INTERACTION OF ZINC AND LEAD ON δ-AMINOLEVULINATE DEHYDRATASE

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Rat erythrocyte δ-aminolevulinate dehydratase (ALA-D) has been confirmed to be an enzyme dependent on dietary zinc. Levels of blood ALA-D decreased substantially during the first five weeks after weaning and remained constant thereafter. Dietary lead (200 ppm) caused a dramatic fall in erythrocyte ALA-D activity and, following the removal of lead exposure, the return of the enzymatic activity toward normal values was quicker in the animals receiving higher zinc than in those on lower zinc diet. Furthermore, in vitro addition of zinc to erythrocyte preparation obtained from rats exposed to dietary lead reactivated ALA-D to control values. Maximal enzymatic activity in both lead-fed and control animals occurred at 10^{-4}M $ZnCl_2$. There seems to be a competition between Pb^{++} and Zn^{++} in binding to the enzyme, with Pb^{++} as inhibitor and Zn^{++} as activator.

Recently we have reported that δ-aminolevulinate dehydratase (ALA-D) activity in rat erythrocyte and liver tissue is dependent on the dietary levels of zinc (1). Other studies support the idea that ALA-D may be a metalloenzyme containing zinc (2,3,4,5). It is also known that this enzyme is rich in sulfhydryl groups (2) and that thiols are required for maximal in vitro enzyme activity (2,6-8). Numerous in vitro and in vivo studies have shown that ALA-D is very sensitive to lead (7, 9-14); DeBruin has reported significant inhibition of this enzyme by Pb at 2 x 10^{-7}M in rabbit blood (14). Many other investigators have reported

negative correlation between ALA-D activity and lead levels in blood (15-18).

Several hypotheses have been raised on the mechanism by which lead inhibits ALA-D. A group of investigators (19,20) suggested that the depression of enzymatic activity by prolonged lead poisoning could be attributed either to a structural change at the level of the enzyme synthesis, or to the production of a thermolabile inhibitor. Their findings seem to indicate that, in chronic exposure to lead, ALA-D inhibition does not result from a direct action of lead on the enzyme as is the case in acute poisoning or *in vitro*. Other studies (7,10,21-23) seem to support the hypothesis that lead inhibits the enzyme by its direct action on the sulfhydryl groups which is reversed by *in vitro* addition of glutathione, cysteine and other thiol reagents.

We herewith report *in vitro* and *in vivo* studies conducted to elucidate the manner by which zinc affects ALA-D activity, and the mechanism by which lead may inhibit this enzyme.

EXPERIMENTAL

Weanling male Sprague-Dawley rats weighing 50-60 g were housed individually in stainless steel wire cages and kept in a special temperature and light-regulated animal room. All animals received a zinc and copper deficient semipurified diet similar to that previously described (1). All rats were given deionized drinking water supplemented with 2.0 ppm copper. Two groups were formed according to the level of zinc supplemented in the drinking water. Group I received 40 ppm and Group II received 2.5 ppm zinc, an amount sufficient to permit small growth response in the rats. During the seventh week of the experiment each of these two groups were subdivided into a

control group and a Pb-treated group. Two hundred ppm Pb (as lead acetate) were added to the drinking water of the rats in the later groups for a two-week period. Blood ALA-D levels were determined at various intervals during the 14 week experiment as shown in Figure 1.

ALA-D activity was assayed by the method of Bonsignore et al. (12) modified as previously reported (1). Metal analyses for zinc and lead were performed by atomic absorption spectrophotometry on diets and red blood cells after wet digestion in acid.

RESULTS

We have plotted erythrocyte ALA-D activity versus time (Figure 1) for two groups of rats kept on different dietary levels of zinc (2.5 and 40 ppm); from the seventh to the ninth

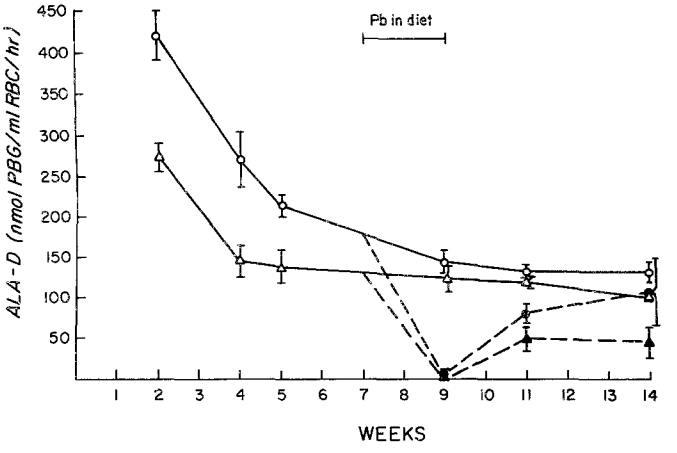

Figure 1. Erythrocyte ALA-D activity of rats fed a semipurified diet low in zinc and supplemented with the following metals in drinking water: 40 µg Zn/ml (o——o); 40 µg Zn/ml + 200 µg Pb/ml, (●---●); 2.5 µg Zn/ml, (Δ——Δ); 2.5 µg Zn/ml + 200 µg Pb/ml, (▲---▲). Lead was given as Pb acetate for two weeks, during the 7th and 8th week of the experiment.

week one half of the animals from each group received 200 ppm Pb^{++} in drinking water.

