Effect of Zinc and Cadmium on δ-Aminolevulinate Dehydratase of Red Blood Cells in Protecting against Enzyme Losses during Storage

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The effect of zinc and cadmium on δ-aminolevulinate dehydratase of bovine erythrocytes stored at −30 °C for different times was determined. The results show a. storage of erythrocytes leads to an enhancement of the enzyme activity, which after six weeks is 165% (500 μM $ZnCl_2$) respectively 220% (100 μM $CdCl_2$) for red blood cells of calves, and after four weeks is 420% respectively 450% (same concentrations) for red blood cells of adult cattle, b. the older the samples are, the higher is the metal concentration, needed for activation.

Introduction

δ-Aminolevulinate dehydratase, E.C. 4.2.1.24. (ALAD) is an enzyme involved in an early step of porphyrin biosynthesis. It catalyzes the condensation of two molecules of δ-aminolevulinate yielding one molecule of porphobilinogen (PBG) and two molecules of H_2O. ALAD shows a good activity in red blood cells and has also been detected in a large variety of other animal and vegetable tissues. The properties of this enzyme are better known from animal sources. Wilson *et al.* [1] reported that ALAD from beef liver possesses 56 sulfhydryl groups per molecule, which can be activated by thiols. Because of the high affinity between sulfhydryl groups and metal ions – especially heavy metal ions – it can be expected that metals may be bound to the enzyme in its natural environment. There are numerous, but sometimes contradicting reports about the metal requirement of this enzyme [2]. Several authors reported copper as a cofactor of ALAD [2, 3]. For monovalent cations Sluiters-Scholten *et al.* [4] could not show any effect of potassium ions on the enzyme activity in leaves of *Phaseolus vulgaris*, although Schneider (spinach) [5] and Shetty and Miller (tobacco) [6] reported inhibition by potassium ions. This is also in contrast to the reported behaviour of ALAD from *Rhodopseudomonas spheroides*, in that the bacterial enzyme exhibited a monovalent cation requirement [7]. Some investigations indicate that zinc is involved in the activity of ALAD. Gurba *et al.* [8] reported that purified ALAD from beef liver contains approximately 1 g atom zinc per 275 000 g of protein. The zinc ion enhanced ALAD activity when incubated with enzyme preparations from rat liver [9]. On the contrary *in vitro* addition of $ZnCl_2$ to erythrocyte preparations, obtained from rats on low zinc diet, only slightly increased the level of the enzyme activity, while *in vivo* studies showed a significant decrease in enzymatic activity in rats fed low zinc diet [2]. From this the authors conclude that the requirement of this metal is at the site of synthesis of the enzyme.

To get more information about the effect of metal ions on ALAD it was the aim of this work to investigate the effect of zinc and that of the chemically related cadmium in protecting against losses of ALAD during storage of red blood cells.

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Materials and Methods

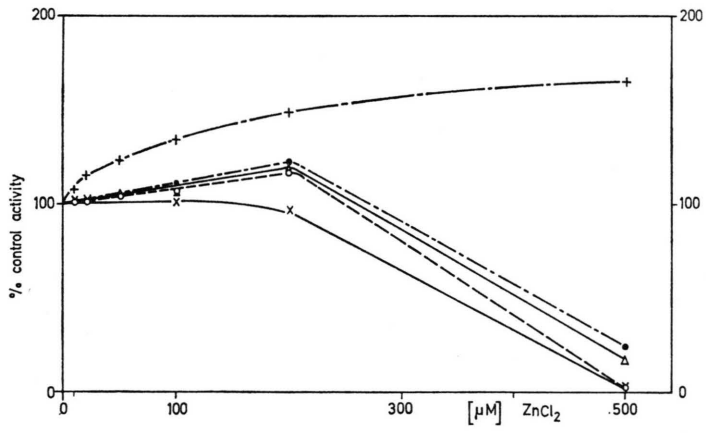
Blood of calves (12 weeks old) and adult cattle (14 years old) was collected with heparin. The plasma was removed and the cells were washed twice with icecold 0.85% NaCl solution. Hemolyzed erythrocytes (rapid freezing and thawing twice) were used as the source of the enzyme. Erythrocytes treated in this way but without storage are called "freshly isolated erythrocytes". For investigations of storage effects, the samples were stored at −30 °C. ALAD activity was tested as reported [10] with slight modifications. The assay mixture contained: HEPES-buffer pH 7,0, 150 μmol; $NaNO_3$, 6 μmol; $MgCl_2$, 6 μmol; ALA, 8 μmol; hemolyzed erythrocytes corresponding to a hemoglobin content of about 25 mg; different concentrations of $ZnCl_2$ and $CdCl_2$ (see results) in a final volume of 3 ml.

The incubation was run for 90 min at 30 °C. It was stopped by addition of 0.5 ml of a mixture containing TCA (28%) and $HgCl_2$ (1.9%). The amount of PBG was estimated according to Mauzerall and Granick [11]. The content of hemoglobin was analyzed with the aid of a Boehringer test combination, the amount of zinc, cadmium, and iron by atomic absorption spectrography. The experiments were repeated at least three times.

