THE ENZYMATIC INCORPORATION OF A TRIPYRRANE INTO UROPORPHYRINOGEN I

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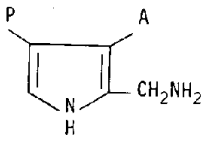
1. Introduction

Porphyrins, chlorins and vitamin B12 share a common metabolic pathway through a long range of initial steps. Of crucial importance to this pathway is the biochemical mechanism involved in the transfortion of porphobilinogen into uroporphyrinogens III and I and into cobyrinic acid. Indeed, this mechanism keeps the secret of why all natural porphyrins, chlorins, and vitamin B12 derivatives are type III porphyrins. The polymerization of four units of porphobilinogen **1** by the combined action of the enzymes porphobilinogen deaminase and uroporphyrinogen III cosynthetase does not afford uroporphyrinogen (– as could be expected from a repetivtive head-to-tail condensation of porphobilinogen – but the isomeric uroporphyrinogen III where an inversion in the order of the β-substituents took place [1] (fig. 1). Porphobilinogen deaminase is the porphobilinogen consuming enzyme but forms only uroporphyrinogen I, while uroporphyrinogen III cosynthetase does not catalyse the condensation of porphobilinogen molecules when the enzyme and the pyrrole are incubated together [1]. Many of the proposals advanced to explain the mechanism of cooperation of the two enzymes suggested that the deaminase formed the 2-aminomethyltripyrrane **2** (fig. 2) which when served as a substrate for the cosynthetase together with the fourth unit of porphobilinogen giving rise to uroporphyrinogen III through a rearrangement step [1,2].

It had been recently demonstrated that while the 2-aminomethyldipyrrylmethane **3** (resulting from a formal head-to-tail condensation of two units of porphobilinogen) was an intermediate in the biosynthesis of uroporphyrinogen I, [3,4], the isomeric 2-aminomethyldipyrrylmethane **4** (resulting from a formal head-to-head condensation of two units of porphobilinogen) was an intermediate in the biosynthesis of



1

A = CH_2CO_2H
P = $CH_2CH_2CO_2H$
R = P; R' = A; uroporphyrinogen III
R = A; R' = P; uroporphyrinogen I

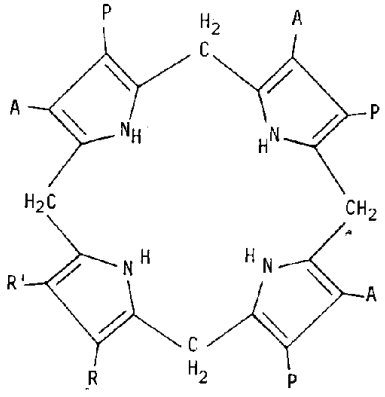


Fig. 1.

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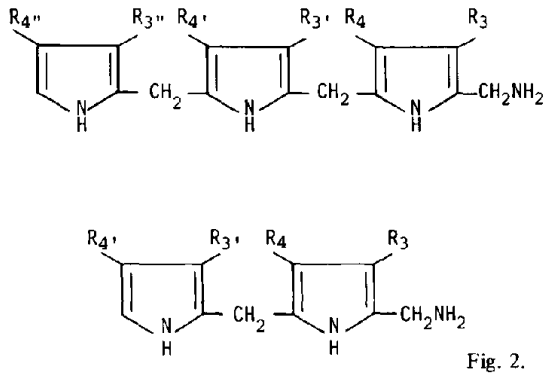

2: $R_3 = R_{3'} = R_{3''} = CH_2CO_2H$;
$R_4 = R_{4'} = R_{4''} = CH_2CH_2CO_2H$.

3: $R_3 = R_{3'} = CH_2CO_2H$;
$R_4 = R_{4'} = CH_2CH_2CO_2H$.

4: $R_3 = R_{4'} = CH_2CH_2CO_2H$;
$R_4 = R_{3'} = CH_2CO_2H$.

Fig. 2.

uroporphyrinogen III [5]. None of them were found to be substrates of cosynthetase in the presence of porphobilinogen. The readily available synthetic 2-aminomethyltripyrrane 2 [6], allowed us to examine its interaction with the enzymes involved in porphobilinogen polymerization and to test the hypotheses on its possible role in uroporphyrinogen biosynthesis.

2. Materials and methods

[^{14}C] Porphobilinogen was obtained by synthesis [7]. The tripyrrane 2 was obtained by unambiguous lineal condensation of a pyrrole and a dipyrrylmethane lactam which followed the synthetic outline described for the synthesis of the dipyrrylmethanes [3]. The full details of the method will be described elsewhere [6]. All other chemicals were reagent grade.

