Biosynthesis of Uroporphyrinogens. Interaction among 2-Aminomethyldipyrrylmethanes and the Enzymatic System†

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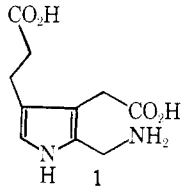
ABSTRACT: The interaction among porphobilinogen deaminase and uroporphyrinogen III co-synthase and 2-aminomethyldipyrrylmethanes formally derived from the polymerization of porphobilinogen was studied. Dipyrrylmethane **5**, formally derived from the "head-to-head" encounter of two porphobilinogen units followed by a migration of the 2-aminomethyl residue to the 5′ free position of the dipyrrylmethane, was by itself not a substrate of deaminase, or deaminase-co-synthase, or of co-synthase in the presence of porphobilinogen. When **5**-^{12}C was added to an enzymatic system which was forming both uroporphyrinogen III and uroporphyrinogen I from porphobilinogen-^{14}C, a decrease in the specific activity of the former was found. When **5**-^{14}C was used in the presence of unlabeled porphobilinogen, an increase in the specific activity of uroporphyrinogen III was observed. These effects were absent in uroporphyrinogen I formation. About 7% of total uroporphyrinogen III was formed at the expense of dipyrrylmethane **5**. In all these measurements, control reactions were prepared to account for the chemical formation of uroporphyrinogen III at the expense of **5**. The results obtained with **5** were compared with studies performed with dipyrrylmethane **7**, a new "nonsense dipyrrylmethane" from the biological standpoint. This last dipyrrylmethane was not incorporated into either isomer and exerted a marked inhibitory effect on the enzymatic formation of isomer III. Studies were also performed with the 5,5′ free dipyrrylmethane **6** (equivalent to **5** without the 2-aminomethyl residue). Under carefully selected conditions the incorporation of **6** into enzymatically formed uroporphyrinogen III could be detected, although it was lower than the incorporation found for **5**.

The enzymatic transformation of porphobilinogen (**1**) (PBG)[1] into uroporphyrinogens III (**2**) and I (**3**) is the reaction by which porphyrins are formed in nature. All the important natural porphyrins (heme, chlorophylls, cytochromes, etc.) are derived from uroporphyrinogen III (**2**) while uroporphyrinogen I (**3**) is the precursor of the more exotic type I porphyrins (Burnham, 1969).



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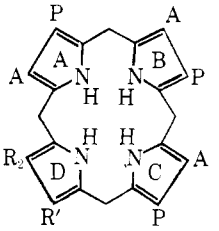
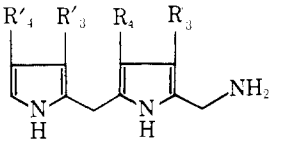


2, R′ = P; R_2 = A
3, R′ = A; R_2 = P

A = CH_2CO_2H; P = $CH_2CH_2CO_2H$

The mechanism by which porphobilinogen (**1**) is transformed into uroporphyrinogen III (**2**) has been the subject of much speculation (see Frydman and Frydman (1975a) for a review on the subject). It is easy to visualize how four units of porphobilinogen (a 5′ free primary Mannich base) self-condense to give uroporphyrinogen I (**3**), but it is not easy to understand how uroporphyrinogen III (**2**) is formed at the expense of **1**. In uroporphyrinogen III the β substituents of ring D are inverted with respect to the sequence of the substituents in the other three rings. The enzymatic polymerization to give **2** is catalyzed by the substrate-consuming enzyme—porphobilinogen deaminase—which by itself gives rise to **3**, and by a second enzyme—uroporphyrinogen III co-synthase—which does not consume **1** but drives the polymerization toward the formation of **2** when it is added to the deaminase.

Many of the hypotheses advanced to explain the formation of **2** proposed that deaminase forms a pyrrylmethane intermediate (most likely a tripyrrane), which the co-synthase takes up and condenses with **1** in a second enzymatic reaction where the inversion of ring D leads to the formation of **2**. The experimental results, however, do not lend support to these hypotheses (Frydman et al., 1978). When we examined the interaction of the 2-aminomethyldipyrrylmethane **4** with the deaminase-co-synthase system, we found that in the presence of **1** it was incorporated only into uroporphyrinogen I (Frydman et al., 1971; 1973), while the 2-aminomethyldipyrrylmethane **5** was incorporated into uroporphyrinogen III (Frydman et al., 1972).



