Evidence of hemin as an end product inhibitor of L-alanine: 4,5-dioxovalerate transaminase in rat liver mitochondria

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This study describes the in vitro and in vivo effect of hemin on L-alanine:4,5-dioxovalerate transaminase activity. Hemin was shown to be an inhibitor of the purified enzyme and this inhibition was proportional to the concentration of hemin. The examined kinetic data with hemin showed uncompetitive inhibition for both alanine and 4,5-dioxovalerate. An apparent K_i of 30 and 42 μM for hemin were obtained with both alanine and 4,5-dioxovalerate, respectively. Moreover, the enzyme activity in livér was considerably decreased after the intravenous hemin administration and such an inhibition is dose and time dependent. Furthermore, maximum inhibition of the enzyme was observed 30 min after hemin injection and 60% enzyme inhibition was achieved with a dose of 1.2 mg/kg body wt of rat. Thus is suggests the important role of this enzyme on heme biosynthesis.

L-Alanine: 4,5-dioxovalerate transaminase *Heme biosynthesis* *Hemin* *End product inhibitor*

1. INTRODUCTION

The formation of δ-aminolevulinic acid (ALA), the first committed precursor of heme, is now considered to be mediated by L-alanine: 4,5-dioxovalerate transaminase (EC 2.6.1.43) in addition to the conventional pathway by ALA synthetase (EC 2.3.1.37) in mammals [1–4]. The enzyme alanine: 4,5-dioxovalerate transaminase which catalyses the formation of ALA via an irreversible transamination reaction is functionally located inside mitochondria and it is more efficient than ALA synthetase pathway, isolated from the same source [1,5]. In addition, Okuno et al. [6] established the enzymatic biosynthesis of 4,5-dioxovalerate by 4-oxo-5-hydroxyvalerate dehydrogenase in rat liver and kidney. Furthermore, incorporation of radioactive 4,5-dioxovaleric acid in porphyrin and heme by intact respiring rat hepatocytes [7] indicates the significant role of alanine: 4,5-dioxovalerate transaminase in heme biosynthesis.

It is also well established that the primary site for regulation of the overall activity of heme biosynthesis in animals is at the level of ALA formation in mitochondria [8,9]. The important fact is that though extensive studies have been done on the regulatory role of ALA synthetase on heme biosynthesis [10–17], nothing is yet known about the regulation of L-alanine: 4,5-dioxovalerate transaminase. Thus, to address this question, we examined the possible regulatory role of hemin, the end product of the mitochondrial L-alanine: 4,5-dioxovalerate transaminase pathway in both in vitro and in vivo rat liver.

2. MATERIALS AND METHODS

2.1. *Chemicals*

δ-Aminolevulinic acid, porphobilinogen, protoporphyrin, hemin and bilirubin were purchased from Sigma (St. Louis, MO). 3,5-Dibromolevu-

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linic acid was from Porphyrin products (Logan, UT). 4,5-Dioxovalerate was synthesized as described in [1].

2.2. *Conditioning of rats*

Male Wistar rats about 60 g were fasted for 24 h prior to injections with free access to water. Hemin was dissolved as described [17] and injected into tail vein within 1 h after preparation. Animals were killed by decapitation.

2.3. *Enzyme source*

Alanine:4,5-dioxovalerate transaminase was purified to homogeneity according to our previous paper [3]. For mitochondrial enzyme the livers were homogenized in 5 vols of 20 mM potassium phosphate buffer, pH 7.4, containing 0.25 M sucrose at 4°C. Then mitochondria were isolated as in [18]. The packed mitochondria were suspended in 20 mM potassium phosphate buffer, pH 7.4, containing 10% glycerol, freeze-thawed, sonicated and centrifuged at 10000 × *g* for 30 min. The supernatant was used for the enzyme assay.

2.4. *Enzyme assay*

Alanine:4,5-dioxovalerate transaminase was assayed as in [1]. Protein was determined by the method in [19].