It is evident in all the animals that a dramatic reduction in erythrocyte ALA-D activity occurred during the first five weeks after weaning. It is also clear that during this period Group II rats, receiving a low zinc diet, show considerably lower enzymatic activity than Group I rats. The addition of lead to the drinking water caused a reduction in enzymatic activity to zero in the low zinc group and almost to zero in the zinc-fortified group. Following the removal of the lead exposure, erythrocyte ALA-D activity in rats receiving the higher level of zinc returned toward normal values more quickly than in those on lower zinc diets. Five weeks after withdrawal of lead the enzyme activity in the Pb-treated low zinc group was still significantly lower than in the control group, whereas in the animals fed a higher zinc diet there appeared to be no significant difference between control and Pb-treated groups.

The effects on erythrocyte ALA-D activity by *in vitro* addition of $ZnCl_2$ to blood obtained from rats fed low and high zinc diet with and without lead exposure are reported in Figure 2. In all cases zinc reactivates the lead-inhibited enzyme. At 10^{-4}M $ZnCl_2$, ALA-D values are similar in lead-exposed and control animals.

In another experiment, rats were fed laboratory chow containing 500 ppm lead (as lead acetate) for one month. Two months after withdrawal of dietary lead the average level of lead in the blood of these animals was about 50 µg/100 ml and ALA-D activity ranged between 10% and 20% of normal. Figure 3 shows the activation of ALA-D in the blood of one of these animals by the *in vitro* addition of $ZnCl_2$. Again we noticed

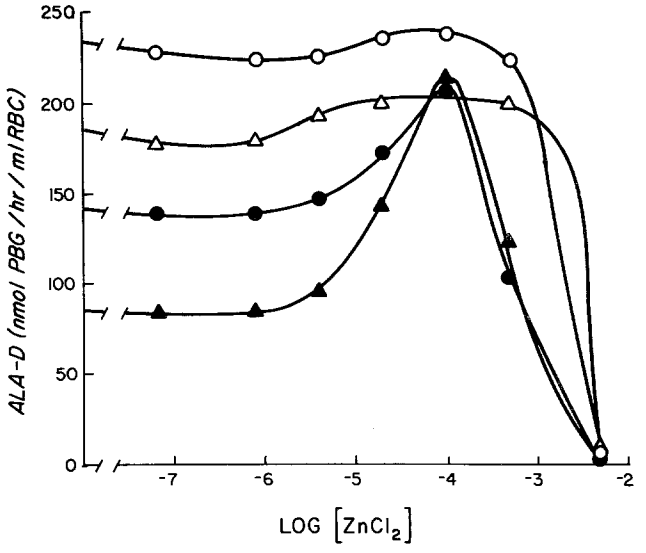

Figure 2. Activation of ALA-D in vitro addition of $ZnCl_2$ to the blood preparations from rats exposed for fourteen weeks to the experimental conditions as shown in Figure 1.

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that ALA-D activity peaked at 10^{-4}M $ZnCl_2$.

Figure 4 shows the activation of ALA-D by $ZnCl_2$ in blood from an adult rat fed laboratory chow with and without the in vitro addition of 10^{-5}M $PbCl_2$. Addition of $ZnCl_2$ not only reversed the inhibition due to lead, but also increased ALA-D activity in the non-leaded mixture by more than 50%. This increase may be explainable by the fact that commercial chow has been found to contain trace amounts of lead. Concentrations of 10^{-5} to 10^{-4}M $ZnCl_2$ completely reversed the inhibitory effect of lead, thus restoring ALA-D activity in full.

DISCUSSION

The dietary levels of zinc affected erythrocyte ALA-D up to the ninth week after weaning; thereafter no significant difference in enzymatic activity was observed between the groups

