THE PORPHOBILINOGEN DEAMINASE - UROPORPHYRINOGEN III COSYNTHETASE SYSTEM (PORPHOBILINOGENASE) FROM BOVINE LIVER. KINETIC STUDIES *

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1. Introduction

The conversion of prophobilinogen (PBG) into uroporphyrinogens is enzymically catalysed by the porphobilinogenase enzyme system which is widely distributed [1–10]. In plant tissue extracts [11] the formation of uroporphyrinogen I from PBG is brought about by PBG deaminase (Uroporphyrinogen I synthetase); in the presence of both PBG deaminase and uroporphyrinogen III cosynthetase (isomerase) uroporphyrinogen III, the physiological intermediate in haem biosynthesis, is formed. Here we report kinetic studies using purified PBG deaminase and porphobilinogenase. The effects of NH_4^+ ion concentrations on the type of uroporphyrinogen formed are also reported. The results indicate that bovine liver porphobilinogenase shows cooperative effects.

2. Materials and methods

PBG was obtained by the method of Sancovich et al. [12] and estimated as described by Mauzerall and Granick [13].

The preparation of both purified bovine liver porphobilinogenase and PBG deaminase and the estimation of enzymic activities, as well as all other methods and materials not specified here were those described in ref. [14].

3. Results and discussion

3.1. *Kinetic studies*

Direct studies were done on porphobilinogenase and PBG deaminase preparations, in the presence and absence of NH_4^+ ions; the latter are known to inhibit porphobilinogenase [15]. Direct studies could not be done on the isomerase because one of its substrates, probably a polypyrrolic compound, has not been defined. The other substrate is PBG.

3.2. *Kinetics of PBG deaminase*

Direct and reciprocal plots of reaction velocity against PBG concentration, both in the absence and the presence of NH_4^+ ions, show classical Michaelis-Menten kinetics. Ammonium ions at concentrations of 0.001 M to 0.2 M behaved as non-competitive inhibitors. For PBG, the apparent $K_m = 5 \times 10^{-6}$ M was independent of NH_4^+ ion concentration and the Hill coefficient [16], n, was near 1 (table 1). For NH_4^+ ions, K_i was estimated to be 0.172 M. However, at concentrations which markedly inhibited uroporphyrinogen I formation, NH_4^+ ions had much less effect on PBG consumption (table 2).

* The trivial name "porphobilinogenase", suggested by Lockwood and Rimington [1] is used to designate the porphobilinogen deaminase-uroporphyrinogen III cosynthetase system.

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Table 1
Hill coefficients and apparent K_m for PBG

Enzyme and addition	Hill coefficient n	Apparent K_m (μM)
1. PBG deaminase		
none	1.0	5.0
NH_4^+ 0.01 M	1.0	5.0
NH_4^+ 0.1 M	0.9	5.0
2. Porphobilinogenase		
none	1.7–1.97	11–14
NH_4^+ 0.0001 M	2.0	10
NH_4^+ 0.001 M	1.85	10
NH_4^+ 0.01 M	1.77	7
NH_4^+ 0.1 M	1.0	5
heated enzyme	1.0	5
dialysed enzyme	1.0	5.5

Activities were assayed as described in Methods [14]. PGB deaminase preparation used as 300-fold purified and porphobilinogenase was a preparation 180-fold purified. "Heated enzyme" was porphobilinogenase heated 20 min at 65–70° and "dialysed enzyme" was the supernatant obtained from a preparation of porphobilinogenase dialysed 24 hr, against glass-distilled water. n was calculated from the slopes of the plot of $\log(v/(V-v))$ against log [PBG].

3.3. *Kinetics of porphobilinogenase*

Plots of the rate of uroporphyrinogen formation against PBG concentration were sigmoidal at all enzyme concentrations studied (fig. 1) and reciprocal plots were not linear. In the absence of NH_4^+ ions, the apparent K_m values for PBG were in the range $(11-14) \times 10^{-6}$ M and n tended to 2 (table 1). However, when the enzyme preparation was heated or dialysed against distilled water, or NH_4^+ ions were added to the reaction mixture, the shape of the substrate saturation curve was altered from sigmoidal to hyperbolic (fig. 1A,B). At the same time there was an increase in the affinity for PBG, the apparent K_m tending to 5×10^{-6} M and n becoming equal to 1 (table 1), the same values as were found for PBG deaminase.

3.4. *The effect of ammonium ions*

The effect of varying NH_4^+ ion concentrations on the porphobilinogenase reaction was studied at fixed PBG concentration (table 2). At 0.2 M NH_4^+ uroporphyrinogen III formation was 89% inhibited while the formation of total uroporphyrinogen was only inhibited by 44% indicating a greater effect on the isomer-

