UROPORPHYRINOGEN III COSYNTHETASE. EVIDENCE FOR THE EXISTENCE OF A POLYPYRROLIC SUBSTRATE IN SOYBEAN CALLUS TISSUE

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1. Introduction

The details of the reactions by which porphobilinogen (PBG) is converted into uroporphyrinogen III are still the subject of speculation. Two enzymes are required together to bring about this conversion, PBG deaminase and uroporphyrinogen III cosynthetase. Deaminase alone converts PBG into uroporphyrinogen I while cosynthetase alone has no action on either PBG or uroporphyrinogen I. Both enzymes have been separated and partially purified from a number of sources (see [1] for a comprehensive bibliography). No intermediate in the overall reaction has yet been identified, although the existence of a di- or tri-pyrrol has been postulated [2] and dipyrrolic compounds with uro-type side chains have been reported in the urine of patients with porphyria [3]. Kinetic data [1, 2] suggest that cosynthetase requires two substrates, PBG and some product of the action of deaminase on PBG.

Here we report for the first time the formation and partial purification of a pyrrolic intermediate formed by the action of porphobilinogenase** on PBG. This intermediate acts as the second substrate, with PBG, in the formation of uroporphyrinogen III by purified cosynthetase preparations from various sources. When it is incubated with PBG and purified deaminase a mixture of urophorphyinogens I and II is formed. Soybean callus deaminase, acting on PBG, produces a different pyrrolic intermediate which also behaves as a second substrate for cosynthetase but with the formation mainly of uroporphyrinogen I.

2. Materials and methods

PBG or ^{14}C-PBG were biosynthetically obtained [5] and estimated by the method of Moore and Labbe [6].

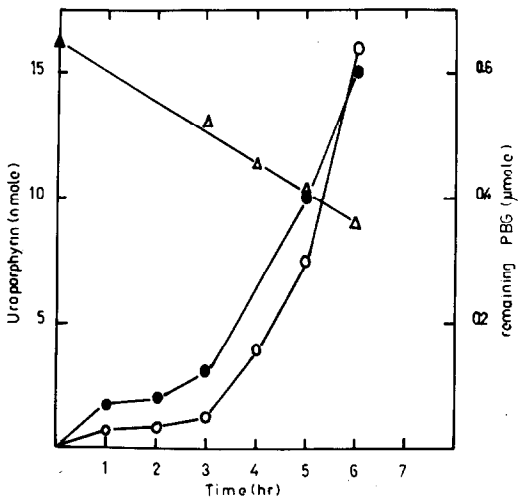

Fig. 1. Porphobilinogen consumption and uroporphyrinogen formation. Phosphate buffer, 0.1 M, pH 7.2. △ PBG consumed when PBG incubated with either porphobilinogenase or deaminase. • Uroporphyrinogen I formed when PBG incubated with deaminase. ○ Uroporphyrinogen III formed when PBG incubated with porphobilinogenase.

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** The trivial name "porphobilinogenase", suggested by Lockwood and Rimington [4] is used to designate the porphobilinogen deaminase-uroporphyronogen III cosynthetase system.

Table 1
Poperties of the supernatants after incubation of porphobilinogen with porphobilinogenase or deaminase.

Fraction	Radioactivity		PBG (μmoles)		Uro* formed (nmoles)	Colour, neutral or acid	Absorption bands (nm)			R_f**
	added (cpm)	remaining (cpm)	added	remaining			neutral	acid	alkaline	
1. P-Supernatant	65437	45980	4.86	2.7	0.131	None	265 (328) 395	275 (328) 407 (480)	290 (332) 395	0.58
2. D-Supernatant	23370	20216	1.76	1.0	0.086	None	280 (330) 390–398	280 (332–338) 407 (470–480)	280 (330) 404	0.67

Incubation mixtures contained: (1) 23 ml soybean callus porphobilinogenase in sodium phosphate buffer pH 7.2, 1.5 ml 0.6 M NaCl, 1.5 ml 0.12 M $MgCl_2$, and ^{14}C-PBG (1.4 ml, 46741 cpm/ml); (2) 7.6 ml soybean callus deaminase in the same buffer and pH and ^{14}C-PBG (0.5 ml, 46741 cpm/ml); volumes were adjusted to 30 ml and 10 ml respectively.

*Uro = total uroporphyrinogens.

**Thin layer chromatography: kieselgel G-H_2O (1 : 2); solvent: n-butanol-acetic acid H_2O (4 : 1 : 5); detection: Ehrlich's reagent; R_f value of PBG = 0.43.

Porphobilinogenase, deaminase and cosynthetase from soybean callus tissues were prepared by the method of Llambías and Batlle [7], the same enzyme fractions from avian erythrocytes as described in ref. [8]. Estimation of their corresponding enzymic activities as well as all other materials and methods not specified here are described in [7] and [8].

3. Results and discussion

3.1. *Rate of uroporphyrinogen formation*

When soybean porphobilinogenase or deaminase is incubated with PBG there is a lag of about 3 hr between the beginning of PBG consumption and the appearance of uroporphyrinogen (fig. 1). After this time uroporphyrinogen formation is rapid. Fig. 1 also shows that the yields of uroporphyrinogens, compared with PBG consumption, are much lower than the stoichiometric values. These results suggest the formation of some intermediate which could be the second substrate for cosynthetase. Attempts were therefore made to isolate and characterize it and to test it as a substrate.

