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## Site of Action of Metals on the Aminolevulinic Acid Dehydratase of Human Erythrocytes

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**Abstract.** The site of action of four metals (copper, lead, nickel and zinc) on the aminolevulinic acid dehydratase of human erythrocytes was studied. Data obtained after denaturation of allosteric sites with heat treatment and after simultaneous incubation with two metals suggest the following conclusions: copper acts on the catalytic site, whereas lead, nickel and zinc act on the allosteric sites; zinc and nickel act on the same allosteric (metallic) site; zinc and lead only partially act on the same metallic site; nickel and lead act on different allosteric sites.

Aminolevulinic acid dehydratase (ALA-D; EC 4.2.1.24) is an allosteric enzyme (10) and its activity is modified by numerous metals (1–3, 7, 8).

Despaux *et al.* (4) think that modifications of the activity of ALA-D are explained by an action of the metals at different levels: thiol groups, allosteric sites, zinc site, and an indirect mechanism at the level of reduced glutathione, indispensable for ALA-D activity *in vivo* (9).

It appears interesting to determine the site of action of different metals on ALA-D to achieve a better knowledge of a possible interaction of metals simultaneously present and their action on the activity of the enzyme. In practice, this occurs, for instance, in several industrial activities (metal workers).

Until now only the site of action of lead has been defined: Vergnano *et al.* (11) found that the site of action of  $6 \times 10^{-6}$  and  $1.5 \times 10^{-6}$  mol/l lead is an allosteric site. Moreover, one must recall the observations (3, 5) that lead and zinc compete for the same (metallic) site.

We will report some observations on the influence of the site of action of four metals of industrial concern, copper, lead, nickel and zinc, on ALA-D activity.

### Methods

ALA-D activity was measured by the method of Bonsignore *et al.* (2). This method measures the enzyme units per milliliter of red blood cells, using as

a substrate 0.01 mol/l aminolevulinic acid (ALA). A Perkin-Elmer model 550 spectrophotometer was used. The substrate is 5-ALA HCl (Merck, Darmstadt, FRG).

Metals at various concentrations were always added to hemolyzed erythrocytes 10 min before adding the substrate by careful mixing.

Denaturation of allosteric sites was performed according to Gerhart and Pardee (6), by heating the hemolysate to 60 °C for 5 min in a thermostated bath and then cooling it in ice. Metals were added after cooling.

For each sample, three measures were carried out; the results are expressed as means  $\pm$  standard deviations; percent variation with respect to the control sample was also calculated. The precision of the method obtained in our laboratory shows a variation of about 5%. In view of this, we do not consider the changes in enzyme activity lower than 5%.

## Results

Variations in ALA-D activity with the addition of nickel at various concentrations were first assessed; the results are reported in table I. Only at very high concentrations is an inhibitory effect evident, which disappears and is

replaced by activation at decreasing metal concentrations. Although  $1.7 \times 10^{-4}$  mol/l is about 1,000 times the nickel concentrations

**Table I.** Variations of ALA-D activity with decreasing concentrations of nickel

Nickel mol/l	ALA-D activity	
	m $\pm$ SD U/ml	%
Control	110 $\pm$ 1.1	100
$1.7 \times 10^{-4}$	26 $\pm$ 2.5	24
$1.2 \times 10^{-4}$	85 $\pm$ 2.6	77
$0.8 \times 10^{-4}$	102 $\pm$ 2.6	93
$0.4 \times 10^{-4}$	115 $\pm$ 2.5	105
$1.7 \times 10^{-5}$	130 $\pm$ 2.1	118
$0.8 \times 10^{-5}$	138 $\pm$ 2.5	125
$1.7 \times 10^{-6}$	128 $\pm$ 2.9	116
$1.7 \times 10^{-7}$	124 $\pm$ 3.1	113
$1.7 \times 10^{-8}$	114 $\pm$ 2.1	104
$1.7 \times 10^{-9}$	110 $\pm$ 1.5	100

Mean  $\pm$  SD was obtained from three samples. Percent activity with respect to the control sample.

**Table II.** Variations of the effects on enzyme activity when metals are added after heat treatment

Metal	Concentration, mol/l	Without heat treatment		After heat treatment	
		m $\pm$ SD U/ml	%	m $\pm$ SD U/ml	%
Control		106 $\pm$ 1.7	100	163 $\pm$ 1.2	100
Cu	$1.5 \times 10^{-4}$	0	0	0	0
	$1.5 \times 10^{-5}$	31 $\pm$ 1.5	29	59 $\pm$ 2.1	36
Pb	$5 \times 10^{-5}$	0	0	5 $\pm$ 0.6	3
	$5 \times 10^{-6}$	36 $\pm$ 1.5	34	101 $\pm$ 1.5	62
Zn	$1.5 \times 10^{-4}$	141 $\pm$ 3.2	133	163 $\pm$ 1.5	100
	$1.5 \times 10^{-5}$	135 $\pm$ 1.2	127	163 $\pm$ 1.6	100
Control <sup>a</sup>		110 $\pm$ 1.1	100	194 $\pm$ 2.1	100
Ni <sup>a</sup>	$1.7 \times 10^{-4}$	26 $\pm$ 2.5	24	105 $\pm$ 1.7	54

For legends, see table I.

