

Effect of heme on the activity of chick embryo liver mitochondrial δ -aminolevulinate synthase

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We have examined the effect of heme on the activity of native δ -aminolevulinate synthase isolated from drug-induced chick embryo liver mitochondria. The enzyme was not inhibited by concentrations of heme up to 1 mM and this finding makes it improbable that heme acts physiologically to control mitochondrial δ -aminolevulinate synthase activity.

<i>Heme</i>	<i>Hemin</i>	<i>δ-Aminolevulinate synthase</i>	<i>Enzyme activity</i>
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1. INTRODUCTION

Hepatic δ -aminolevulinate (ALA) synthase is the first and rate-limiting enzyme in the heme biosynthetic pathway, and its level is tightly regulated by heme, the end-product of the pathway [1]. The regulation by heme has been proposed to occur at 3 levels: repression of ALA-synthase mRNA synthesis [2], inhibition of translocation of enzyme into mitochondria [3] and inhibition of mitochondrial ALA-synthase activity [4,5]. The latter role for heme in inhibiting the activity of ALA-synthase is attractive since both ALA-synthase and heme synthase, the enzyme which catalyses the final step of heme biosynthesis, are located in the mitochondria.

However, there have been conflicting views [4–7] regarding such a role for hepatic mitochondrial heme and the issue has not been resolved. In studies where this proposed role for heme has been investigated, the preparations of ALA-synthase used have not been those of the native mitochondrial enzyme. Authors in [4] used partially purified rat cytosolic enzyme while authors in both [6] and [5] used mitochondrial enzyme preparations which are now known to have been breakdown products [8]. Moreover, in these studies, the effect on ALA-

synthase activity was investigated using hemin (ferriprotoporphyrin IX) rather than heme (ferriprotoporphyrin IX). In the final step of heme biosynthesis, heme synthase catalyses the incorporation of Fe^{2+} into protoporphyrin IX and it seems likely, therefore, that it is heme (rather than hemin) which exists in mitochondria.

Recently, we purified to homogeneity the native intact form of ALA-synthase from chick embryo liver mitochondria [8]. We report here the effect of heme and hemin on the activity of this enzyme.

2. MATERIALS AND METHODS

Succinyl-CoA, pyridoxal 5'-phosphate, glycine and trizma-base were purchased from Sigma (St. Louis, MO). Hemin (ferriprotoporphyrin IX chloride) was purchased from Porphyrin Products, USA. All other reagents were of the purest grade available.

Mitochondrial ALA-synthase was purified to near homogeneity from drug-induced chick embryo liver as in [8] except that the activity from the chromatofocusing step was collected over a narrow range and the final step of CoA-affinity chromatography was omitted. The enzyme was judged to be at least 95% pure by SDS-polyacrylamide gel electrophoresis with a single major protein band of minimum M_r 68 000.

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ALA-synthase activity was measured colorimetrically [9] using succinyl-CoA (100 μ M) together with Tris-HCl (100 μ M), glycine (100 mM) and pyridoxal 5'-phosphate (250 μ M) in a total volume of 150 μ l at pH 7.6 and incubated for 10 min at 37°C. Under these conditions the assay was linear for at least 15 min. Separate experiments established that there was no degradation of the purified ALA-synthase during the course of enzyme activity assays.

Hemin was solubilized immediately prior to use as in [10]. Hemin in the assay mix was reduced quantitatively to heme (ferroprotoporphyrin IX chloride) using 2 mM sodium dithionite and the conversion estimated by the pyridine hemochrome assay [11].

3. RESULTS AND DISCUSSION

ALA-synthase was purified from liver mitochondria of drug-induced chick embryos (see section 2). The enzyme had a minimum M_r of 68 000 and we have previously established [8] that this form of the enzyme is the native undegraded form.

The purified enzyme was markedly stimulated by either Mg^{2+} or Na^+ (fig.1). A 7-fold activation by Mg^{2+} was observed at 250 mM while Na^+ at 500 mM gave a 5-fold stimulation. This degree of activation by these cations is greater than that reported in [4,5] for other preparations of ALA-synthase (details will be published elsewhere).

