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Disturbances on Delta aminolevulinate dehydratase (ALA-D) enzyme activity by Pb^{2+}, Cd^{2+}, Cu^{2+}, Mg^{2+}, Zn^{2+}, Na^{+}, K^{+} and Li^{+}: analysis based on coordination geometry and acid–base Lewis capacity

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Abstract

ALA-D (EC 4.2.1.24) is the first cytosolic enzyme in the haem metabolic pathway. Some metals compete with its major cofactor Zn^{2+}, modifying both enzyme structure and function. Our purpose was to contribute to the understanding of the biochemical role of metals such as Pb^{2+}, Cd^{2+}, Cu^{2+}, Mg^{2+}, Zn^{2+}, Na^{+}, K^{+} and Li^{+} on ALA-D, using chicken embryos as experimental model. Mg^{2+} and Zn^{2+} showed enzyme activation in yolk sac membrane (YSM) (113% at 10^{-5} M Mg^{2+} and from 10^{-4} M Zn^{2+}), and slight inactivation in liver. Cd^{2+} and Cu^{2+} caused a non allosteric inhibition in both tissues (100% from 10^{-4} M). Surprisingly Pb^{2+} was not such a strong inhibitor. Interference of cations during the Schiff base formation in enzymatic catalysis process is explained considering their Lewis acid–base capacity, coordination geometry and electron configuration of valence. Interactions among monovalent cations and biochemical substances are governed chiefly by its electrostatic potential. 0.1 M K^{+} and 0.4 M Na^{+} produced 30% of enzymatic inhibition by the interference on interactions among the functional subunits. Li^{+} activated the YSM enzyme (130% at 10^{-5} M) due to a more specific interaction. This study may contribute to elucidate for the first time the possible structural differences between the YSM and liver enzymes from chicken embryo.

Keywords: Acid–base Lewis capacity; ALA-D; Coordination geometry; Haem; Metals

1. Introduction

Delta aminolevulinate dehydratase (ALA-D) is the first cytosolic enzyme in haem metabolic pathway and catalyses the synthesis of PBG. The enzyme is an oligomer formed by eight similar subunits of molecular weight (Mw) of approximate 35 k each (Mw 280 k) [1–3]. In most species, ALA-D appears as a metalloenzyme [4] with a highly preserved structure [5]. The metallic ion used varies according to the species [6,7], but most animal ALA-D use Zn^{2+} as metallic cofactor in catalysis [8].

The association of metals with macromolecules appears to subserve their biologic roles. The mechanism of action of heavy metals lies on their strong bonds with bases and phosphates from nucleic acids, and with –SH groups from proteins, modifying both their structure and functions [9].

Pb, Cd and Cu were selected for our study because they are well known environmental pollutants and highly toxic for living organisms [10–15]. In particular, the most prominent effect of Pb^{2+} poisoning is exerted

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on haem biosynthesis, producing symptoms similar to those observed in individuals with acute porphyria [16]. Levels of Pb^{2+} in the body are related to blood ALA-D activity [16]. On the other hand, despite its toxicity, Cu^{2+} is an essential cation with many physiological functions as a trace element [17–19]. Other cations chosen for our study, such as Mg^{2+} and Zn^{2+}, are essential trace minerals required for proper development in birds and mammals [20,21]. All these cations are, in general, structural components of several proteins and enzymes that stabilize the macromolecular structure or take part in catalysis.

Among monovalent cations with physiological roles, Na^+ and K^+ are the most abundant in mammalian species [22]. Li^+ is generally used in the treatment of some neurological disorders and is able to inhibit many enzymes [23]. Since some porphyrias present neurological manifestations, we believe it was interesting to evaluate the effect of Li^+ on ALA-D.

A number of studies tried to correlate the affinity and the coordination geometry of the metallic cation with the macromolecule binding site, and thus to establish the preferred configurations [24–28]. It has been proposed that elements with similar physicochemical properties would be biological antagonists [29]. However, the biochemical bases of such significant antagonism are largely unexplored. The aim of this work is to contribute to the understanding of the biochemical interactions of metals with ALA-D, using chicken embryos as experimental model. Studies carried out in chick embryos will be helpful in correlating data with effects of drugs in humans, since haem metabolic pathway is similar to that in birds [30]. We made our studies on YSM because it is one of the main sites of haem synthesis and erythropoiesis in the bird embryo, and a further site of haem production is the liver, playing a minor role in maintaining haemopoiesis in the embryo [31].

2. Materials and methods

2.1. Chemicals

ALA hydrochloride and reduced glutathione were purchased from Sigma Chemical Co. (St. Louis) MO, USA. Sephadex from Pharmacia (Upssala, Sweden). Disodium EDTA from Mallinckrodt. All other chemicals were of analytical grade.

2.2. Animals

Fertilized eggs from White Leghorn hens were supplied by the "Tres Arroyos Farm". They were incubated in a manual incubator, at 37 ± 1 °C and proper humidity conditions, 1 day after being laid. The eggs were turned twice a day.

Chicken embryos of 12 days of development were used. The YSM was separated and the yolk was removed by opening the sac. Livers were carefully removed from the embryo and both tissues were washed with Ringer solution (NaCl 9 g/l, KCl 0.4 g/l, $CaCl_2$ 0.25 g/l, pH 7.5) and dried smoothly on filter paper. Each liver and YSM was homogenized with 5 volumes of 0.25 M sucrose, and then centrifuged at 11,000*g* for 20 min at 4 °C. Supernatants were used as enzyme source.

2.3. Enzyme activity

Enzyme activity was measured according to Pauza [32], with the following modifications: Tris–HCl Buffer, 5 mM, pH 7, was used instead of Phosphate Buffer, because there are not known interference between Tris–HCl buffer and the cations studied, at the concentrations and pH used; 2-mercaptoethanol was eliminated and samples were incubated with metals for 5 min at 0 °C, prior to their incubation with the substrate. pH was tested before and after the addition of metal solution to the incubation mixture.

All assays were carried out in metal-free plastic test tubes.

2.4. Metal solutions

Na^+, K^+ and Mg^{2+} were used as chlorides; Cd^{2+} and Pb^{2+}, as acetates; and Li^+, Zn^{2+} and Cu^{2+}, as sulfates.

2.5. Demetalization and remetalization

The homogenate used as enzymatic source was incubated for 5 min at 0 °C with 0.1 M EDTA in order to sequester the metal from the holoenzyme. Reagent excess was eliminated by a Sephadex G-25 column, and separated in two portions. The apoenzyme was remetalized either with the corresponding Zn^{2+} or Mg^{2+} solutions.

2.6. Protein assay

Proteins were determined according to Bradford [33], using bovine serum albumin as standard.

2.7. Data analysis

- Linear regression analysis was performed using *Origin 6.0* Software.
- Determination of C_{50}: C_{50} values were calculated from percentage of inhibition vs –log metal concentration plots.
- Hill coefficients were obtained from Hill equation: $\log v/(V - v) = n \log (S) - \log K$ in absence and in presence of metal ions.