4, R_3 = R′$_3$ = CH_2CO_2H; R_4 = R′$_4$ = $CH_2CH_2CO_2H$
5, R_3 = R_4 = $CH_2CH_2CO_2H$; R_4 = R′$_3$ = CH_2CO_2H
7, R_3 = R′$_3$ = $CH_2CH_2CO_2H$; R_4 = R′$_4$ = CH_2CO_2H

The dipyrrylmethane **4** could formally be formed by a "head-to-tail" condensation of two porphobilinogen units (the 2-aminomethyl group of one unit attacking the free C-5 position of the other unit), while the dipyrrylmethane **5** could formally arise from a "head-to-head" encounter of two porphobilinogen units followed by a migration of the 2-aminomethyl residue. On the basis of these studies and of the studies carried out on the properties of the enzymes, we proposed (Frydman et al., 1971, 1975) that the deaminase and the co-synthase form a close association, with the co-synthase acting as a "specifier protein" of the deaminase. This proposal, which

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[1] Abbreviations used are: PBG, porphobilinogen; TLC, thin-layer chromatography.

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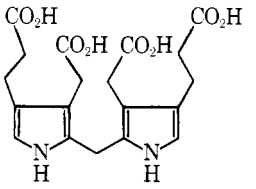
has been gaining in favor recently (Shemin, 1975; Higuchi and Bogorad, 1975), precludes the formation of free pyrrylmethane intermediates in the reaction medium, and suggests that the biosynthetic pathway toward uroporphyrinogen III and uroporphyrinogen I diverges from the beginning of the enzymatic polymerization of porphobilinogen. The deaminase gives rise to **3** through the enzyme-bound intermediate **4**, and the deaminase-co-synthase gives rise to **2** through the enzyme-bound intermediate **5**. The experimental procedures used to study the interaction of the 2-aminomethyldipyrrylmethanes with the enzymatic system were made difficult by the chemical self-condensation of the former to give uroporphyrinogens. These chemically formed uroporphyrinogens have to be deducted in control runs from the uroporphyrinogen formed enzymically at the expense of the 2-aminomethyldipyrrylmethanes. Scott reported recently (Scott et al., 1976) that the 5,5′ free dipyrrylmethane **6** is also incorporated into **2**, with



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the advantage that no chemical uroporphyrinogen was formed at the expense of **6**. Since the "headless" dipyrrylmethane **6** is closely related to **5**, the interaction of both with the deaminase-co-synthase system was examined, and the results will be discussed below. The new experimental data obtained with **5** reinforce our previous results (Frydman et al., 1972) and will be discussed in the same framework as the data obtained with a "nonsense" (from the biosynthetic standpoint) dipyrrylmethane **7** which will serve as a reference compound in this study.

