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Allosteric Inhibition of Rat Liver and Kidney Arginase by Copper and Mercury Ions

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Two isozyme forms of arginase are found in the rat. All arginases are metalloenzymes which require manganese for activity. Many arginases are activated by cobalt and nickel ions and inhibited by heavy metal ions. The purpose of this study was to compare the effect of other heavy metal ions on the rat liver isozyme (arginase I) and the rat kidney isozyme (arginase II). The activation and inhibition of arginase I and II by metal ions were different. However, both isozymes were strongly inhibited by cupric and mercuric ions. The inhibition of arginase I by cupric and mercuric ions was increased greatly by preincubation of the enzyme with the metal ions. However, preincubation of arginase II by cupric and mercuric ions had little effect on the inhibition of the enzyme. Under certain conditions the kinetics of the inhibition of both arginases I and II by cupric and mercuric ions was nonlinear allosteric.

Keywords: Arginase; Allosteric kinetics; Rat liver and kidney; Enzyme inhibition; Copper and mercury

INTRODUCTION

Two isozymes forms of arginase (L-arginine ureohydrolase, EC 3.5.3.1) are found in the rat.[1] Arginase I is found mostly in rat liver cytosol where it participates in the urea cycle whereas arginase II is found in several tissues, especially in the kidney mitochondria, where it functions in the formation of glutamate, proline, and polyamines from ornithine. Rat arginases are trimeric enzymes which have a binuclear manganese cluster at the active site.[2]

Arginases from various species of animals differ in the effect of metal ions on the enzyme activity. Since at least 1945,[3] it has been known that liver arginase from beef, rat, dog, and rabbit is activated by manganese, nickel, and cobalt ions and is resistant to inactivation by other heavy metal ions. However, Mexican axolotl (a salamander) liver arginase is strongly inhibited by heavy metal ions.[4] Tormanen[5] reported that zebra mussel arginase is also strongly inhibited by heavy metal ions and is activated by manganese, nickel, and cobalt ions. More recently Colleluori *et al.*[6] reported that partially purified recombinant human arginase II is activated by cobalt and nickel

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ions and inhibited by magnesium, zinc, cadmium, and cupric ions.

Numerous papers have been published on the effect of metal ions on rat arginases. For example, Kaysen and Strecker[7] reported that purified rat kidney arginase activity is not affected by incubation with manganese, magnesium, or cobalt ions. However, incubation with 10 mM mercuric, cadmium, ferrous, and nickel ions causes significant inhibition. Tarrab *et al.*[8] reported that purified rat liver arginase activity is not affected significantly by incubation with manganese, cobalt, nickel, ferrous, cadmium, or zinc ions. However, Konarska and Tomaszewski[9] reported that arginase from dialyzed extract from rat small intestine is inhibited by cobalt, nickel, and cadmium ions. Bond *et al.*[10] reported that arginase in rat liver cytosol is activated 2.6-fold by 1 mM manganese ion, but cobalt, nickel, zinc, ferric, ferrous, cupric, calcium, and magnesium ions have no effect on the activity. However, Gasiorowska *et al.*[11] reported that rat liver arginase in dialyzed extracts is activated from 1.3- to 2.3-fold by incubation with manganese, magnesium, cobalt, cadmium, and nickel ions.

There are no reports in the literature directly comparing the effect of metal ions on the activity of rat liver and kidney arginases under the same conditions. Therefore, this study was done to determine the effect of metal ions on arginase from rat liver and kidney extracts under the same assay conditions. Because of the strong inhibition of rat arginase by cupric and mercuric ions, the inhibition by those ions was investigated in more detail. Under certain conditions the inhibition of rat liver and kidney arginase by cupric and mercuric ions was found to be allosteric.

MATERIALS AND METHODS

Preparation of Rat Liver and Kidney Extracts

An adult male Sprague–Dawley rat was sacrificed by decapitation. The liver was removed and homogenized in 1 mM Tris buffer, pH 7.0 containing 0.154 M KCl using a Sorvall blender. The 20% liver homogenate was centrifuged at 15,000 × *g* for 15 min at 4°C. The supernatant was centrifuged at 105,000 × *g* for 60 min at 4°C. The supernatant fraction containing cytosolic arginase I isozyme was stored at −20°C.