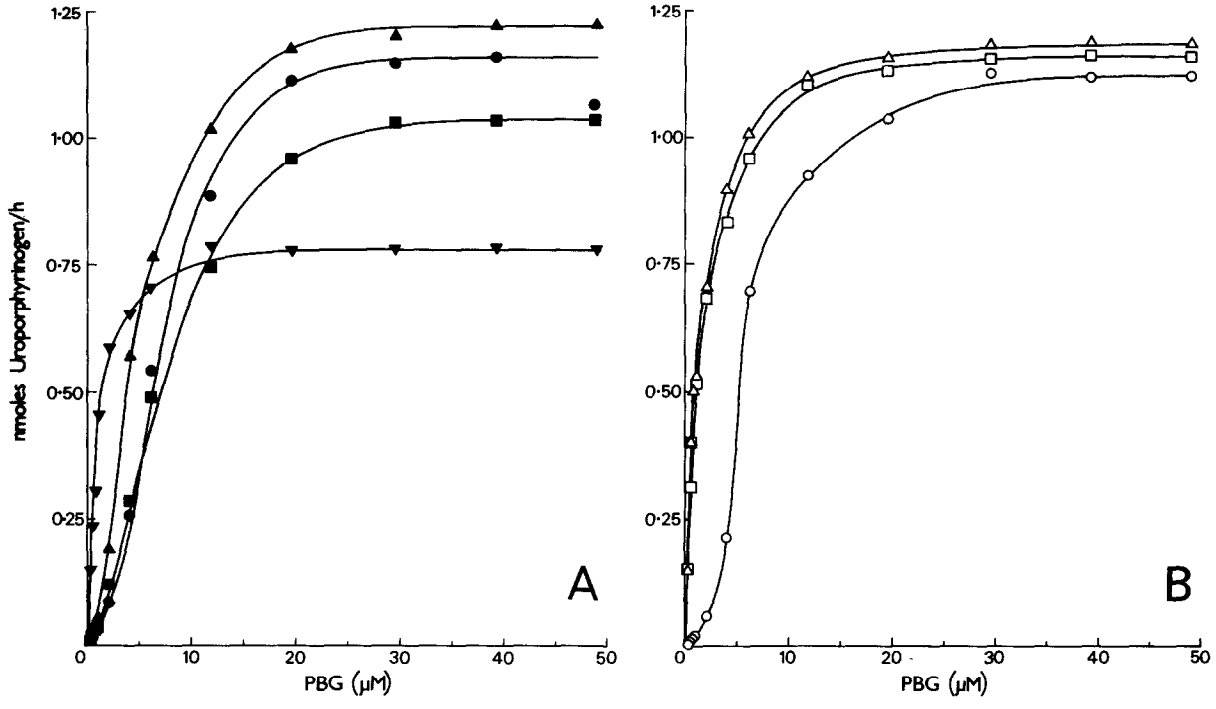

Fig. 1. Effect of porphobilinogen (PBG) concentration on the activity of porphobilinogenase. A, at different NH_4^+ concentrations: ▼—▼, 0.1 M NH_4^+; ■—■, 0.01 M NH_4^+; ●—●, 0.001 M NH_4^+; ▲—▲, 0.0001 M NH_4^+. B, ○—○, no addition; □—□, enzyme heated for 20 min at 65–70°; △—△, enzyme dialysed against glass-distilled water.

Table 2
Effects of ammonium ions

Enzyme and addition	PBG consumption (%)	Porphyrinogen yields (%)	Total porphyrinogen formed		Uroporphyrinogen III formed	
			nmoles/h	inhibition (%)	nmoles/h	inhibition (%)
1. PBG deaminase						
None	100	100				
NH_4^+ 0.01 M	100	78				
0.05 M	95	70				
0.10 M	92	66				
0.20 M	85	57				
2. Porphobilinogenase						
None			0.530	0	0.530	0
NH_4^+ 0.2 M			0.298	44	0.060	89
0.1 M			0.349	34	0.087	84
0.05 M			0.369	30	0.143	73
0.01 M			0.465	12	0.278	48
0.005 M			0.520		0.360	32
0.001 M			0.536		0.428	19
0.0005 M			0.537		0.537	
0.0001 M			0.575		0.575	
0.00001 M			0.530		0.530	

The PBG concentration was 30 μM in the PBG deaminase and in the porphobilinogenase experiments. PBG consumption of control was taken as 100%. PBG deaminase and porphobilinogenase 150-fold purified preparations were used. Assay conditions were as described [14]. Identification, quantitative determination, and isomeric analysis of uroporphyrinogens formed were carried out by the usual methods [14].

ase than on the PBG deaminase parts of the prophobilinogenase system. The inhibition of the isomerase was competitive, K_i = 0.01 M, and could be reversed by removing NH_4^+ ions.

3.5. *Conclusions*

The most important fact which has emerged from the experiments reported here is that, for bovine liver porphobilinogenase, the rate of uroporphyrinogen III formation is sigmoidally related to the PBG concentration, in the absence of NH_4^+ ions. Since no cooperative effects were observed in the PBG deaminase reaction, it is suggested that the cooperative effects seen in the porphobilinogenase reaction facilitate the binding of PBG to the isomerase part of the system.

When NH_4^+ ions were present, or when the porphobilinogenase was heated or dialysed against glass-distilled water, the homotropic interactions were lost, the affinity of the enzyme for PBG was increased and uroporphyrinogen I was formed instead of uroporphyrinogen III. Ammonium ions inhibited the PBG deaminase part of the system non-competitively but inhibited the isomerase part competitively.

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