Materials and Methods

Porphobilinogen (**1**) and porphobilinogen-^{14}C were prepared by synthesis (Frydman et al., 1969). The label was at C-3 of the propionic acid side chain. 2-Aminomethyldipyrrylmethane **5** and 2-aminomethyldipyrrylmethane **7** were obtained by synthesis (Valasinas et al., 1974). **6** and **6**-^{14}C (1000 cpm/mmol) were prepared by synthesis (Valasinas and Frydman, 1976). **5**-^{14}C (3800 cpm/nmol) carried the label on the methylene bridge. All the chemicals used were reagent grade. Porphobilinogen deaminase and uroporphyrinogen III co-synthase were isolated and purified from either wheat germ or human erythrocytes (Frydman and Frydman, 1970; Frydman and Feinstein, 1974), and were recombined to afford the desired isomer composition in the reaction products. The standard incubation mixture contained (in a final volume of 100 μL): 10 μmol of phosphate buffer (pH 7.4), 9 nmol of porphobilinogen-^{12}C (unless otherwise stated) or 9 nmol of porphobilinogen-^{14}C, 25 μL of porphobilinogen deaminase, 50 μL of uroporphyrinogen III co-synthase (when needed to form isomer III), and the indicated amount of dipyrrylmethane used in each case. The incubations were carried out at 37 °C during 60 min; the reaction was stopped by addition of a 1% aqueous iodine solution and the solution evaporated to dryness in vacuo at 20°C. When the total amount of uroporphyrinogen formed in the reaction was estimated, evaporation to dryness was omitted; the excess of iodine was destroyed by adding a 2% sodium thiosulfate solution, and uroporphyrins were estimated spectrophotometrically as described elsewhere (Frydman and Frydman, 1970). When isomers were estimated, the residue obtained in the evaporation described above was esterified with 5% sulfuric acid in methanol, and the octamethyl esters were extracted and decarboxylated to the corresponding coproporphyrins (Edmondson and Schwartz, 1953). The coproporphyrin isomers of types I, II, and III/IV were separated by TLC on cellulose coated plates (E. Merck, DC-Fertigplatten, Schichtdicke 0.1 mm) using 2,6-lutidine–11 N ammonia (40:28, v/v). Coproporphyrins III and IV were distinguished using TLC on cellulose as described elsewhere (Frydman and Frydman, 1975b). The coproporphyrin isomers were located by fluorescence, and eluted with a 0.7 M ammonia solution. The porphyrin concentration in the eluates was determined by a spectrophotometric method; the eluates were then plated on planchets and counted in a gas-flow counter.

The control runs for the incorporation experiments described in Table II were prepared by mixing two separate incubation mixtures. One contained (in a final volume of 100 μL), 10 μmol of phosphate buffer (pH 7.4), 9 nmol of porphobilinogen-^{14}C (3500 cpm/nmol), 25 μL of porphobilinogen deaminase, and 50 μL of uroporphyrinogen III co-synthase. The second mixture contained the indicated amount of dipyrrylmethane **5**, porphobilinogen-^{14}C (9 nmol), and phosphate buffer (pH 7.4) (10 μmol), all in a final volume of 100 μL. Both mixtures were incubated separately at 37 °C during 60 min and stopped by addition of a 1% aqueous iodine solution, and the resulting uroporphyrin solution was worked up, as described above. The obtained octamethyl esters were mixed, and the mixture was decarboxylated as described. The controls prepared in this way accounted for the uroporphyrinogens formed enzymically from porphobilinogen-^{14}C and the uroporphyrinogens formed by the chemical polymerization of dipyrrylmethane **5**. When the incorporation of **5**-^{14}C into both uroporphyrinogen isomers was measured (Table III), the control runs were prepared as follows. A mixture containing phosphate buffer (pH 7.4) (10 μmol), porphobilinogen (13 nmol), co-synthase (50 μL), and **5**-^{14}C (3800 cpm/nmol) was incubated as described above. The obtained uroporphyrin octamethyl esters were mixed with the octamethyl esters obtained from a standard incubation mixture prepared by using 13 nmol of porphobilinogen. The ester mixture was worked up as described above to obtain the coproporphyrins. By doing so the control runs contained the amount of uroporphyrinogen-^{14}C formed in the chemical polymerization of **5**-^{14}C, which could thus be deducted from the enzymatic reaction.

Results

*Enzymatic Incorporation of Dipyrrylmethane **5** into Uroporphyrinogen III.* When measuring the enzymatic incorporation of dipyrrylmethane **5** into uroporphyrinogens, it is necessary to take into account its chemical dimerization at 37 °C and pH 7.4 to form uroporphyrinogens. Under these conditions uroporphyrins are formed in about 10% yield, after the uroporphyrinogen mixture was oxidized with iodine. Almost 95% of the product is uroporphyrin II (Table I). This is what could be expected when the two possible dimerization reactions of dipyrrylmethane **5** were considered (Figure 1). A "head-to-tail" condensation of two dipyrrylmethane units will give rise to a 2-aminomethylbilane (reaction A) which will then cyclize to form uroporphyrinogen II. Alternatively, a "head-to-head" condensation of two dipyrrylmethanes will liberate formaldehyde and form a 5,5′ free bilane (reaction B). By recombination of both the formaldehyde and the bilane, uroporphyrinogen II will be formed as well.

