Journal of Enzyme Inhibition and Medicinal Chemistry, February 2006; 21(1): 119–123

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Inhibition of rat liver and kidney arginase by cadmium ion

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(Received 22 August 2005; in final form 27 October 2005)

Abstract

Cadmium ion activates arginase from many species of organisms but is an inhibitor of arginase from many other species. The purpose of this study was to investigate the inhibition of rat liver and kidney arginase by cadmium ion. Rat kidney arginase was inhibited by much lower concentrations of cadmium ion than rat liver arginase. Cadmium ion was a mixed noncompetitive inhibitor of both rat liver and kidney arginase. Cadmium ion enhanced the substrate activation of rat kidney arginase while still inhibiting the enzyme. Cadmium ion prevented the substrate inhibition of rat kidney arginase by fluoride while still inhibiting the enzyme. Cadmium ion also inhibited rat kidney arginase in the presence of manganese ion.

Keywords: *Arginase, cadmium, inhibition, rat liver, rat kidney*

Introduction

Cadmium is a toxic heavy metal environmental pollutant. Cadmium binds to thiol groups on proteins and can displace metal ion cofactors required by proteins [1]. Cadmium may also bind to histidine and aspartate residues on proteins [2]. Cadmium is an inhibitor of many enzymes such as rat brain synaptosomal ATPase [3], electric eel creatine kinase [4], wheat peroxidase [5], yeast hexokinase [6], amphibian glyceraldehyde 3-phosphate dehydrogenase [7], horseradish peroxidase [8], spinach ferridoxin:$NADP^+$ oxidoreductase [9] and yeast DNA mismatch repair MSH2-MSH6 ATPase [10].

Arginase (L-arginine ureohydrolase EC 3.5.3.1) is a manganese-requiring enzyme that catalyzes the hydrolysis of L-arginine to L-ornithine and urea [11]. Arginase is activated by cadmium ion in tissue extracts from different organisms such as hepatopancreas of land snail [12], silk moth [13], bovine brain [14], rat liver, kidney, submaxillary gland, and brain [15,16], frog liver [17], sea mollusk *Chiton latus* [18] and bivalve clam *Semele solida* [19]. However, arginase is inhibited by cadmium ion in tissue extracts from axolotl liver [20], lupin [21], rat kidney [22], rat small intestine [23], barnacle rock shell *Concholepas concholepas* [24], common bean *Phaseolus vulgaris* [25] and zebra mussel *Dreissena polymorpha* [26].

Since cadmium can be an activator or inhibitor of arginase depending upon the source of the enzyme or the conditions of the assay, the purpose of this investigation was to compare the kinetics of the inhibition of rat liver and kidney arginase by cadmium ion under the same conditions. Arginase in the extracts of rat liver and kidney was studied without purification and heat activation. The kinetics of the inhibition or rat liver and kidney arginase by copper and mercury ions has been reported by Tormanen [27].

Materials and methods

Preparation of rat liver and kidney extracts

Extract of rat liver homogenate (20% by weight) in 1 mM Tris buffer, pH 7.0, containing 0.154 M KCl, was prepared as described by Tormanen [27]. Rat kidney mitochondrial soluble extract was prepared using Zwittergent 3-14 as described by Tormanen [27].

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ISSN 1475-6366 print/ISSN 1475-6374 online © 2006 Taylor & Francis
DOI: 10.1080/14756360500483420

Assay of arginase activity

Arginase activity was determined by measurement of L-ornithine as described by Tormanen [26], except that the L-arginine substrate was dissolved in 0.10 M 3-(N-morpholino)propanesulfonate (MOPS) buffer, and the pH was adjusted to 7.0. One unit of arginase activity was the formation of 1 umole L-ornithine per hour at 37°C at pH 7.0. The assays were performed in duplicate.

Inhibition by cadmium

Prior to assay, the liver extract was diluted from 20% by weight to 0.02–0.06% with 0.10 M MOPS buffer, pH 7.0. The surfactant-solubilized mitochondrial kidney extract was diluted from 20% by weight to 1–4% with 0.10 M MOPS buffer, pH 7.0. Stock solutions of reagent grade cadmium chloride (Fisher Scientific Company) were prepared in deionized water.