3.2. *Separation and properties of the intermediate(s)*

PBG or ^{14}C-PBG were incubated either with soybean porphobilinogenase or with soybean deaminase for 3 hr. Protein was then precipitated by acidification and separated by centrifugation. The properties of the resulting supernatants, P-supernatant from the porphobilinogenase incubation and D-supernatant from the deaminase incubation, are shown in table 1. Since the uroporphyrinogens formed during the incubations only accounted for part of the PBG consumed, and from the amount of radioactivity remaining in the supernatants, it was concluded that some product(s) other than uroporphyrinogens had been formed from PBG. If these products were polypyrrolic compounds, the fact that the solutions were colourless suggests that they were methane polypyrrols with no conjugation between the rings. The UV absorption spectra of the supernatants were similar to those of monopyrrols but there were slight differences between the P- and D-supernatants.

The supernatants were loaded onto a G-15 Sephadex column (1.4 × 110 cm) and eluted with 0.02 M borate pH 8.0. The void volume was 55 ml, PBG, m.w. 226, eluted at 84 ml and the unknown

Table 2
Synthesis of uroporphyrinogens from the new intermediate.

	Supernatant	Experimental conditions: PBG	Deaminase	Cosynthetase	Porphobilinogenase	Uroporphyrinogens formed: nmoles	cpm	Isomer type: I %	III %
(A) *Soybean callus enzymes*									
1.	P	+	–	+	–	14.6		0	100
2.	P (^{14}C)	+	–	+	–	18.6	1534	0	100
3.	P	+	+	–	–	2.6		31	69
4.	P	+	+	+	–	15.3		10	90
5.	D	+	–	+	–	2.8		70	30
6.	D	+	+	–	–	2.3		100	0
7.	P	+	–	–	–	1.5		49	51
8.	D	+	–	–	–	1.0		70	30
9.	–	+	–	+	–	0.7		0	100
10.	–	+	+	–	–	0.9		100	0
11.	P	–	–	+	–	1.1		20	80
12.	P	–	+	–	–	0.8		25	75
13.	P	–	–	–	–	0.7		–	–
14.	–	+	–	–	–	0.2		–	–
15.	–	+ (^{14}C)	–	–	+	20.4	40980	10	90
16.	P	+ (^{14}C)	–	–	+	28.6	10370	0	100
(B) *Avian erythrocyte enzymes*									
1.	P	+	–	+	–	70.6		0	100
2.	P (^{14}C)	+	–	+	–	40.5	1440	0	100
3.	P	+	+	–	–	75.6		40	60
4.	D	+	–	+	–	42.0		67	33
5.	D	+	+	–	–	75.6		100	0
6.	P	+	–	–	–	0.5		50	50
7.	D	+	–	–	–	0.5		70	30
8.	–	+	–	+	–	3.6		0	100
9.	–	+	+	–	–	12.8		80	20
10.	P	–	–	+	–	1.2		0	100
11.	P	–	+	–	–	30.4		87	13
12.	P	–	–	–	–	0.7		–	–
13.	–	+	–	–	–	0.1		–	–

Incubations: (A) soybean callus enzymes in 0.1 M phosphate pH 7.2; and, when present, PBG, 20 μg/ml; 37°C for 6 hr; (B) avian erythrocyte enzymes in 0.1 M phosphate pH 7.4; and, when present, PBG, 10 μg/ml; 37°C for 4 hr. In both (A) and (B), when presnet, P- or D-supernatants, 4 ml; deaminase, 1 mg; cosynthetase, 5 mg. All incubations were anaerobic in the dark; total volume 10 ml. Uroporphyrinogen analyses and identifications by standard methods [9, 10]. Added radioactivity 4840 cpm in (A)2 and (B)2, 65437 cpm in (A)15 and (A)16.

compounds at 60–65 ml, corresponding to estimated molecular weights between 600 and 700, suggesting they might be tripyrrols.

3.3. *Synthesis of uroporphyrinogens from the intermediate(s)*

The possibility that the supernatants described

above might contain the second substrate for the cosynthetase was tested by using them, after neutralization, in the experiments shown in table 2.

Cosynthetase was able to bring about the synthesis of uroporphyrinogens from PBG and either the P- or the D-supernatant, the presence of one of the latter being necessary. Further, when ^{14}C-PBG was incubated with porphobilinogenase, the complete enzyme system, the amount of uroporphyrinogen III formed was increased by addition of P-supernatant to the incubation but the incorporation of radioactivity into uroporphyrinogen III was then markedly diminished. This would support the idea that P-supernatant contained an intermediate of uroporphyrinogen biosynthesis.

As would be expected, neither deaminase nor cosynthetase alone brought about significant uroporphyrinogen synthesis from either PBG or P-supernatant alone.

That the intermediates in the P- and D-supernatants were not the same, is indicated by the proportions of the I and III uroporphyrinogen isomers produced when either supernatant was incubated with PBG and cosynthetase.

On the basis of these and other results [7] a mechanism for the conversion of PBG into uroporphyrinogens will be proposed [11].

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