The kidneys were removed and homogenized in 1 mM Tris buffer, pH 7.4 containing 0.25 M sucrose using a Potter–Elvehjem tissue homogenizer. The 20% kidney homogenate was centrifuged at 15,000 × *g* for 15 min at 4°C. The mitochondrial pellet was resuspended in 1 mM Tris buffer, pH 7.4 containing 0.25 M sucrose and 0.3% Zwittergent 3-14 surfactant at 4°C. The surfactant-treated resuspended pellet was centrifuged at 15,000 × *g* for 15 min at 4°C. The supernatant fraction containing mitochondrial arginase II isozyme was stored at −20°C.

Assay of Arginase Activity

The arginase activity was determined by measurement of L-ornithine produced as described by Tormanen[5] except that the L-arginine was dissolved in 0.10 M MOPS buffer, pH 7.0. One unit of arginase activity was the formation of one micromole of L-ornithine per hour at 37°C. The assays were performed in duplicate.

Effect of Metal Ions on Arginase Activity

Prior to assay, the liver cytosolic arginase I extract was diluted from 20 to 0.03% with 0.10 M MOPS buffer, pH 7.0, and the kidney mitochondrial arginase II surfactant-solubilized extract was diluted from 20 to 1.0% with 0.10 M MOPS buffer, pH 7.0.

The metal ion solutions were prepared from reagent grade metal chloride salts dissolved in water. An equal volume of enzyme was added to the metal ion solution prior to adding substrate to start the reaction. In some experiments, the

enzyme was preincubated with the metal ion for 10 min at 0°C prior to adding substrate. The liver arginase was incubated with 20 mM L-arginine substrate for 10 min at 37°C, and the kidney arginase was incubated with 20 mM L-arginine substrate for 60 min at 37°C. The remainder of the assay was as described above.

For the kinetic experiments, the rat liver extract was diluted to 0.05% and the rat kidney extract was diluted to 2.0%. The extracts were preincubated with or without metal ions for 10 min at 0°C. The reaction was started by the addition of substrate. For experiments without preincubation, the reaction was started by the addition of enzyme. The concentration of L-arginine was varied from 1 to 4 mM. Double reciprocal plots of the velocity of the reaction versus substrate concentration were made using the method of Lineweaver–Burk.

RESULTS

Effect of Metal Ions with Preincubation

The results shown in Table I indicate that rat liver and kidney arginase were affected differently by metal ions when assayed under the same conditions. Arginase II was activated by manganese and cobalt ions while arginase I was not. Arginase II was more sensitive to inhibition by heavy metal ions than arginase I.

TABLE I The effect of metal ions on rat liver and kidney arginase activity*

Metal ion added	Liver arginase (I) (relative activity)	Kidney arginase (II) (relative activity)
None	100	100
Mn^{2+}	117	166
Ni^{2+}	95	89
Co^{2+}	100	146
Zn^{2+}	87	25
Cd^{2+}	90	14
Cu^{2+}	43	31
Fe^{3+}	88	64
Al^{3+}	95	67
Ca^{2+}	104	100
Mg^{2+}	104	105
Hg^{2+}	20	4
Pb^{2+}	26	8

*The enzyme was preincubated for 10 min at 0°C with 2 mM metal ion prior to adding substrate. The final concentration of the metal ion was 1 mM and L-arginine was 20 mM.

Inhibition With Cupric and Mercuric Ions with or without Preincubation

The results reported in Table II and shown Fig. 1 indicate that the inhibition of arginase I by cupric and mercuric ions was much greater when the enzyme was preincubated with the metal ions. However, the inhibition of arginase II by cupric and mercuric ions was not significantly affected by preincubation with the ions (Table II).

Kinetics of Inhibition with Preincubation

When preincubated with cupric ion, the kinetics of the inhibition of arginase I was found to be linear mixed noncompetitive (Fig. 2A). However, when preincubated with cupric ion, the inhibition of arginase II was found to be nonlinear (Fig. 2B). The upward curving lines on the Lineweaver–Burk plot indicates that the inhibition by cupric ion is allosteric.

When preincubated with mercuric ion, the inhibition of arginase I was found to be nonlinear (Fig. 3A). However, the inhibition of arginase II when preincubated with mercuric ion was found to be linear mixed noncompetitive (Fig. 3B).