A small amount of uroporphyrinogen III (or IV) is, however, formed during the reaction (Table I), very likely through further attack of **5** on the uroporphyrinogen II ring (see

TABLE I: Chemical Dimerization of Dipyrrylmethanes **5** and **7**.[a]

Dipyrryl-methane	Concn (nmol)	Addition	Isomers formed: I nmol	I %[b]	III/IV nmol	III/IV %[b]	II nmol	II %[b]
5	30				0.10	6	1.50	94
5	30	PBG			0.13	7	1.67	93
5	30	PBG + co-synthase			0.10	6	1.67	94
7	10		1.0	90	0.13	10		
7	10	Deaminase	0.94	85	0.20	15		
7	10	PBG + co-synthase	1.10	85	0.20	15		

[a] The polymerizations of **5** and **7** were carried out by heating during 60 min at 37 °C the indicated amounts of **5** and **7** in 0.1 M phosphate buffer (pH 7.4) in a final volume of 100 μL. Isomers were estimated as coproporphyrins. [b] Percent of total.

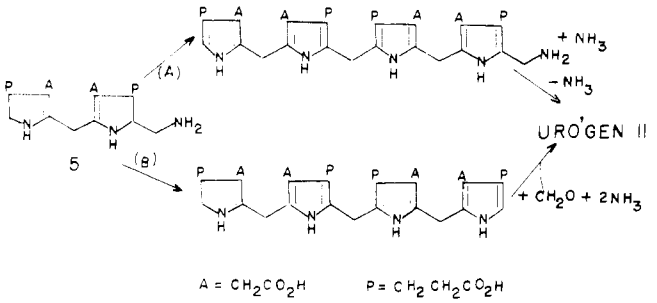

FIGURE 1.

Whitlock et al., 1974). This formation of uroporphyrinogen III (or IV) must be taken into account when considering a possible enzymatic incorporation of dipyrrylmethane **5** into the uroporphyrinogen isomers.

The dipyrrylmethane is by itself not a substrate of deaminase or deaminase-co-synthase and it is not a substrate of the co-synthase in the presence of porphobilinogen either (Table I). To examine a possible incorporation of dipyrrylmethane **5** into either uroporphyrinogens III or I in the presence of porphobilinogen (the natural substrate), dipyrrylmethane-^{12}C was added to an incubation mixture forming uroporphyrinogen-^{14}C at the expense of porphobilinogen-^{14}C. A dilution in the specific activity of either isomer will indicate an incorporation of the dipyrrylmethane into the reaction products. This technique (as well as any incorporation study using labeled **5** and unlabeled porphobilinogen) requires, however, strict control reactions to avoid deceptive results. They have to account for the chemical formation of uroporphyrinogen III from dipyrrylmethane **5** and porphobilinogen (see above). This reaction will decrease the specific activity of the product, and can mistakenly be taken as an enzymatic incorporation. To avoid this error, control reactions had to be prepared including both the chemical reaction and the enzymatic formation of uroporphyrinogens at the expense of the labeled substrate. The specific activities of the uroporphyrinogens obtained in these control reactions were then compared with the specific activities of the same isomers obtained when the unlabeled dipyrrylmethane was incubated with the enzymatic system in the presence of porphobilinogen-^{14}C. If the specific activities of the uroporphyrinogens obtained in the latter incubations were lower than those of the control reactions, the enzymatic incorporation of the dipyrrylmethane was taking place. The actual enzymatic incorporation values of the dipyrrylmethane might still be higher than the values obtained by subtracting from the control reactions, but in this way the lowest possible enzymatic incorporation values are obtained with the smallest chance of error.

