable to inhibit active transport of metabolites through a membrane. Cd^{2+} in vitro (1–100 μM) is capable to activate Ca^{2+}-dependent K^+ channels (Vijverberg et al., 1994). Apparently, Cd^{2+} cations are capable to replace Ca^{2+} cations in order to activate Ca^{2+}-dependent K^+ channels. However, in high concentrations, Cd^{2+} displays blocking properties, reducing the frequency of channels opening and of the signal amplitude. Cadmium ions in vitro inhibit the work of C-proteinase (Hojima et al., 1994), glucose-6-phosphatedehydrogenase (Boadi et al., 1992) and succinate dehydrogenase (Jay et al., 1991) as well. We make no pretense of full reviews of the effects of cadmium ions in vitro on membrane-bound enzymes. Anyway we can conclude from this mini-review that cadmium ions can disturb the activity of membrane-bound enzymes, which work with ions as a substrate or fulfil ATP- or ion-dependent transport metabolites through the membrane. Maybe, therefore we could not clearly see the direct effect of cadmium ions to the activity of NPase. The statistical analysis of NPase activity has shown that cadmium ions (in this concentration) in vitro did not change significantly the kinetic parameters of the enzyme from unexposed and cadmium adapted mussels.

The microsomal NPase activity in both unexposed and cadmium adapted mussels was inhibited by in vitro solubilization of microsomes with a non-ionic detergent (Triton X100, 0.01%). The detergent was used because the 'target' of its effect is the lipid matrix of membrane. NPase is a membrane-bound enzyme and the structure of the membrane surroundings (specific lipids, microviscosity, etc.) regulate its activity in a similar way as for G6Pase (Duttera et al., 1968; Zakim, 1970; Mithieux et al., 1998). For microsomes of unexposed mussels, uncompetitive inhibition was observed, because V_m and K_m were decreased approximately to the same extent and the slope does not change seriously. For cadmium adapted mussels, mixed inhibition was observed, V_m and K_m were changed in a different manner and more seriously. In terms of enzyme kinetics, K_m was lower. It means that the affinity of the enzyme to the substrate in cadmium adapted mussels was higher in the presence of the membrane disordering agent. Therefore the enzyme will work with full capacity in the physiological range of the substrate (Hochachka and Somero, 1984) under these conditions. Different types of alterations by detergent in unexposed and cadmium adapted mussels show that some changes of membrane–enzyme complex occurred during adaptation to cadmium accumulation. These changes are not beneficial for mussels, because the adaptation capacity of this enzyme in cadmium adapted mussels is reduced.

The simultaneous actions of detergent and cadmium ions did not alter NPase activity significantly in comparison with the effect of detergent alone. Probably due to the same reasons which were mentioned above.

Unfortunately, we did not have enough material in order to do isozymes assay and determine K_i of the detergent, but we are sure it will be the next step of our work.

5. Conclusions

Thus, we can assume that cadmium accumulation is an indirect inductor of the increase in NPase concentration in the microsomal fraction of digestive glands of mussels. Cadmium ions in vitro do not significantly alter affinity of the enzyme to the substrate in unexposed or cadmium-adapted mussels. Differences in the types and in the extent of inhibition of enzyme activity by the membrane disordering agent (Triton X100); in the slope of the equations between unexposed and cadmium adapted mussels show that the enzyme–

membrane complex (NPase) has been altered as a result of adaptation to cadmium accumulation. Therefore, we can assume that the mussels produced a new enzyme–membrane complex with the same K_m, but with other chemical properties (detergent sensitivity, for instance) in greater amounts, as result of the adaptation to cadmium accumulation. These changes are not beneficial for mussels, since they reduce the adaptation capacity of this enzyme system.

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