NEGATIVE HOMOTROPIC KINETICS OF SOYBEAN CALLUS PORPHOBILINOGEN DEAMINASE-UROPORPHYRINOGEN III COSYNTHETASE*

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1. Introduction

The conversion of porphobilinogen (PBG) into uroporphyrinogens is catalyzed by two enzymes, PBG deaminase and uroporphyrinogen III cosynthetase. Deaminase alone forms uroporphyrinogen I from PBG, while cosynthetase alone has no action on either PBG or uroporphyrinogen I; in the presence of both deaminase and cosynthetase, uroporphyrinogen III is formed.

The sigmoid response of bovine liver porphobilinogenase [2] has prompted Sancovich, Batlle and Grinstein [2, 3] to classify it as an allosteric enzyme, indicating that porphobilinogenase is well adapted for regulation by its substrate. Data described in this report are in agreement with this assumption, but they also show many of the unusual characteristics of negative cooperativity [4–6].

Here we report kinetic studies using purified deaminase and porphobilinogenase, in the presence and absence of ammonium ions, which are known to inhibit these enzymes [2, 3].

2. Material and methods

PBG was obtained biosynthetically [7]. For measurement of enzyme activity, the incubation system contained: the enzyme preparation (usually 8 ml) together with 0.1 M tris-HCl buffer, pH 7.2, and PBG, with or without the addition of ammonium ions, in a final volume of 10 ml. Incubations were anaerobic, in the dark, with mechanical shaking, at 38°. Blanks were run with PBG and without enzyme. After incubation trichloroacetic acid was added to precipitate the protein (final concentration 5%, w/v), the mixture was then exposed to 50 ft. candella of white light for 20 min to oxidize phyrinogens, the protein precipitate was filtered off and total porphyrins estimated in the resulting solution. Reaction velocity is expressed as nmoles of uroporphyrinogens formed in one hr.

The purification procedures for porphobilinogenase and deaminase, as well as all other methods and materials not specified here, were those described in [8 and 9].

3. Results and discussion

3.1. *Saturation kinetics*

The saturation curves of porphobilinogenase and deaminase were quite unusual (fig. 1). In the absence of ammonium ions, saturation kinetics of both enzymes were much alike; at low substrate concentrations Michaelis-Menten type curves were observed while at higher PBG concentrations second curves were also seen; these data suggest the occurrence of two reactions, one saturating at low PBG concentrations and the other saturating at higher concentrations of PBG. Further even at high PBG concentrations, the

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

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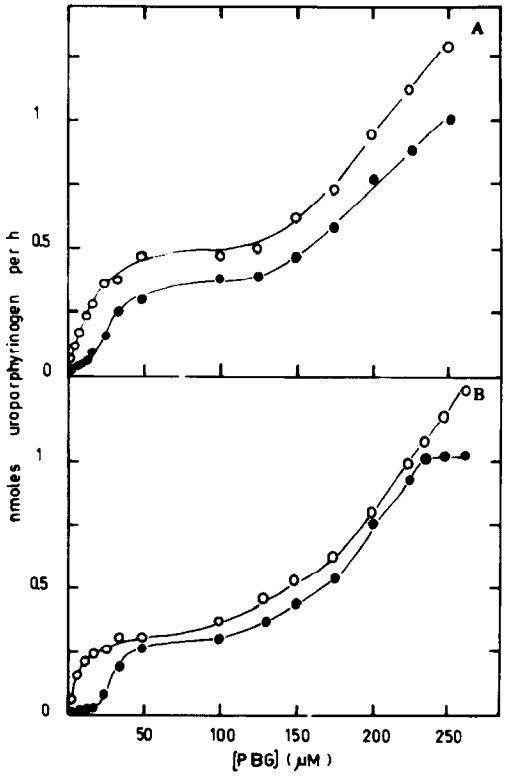

Fig. 1. The effect of PBG concentration on the rate of uroporphyrinogen formation catalysed by A) 36-fold purified deaminase and B) 30-fold purified porphobilinogenase from soybean callus, in the absence (o——o) and the presence (•——•) of 0.1 M ammonium ions. Assay conditions were as described in the text.

enzymes did not become saturated since the velocities continued to increase. In the presence of ammonium ions, the rate-concentration plots become sigmoidal, as far as the first reactions were concerned, but the effect on the second reactions was different, thus a second velocity plateau was reached with porphobilinogenase at high concentrations of PBG (fig. 1B), but saturation was not attained by the deaminase (fig. 1A).

