EFFECT OF CADMIUM ON ALKALINE PHOSPHATASE ACTIVITY IN RENAL BRUSH BORDER MEMBRANES ISOLATED FROM SQUALUS ACANTHIAS

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The alkaline phosphatase found in high concentrations in renal and intestinal brush border membranes is an integral membrane protein supposedly involved in the dephosphorylation of proteins phosphorylated during the regulation of their activity by cAMP or cGMP [R. Kinne et al., J. Membrane Biol. 24: 145-159, 1975]. The enzyme is known to be activated by zinc ions binding to a catalytic and/or allosteric site of the protein [K.-D. Gerbitz, Hoppe-Seyler's Z. Physiol. Chem. 358: 1491-1497, 1977]. Due to the importance of the enzyme in regulatory processes and the chemical similarity between zinc and cadmium we investigated the effect of cadmium on the activity of alkaline phosphatase and possible interactions between the two divalent cations.

Brush border membranes were isolated from the kidney of male or female dogfish (Squalus acanthias) by differential precipitation using calcium as described previously [C. Bevan et al., J. Comp. Physiol. B 159: 339-347, 1989]. Enzyme activity was determined by measuring the amount of p-nitrophenol released during a 15 min incubation at 25°C in glycine buffer (pH 10.5) [ibid.].

Isolated renal brush border membranes showed a specific activity of alkaline phosphatase of 34 ± 0.3 μmoles/h x mg protein ($\bar{x}$ ± SD, n = 4). In the presence of 0.1 mM $ZnCl_2$ in the assay medium, $CdCl_2$ (2×10^{-6} M - 1×10^{-3} M) increasingly inhibited the enzyme activity. The first significant inhibition was observed with 2×10^{-5} M, maximal inhibition was 63 ± 2% at 1×10^{-3} M $CdCl_2$ (see Table 1). From these experiments an IC_{50} of 2×10^{-4} M could be estimated. In the absence of $ZnCl_2$ in the assay medium activity dropped to 41.5% of the activity in the presence of $ZnCl_2$. Under these experimental conditions $CdCl_2$ lost almost completely its inhibitory potency. Even at 1×10^{-3} M $CdCl_2$ only a reduction of 10% was observed. These data demonstrate that the enzyme requires the presence of Zn^{++} for optimum activity. The interaction between free Zn^{++} in the assay medium and the alkaline phosphatase is impaired by Cd^{++}. Therefore, an inhibition is observed in the presence of Zn^{++} but not in its absence. In order to further elucidate the mode of action of cadmium, the dose response curve for cadmium inhibition was determined at different zinc concentrations.

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Table 1
Effect of cadmium on alkaline phosphatase activity (as % of control) in brush border membranes isolated from dogfish kidney in the presence and absence of zinc in the assay medium

Cd [M]	0.1 mM Zn^{2+}	no Zn^{2+}
none	100.0	100.0
1×10^{-3}	37.2 ± 1.48*	92.2 ± 0.84
5×10^{-4}	41.1 ± 2.19*	88.9 ± 2.72
2×10^{-4}	50.7 ± 1.43*	91.1 ± 0.77
5×10^{-5}	74.4 ± 0.98*	89.2 ± 1.12
2×10^{-5}	87.3 ± 0.93*	88.4 ± 1.89
5×10^{-6}	100.1 ± 0.64	94.0 ± 0.84
2×10^{-6}	104.5 ± 1.08	97.6 ± 1.05

Enzyme activity was measured at 25°C in lyophilized renal brush border membranes in an assay medium containing 100 mM glycine, 1 mM $MgSO_4$, 5 mM p-nitrophenylphosphate, pH 10.5, and in the presence of the indicated nominal cadmium concentrations. Enzyme activity is expressed as percentage of the enzyme activity measured in the absence of cadmium. Nominal cadmium concentrations have been calculated according to the amount of cadmium added to the assay media. No preincubation of the membranes was performed. In the absence of cadmium average enzyme activity amounted to 34.43 ± 0.35 µmoles/h·mg protein when 0.1 mM zinc was added to the media, and to 14.29 ± 0.42 µmoles/h·mg protein when no zinc was added to the media. Mean values ± SD from 4 experiments performed in triplicates are given (*$p < 0.01$).

Increasing zinc from 0.1 mM to 5 mM decreased the inhibitory potency of 1×10^{-3} M cadmium from 63% to 13%, thus zinc exerted a strongly protective effect on the enzyme (see Table 2). Preliminary data suggest a competition between zinc and cadmium for the binding site at the enzyme with an about equal affinity. The zinc enzyme complex is biologically active, the cadmium enzyme complex is biologically inactive.

These data stronly suggest that a number of metalloenzymes containing Zn^{++} may be potential targets in cadmium toxicity. Such enzymes include superoxide oxidoreductase, pyridoxal kinase, dipeptidases, carboxypeptidases, carbonic anhydrase, and DNA polymerase. In addition, as already suggested from studies on the role of metallothionein in cadmium toxicity [X.Y. Liu et al., Toxicol. Appl. Pharmacol. 114: 239-245, 1992] the amount of the tracer metal zinc available to the organism can strongly modify the toxic effects of cadmium.

Table 2
Effect of zinc on cadmium-induced inhibition of alkaline phosphatase activity (as % of control) in brush border membranes isolated from dogfish kidney

	Zn^{2+}		
Cd [M]	0.1 mM	1.0 mM	5.0 mM
1×10^{-3}	37.4 ± 1.47	64.8 ± 1.70	87.4 ± 1.81

Alkaline phosphatase activity was determined in lyophilized renal brush border membranes in the presence of varying concentrations of zinc at a nominal cadmium concentration of 1×10^{-3} M. No preincubation of the membranes was performed. Enzyme activity is expressed as percentage of the enzyme activity in the absence of cadmium. Average enzyme activity in the presence of 0.1 mM zinc amounted to 34.43 ± 0.35 μmoles/h·mg protein; in the presence of 1.0 mM zinc to 30.91 ± 0.64 μmoles/h·mg protein; and in the presence of 5.0 mM to 32.20 ± 0.71 μmoles/h·mg protein. Mean values ± SD from 4 experiments performed in triplicates are given ($p < 0.05$).

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