The effect of heme and hemin on the activity of ALA-synthase was investigated under optimal conditions of Na^+ or Mg^{2+} (table 1). Hemin had no effect on activity at concentrations up to 25 μ M. Some inhibition was observed at higher concentrations but this effect plateaued and the enzyme was inhibited by only 25% with 1 mM hemin. When hemin was reduced to heme [11] and the effect of the latter on ALA-synthase activity determined, no inhibition was observed at concentrations up to 1 mM. The possibility that metal ions might somehow prevent an inhibitory action of heme (or hemin) was eliminated by separate experiments which showed that, in the absence of activating metal ions, ALA-synthase activity was not affected by concentrations of heme (or hemin) up to 1 mM (not shown).

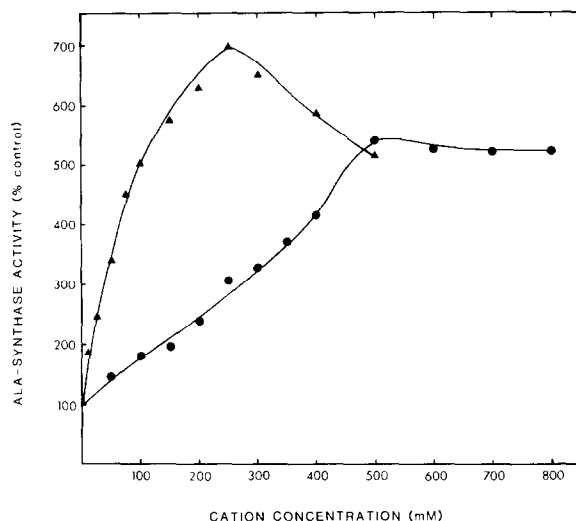


Fig. 1. Effect of $MgSO_4$ ($\blacktriangle$) and $NaCl$ ($\bullet$) on ALA-synthase activity. Enzyme was assayed in the presence of increasing $MgSO_4$ or $NaCl$ concentrations. Control enzyme samples were assayed without $MgSO_4$ or $NaCl$. These values represent the means of at least 3 separate determinations.

Table 1

Effect of hemin and heme on ALA-synthase activity

Hemin	ALA-synthase activity (% control)		Heme	ALA-synthase activity (% control)	
	A	B		A	B
0	100	100	0	100	100
100 nM	104	102	100 nM	103	102
500 nM	100	100	500 nM	98	106
1 μ M	100	101	1 μ M	97	109
10 μ M	101	107	10 μ M	100	103
25 μ M	102	91	25 μ M	101	104
50 μ M	86	88	50 μ M	98	103
100 μ M	75	76	100 μ M	101	100
1 mM	74	77	1 mM	96	97

Enzyme was assayed in the presence of either 250 mM $MgSO_4$ (A) or 500 mM $NaCl$ (B). Hemin in the assay mix was reduced to heme using 2 mM sodium dithionite. Control enzyme samples were assayed without hemin or with 2 mM sodium dithionite alone. The values represent the means of at least 3 separate determinations

Although the concentration of free heme that exists in the mitochondria is not known, the level of free heme in liver cytosol has been estimated to be about 10 nM [12]. The synthesis of ALA-synthase by isolated chick embryo hepatocytes is inhibited by concentrations of this order [13]. At high concentrations of hemin ($> 20 \mu\text{M}$) there is a general toxic effect on protein synthesis in isolated chick embryo hepatocytes (unpublished) and it seems, therefore, unlikely that heme concentrations of this magnitude exist in mitochondria. Moreover, the results in [7] do not support the idea of ALA-synthase being inhibited by high local concentrations of heme.

Here, heme concentrations up to 1 mM have no effect on that activity of ALA-synthase and since heme levels in mitochondria are likely to be considerably less than this, it can be concluded that heme *in vivo* does not act as a feedback inhibitor on the activity of ALA-synthase.

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