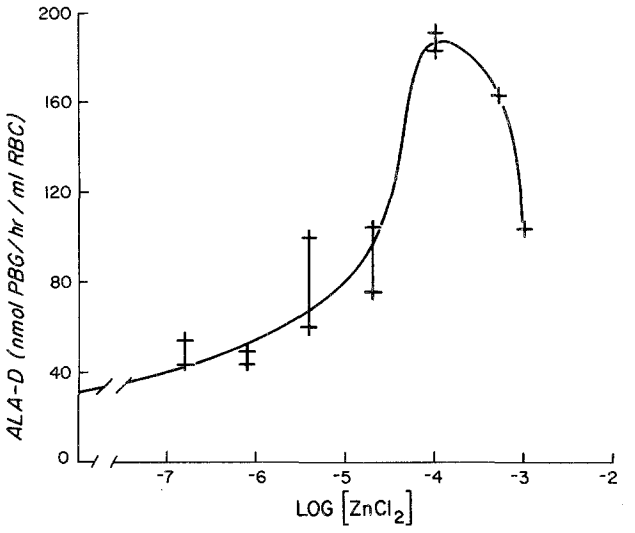

Figure 3. Activation of ALA-D by $ZnCl_2$ in blood from a Pb-exposed rat with blood lead concentration of 50 µg/100 ml.

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receiving high and low levels of zinc. A drop in ALA-D activity during the first five weeks after weaning confirmed our early findings (1), that is, adult rats weighing 200 g or more showed approximately half of the erythrocyte ALA-D activity of younger animals weighing 100 g or less.

Exposure of the animals to 200 ppm lead in the diet for two weeks caused a complete inhibition of ALA-D in the blood of the animals fed low zinc; however, residual activity was still present in the blood of the high zinc group. This observation and the rapid return of enzymatic activity toward normal values in the rats on the high zinc diet suggest a protective role of zinc on the inhibitory effect of lead.

Until recently there existed doubts as to whether the measured inhibition of ALA-D in blood of lead exposed individuals reflectd an *in vivo* effect or was an artefact created in the

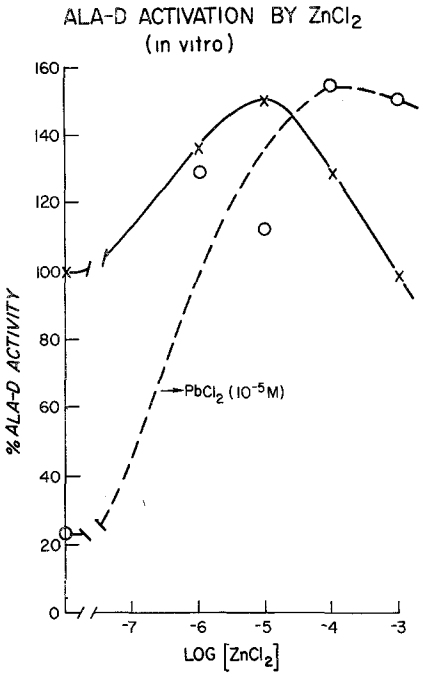

Figure 4. Activation of ALA-D by $ZnCl_2$ in adult rat blood with and without addition of 10^{-5}M $PbCl_2$.

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test tube assay as a result of lead redistribution due to the hemolysis. A recent publication of Roels et al. (24) cleared these doubts by reporting that at blood lead concentration < 120 µg/100 ml the measured ALA-D activity represents the true in vivo activity. This information and the results reported here establish the fact that ALA-D is very sensitive to lead in vivo.

ALA-D activity in the erythrocyte preparations peaked in all cases when 10^{-4} $ZnCl_2$ was added to the mixture; therefore, the increase in ALA-D activity obtainable by the addition of 10^{-4}M Zn^{++} to a blood preparation may be a useful diagnostic tool for the measurement of Pb-inhibited enzyme.

The high sensitivity of this enzyme *in vivo* and *in vitro* to low lead concentrations suggests a specific binding of this metal to the enzyme active sites. The reported high contents of -SH groups in this enzyme, and the reversal of the lead inhibition by addition of thiols or $ZnCl_2$ also suggest the involvement of zinc at the active site, possibly bound to -SH groups. However, other heavy metals such as Hg^{++}, CH_3Hg^{+}, Cu^{++} and Cd^{++}, with affinity for -SH group higher than or similar to Pb^{++}, show much less ALA-D inhibition. Therefore, the preferential affinity of lead to ALA-D may be due to the steric configuration of the protein molecule and/or to the presence in the biological system of other ligands (proteins, amino acids, etc.) with higher binding capacity for the other metals.

The stability constant of the Zn-apoenzyme complex may be relatively low, since it has been reported by Cheh and Neilands (3) that the enzyme lost zinc during isolation and purification steps. These investigators pointed out that precipitation of the protein with $(NH_4)_2SO_4$ tended to alter the metal content of ALA-D by lowering the zinc and raising copper and iron which were present as trace impurities in ammonium sulfate. The requirement of relatively high concentration of Zn^{++} to reactivate ALA-D inhibited by much lower amount of Pb^{++} also suggests that the enzyme has greater affinity for lead than zinc. More studies have to be done to establish whether ALA-D is a zinc containing metalloenzyme or a zinc activated enzyme.

These findings may be of importance in improving plumbism therapy and in determining the exposure level of the toxicant in relation to the nutritional state of the exposed population.

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