<sup>a</sup> Performed separately, with autonomous control values.

one can find *in vivo*, this inhibitory concentration was used for the following investigations.

The variations in effect obtained by heating are reported in table II. Heat treatment prevents the activation produced by zinc at both concentrations studied ( $1.5 \times 10^{-4}$  and  $1.5 \times 10^{-5}$  mol/l) and partly prevents the inhibition produced by  $5 \times 10^{-6}$  mol/l lead and  $1.7 \times 10^{-4}$  mol/l nickel, but it has no effect on the

inhibition caused by  $5 \times 10^{-5}$  mol/l lead and  $1.5 \times 10^{-4}$  and  $1.5 \times 10^{-5}$  mol/l copper.

The results obtained when adding two metals at the same time are reported in table III; they will be considered in more detail below.

### Discussion

The results of these *in vitro* experiments show that simultaneous absorption of various metals *in vivo* can give rise to interferences likely to modify the effect of metals on enzyme activity.

These interferences are better understood if one can define the site of action of each metal involved.

As concerns ALA-D activity, we could show that the denaturation of allosteric sites has no effect on the inhibition caused by copper, but, on the contrary, entirely prevents the activation caused by zinc, and, at least partially, the inhibition caused by lead and nickel.

These results suggest that copper acts on the catalytic site at all the concentrations studied, whereas activation related to zinc and inhibition related to nickel and lead at the concentration of  $5 \times 10^{-6}$  mol/l are mediated via allosteric sites.

It is worthwhile to note that these effects occur, except for nickel, at metal concentrations one can find *in vivo* ( $5 \times 10^{-5}$  mol/l lead also acts on the catalytic site, but such a concentration is practically impossible *in vivo*).

The combined effects of two metals (table III) appear at different sites of action, as described above, and give further information.

As zinc, acts on an allosteric (metallic) site, it prevents neither the inhibition caused by ( $1.5 \times 10^{-4}$  and  $1.5 \times 10^{-5}$  mol/l) copper nor that caused by  $5 \times 10^{-5}$  mol/l lead: these inhibitions involve the catalytic site. On the other

**Table III.** ALA-D activity when two metals were combined

Metal	Concentration mol/l	ALA-D activity	
		m $\pm$ SD U/ml	%
Control		106 $\pm$ 1.7	100
Cu	$1.5 \times 10^{-5}$	31 $\pm$ 1.5	29
Pb	$5 \times 10^{-6}$	36 $\pm$ 1.5	34
Together		10 $\pm$ 1.3	9
Cu	$1.5 \times 10^{-5}$	31 $\pm$ 1.5	29
Ni	$1.7 \times 10^{-4}$	26 $\pm$ 1.2	24
Together		3 $\pm$ 0.4	3
Cu	$1.5 \times 10^{-4}$	0	0
Zn	$1.5 \times 10^{-4}$	141 $\pm$ 3.2	133
Together		0	0
Cu	$1.5 \times 10^{-5}$	31 $\pm$ 1.5	29
Zn	$1.5 \times 10^{-4}$	141 $\pm$ 3.2	133
Together		31 $\pm$ 1.2	29
Ni	$1.7 \times 10^{-4}$	26 $\pm$ 1.2	24
Pb	$5 \times 10^{-6}$	36 $\pm$ 1.5	34
Together		0	0
Ni	$1.7 \times 10^{-4}$	26 $\pm$ 1.2	24
Zn	$1.5 \times 10^{-4}$	141 $\pm$ 3.2	133
Together		114 $\pm$ 2.1	108
Pb	$5 \times 10^{-5}$	0	0
Zn	$1.5 \times 10^{-4}$	141 $\pm$ 3.2	133
Together		2 $\pm$ 0.5	2
Pb	$5 \times 10^{-6}$	36 $\pm$ 1.5	34
Zn	$1.5 \times 10^{-4}$	141 $\pm$ 3.2	133
Together		53 $\pm$ 1.6	50

For legends, see table I.

hand, zinc brings back to normal the enzyme activity inhibited by  $1.7 \times 10^{-4}$  mol/l nickel: this indicates that nickel acts on the same site as that activated by zinc.

Enzyme inhibition caused by  $5 \times 10^{-6}$  mol/l lead is only partially restored by  $1.5 \times 10^{-4}$  mol/l zinc: this suggests a prevalent inhibition of the allosteric sites not influenced by zinc (likely the thiol groups; 3, 5) with only a minor involvement of allosteric sites, for which zinc efficiently competes.

Finally, the additive inhibition observed when combining  $1.5 \times 10^{-5}$  mol/l copper and  $5 \times 10^{-6}$  mol/l lead;  $1.5 \times 10^{-5}$  mol/l copper and  $1.7 \times 10^{-4}$  mol/l nickel, and  $1.7 \times 10^{-4}$  mol/l nickel and  $5 \times 10^{-6}$  mol/l lead agrees with the simultaneous inhibition of different sites of the enzyme brought about by different metals.

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