Kinetics of Inhibition without Preincubation

When arginase I was assayed with various concentrations of cupric ion without preincubation, the inhibition was nonlinear (Fig. 4A). However, when arginase II was assayed without preincubation with various concentrations of cupric ion, the Lineweaver–Burk plots were more linear (Fig. 4B).

When arginase I was assayed without preincubation with various concentrations of mercuric ion, the inhibition was nonlinear (Fig. 5A). However, when arginase II was

TABLE II The effect of preincubation on the inhibition of rat liver and kidney arginase by cupric and mercuric ions*

Arginase	Metal ion	IC_{50}† with no preincubation (mM)	IC_{50}† with 10 min preincubation at 0°(mM)
Rat liver (I)	Cu^{2+}	4.5	0.016
	Hg^{2+}	1.8	0.054
Rat kidney (II)	Cu^{2+}	0.69	0.30
	Hg^{2+}	0.022	0.019

*The concentration of L-arginine was 20 mM in the incubation volume.
†The concentration of metal ion causing 50% inhibition of arginase activity.

assayed without preincubation with various concentrations of mercuric ion, the inhibition was linear mixed noncompetitive (Fig. 5B).

The allosteric inhibition of arginase I by cupric ion and mercuric ion without preincubation was more clearly shown when Michaelis–Menten plots were made of the activity of arginase I with various concentrations of cupric ion (Fig. 6A) and mercuric ion (Fig. 6B). With increasing concentrations of cupric or mercuric ions, the plots became increasingly sigmoidal.

DISCUSSION

The effect of metal ions on rat liver arginase reported in this paper are in general agreement with previously published research.[3,8,10,11] There was little or no activation of arginase I by manganese, nickel, or cobalt ions (Table I). Also, the liver isoenzyme was not inhibited significantly by zinc, calcium, aluminum, cadmium, and ferric ions. However, there was significant inhibition of arginase I by cupric, mercuric, and lead ions (Table I). The effect of aluminum, calcium, or lead ions on liver arginase had not been reported previously.

There has been only one report in the literature on the effect of metal ions on rat kidney arginase. Kaysen and Strecker[7] did not find activation by manganese, nickel, or cobalt ions. In this report, manganese and cobalt activated arginase II by 66 and 46%, respectively (Table I). Arginases from many species of animals have been reported to be activated by manganese and cobalt ions.[3] Recently, Tormanen[5] reported that zebra mussel arginase is activated several fold by manganese, nickel, and cobalt ions. Kaysen and Strecker[7] reported

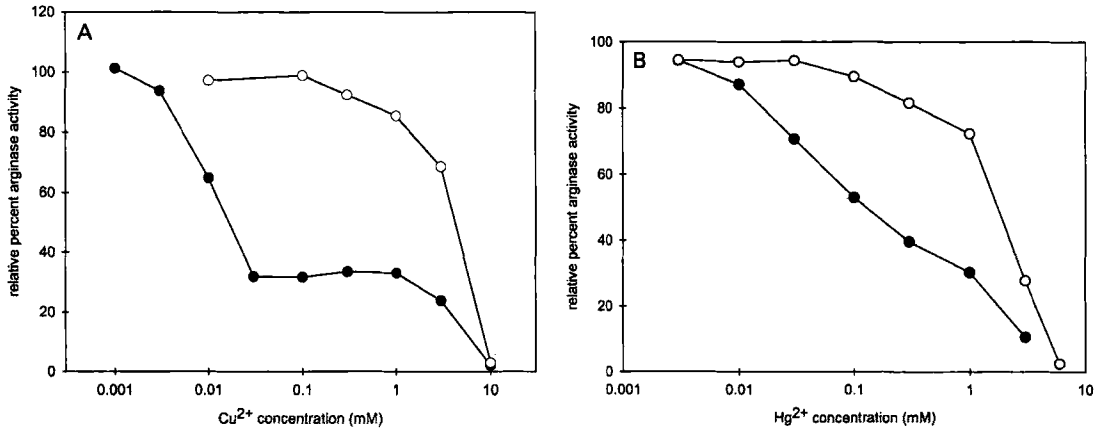

FIG. 1 Effect of preincubation on the inhibition of rat liver agrinase by (A) cupric ion and (B) mercuric ion: •, preincubated 10 min; O, no preincubation.

