Pre-uroporphyrinogen: a Substrate for Uroporphyrinogen III Cosynthetase

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Summary Pre-uroporphyrinogen, produced from porphobilinogen by the action of porphobilinogen deaminase has been shown to act as a substrate for the enzyme uroporphyrinogen III cosynthetase, being converted in high yield into uroporphyrinogen III; in the absence of cosynthetase, pre-uroporphyrinogen rearranges chemically to afford exclusively uroporphyrinogen I, and evidence is also presented which demonstrates that the deaminase and cosynthetase function independently and sequentially in the overall conversion of porphobilinogen into uroporphyrinogen III.

TWENTY years ago the dual action of porphobilinogen (PBG) deaminase and uroporphyrinogen (uro'gen) III cosynthetase for the enzymic synthesis of uro'gen III (**2a**)† was established.[1] Since then a large number of hypotheses have been put forward to explain the mechanism and timing of the rearrangement which occurs in ring D, most of them involving the action of the deaminase and cosynthetase together in the form of an 'enzyme complex'.[2,3,4] More recently the interesting approach of using dipyrromethanes[3,5] and related compounds[6] which, however, are not physiological substrates has led to results which, by virtue of combining *in vitro* and *in vivo* reactions, are difficult to interpret with respect to the stage at which the rearrangement occurs in the overall sequence from porphobilinogen (PBG) (**1**)† to uro'gen III. In spite of the useful information provided by the use of such synthetic substrates, the mechanism by which PBG is incorporated into uro'gen III under normal conditions is largely unknown because no free intermediate between PBG and uro'gen has been identified by isolation.

Our observation from ^{13}C n.m.r. spectroscopy of pre-uro'gen, the first detectable compound formed by PBG deaminase, led us to examine the possibility that under the influence of uro'gen III cosynthetase, rearrangement of this intermediate may be directed to form the isomer III.

Accordingly, PBG deaminase (80 units/mg) was incubated with PBG (see previous communication) and at various times aliquots were filtered to afford a deaminase-free solution of the pre-uro'gen (stage 1). The concentration of pre-uro'gen reached a maximum after *ca.* 50% of the PBG had been consumed (15 min) and decreased to zero after 40 min, as determined by ^{13}C n.m.r. spectroscopy (previous communication) and by the differential reactivity between uro'gen I and pre-uro'gen to several oxidizing agents.[7] The pre-uro'gen filtrates were incubated with uro'gen III cosynthetase[8] (16 units/ml, 37 units/mg)‡ together with the appropriate controls (stage 2) which showed the complete absence of deaminase, since during stage 2 there was no further consumption of PBG. The amounts of uro'gen III formed during these experiments are shown in the Table.

† For the structures discussed herein, see previous communication.

‡ One unit of uro'gen III cosynthetase is equivalent to 1 μmol pre-uro'gen per hour determined under standard uro'gen III cosynthetase assay conditions.

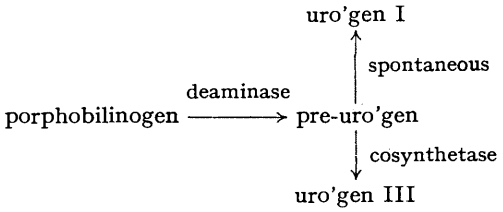
TABLE

Incubation time/min Stage 1	Incubation time/min Stage 2	% Isomer III formed		% of pre-uroporphyrinogen present	% Conversion of pre-uroporphyrinogen into uroporphyrinogen III
Deaminase	Cosynthetase	Cosynthetase	Buffer		
11	10	37[a]	0[a]	43[c]	86
23	10	21[a]	0[a]	24[c]	87
37	10	7[a]	0[a]	—	—
60	10	0[a]	0[a]	0[c]	—
30	10	13[b]	0[b]	—	—

[a] Determined by h.p.l.c. of uroporphyrin methyl esters by modification of the method of J. C. Bommer, B. F. Burnham, R. E. Carlson, and D. Dolphin, *Analyt. Biochem.*, in the press, μ porasil column; solvent: heptane–acetic acid–acetone–H_2O (90:60:30:0·5). [b] Determined by h.p.l.c. of coproporphyrin methyl esters. [c] Determined by ^{13}C n.m.r. spectroscopy in a separate experiment (see previous communication).

The data clearly demonstrate several important features of the uro'gen III synthesizing system. (i) Pre-uro'gen is formed transiently by the action of PBG deaminase and, in the absence of uro'gen III cosynthetase, is transformed into uro'gen I in acidic, basic, or neutral media. (ii) The formation of uro'gen I from pre-uro'gen occurs in the absence of deaminase. (iii) Pre-uro'gen is converted into uro'gen III in high yield by uro'gen III cosynthetase alone. (iv) PBG Deaminase is not required for the conversion of pre-uro'gen into uro'gen III. (v) Pre-uro'gen is tetrapyrrolic since PBG is not consumed during its conversion into uro'gen III. (vi) Uro'gen I does not act as a substrate for uro'gen III cosynthetase.

The available data from this and the preceding communication suggest that a hitherto unsuspected pathway for the biosynthesis of uro'gen III is operative in which the enzymes act sequentially and independently as shown in the Scheme, with pre-uro'gen as the key intermediate. The discovery and nature of pre-uro'gen as the true substrate for cosynthetase requires that the rearrangement takes place after head-to-tail assembly[5] and not, as previously suggested, at an earlier stage.[3,6]



SCHEME

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[1] L. Bogorad, *J. Biol. Chem.*, 1958, **233**, 501, 510, and 516.
[2] M. Higuchi and L. Bogorad, *Ann. New York Acad. Sci.*, 1975, **244**, 401.
[3] B. Frydman and R. B. Frydman, *Accounts Chem. Res.*, 1975, **8**, 201 and references therein.
[4] A. R. Battersby and E. McDonald, *Phil. Trans.*, 1976, **273**, 161 and references therein.
[5] A. R. Battersby, D. W. Johnson, E. McDonald, and D. C. Williams, *J.C.S. Chem. Comm.*, 1977, 117.
[6] A. I. Scott, K. S. Ho, M. Kajiwara, and T. Takahashi, *J. Amer. Chem. Soc.*, 1976, **98**, 1589.
[7] A. I. Scot t, G. Burton, and P. M. Jordan, unpublished results.
[8] A. I. Scott, H. Nordlöv, P. M. Jordan, M. M. Schneider, S. Hosozawa, and L. Pryde, manuscript in preparation.