Porphobilinogen deaminase and uroporphyrinogen III cosynthetase were isolated and purified from wheat germ and from human erythrocytes [8,9]. The incubation system contained in a final volume of 100 μl: 10 μmoles of phosphate buffer (pH 7.4), 6 nmoles of [^{14}C] porphobilinogen (2500 cpm/nmole) 20 μl of purified wheat germ deaminase or purified deaminase-cosynthetase from wheat germ or human erythrocytes, and the indicated amounts of tripyrrane 2. Incubations were carried out at 37°C during 60 min. Controls were prepared by mixing the incubation mixtures in which tripyrrane was omitted with blank obtained by incubating at 37°C during 60 min a 100 μl mixture containing 10 μmoles of phosphate buffer (pH 7.4), 6 nmoles of [^{14}C] porhobilinogen and the indicated amounts of tripyrrane 2. Uroporphyrinogen formation was estimated by decarboxylating it to coproporphyrin and by measuring the specific activity

Table 1
Chemical polymerization of tripyrrane.

System	Uroporphyrinogen formed		Isomer composition			
			I		III	(IV)
	(nmoles)	(%)	(nmoles)	(%)	(nmoles)	(%)
Tripyrrane[a]	4.75	3.4	2.4	50.5	2.35	49.5
Deaminase + tripyrrane[b]	3.28	4.6	1.4	43	1.88	57
Deaminase + tripyrrane[a]	4.22	3	2.07	49.5	2.15	51
Tripyrrane[b] + porphobilinogen	2.76	4	1.58	57	1.18	43
Tripyrrane[a] + porphobilinogen	5.50	3.95	3.15	57	2.35	43

The incubation mixture and conditions were described in Materials and methods. Isomer analysis was performed as described elsewhere [3].
[a]Tripyrrane, 140 nmoles were used.
[b]Tripyrrane, 70 nmoles were used.

Table 2
Tripyrrane polymerization in the presence of urophorphyrinogen III cosynthetase.

System	Isomers formed			
	I		III	
	(nmoles)	(cpm)	(nmoles)	(cpm)
Run 1				
Tripyrrane + [^{14}C] porphobilinogen	0.32	715	0.24	302
Cosynthetase[a] + tripyrrane + [^{14}C] porphobilinogen	0.33	668	0.22	425
Run 2				
Deaminase[b] + cosynthetase[b] + [^{14}C] porphobilinogen	0.136	925	0.215	1500
Deaminase + cosynthetase + tripyrrane + [^{14}C] porphobilinogen	1.43	1990	1.0	860
cosynthetase + tripyrrane + [^{14}C] porphobilinogen	1.06	830	0.91	650

The incubation mixture and conditions were described in Materials and methods. Run 1: 25 nmoles of tripyrrane were used. Run 2: 70 nmoles of tripyrrane were used.
[a]Human erythrocyte cosynthetase was used.
[b]Wheat germ purified deaminase (5 μl) and cosynthetase were used.

of the latter [3]. Radioactivity was measured with a gas-flow counter.

3. Results

Heating of the tripyrrane under the usual incubation conditions (37°C during 60 min at pH 7.4) resulted in uroporphyrin formation after oxidation of the corresponding uroporphyrinogens (table 1). Uroporphyrins were formed in 3 to 4% yield and the isomer analysis when performed at the coproporphyrin stage indicated that only isomer I and III (or IV) were formed. This porphyrin formation must be rationallized by accepting an initial dimerization (head-to-head and head-to-tail) to an hexapyrrylmethane followed by bond breaking at the thermodynamic favourable cyclic tetrapyrrole site. Addition of porphobilinogen deaminase to the tripyrrane incubation of isomers in the reaction mixture. The addition of porphobilinogen to the tripyrrane incubation mixture produced a small increase in the amount of isomer I formed.

Incubation of uroporphyrin III cosynthetase with tripyrrane in the presence of [^{14}C] porphobilinogen did not affect the uroporphyrin isomer pattern obtained by the chemical polymerization of tripyrrane in the presence of porphobilinogen (table 2, run 1). When a small amount of deaminase was added to the same incubation mixture a decrease in the amount of uroporphyrinogen III was detected together with a simultaneous increase in isomer I formation (table 2, run 2). No enzymatic formation of uroporphyrinogen III at expense of the tripyrrane could be detected. Addition of tripyrrane to an enzymatic system forming both uroporphyrinogen I and uroporphyrinogen III had a marked influence on the total porphyrin formation and the isomer distribution (fig. 3). The proportion of uroporphyrinogen I increased, while a parallel decrease was observed in the amount of uroporphyrinogen III and of total porphyrin. The former effect was reminiscent of the influence of dipyrrylmethane 3 on the isomer distribution [3]. Incorporation of tripyrrane 2 into uroporphyrinogen I could be detected using wheat germ deaminase in the presence of porphobilinogen (table 3). A large excess of tripyrrane must be used to detect a small but significant (2% to 8%) enzymatic formation of uroporphyrinogen I at expense of the tripyrrane. The effect was more pronounced (14% incorporation) when glycerol was added to the incubation mixture. Glycerol was found to affect deaminase activity [10].