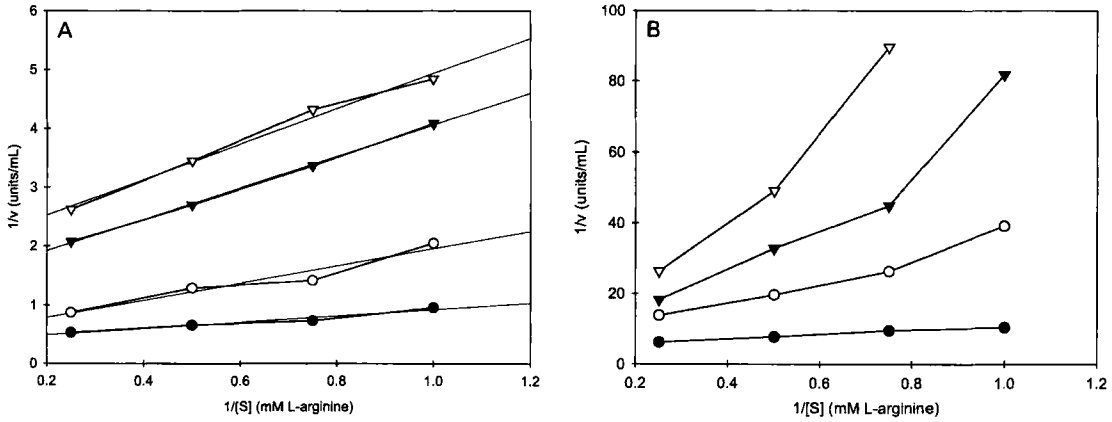

FIG. 2 Inhibition of rat (A) liver and (B) kidney arginase preincubated 10 min with various concentrations of cupric ion: (A) •, control; ○, 0.01 mM; ▼, 0.02 mM; ▽, 0.03 mM. (B) •, control; ○, 0.1 mM; ▼, 0.2 mM; ▽, 0.3 mM.

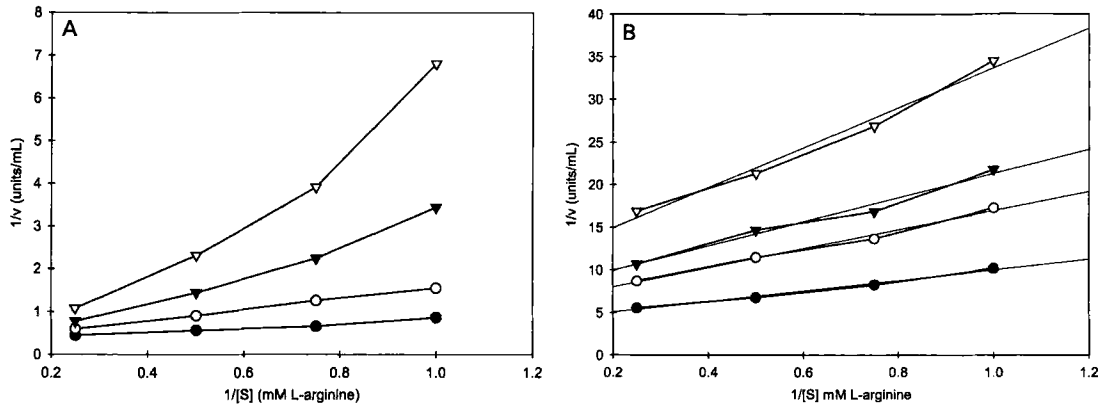

FIG. 3 Inhibition of rat (A) liver and (B) kidney arginase preincubated 10 min with various concentrations of mercuric ion: (A) •, control; ○, 0.03 mM; ▼, 0.05 mM; ▽, 0.07 mM. (B) •, control; ○, 0.02 mM; ▼, 0.03 mM; ▽, 0.05 mM.