3.2. *Lineweaver-Burk plots, Hill coefficients and R_s values*

Double reciprocal plots (fig. 2) were non-linear. They appear to consist of two portions with different slopes that yielded two apparent K_m values at the extremes of PBG concentration (table 1). From data in

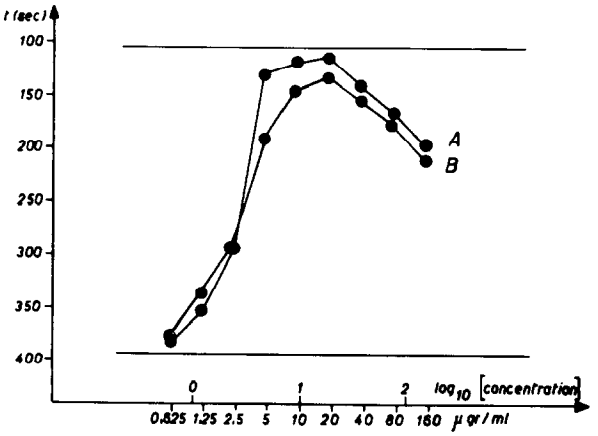

Fig. 2. Double reciprocal plots of the rate of uroporphyrinogen formation with respect to PBG concentration catalysed by A) 36-fold purified deaminase and B) 30-fold purified, porphobilinogenase from soybean callus in the absence (o——o) and presence (•——•) of 0.1 M ammonium ions. Assay conditions were as described in the text.

fig. 2B, it can be seen that for porphobilinogenase, K_m values for the second reaction, $K_{m_2} = 5 \times 10^{-4}$ M, were the same in the absence and presence of ammonium ions, but ammonium ions changed K_{m_1} without modifying V_{max}, showing clear competitive inhibition.

Table 1
Kinetic constants of soybean callus porphobilinogenase and deaminase.

Enzymes and addition	Kinetic constants	
	K_{m1} (M)	K_{m2} (M)
Deaminase		
None	3.2×10^{-5}	1.9×10^{-4}
0.1 M ammonium ions	3.8×10^{-5}	5.0×10^{-4}
Porphobilinogenase		
None	4.5×10^{-6}	5.0×10^{-4}
0.1 M ammonium ions	3.0×10^{-5}	5.0×10^{-4}

Activities were assayed as described in material and methods. 35–40 fold purified deaminase and 30–35 fold purified porphobilinogenase preparations were used. Michaelis constants were calculated from Lineweaver-Burk plots and they are mean values of six determinations.

For deaminase (fig. 2A), K_{m_1} was practically the same in the presence or absence of inhibitor, but V_{max} was reduced to one third in the presence of ammonium ions, showing that the inhibition is apparently non-competitive for the low K_m site, suggesting that the inhibitor occupies site(s) different from PBG site(s). On the other hand, ammonium ions have the effect of shifting K_{m_2} from 1.9×10^{-4} to 5×10^{-4} M and decreasing V_{max}; this behaviour is essentially of the non-competitive type, also indicating that the inhibitor site(s) is distinct from the PBG site(s). From these data we cannot decide whether the same site or sites are involved in the first and second reaction.

It is important to add, that saturation curves and double reciprocal plots for deaminase and porphobilinogenase both in the absence and presence of ammonium ions, when velocity was measured in terms of PBG consumed per hr, also showed a rather complex pattern of the same kind as that described above; the occurrence of two reactions was also clearly indicated and K_m values were of the same magnitude.

Hill plots [10] for deaminase either in the absence or presence of ammonium ions, resulted in a slope approaching 1 at very low PBG concentrations, and almost 2 at high concentrations of substrate; however Hill coefficients for porphobilinogenase were about 1 at high concentrations of PBG but less than 1 at low substrate concentrations.

The deviation from a Michaelis-Menten pattern can also be seen by calculating the R_s values [4], both for deaminase and porphobilinogenase, R_s values greater than 81 were obtained.

Although unusual, there are several examples in the literature of the kind of kinetics exhibited by soybean callus porphobilinogenase and deaminase (see [6] for a comprehensive biliography) and it has been noted by Levitzky and Koshland [6], that the sequential model for subunit interactions [4, 5, 11] is ideally suited to explain the complex phenomena observed with these enzymes. Applying Levitzky and Koshland's criteria [6] to the soybean callus enzymes, many of the characteristics of negative cooperativity are seen to be present; that is, high R_s values, biphasic double reciprocal plots and in some cases Hill coefficients less than 1.

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