When **5**-^{12}C was added to an enzymatic system which was

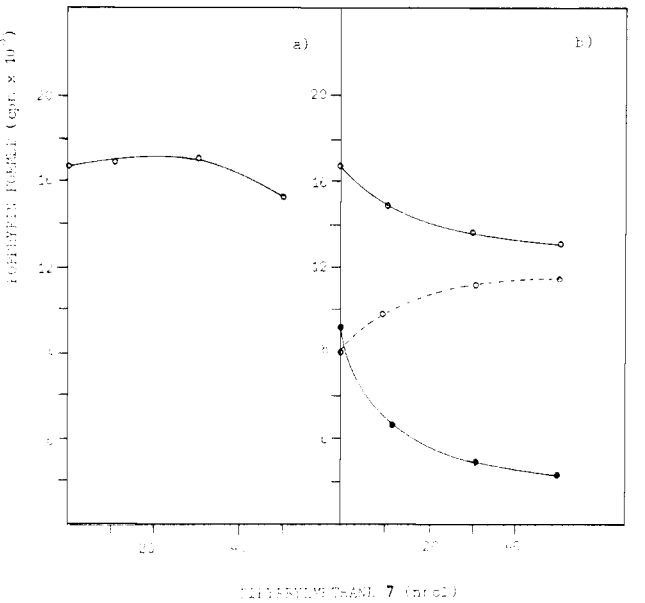

FIGURE 2: Effect of dipyrrylmethane **7** on the activity of: (a) porphobilinogen deaminase measured as uroporphyrin I; (b) porphobilinogen deaminase-uroporphyrinogen III co-synthase; (O—O) total porphyrins; (O- - -O) uroporphyrin I; (●—●) uroporphyrin III.

forming both uroporphyrinogen III and uroporphyrinogen I from porphobilinogen-^{14}C, a decrease in the specific activity of the former was found (Table II). The specific activity of uroporphyrinogen I remained unaffected, as compared to the control reaction.

When the experiment was carried out by using **5**-^{14}C in the presence of unlabeled porphobilinogen, an increase in the specific activity of uroporphyrinogen III was observed as compared to a control reaction which accounted for the chemical dimerization of dipyrrylmethane **5** (Table III). This incorporation effect was absent in isomer I formed during this reaction. These results are in full agreement with our previous observations (Frydman et al., 1972) and can only be explained by assuming that dipyrrylmethane **5** is incorporated only into isomer III, while it is not incorporated into isomer I. The results obtained with dipyrrylmethane **5** were compared with the results obtained by studying the interaction among dipyrrylmethane **7** and the enzymatic system in order to ascertain their specificity. Dipyrrylmethane **7** cannot formally be derived from **1** and is hence a "nonsense dipyrrylmethane". Its chemical dimerization at 37 °C and pH 7.4 under the standard incubation conditions formed uroporphyrinogens in about 20% yield. Two isomers were present in the reaction product: uroporphyrinogen I and uroporphyrinogen IV. The relative proportions of both varied from 70 to 90% for isomer I and cor-

TABLE II: Enzymatic Incorporation of Dipyrrylmethane **5** into Uroporphyrinogen III.[a]

System	[Dipyrrylmethane] (nmol)	Uroporphyrinogens: Isomer I nmol	Isomer I cpm	Isomer I Sp act. (cpm/nmol)	Isomer III nmol	Isomer III cpm	Isomer III Sp act. (cpm/nmol)	Sp act. (Δ)
Control	18	0.55	7120	12 940	1.15	14 900	12 950	1050
Incubated	18	0.53	6840	12 900	0.81	9 700	11 900	
Conlrol	36	0.36	4600	12 780	1.53	20 740	13 550	3750
Incubated	36	0.50	6700	13 400	1.0	9 800	9 800	

[a] The incubated system contained PBG-^{14}C (9 nmol, 3500 cpm/nmol), wheat germ deaminase-co-synthase, and the indicated amounts of dipyrrylmethane. The control system was prepared by mixing the reaction products obtained from separate incubations of PBG-^{14}C with deaminase-co-synthase, and of dipyrrylmethane (the indicated amounts), and PBG-^{14}C. The incubation conditions and isomer estimation were performed as described under Materials and Methods.

TABLE III: Enzymatic Incorporation of **5**-^{14}C into Uroporphyrinogen III.[a]

System	[Dipyrrylmethane] (nmol)	Uroporphyrinogens: Isomer I nmol	Isomer I cpm	Isomer I Sp act. (cpm/nmol)	Isomer III nmol	Isomer III cpm	Isomer III Sp act. (cpm/nmol)	Isomer III Sp act. (Δ)	Isomer II nmol	Isomer II cpm
Incubated		0.81			0.48					
Incubated	30	1.43	590	412	0.51	1176	2300	590	1.50	11 250
Control	30	1.55	770	497	0.84	1436	1710		1.64	12 300

[a] The incubated system contained PBG-^{12}C (13 nmol), wheat germ deaminase-co-synthase, and, when indicated, **5**-^{14}C (3800 cpm/nmol). The control system was prepared by mixing the reaction products obtained from separate incubations of PBG-^{12}C, co-synthase, and **5**-^{14}C, and of PBG-^{12}C and deaminase-co-synthase. The incubation conditions and isomer estimation were performed as described under Materials and Methods.