Results and Discussion

Fig. 1 shows the activity of ALAD from calf erythrocytes stored different times at −30 °C in relation to the zinc concentration. For freshly isolated erythrocytes there is no effect up to 100 μM Zn^{2+}; raising the zinc concentration results in a loss of activity, reaching zero at 500 μM Zn^{2+}. This finding is in accordance with results of other authors [2, 5]. Storage of erythrocytes leads to an enhancement of enzyme activity by Zn^{2+}, which is about 20% (200 μM Zn^{2+}) in samples stored up to three weeks. ALAD of six weeks old samples shows a more intensive activation by zinc ions. The highest PBG production (165% of the control) is mediated by a Zn^{2+} concentration of 500 μM that, by using freshly isolated erythrocytes, is followed by a complete inhibition. The effect of Cd^{2+} is still more pronounced (Fig. 2 a). For this ion an enhancement of PBG synthesis was measured in freshly isolated erythrocytes. The older the samples are, the more pronounced is the enhancement, and the higher is the optimum Cd^{2+} concentration. While the most intensive enhancement with fresh enzyme is at a Cd^{2+} concentration of 20 μM, after a week it is 50 μM, and after two weeks it is 100 μM (Fig. 2 b). In relation to this, the inhibition by higher Cd^{2+} concentrations is reduced: While a 200 μM Cd^{2+} solution at the beginning causes an inhibition of

Fig. 1. Effect of zinc on ALAD (calf). Age of erythrocytes (stored at −30 °C): ×, without storage; ○, 1 week; △, 2 weeks; ●, 3 weeks; +, 6 weeks.



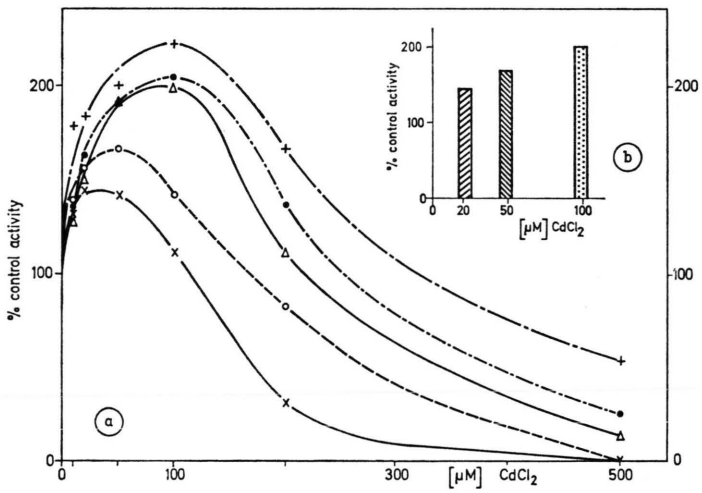


Fig. 2. a. Effect of cadmium on ALAD (calf). For symbols see Fig. 1; b. the respectively most effective cadmium concentration in relation to the age of erythrocytes (calf): ▨, without storage; ▧, 1 week; ⁞⁞⁞, 2 weeks.

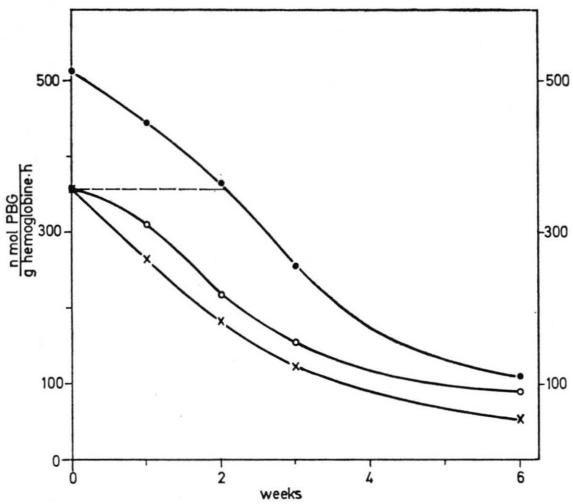
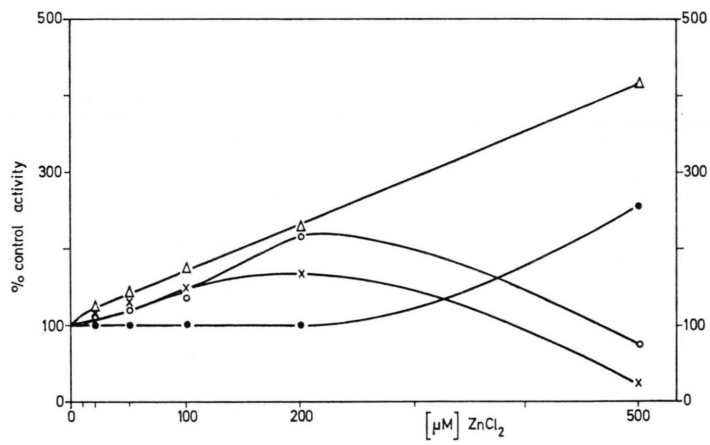