For studies conducted without preincubation, 0.250 mL of substrate was added to 0.125 mL of cadmium chloride at the appropriate concentration. The reaction was started by the addition of 0.125 mL of rat liver or kidney extract. The samples were incubated for 10 min at 37°C for liver extract and for 60 min at 37°C for kidney extract. The remainder of the assay was as described above. For kinetic studies, the concentration of L-arginine substrate varied from 1 to 4 mM in the incubation mixture. Substrate concentrations above 4 mM caused activation of rat kidney arginase.

For studies conducted with preincubation, 0.125 mL of liver or kidney extract was added to 0.125 mL of cadmium chloride at appropriate concentrations. The samples were preincubated at 0°C for 10 min. The reaction was started by the addition of 0.250 mL of 40 mM L-arginine substrate in 0.10 M MOPS buffer, pH 7.0. The remainder of the assay was as described above.

In studies on the effect of fluoride, potassium fluoride was added to the incubation mixture at a final concentration of 10 mM. In studies on the effect of manganese, manganese chloride was added to the incubation mixture at a final concentration of 1 mM.

Results

Rat kidney arginase is inhibited by much lower concentrations of cadmium ion than rat liver arginase (Figures 1 and 2). With 20 mM L-arginine substrate, fifty percent inhibition of rat liver arginase occurred at 16 mM cadmium ion and 50% inhibition of rat kidney arginase occurred at 0.092 mM cadmium ion. Preincubation of rat liver arginase with cadmium ion caused a small increase in the inhibition (Figure 1). As shown in Figure 2, preincubation with cadmium ion did not affect the inhibition of rat kidney arginase.

As shown in Figures 3 and 4, cadmium ion is a mixed noncompetitive inhibitor of both rat liver and kidney arginase with a concentration of L-arginine of from 1–4 mM.

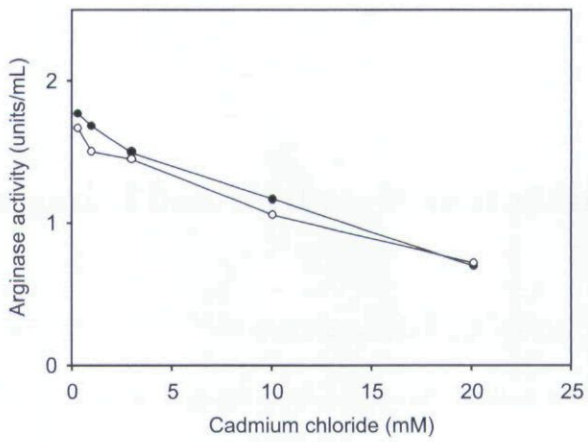

Figure 1. Effect of preincubation on the inhibition of rat liver arginase by cadmium chloride: no preincubation (closed circle) and preincubated 10 min (open circle).

As shown in Figure 5, cadmium ion enhanced the substrate activation of rat kidney arginase while still causing inhibition. Also, cadmium ion prevented the substrate inhibition by fluoride while still causing inhibition of arginase (Figure 5).

As shown in Figure 6, manganese ion enhanced the substrate activation of rat kidney arginase. Cadmium ion inhibited rat kidney arginase in the presence of manganese ion and did not prevent the substrate activation.

Discussion

Rat liver arginase has 3 cysteine residues and 8 histidine residues [28] while rat kidney arginase has 5 cysteine residues and 16 histidine residues [29]. Since cadmium binds to the thiol group of cysteine residues and imidazole nitrogen atoms of histidine residues, it is not surprising that rat liver arginase is not inhibited

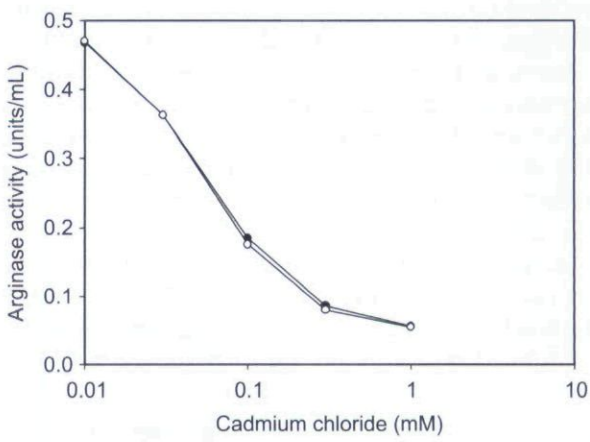

Figure 2. Effect of preincubation on the inhibition of rat kidney arginase by cadmium chloride: no preincubation (closed circle) and preincubated 10 min (open circle).