TABLE IV: Interaction between **6**-^{14}C and the Enzymatic System.[a]

System	[Dipyrrylmethane] (cpm × 10^3)	Uroporphyrinogens: Isomer I nmol	Isomer I cpm	Isomer III/IV nmol	Isomer III/IV cpm	Isomer II nmol	Isomer II cpm
Incubated[b]		1.37					
Incubated	20	0.77	104	0.25	315	1.49	2906
Incubated	30	0.67	131	0.23	274	1.92	3585
Incubated[c]		0.46		0.512			
Incubated	20	0.25	120	0.17	155	0.87	1550
Incubated	30	0.13	62	0.23	250	1.4	240
Blank[d]	30		66	0.16	274	0.77	1400

[a] The incubation mixtures and conditions were as described under Materials and Methods. Isomers were measured as coproporphyrins. [b] Porphobilinogen deaminase. [c] Deaminase-co-synthase. [d] Co-synthase + porphobilinogen (9 nmol) + **6**-^{14}C (1000 cpm/nmol).

respondingly from 30 to 10% for isomer IV (Table I). The formation of both isomers can be visualized by two dimerization reactions which follow the pattern outlined above for dipyrrylmethane **6** (Figure 1). The dipyrrylmethane **7** was not a substrate of deaminase or co-synthase in the presence of porphobilinogen (Table I). When **7** was added to an incubation mixture which formed porphobilinogen-^{14}C, it did not affect the specific activity of either isomer when the appropiate control reactions were run simultaneously. The dipyrrylmethane **7** exerted a marked inhibitory effect on the enzymatic isomer III formation, while it only had a slight inhibitory effect on porphobilinogen deaminase (Figure 2). It was evident that **7** was not incorporated into either isomer, and exerted an inhibitory effect on the deaminase-co-synthase system.

Interaction between Dipyrrylmethane **6** *and the Enzymatic System.* When **6**-^{14}C (labeled at the methylene bridge carbon) was incubated under the usual conditions (37 °C, pH 7.4) in the presence of porphobilinogen it gave rise to a mixture of uroporphyrins I, II, and III (or IV), all labeled with ^{14}C (Table IV, blank reaction). When deaminase or deaminase-co-synthase was added to the incubation mixture, no significant increase in the label of the isomer III fraction was observed with respect to the blank reaction containing dipyrrylmethane-^{14}C, porphobilinogen-^{12}C, and co-synthase (Table IV).

We assumed that **6**-^{14}C was being destroyed by self-radiolysis and that the recombination of the fragments was producing a random mixture of isomers with predominance of isomer II. Hence, the experiment was repeated with unlabeled dipyrrylmethane **6** in the presence of porphobilinogen-^{14}C. Under these conditions no chemical uroporphyrinogen formation at the expense of **6** was observed and larger amounts of the dipyrrylmethane could be used. When this was done, a decrease in the specific activity of isomer III was found while no significant decrease was found in the specific activity of isomer I (Table V). The dipyrrylmethane **6** exerted a strong inhibitory effect on porphobilinogen deaminase, as well as on

TABLE V: Interaction between Dipyrrylmethane **6** and the Enzymatic System.[a]

System	[Dipyrrylmethane] (nmol)	Uroporphyrinogen Isomer I nmol	Isomer I cpm	Isomer I Sp act. (cpm/nmol)	Isomer III nmol	Isomer III cpm	Isomer III Sp act. (cpm/nmol)	Sp act. (Δ)
Incubated[b]		1.26	25 770	20 450				
Incubated	48	0.90	18 500	20 500				
Incubated	80	0.75	14 540	19 400				
Incubated	160	0.50	10 000	20 000				
Incubated[c]		0.73	13 500	18 500	0.66	12 200	18 500	
Incubated	48	0.49	8 950	18 260	0.37	6 670	18 030	470
Incubated	80	0.50	9 400	18 800	0.32	5 350	16 720	1780
Incubated	160	0.37	6 870	18 570	0.17	2 850	16 760	1740

