KINETICS OF METAL CHELATASE FROM RAT LIVER MITOCHONDRIA

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1. Introduction

The name metal chelatase has been deliberately chosen for the enzyme ferrochelatase (protohaem ferro-lyase, EC 4.99.1.1.). This enzyme preferentially catalyses the insertion of Fe^{2+} ions into porphyrins to form haems. However, Co^{2+} and Zn^{2+} are almost as active as Fe^{2+}. Many divalent cations including Mg^{2+}, Ca^{2+}, Ni^{2+}, Cd^{2+}, Pb^{2+} and Hg^{2+} inhibits this enzyme process [1–4]. The rate of non-enzymic incorporation of metal ions under the same experimental conditions is practically zero.

Metal chelatase has a wide distribution in a great number of aerobic cells. Its purification, however, is difficult and this was attributable to its chemical nature. Metal chelatase appears to be a structure-bound lipoprotein although a second water soluble enzyme has been detected in *Rhodopseudomonas spheroides* [5]. A 40 fold enrichment was obtained from rat liver mitochondria employing a rather time consuming procedure [6] and a similar degree of purification is reported here using the inner membrane fraction of freshly isolated rat liver mitochondria. Kinetic studies were performed and are of interest in relation to different K_m values reported for the insertion of Fe^{2+} into either protoporphyrin (8 μM) or mesoporphyrin (36 μM). These data suggest an influence of the porphyrin binding site on the metal binding site. The measurements, however, had to be carried out under anaerobic conditions which were not always reproducible. Therefore, Co^{2+} was employed and protoporphyrin IX served as the cosubstrate. This permitted assay of the metal chelatase activity much more precisely since anaerobic precautions could be omitted. The maximum deviation of this assay did not exceed 7%. The experimental data presented in this report strongly suggest a random binding of either substrate – i.e. Co^{2+} or protoporphyrin IX – to the metal chelatase. The respective K_m values proved to be independent of the concentration of the cosubstrate. The numerical K_m values were 8 μM for Co^{2+} and 3.6 μM for protoporphyrin IX.

2. Materials and methods

Female albino rats (Sprague-Dawley) weighing 150 ± 30 g were kept under normal laboratory conditions and were used without further treatment. Mitochondria were isolated in 0.25 M sucrose + 0.04 M tris–HCl buffer pH 7.2 [8]. The inner membrane fraction was prepared as in [9]. If this fraction was used for the metal chelatase preparation [10, 11], a 40 fold enrichment of this enzyme compared to the whole liver homogenate was obtained.

Haemin was isolated from bovine blood according to [12] and subsequent isolation of protoporphyrin was carried out exactly as described in [2, 13]. Tween 80, pure, and Triton X-100 were obtained from Serva, Heidelberg. All other chemicals were of analytical purity. Protein was estimated using the Lowry procedure [14]. Metal chelatase activity was determined by the dual wavelength assay developed by Jones [15]. The reaction rate was detected by

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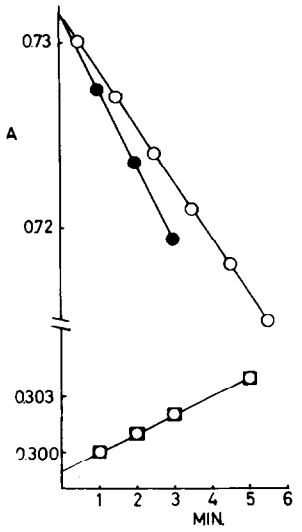

Fig. 1. Time dependence of metal chelatase reaction. 2.50 ml of the incubation medium contained: 9.2 mg mitochondrial metal chelatase from rat liver; 0.2 M tris–HCl buffer, pH 8.2; 1% Tween 80; 0.126 μM protoporphyrin IX. After 10 min of preincubation at 38° the reaction was started by adding 0.02 μmoles Co^{2+} dissolved in 0.02 ml H_2O. A_{505}(o—o) and A_{521}(□—□) were recorded after each min. Δ ($A_{505}-A_{521}$) (●—●) was determined from the slopes of absorbance at either wavelength. In accordance with [15] a differential millimolar coefficient of absorbance, ΔE, was calculated to be 7.32 $mM^{-1} \times cm^{-1}$. The specific activity was 0.43 nmole per min per mg protein.

measuring the absorbance at 505 nm and 521 nm in a Zeiss PMQ II spectrophotometer using a 1 cm light path cell. Co^{2+} and protoporphyrin IX served as substrates. The absorbance was recorded every min allowing a 30 sec interval for a convenient reading at each wavelength.

3. Results

The dependence of the time and the concentration of metal chelatase on the binding of Co^{2+} to protoporphyrin was examined in the first series of experiments. Enzyme concentrations up to 10 mg proved to synthesize haem linearily with the amount of added metal chelatase. Moreover, the reaction rate proceeded linearlily with time (fig. 1), which allowed an exact determination of the initial velocity V.