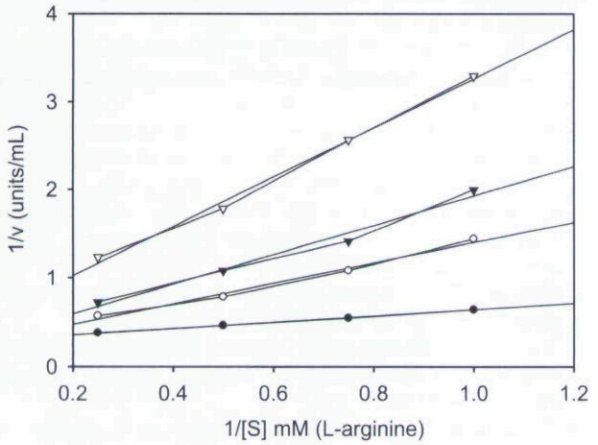

Figure 3. Lineweaver-Burk plot of the inhibition of rat liver arginase by cadmium ion. Various concentrations of cadmium chloride: none (closed circle), 2 mM (open circle), 5 mM (closed triangle), and 10 mM (open triangle).

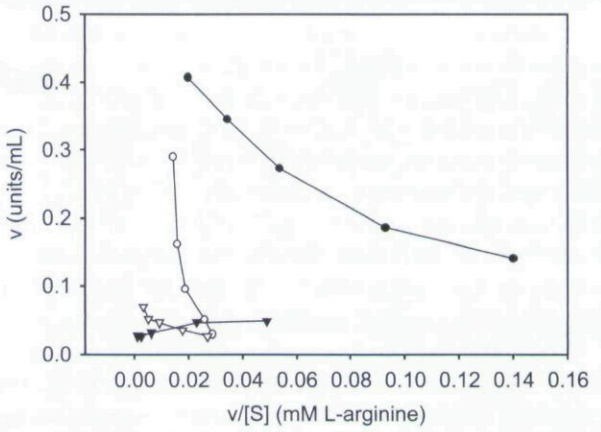

Figure 5. Eadie-Hofstee plot of the inhibition of rat kidney arginase by cadmium ion and fluoride ion: no cadmium or fluoride ion added (closed circle), 0.03 mM cadmium chloride (open circle), 10 mM potassium fluoride (closed triangle), and 0.03 mM cadmium chloride and 10 mM potassium fluoride (open triangle).

as strongly by cadmium ion as is rat kidney arginase (Figure 1).

Rat arginase does not have cysteine residues at the active site [30]. Therefore, it could be expected that the inhibition by cadmium ion would not be competitive if cadmium ion is binding to cysteine residues. As shown in Figures 3 and 4 the inhibition of both rat liver and kidney arginase by cadmium ion is mixed noncompetitive. However, the binding of cadmium ion at histidine or aspartate residues of arginase cannot be excluded. The binding of cadmium to purified crystalline arginase has not been studied.

L-arginine at concentrations above 4 mM causes substrate activation of rat kidney arginase [31]. Cadmium ion increased the substrate activation of rat kidney arginase as shown in Figure 5.

Fluoride prevents the substrate activation of rat kidney arginase [31]. Cadmium ion blocked the substrate inhibition of rat kidney arginase by fluoride as shown in Figure 5. It has recently been reported that two fluoride ions bind to the manganese binuclear cluster in rat liver arginase [32].

Manganese ion enhanced the substrate activation of rat kidney arginase. There have been no reports in the literature of a second binding site for L-arginine in rat arginase. However, Bewley et al. [33] have reported that *Bacillus caldovelox* has a second binding site for L-arginine. They have proposed that the second binding site, which is not near the active site, may have a regulatory role.