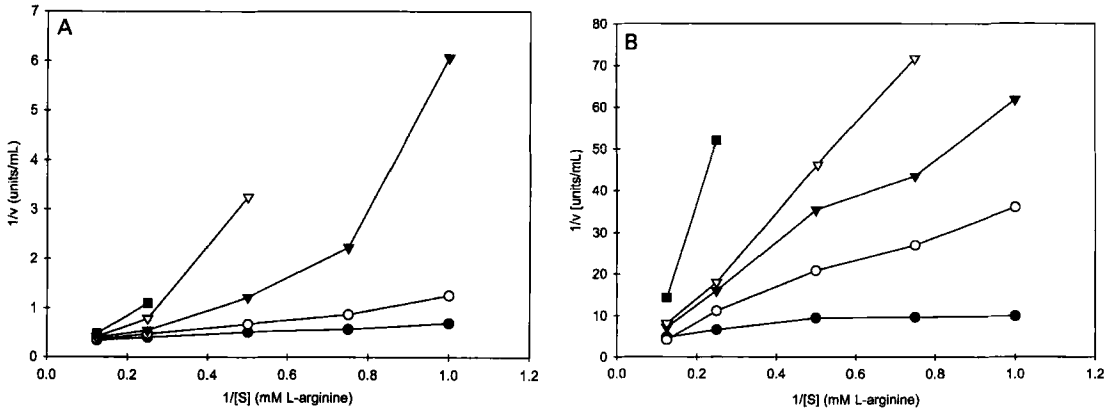

FIG. 4 Inhibition of rat (A) liver and (B) kidney arginase without preincubation with various concentrations of cupric ion: (A) •, control; ○, 0.1 mM; ▼, 0.3 mM; ▽, 0.6 mM; ■, 1.0 mM. (B) •, control; ○, 0.1 mM; ▼, 0.2 mM; ▽, 0.3 mM; ■, 1.0 mM.

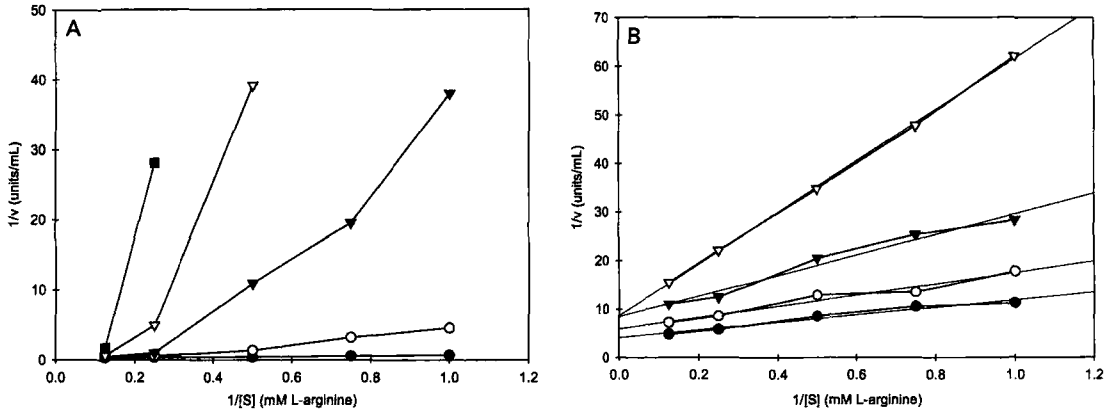

FIG. 5 Inhibition of rat (A) liver and (B) kidney arginase without preincubation with various concentrations of mercuric ion: (A) •, control; ○, 0.1 mM; ▼, 0.3 mM; ▽, 0.6 mM; ■, 1.0 mM; (B) •, control; ○, 0.02 mM; ▼, 0.03 mM; ▽, 0.05 mM.

