Enzymic Formation of the Type-III Porphyrin Macrocycle from Unrearranged AP·AP Pyrromethane

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Summary The unrearranged [^{14}C]-AP·AP pyrromethane (**2**) is converted by deaminase–cosynthetase into uro'gen-III (**7**), thus supporting initial *head-to-tail* combination of porphobilinogen units for biosynthesis of the natural type-III porphyrins.

THE significance of uro'gen-III (**7**) was emphasised in the preceding communication; its biosynthesis from 4-molecules of porphobilinogen (PBG) (**1**) is catalysed by deaminase with cosynthetase. No intermediates between PBG (**1**) and uro'gen-III (**7**) have been detected under normal conditions.

The building process can be viewed as a stepwise one in which the catalysed combination of the first two PBG units yields an unrearranged structure (**2**, or in enzyme-bound form with NH_2 replaced by enzyme) or a rearranged one (*e.g.* **3**, or with NH_2 replaced by enzyme). Enzymic attachment of two further PBG units to such a pyrromethane would then yield a bilane (*e.g.* **5**, or with NH_2 replaced by enzyme) ready for formation of the porphyrinogen macrocycle.

Experiments were carried out [1,2] to test as precursors of uro'gen-III (**7**) various isomeric aminomethylpyrromethanes, derivable in principle from PBG (**1**). Two candidates were eliminated[1,2] and attention was focussed on unrearranged AP·AP (**2**) formed by *head-to-tail* combination of PBG units and on the rearranged PA·AP (**3**), produced by initial *head-to-head* reaction of PBG units with migration of a C_1-residue. The reports from other laboratories supporting the latter possibility have been summarised in the preceding paper and elsewhere.[1,3]

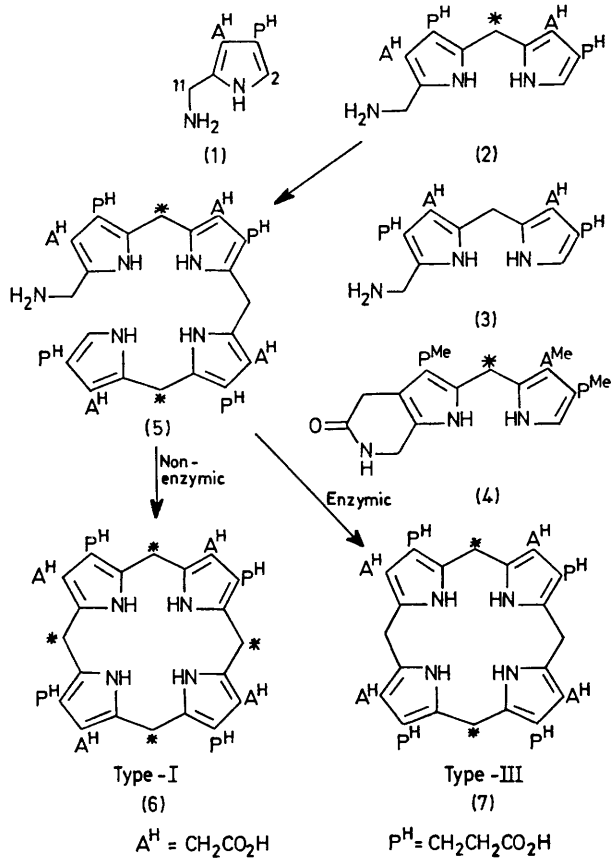

TABLE. Radioactivity of the uro'gen isomers formed from AP·AP (**2**)

	Uro'gens formed[a] (% of total)			
	Type-I	Type-II	Type-III	Type-IV
Blank run ..	68 ± 2	1 ± 0·5	3 ± 2	28 ± 2
Enzyme run ..	15 ± 1	2 ± 1	54 ± 2	29 ± 2

[a] Analysed as the corresponding coproporphyrin tetramethyl esters by h.p.l.c.

The present results show that uro'gen-III (**7**) is formed from *unrearranged* AP·AP (**2**) far more efficiently in the presence of a concentrated, purified deaminase–cosynthetase enzyme preparation than in a parallel blank experiment. This clear distinction between enzymic and blank runs was not possible with our earlier enzyme preparations.[2c]

The AP·AP lactam ester (**4**) was synthesised as earlier[4] ^{14}C-labelled at the position marked * and h.p.l.c. proved this product to be free from isomers.[2c] This product (15 mg) was hydrolysed with alkali to yield the AP·AP system (**2**) which was incubated for 16 h with deaminase–cosynthetase (*ca.* 38,000 units) at 37 °C and pH 7·2. The experiment was completed by addition of a mixture of uro'gens-I (**6**), -II, -III (**7**), and -IV (0·5 mg each), freshly prepared by reduction of the corresponding mixture of porphyrins; an excess of iodine was then immediately added to aromatise all the porphyrinogens to porphyrins. Work-up was as earlier[5] and the coproporphyrin esters† were separated preparatively by h.p.l.c.[6]