[a] The incubation mixtures and conditions were as described under Materials and Methods. Isomers were estimated as coproporphyrins. [b] Deaminase and porphobilinogen-^{14}C (9 nmol, 5000 cpm/nmol) were used. [c] Deaminase-co-synthase was used.

the deaminase-co-synthase system (Tables IV and V). These results lend support to the suggestion that dipyrrylmethane **6** is incorporated into uroporphyrinogen III although to a lesser extent than dipyrrylmethane **5**.

Discussion

In our previous work (Frydman et al., 1971, 1973) we have observed that the 2-aminomethyldipyrrylmethane **4** was not enzymatically incorporated in the presence of porphobilinogen into uroporphyrinogen III. Under the same conditions it was, however, incorporated into uroporphyrinogen I. The deaminase-co-synthase system did not behave as a dimerizing enzyme, since in the absence of porphobilinogen no enzymatic formation of uroporphyrinogen at the expense of **4** was observed (Frydman et al., 1973; Pluscec and Bogorad, 1970). Dipyrrylmethane **4** undergoes, however, a chemical dimerization reaction which forms uroporphyrinogens I and IV (Frydman et al., 1976). The mechanism of this dimerization is similar to that described for **5** (Figure 1), and involves the formation of a 2-aminomethylbilane (the chemical cyclization of which leads to uroporphyrinogen I), and a 5,5′ free bilane which cyclizes to uroporphyrinogen IV (for a discussion see Frydman et al., 1973).

It has been recently briefly reported (Battersby et al., 1977) that the 2-aminomethylbilane originated in the aforementioned chemical dimerization of **4** is transformed by a deaminase-co-synthase system into uroporphyrinogen III. Thus, although the direct enzymatic dimerization of **4** is also discarded by Battersby et al., the claim is made that **4** is indirectly incorporated into uroporphyrinogen III through the enzymatic incorporation of the 2-aminomethylbilane into the former. The experimental data advanced to support this assertion (Battersby et al., 1977) do not show any increase in the total amount of uroporphyrinogens formed at the expense of the 2-aminomethylbilane in the presence of deaminase-co-synthase, as compared with the chemical reaction. An increase should, however, be expected, if an enzymatic reaction is taking place in the presence of the large excess of substrate, 70% of which remains unaltered after the prolonged incubations. On the other hand, our previous results indicated that the deaminase-co-synthase did not interact with the chemical dimerization of **4** in the absence of porphobilinogen, and that in the presence of the latter, the dipyrrylmethane **4** exerted a strong inhibitory effect on the enzymatic formation of uroporphyrinogen III (**2**) (Frydman et al., 1973). We must then conclude that **4** is not incorporated into **2** either directly or through any of its chemically formed polymers.

The data presented in Tables II and III clearly indicate that the rearranged dipyrrylmethane **5** is incorporated into uroporphyrinogen III, and these results were further confirmed by a study with tripyrranes (see Frydman et al., 1978). Furthermore, the "headless" dipyrrylmethane **6** was also found to be incorporated to a certain extent into uroporphyrinogen III (Table V). The "nonsense" dipyrrylmethane **7** was not incorporated into either isomer, thus lending support to the biological significance of the data obtained with dipyrrylmethane **5**.

The incorporation of dipyrrylmethane **5** into uroporphyrinogen III in the presence of porphobilinogen was low; about 7% of total uroporphyrinogen III was formed at the expense of dipyrrylmethane **5**. The incorporation of dipyrrylmethane **6** gave lower values. These results agree with the general picture of an enzymatic polymerization reaction which takes place on a protein surface, and which does not release pyrrylmethane intermediates into the reaction medium. The deaminase-co-synthase system must direct the enzymatic polymerization of porphobilinogen toward the formation of uroporphyrinogen III from the beginning of the reaction. The "head-to-head" encounter of two porphobilinogen units will give rise to the enzyme-bound dipyrrylmethane **5**, where the displaced aminomethyl chain remains bound to the α-free position of the dipyrrylmethane (Frydman et al., 1976). The incorporation of dipyrrylmethane **6** into uroporphyrinogen III might indicate that this first step in the biosynthesis of uroporphyrinogen III takes place in two stages, i.e., a prior formation of the 5,5′ free dipyrrylmethane **6** followed by a recombination of **6** and the "active" aminomethyl residue to give dipyrrylmethane **5**.