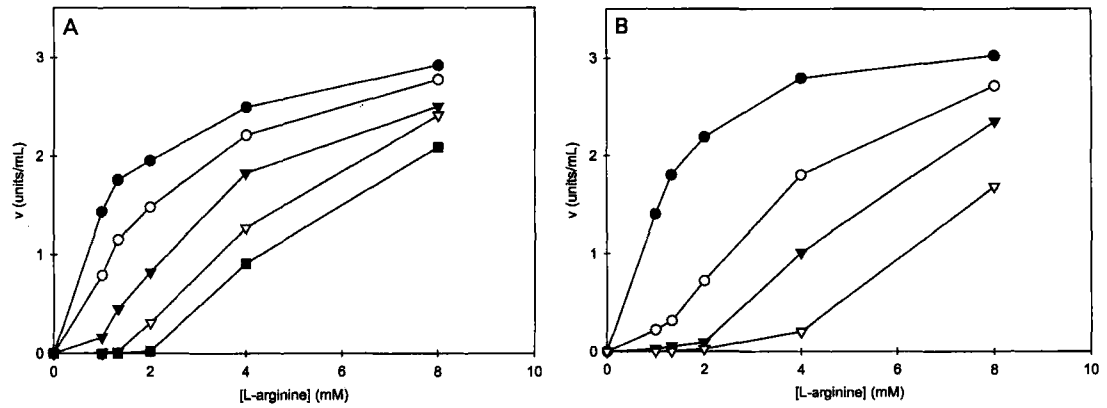

FIG. 6 Michaelis–Menten plot of the activity of rat liver arginase without preincubation with various concentrations of (A) cupric ion: •, control; ○, 0.1 mM; ▼, 0.3 mM; ▽, 0.6 mM; ■, 1.0 mM. (B) mercuric ion: •, control; ○, 0.1 mM; ▼, 0.3 mM; ▽, 0.6 mM.

inhibition of rat kidney arginase by mercuric, cadmium, and ferrous ions. Inhibition of arginase II by mercuric and cadmium ions was also reported in this paper (Table I). In addition, this study found inhibition of rat arginase II by zinc, cupric, ferric, aluminum, and lead ions. Colleluori *et al.*[6] have reported that human arginase II is also inhibited by zinc and cupric ions.

The inhibition of rat liver arginase by cupric and mercuric ions was increased greatly by preincubation with the metal ion (Table II, Fig. 1). The effect was not observed with rat kidney arginase. Apparently, the presence of substrate protects arginase I from inhibition by low concentrations of cupric and mercuric ions. Arginase I may have binding sites for cupric and mercuric ions at the active site and at other sites on the enzyme. The binding sites for cupric and mercuric ions for arginase II must not include the active site.

The kinetics of the inhibition of arginase I and II by cupric ion with preincubation are different. Cupric ion was a linear mixed noncompetitive inhibitor of rat liver arginase (Fig. 2A). However, the inhibition of rat kidney arginase by cupric ion was nonlinear (Fig. 2B). The inhibition of arginase II by cupric ion increased greatly at low concentrations of L-arginine.

The kinetics of the inhibition of arginase I and II by mercuric ion with preincubation are also different. The inhibition of rat liver arginase by mercuric ion was nonlinear (Fig. 3A). The inhibition of arginase I by mercuric ion increased at low concentration of L-arginine. However, the inhibition of rat kidney arginase by mercuric ion was linear mixed noncompetitive (Fig. 3B).

The kinetics of the inhibition of arginase I and II by cupric and mercuric ions without preincubation are also different (Figs. 4 and 5). The inhibition of arginase I by cupric ion changed from linear mixed noncompetitive with preincubation (Fig. 2A) to nonlinear allosteric without preincubation (Fig. 4A). However, for arginase II, the inhibition by cupric ion changed from nonlinear allosteric with preincubation (Fig. 2B) to more linear without preincubation (Fig. 4B). The kinetics of the inhibition of arginase I and II with mercuric ion without preincubation (Fig. 5) were similar to the inhibition of arginase I and II with mercuric ion with preincubation (Fig. 3).

The different inhibitor profiles suggest that cupric and mercuric ions are binding to arginase at different sites on each isozyme. Heavy metals can bind to thiol groups on enzymes.[12] Mammalian arginases do not have a cysteine residue at the active site.[13] Rat arginase I has three cysteine residues (119, 168, and 303),[14] and rat arginase II has five cysteine residues (63, 113, 138, 187, and 351).[15]

Metal ions also can bind to histidine residues on enzymes.[12] Rat arginase I has eight histidine residues [three are at the active site (101, 126, and 141)].[14,16] Rat arginase II has 16 histidine residues (12, 24, 64, 120, 130, 133, 134, 145, 160, 206, 247, 284, 300, 331, 336, and 344).[15] The increased number of histidine residues in arginase II compared to arginase I is consistent with the greater sensitivity of arginase II to inhibition by heavy metal ions reported in this paper.

The allosteric inhibition of arginase I by cupric and mercuric ions and arginase II by cupric ions shown in this report is possible because arginase is a trimeric enzyme. The heavy metal ions may be binding to cysteine or histidine residues on the surface of the enzyme subunits. Lavulo *et al.*[17] have recently reported that rat liver arginase residue arginine-308 is involved in the subunit-subunit interactions of the enzyme. This residue is in a S-shaped motif at the carboxyl terminus of the enzyme. This motif also contains a histidine residue (histidine-312)[17] which could interact with cupric ions. The S-shaped motif does not contain cysteine residues.[17]

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