An attempt has been made to elucidate the reaction

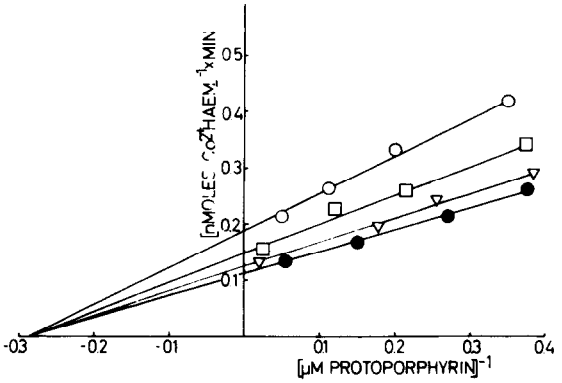

Fig. 2. Effect of varied protoporphyrin IX concentrations on the initial velocity of Co^{2+} haem synthesis in the presence of different Co^{2+} concentrations. Total incubation volume 2.52 ml; metal chelatase fraction (7.6 mg protein); 0.2 M tris–HCl buffer, pH 8.2; 1% Tween; temperature 38°; 10 min preincubation; Co^{2+} concentrations (μM): 4 o—o, 6 □—□, 8 ▽—▽, 10 ●—●.

mechanism of the Co^{2+} haem formation catalysed by metal chelatase. Differences of the initial velocity of this enzymic process were detected by varying the concentration of either substrate while the other remained constant [16]. Reciprocal numerical values of the initial velocity and of Co^{2+} of protoporphyrin concentrations were plotted in figs. 2 and 3. Obviously all straight lines are focussed in both diagrams on the abscissa. This would strongly suggest a random mechanism [17] of the Co^{2+} haem synthesis, which

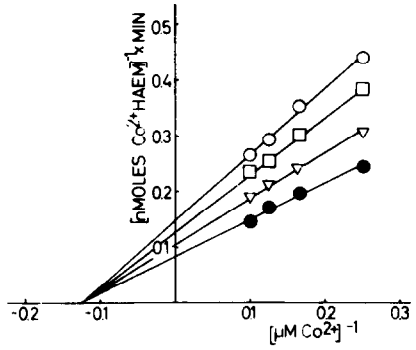

Fig. 3. Effect of varied Co^{2+} concentrations on the initial velocity of metal chelatase catalysed Co^{2+} haem formation, in the presence of different constant protoporphyrin IX concentrations. Protoporphyrin IX concentrations (μM): 2.5 o—o, 3.3 □—□, 5 ▽—▽, 10 ●—●; further incubation conditions were exactly the same as described in fig. 2.

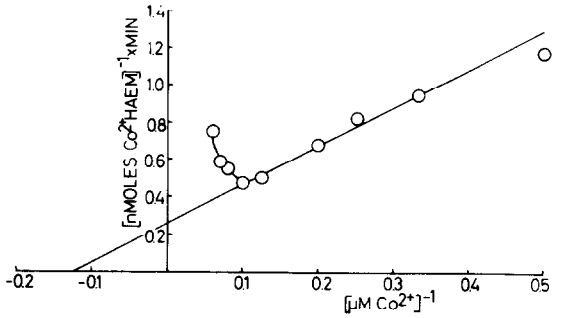

Fig. 4. Co^{2+} induced inhibition of Co^{2+} haem synthesis catalysed by mitochondrial metal chelatase isolated from rat liver. For incubation conditions see figs. 1 and 2. The concentration of protoporphyrin IX was 0.2 μM and 9.2 mg of the metal chelatase preparation was used.

means, the attachment of either substrate to the enzyme is arbitrary. The respective K_m values remained constant if the concentrations of the cosubstrates were changed.

The numerical K_m values were graphically determined; the K_m or protoporphyrin IX was 3.6 μM and that of Co^{2+} was 8 μM. Co^{2+} concentrations above 10 μM caused an inhibition of this enzymic reaction (fig. 4).

4. Discussion

The two step procedure employing the isolation of the inner mitochondrial membrane fraction and the subsequent detachment of metal chelatase, yielded a 40 fold enriched enzyme. A further purification of the inner mitochondrial membrane fraction would most probably improve the purity of metal chelatase. This is a convenient method compared to the procedure involving ammonium sulfate precipitation, gel filtration and DEAE chromatography described by Mazanovska et al. [6], which resulted in approximately the same enrichment. The kinetic data presented here, appear to exclude an influence of the porphyrin binding site on the metal binding site of the enzyme, since evidence for a random mechanism is quite convincing. The reaction mechanism and the K_m values for Co^{2+} (11 μM) and mesoporphyrin (5 μM), using a bacterial enzyme, [5] show striking indentity with our data obtained from studies with metal chelatase isolated from rat liver mitochondria.

Acknowledgement

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