The results reported here do not support the results reported by Gasiorowska et al. [15] where cadmium ion was found to activate rat liver and kidney arginase. However, Gasiorowska used dialyzed tissue extracts and 5 mM cadmium ion was added to the extracts after 18 hours of dialysis. The dialysis may cause the loss of

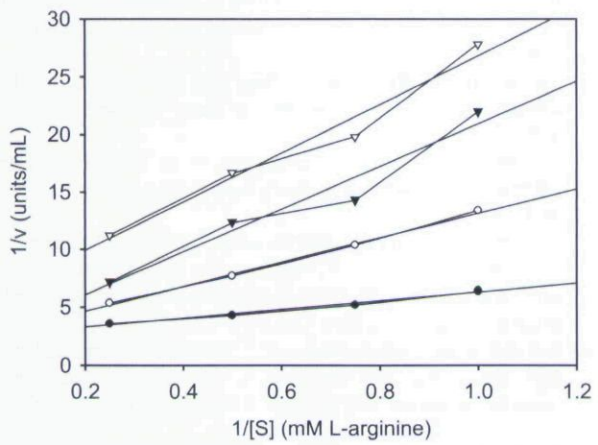

Figure 4. Lineweaver-Burk plot of the inhibition of rat kidney arginase by cadmium ion. Various concentrations of cadmium chloride: none (closed circle), 0.03 mM (open circle), 0.06 mM (closed triangle), and 0.09 mM (open triangle).

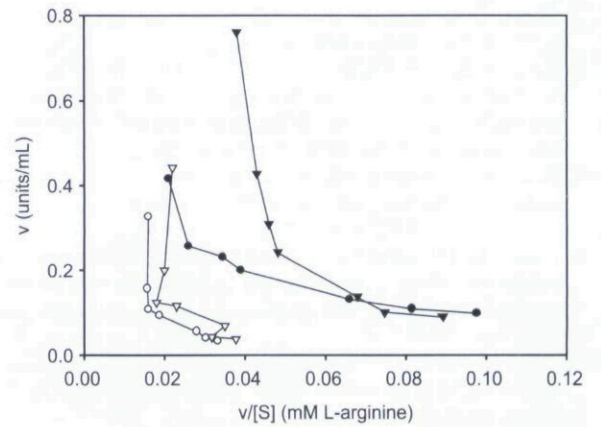

Figure 6. Eadie-Hofstee plot of the inhibition of rat kidney arginase by cadmium ion with or without manganese ion: no cadmium or manganese ion added (closed circle), 0.03 mM cadmium chloride (open circle), 1 mM manganese chloride (closed triangle), and 0.03 mM cadmium chloride and 1 mM manganese chloride (open triangle).

bound manganese ion and the cadmium ion may be substituting for the manganese ions at the active site of arginase. Interestingly, Tarab et al. [34] found that 1 mM cadmium ion did not activate or inhibit dialyzed rat liver arginase. Carvajal et al. [25] have reported that common bean arginase is inhibited by cadmium ion, although the addition of cadmium ion to inactivated arginase caused a partial recovery of activity. Also, Fuentes et al. [30] have shown that cadmium ion can substitute for manganese ion to recover much of the activity of dialyzed rat mammary gland arginase.

The metal-depleted recombinant H101N mutant of rat liver arginase is activated by cadmium ion [35]. The cadmium ion substitutes for the second manganese ion that is missing in the H101N mutant. The x-ray crystal structure of a mixed metal hybrid arginase has not been reported by Scolnick et al. [36]. Also, Patchett et al. [37] have reported that cadmium ion can substitute for manganese ion to produce an active arginase in the thermophilic bacteria *Bacillus caldovelox*.

Copper and mercury ions cause nonlinear allosteric inhibition of rat liver and kidney arginase [27]. However, the inhibition of rat liver and kidney arginase by cadmium was linear mixed noncompetitive (Figures 3 and 4). Also, preincubation of rat liver arginase by copper and mercury ions caused greater inhibition [27]. However, preincubation of rat liver arginase by cadmium ion had little effect on the inhibition (Figure 1). Therefore, cadmium ion may be binding at different amino acid residues of rat arginase than either copper or mercury ions.

Further studies will require purified crystalline arginase to determine where on the arginase molecule the cadmium ion is binding when it is not substituting for manganese ion at the active site.

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