4. Discussion

The obtained results indicated that the tripyrrane derived from the formal head-to-tail condensation of three units of porphobilinogen by the loss of two moles of ammonia was not a substrate of uroporphyrinogen III cosynthetase in presence or absence of porphobilinogen. It was neither a substrate of porphobilinogen deaminase in the absence of porphobilinogen. Moreover, it inhibited the enzymatic formation of uroporphyrinogen III when added in a relatively large excess. It was incorporated into uropor-

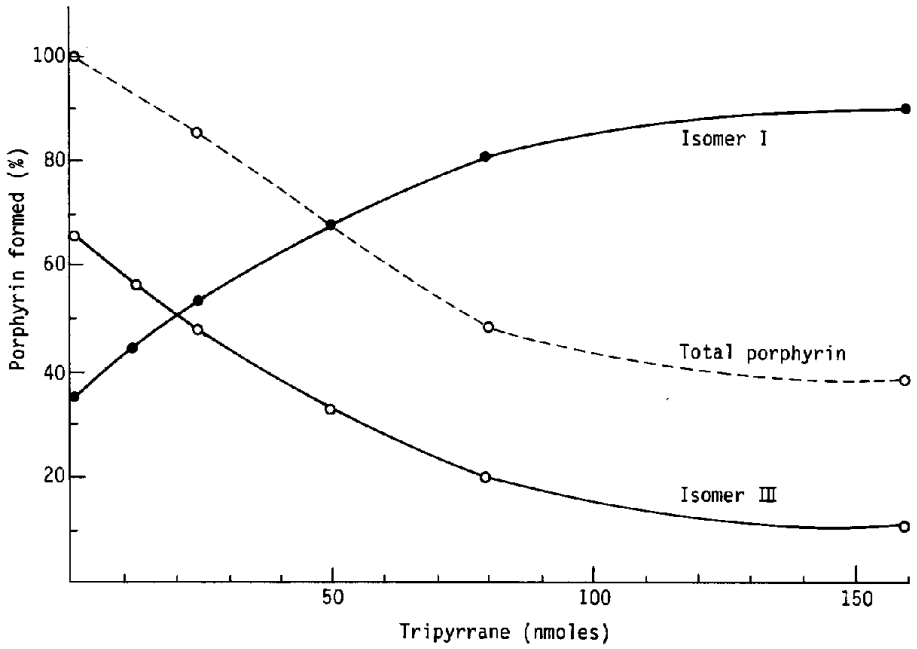

Fig. 3. Effect of tripyrrane on the enzymatic formation of uroporphyrinogens and on its isomer composition. The incubation mixture contained in a final volume of 100 μl; 10 μmoles of buffer phosphate (pH 7.4), 6 nmoles of [^{14}C] porphobilinogen (Sp. Act., 2500 cpm/nmol); 20 μl of wheat germ deaminase-cosynthetase and the indicated amount of tripyrrane. Incubations were performed at 37°C during 60 min. Uroporphyrin formed chemically (see text) was deducted at each run. Isomers were estimated as coproporphyrins [3].

Table 3
Enzymatic incorporation of tripyrrane into uroporphyrinogen I.

System	Tripyrrane added	Uroporphyrinogen I		
	(nmoles)	(nmoles)	(cpm)	Specific activity	Decrease in Sp. Act.
Run 1					
Control	12	0.70	5600	8000	
Incubated	12	0.53	4400	8200	–
Control	25	0.90	7150	7900	
Incubated	25	0.44	3470	7900	–
Control + Glycerol	25	0.25	2120	8300	
Incubated + Glycerol	25	0.34	2120	6200	2100
Run 2					
Control	70	2.45	10750	4350	
Incubated	70	2.00	8300	4150	200
Control	140	3.30	9174	2800	
Incubated	140	3.30	6700	2040	760
Control + Glycerol	70	1.90	7300	3800	
Incubated + Glycerol	70	1.60	5500	2300	400
Control + Glycerol	140	2.00	5800	2900	
Incubated + Glycerol	140	1.90	2830	1500	1400

The incubated system and the controls were described in Materials and methods. Where glycerol was added, a 10% final concentration was used, and both the incubated and the blank contained glycerol during the incubation period.

phyrinogen I to a very small extent and cannot then be considered as a substrate of porphobilinogen deaminase in the presence of porphobilinogen either; although it could be considered as an intermediate between porphobilinogen and uroporphyrinogen I. These results agree with our former data on the enzymatic incorporation of dipyrrylmethanes into uroporphyrinogens, and lend support to our proposals, [3, 5], that the entire process takes place on the enzyme's surface, with no liberation of free pyrrylmethane intermediates at any stage of the biosynthetic process.

Acknowledgement

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