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Biosynthesis of Uroporphyrinogens. Interaction among 2-Aminomethyltripyrranes and the Enzymatic System†

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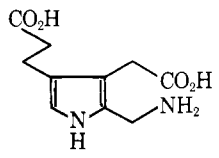
ABSTRACT: Many hypotheses on uroporphyrinogen biosynthesis advanced the possibility that 2-aminomethyltripyrranes formed by porphobilinogen deaminase are further substrates of uroporphyrinogen III co-synthase in the presence of porphobilinogen. These proposals were put to test by employing synthetic 2-aminomethyltripyrranes formally derived from porphobilinogen. None of them was found to be by itself a substrate of deaminase or of co-synthase in the presence of porphobilinogen. The tripyrranes chemically formed uroporphyrinogens by dimerization reactions, and the latter had to be deducted in control runs during the enzymatic studies. Two of the tripyrranes examined, the 2-aminomethyltripyrrane **7** and the 2-aminomethyltripyrrane **8,** were found to be incorporated into enzymatically formed uroporphyrinogen III in the presence of porphobilinogen and of the deaminase-co-synthase system. While the former gave only a slight incorporation, the latter was incorporated in about 16%. No incorporation of **8** into uroporphyrinogen I was detected. On the basis of these results, and of the previous results obtained with 2-aminomethyldipyrrylmethanes, an outline of the most likely pathway of uroporphyrinogen III biosynthesis from porphobilinogen is given.

Porphobilinogen (**1**) (PBG)[1] is transformed into uroporphyrinogen III (**2**) and into uroporphyrinogen I (**3**) in an enzymatic reaction which is catalyzed by two enzymes: porphobilinogen deaminase and uroporphyrinogen III co-synthase (Bogorad, 1958a,b).



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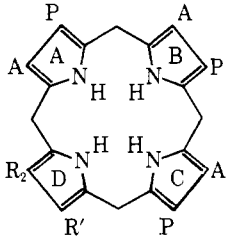

2, R′ = P; R_2 = A
3, R′ = A; R_2 = P

A = CH_2CO_2H; P = $CH_2CH_2CO_2H$

The deaminase is the substrate-consuming enzyme and it gives rise to uroporphyrinogen I (**3**). The enzymatic reaction proceeds with the loss of 4 mol of ammonia, and a stoichiometric relation of 4 mol of substrate consumed per mol of product formed is obtained. Hence, the reaction mechanism can be easily understood as a "head-to-tail" self-polymerization of the natural Mannich base. In the presence of the co-synthase, however, the reaction is driven toward the formation of uroporphyrinogen III (**2**). The mechanism of this reaction has been the subject of much speculation (see Frydman and Frydman (1975a) for a review of the problem). Bogorad (1958a,b) found that the co-synthase is not a substrate-consuming enzyme but takes part somehow in the process and drives it toward uroporphyrinogen III formation instead of uroporphyrinogen I formation. Since co-synthase affects the kinetic constants of the deaminase (Bogorad, 1958a,b; Frydman and Feinstein, 1974), many hypotheses on this reaction mechanism have advanced the idea that the deaminase forms a pyrrylmethane intermediate which serves as a further substrate of the co-synthase together with porphobilinogen. It is during this second enzymatic reaction that the rearrangement would take place leading to the formation of uroporphyrinogen III instead of uroporphyrinogen I. It has thus been proposed (Radmer and Bogorad, 1972; Dalton and Dougherty, 1969; Conford, 1964) that deaminase forms the tripyrrane **4** through a sequential "head-to-tail" polymerization. The tripyrrane **4** is then taken up by the co-synthase and condensed with the fourth molecule of porphobilinogen to give uroporphyrinogen III.

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[1] Abbreviations used are: PBG, porphobilinogen; TLC, thin-layer chromatography.

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