Table 1

Effect of some intermediary compounds of heme metabolism on the activity of alanine:4,5-dioxovalerate transaminase[a]

Metabolite	Concentration (μM)	Specific activity (μmol ALA formed/mg protein per 30 min)
–	–	13.36
Aminolevulinic acid	50	12.70 (5)
	100	12.41 (7)
	200	12.33 (7)
Porphobilinogen	50	13.19 (1)
	100	13.00 (3)
	200	12.82 (4)
Protoporphyrin	50	12.80 (4)
	100	12.14 (9)
	200	11.37 (15)
Bilirubin	50	13.36 (0)
	100	11.82 (12)
	200	10.94 (22)

[a] The purified enzyme was first preincubated for 5 min at 37°C with the metabolites. The treated fractions were immediately diluted with the assay substrates. The conditions were the same as mentioned in section 2

Numbers in parentheses indicate % inhibition

3. RESULTS AND DISCUSSION

3.1. *Effect of some intermediate compounds of heme metabolism on purified alanine:4,5-dioxovalerate transaminase*

This study has been undertaken to determine the effect of some of the intermediate compounds of heme metabolism on purified alanine:4,5-dioxovalerate transaminase (table 1). Appropriate control reactions were carried out simultaneously. Our findings clearly indicate that none of these compounds have any significant effect on purified alanine:4,5-dioxovalerate transaminase activity.

3.2. *Inhibition of purified alanine:4,5-dioxovalerate transaminase by hemin*

Several experiments were carried out in which effect of hemin was studied on the activity of alanine:4,5-dioxovalerate transaminase. As shown in fig.1, hemin inhibited the purified enzyme and such inhibition was greater at higher concentrations of hemin. An inhibition of 20% was observed with the addition of 5 μM hemin whereas 50% inhibition was obtained with 50 μM of hemin addition.

When ALA formation was measured as a function of alanine or 4,5-dioxovalerate concentrations in the presence of hemin at either 0.05 or 0.1 mM, parallel lines typical of uncompetitive inhibition for both the substrates were generated (fig.2a,b). Hemin for alanine:4,5-dioxovalerate transaminase exhibited a K_i of 30 μM with different concentrations of alanine, at fixed 4,5-dioxovalerate concentration. However, when 4,5-dioxovalerate concentration was varied, at fixed alanine concentration, the K_i was 42 μM. It is evident from our studies that only hemin among the other intermediate metabolites acts as inhibitor of this enzyme.

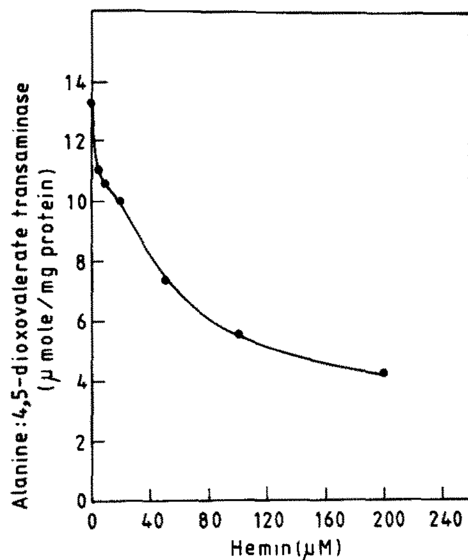

Fig.1. Inhibition of purified alanine:4,5-dioxovalerate transaminase activity by exogenous hemin. The enzyme was preincubated with different concentrations of hemin for 5 min and was assayed.

3.3. *Effect of hemin administration on the activity of L-alanine:4,5-dioxovalerate transaminase in rat liver mitochondria*

To examine whether the observed regulatory action of hemin really has any physiological significance in normal rats, the effect of hemin administration on the activity of alanine:4,5-dioxovalerate transaminase has been studied. Hemin at 0.8 mg/kg body wt was administered intravenously to rats and the time course study was carried out up to 2 h (fig.3). The activity of the enzyme decreased rapidly and the maximum inhibition of 42% was observed after 30 min. Furthermore, when rats were given various doses of hemin injections the enzyme activity decreased progressively with the increasing hemin doses (fig.4) and such inhibition appeared to be nearly maximal at a hemin dose of 1.2 mg/kg body wt.

This is the first report which shows in vitro and in vivo inhibition of alanine:4,5-dioxovalerate transaminase by hemin, the end product, suggesting the regulatory role of this enzyme on heme biosynthesis. It is important to mention the previous findings [20,21] that the administration of hemin resulted in a rapid decline of ALA in serum. Therefore, the inhibition of alanine:4,5-dioxovalerate transaminase by heme, which is synthesized intramitochondrially [22], may be a potential regulatory factor for heme biosynthesis by means of feedback inhibition. Moreover, generation of heme in mitochondrial preparations [23] at least 75-times the basal rate occurring in vivo does not change the ALA synthetase activity and it was concluded that end product inhibition of ALA synthetase activity by heme is not an important physiological mechanism for regulation of hepatic heme biosynthesis. Thus, considering the above reports it may be reasonable to assume that inhibition of alanine:4,5-dioxo-