Fig. 3. Decrease of ALAD activity of untreated samples during storage, and the effect of zinc and cadmium (calf): ×, control; ○, zinc; ●, cadmium.

about 70%, the same concentration is still enhancing the enzyme of six weeks old erythrocytes. Fig. 3 shows the decrease of the PBG synthesis of the untreated controls during storage. This decrease of PBG synthesis can be reduced by Zn^{2+}, but not compensated. However, incubation with Cd^{2+} results in an enzyme activity that exceeds the control up to a period of two weeks. Only after this time the effect of storage exceeds the enhancement induced by Cd^{2+}. In Figs 4 – 6 the results of similar investigations, done with erythrocytes of adult cattle, are given. The effect of the metal ions is much more pronounced. For the range of concentrations investigated, a maximum of enhancement (420%) was found with 500 μM Zn^{2+} (Fig. 4). It is interesting that in this case the PBG production by freshly isolated erythrocytes is enhanced by Zn^{2+} (170% of the control) (Figs 4, 6). The change of

Fig. 4. Effect of zinc on ALAD (adult cattle). Age of erythrocytes (stored at −30 °C): ×, without storage; ○, 1 week; △, 2 weeks; ●, 4 weeks.



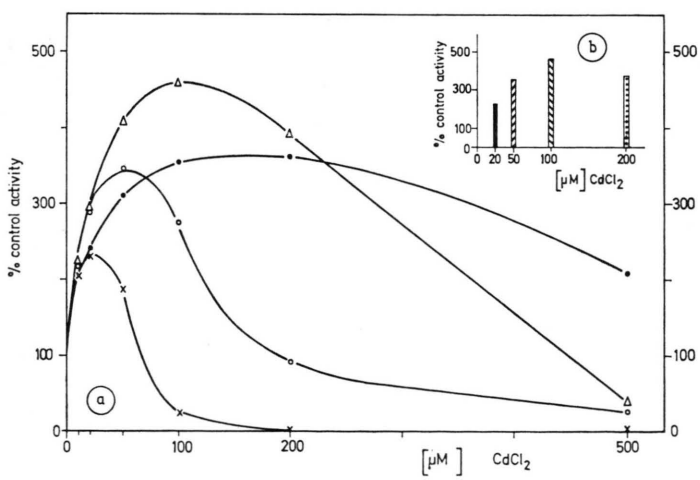


Fig. 5. a. Effect of cadmium on ALAD (adult cattle). For symbols see Fig. 4; b. the respectively most effective cadmium concentration in relation to the age of erythrocytes (adult cattle): ■, without storage; ▨, 1 week; ▧, 2 weeks; ⁞⁞, 4 weeks.

the cadmium effect is easy to recognize (Figs 5 a, b). A Cd^{2+} concentration of 200 μM that induced a complete inhibition using a freshly prepared suspension, two weeks later leads to an activity, which is 400% of the corresponding control (Fig. 5 a).

The results show a. that besides a possible essential role in catalysis the zinc ion probably acts in protecting against enzyme losses during storage and b. that this effect is not only restricted to zinc, but is still more pronounced using cadmium. This protection possibly could be due to a replacement

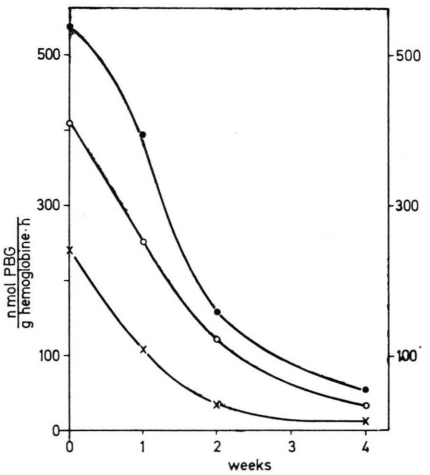


Fig. 6. Decrease of ALAD activity of untreated controls during storage, and the effect of zinc and cadmium (adult cattle): ×, control; ○, zinc; ●, cadmium.

of zinc ions lost during storage by added zinc and cadmium – but there was no significant enrichment of zinc in the suspension medium after thawing.

Table I. Fe, Zn, and Cd in washed red blood cells of calves, and adult cattle in relation to the hemoglobin content.

μg/g hemoglobin	Fe	Zn	Cd
calf (12 weeks)	7.0	0.2	–
adult cattle (14 years)	6.4	1.0	–

The quantitatively different reaction of the erythrocyte ALAD of different aged cattle is interesting too. In comparison Table I shows the content of Fe, Zn, and Cd of both samples of blood with respect to hemoglobin. While the content of iron is similar in both samples and cadmium could not be detected, the amount of zinc in washed erythrocytes differs. For the adult it is about five times higher than for the calf.

The mode of action of these quantitative different protection effects – perhaps due to an adaption to the higher zinc content of the red blood cells of adult cattle – must be subject of further investigations.

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