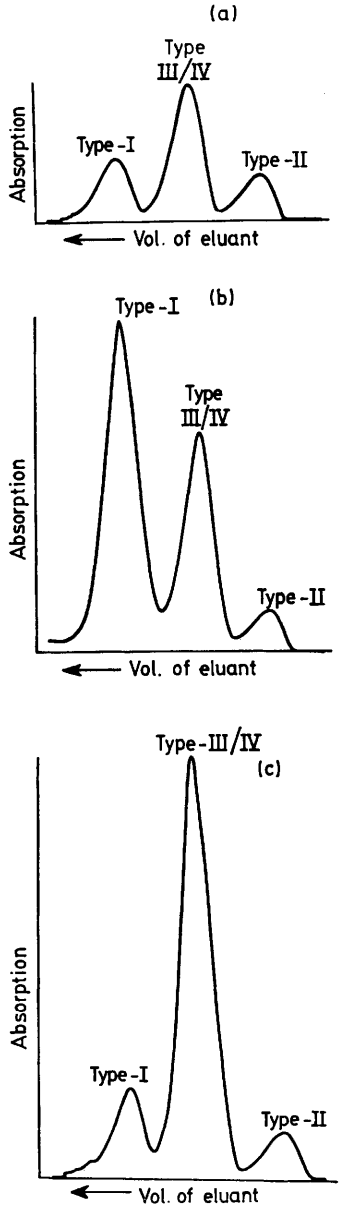

FIGURE. H.p.l.c. traces for analyses of coproporphyrin esters run on CN normal phase column; u.v. analyser set at *ca.* 390 nm. (a) Carrier material added; (b) blank run; (c) enzyme run.

† Structures of the four coproporphyrin isomers are drawn in the following communication.

A parallel blank run was carried out using boiled (inactivated) enzyme.

The Figure (a) shows the h.p.l.c. trace for an equimolar mixture of the four coproporphyrin methyl esters; the coproporphyrin esters which represent the macrocyclic products from the blank (Figure, b) and enzymic (Figure, c) runs are superimposed on this Figure (a) curve for added carrier material. The Figure (b) shows that in the blank run, a large amount of type-I isomer has been formed together with a smaller quantity of type-III and/or type-IV. In sharp contrast the Figure (c) shows that the major enzymic product is the type-III isomer *formed at the expense of the type-I isomer*. Preparative h.p.l.c. on a different column[6] of the central peak from blank and enzymic runs proved that type-III plus the type-IV isomer were present and radio-assay of all four isomers gave the complete analysis in the Table; this should be viewed in conjunction with the Figure (a—c).

Note that the amounts of the type-IV and type-II isomers formed chemically[1,2] from AP·AP (**2**) are unaffected by the enzyme but, importantly, the major part (*ca.* 78%) of the reaction sequence which in the blank run leads to uro'gen-I (**6**) is diverted by deaminase–cosynthetase to form uro'gen-III (**7**). The absolute incorporation of AP·AP (**2**) into uro'gen-III (**7**) is 17% corresponding to an isolated quantity of 2·5 mg of enzymically formed uroporphyrin-III.

These results and all our knowledge of this area fall into place on the view that in the above experiments AP·AP (**2**) first yields the aminomethylbilane (**5**) in a chemical step and, as it is formed, the bilane is steadily converted by deaminase–cosynthetase into uro'gen-III (**7**). The supporting points are: (a) chemical formation of uro'gen-I (**6**) must involve prior formation of unrearranged bilane (**5**) (b) the formation of closely related bilanes from aminomethylpyrromethanes at pH 8·2 *in vitro* is known,[7] and (c) the aminomethylbilane (**5**) is accepted by deaminase–cosynthetase and is converted efficiently thereby into uro'gen-III[5] (**7**).

These high enzymic incorporations of AP·AP (**2**) into uro'gen-III (**7**) contrast with the very low 'incorporations' found earlier[2c] in tests of [^{14}C]-PA·AP (**3**) into the type-III macrocycle for long or short incubations, with or without PBG (**1**). Further support for the above results comes from $^{13}C_2$-labelling studies outlined in the following Communication which establish the *mode* of incorporation of AP·AP (**2**).

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[1] B. Frydman and R. B. Frydman, *Accounts Chem. Res.*, 1975, **8**, 201; B. Frydman, R. B. Frydman, A. Valasinas, E. S. Levy, and G. Feinstein, *Phil. Trans. Roy. Soc.* (*B*), 1976, **273**, 137.

[2] (a) A. R. Battersby, 23rd International Congress of Pure and Applied Chemistry, Special Lectures, 1971, vol. 5, p. 1; (b) A. R. Battersby, K. H. Gibson, E. McDonald, L. N. Mander, J. Moron, and L. N. Nixon, *J.C.S. Chem. Comm.*, 1973, 768; (c) A. R. Battersby, and E. McDonald, *Phil. Trans. Roy. Soc.* (*B*), 1976, **273**, 161.

[3] A. I. Scott, K. S. Ho, M. Kajiwara, and T. Kakahashi, *J. Amer. Chem. Soc.*, 1976, **98**, 1589.

[4] A. R. Battersby, D. A. Evans, K. H. Gibson, E. McDonald, and L. N. Nixon, *J.C.S. Perkin I*, 1973, 1546.

[5] A. R. Battersby, E. McDonald, D. C. Williams, and H. K. W. Wurziger, preceding Communication; *cf.* ref. 7.

[6] A. R. Battersby, D. G. Buckley, G. L. Hodgson, R. E. Markwell, and E. McDonald in 'High Pressure Liquid Chromatography in Clinical Chemistry,' eds. P. F. Nixon, C. H. Gray, C. K. Lim, and M. S. Stoll, Academic Press, London, 1976, p. 63.

[7] H.-O. Dauner, G. Gunzer, I. Heger, and G. Müller, *Z. physiol. Chem.*, 1976, **357**, 147.