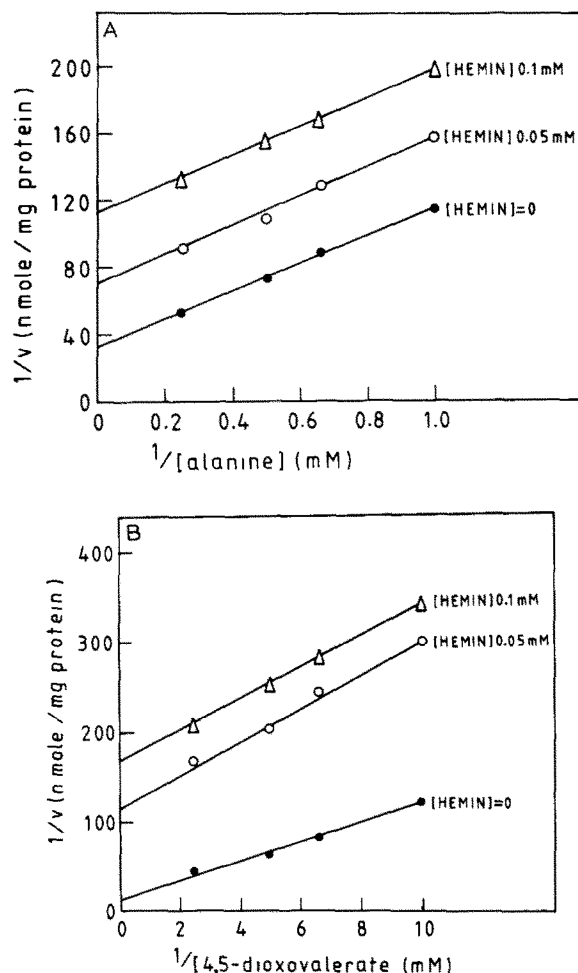

Fig.2. Hemin as an end product inhibitor: (a) plot of $1/v$ against 1/(alanine),(dioxovalerate) = 0.2 mM. (b) Plot of $1/v$ against 1/(dioxovalerate),(alanine) = 20 mM.

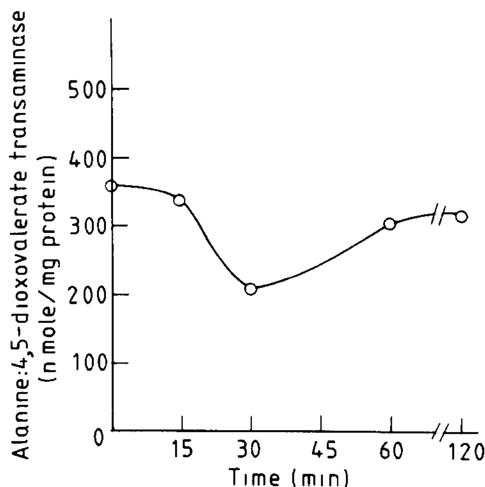

Fig.3. Effect of hemin administration on L-alanine:4,5-dioxovalerate transaminase activity in rat liver mitochondria. Rats were given (0.8 mg/kg body wt) injections intravenously and were killed for enzyme assay at the times indicated. Each point represents the value obtained for pooled livers of 6 rats.

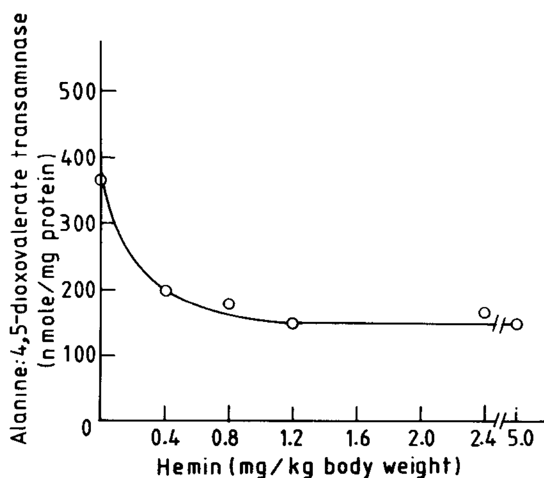

Fig.4. Effect of hemin administration on L-alanine:4,5-dioxovalerate transaminase activity in rat liver mitochondria. Rats were given indicated doses of hemin intravenously and sacrificed 30 min later for enzyme assay. Other conditions are as described for fig.3.

valerate transaminase by heme is an important physiological mechanism for regulation of hepatic heme biosynthesis.

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