Biosynthesis of Natural Porphyrins: Enzymic Experiments on Isomeric Bilanes

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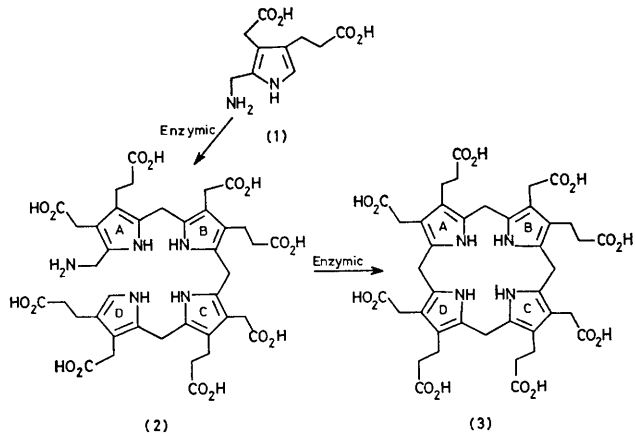
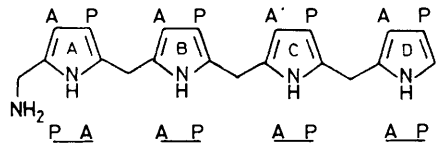
Summary The deaminase–cosynthetase enzyme system ring closes the unrearranged bilane (**2**) far more efficiently than it closes any of five synthesised isomeric bilanes, thus supporting (**2**) as the key intermediate of type-III porphyrin biosynthesis.

EARLIER ^{14}C- and ^{13}C-labelling experiments with pyrromethanes[1] and with the unrearranged bilane[2,3] (**2**) showed that the biosynthetic pathway to the natural, type-III porphyrins involves head-to-tail joining of 4 units of porphobilinogen (**1**) (PBG) by the deaminase–cosynthetase enzyme complex to form the unrearranged bilane† (**2**) *followed by* a single rearrangement step,[4] the intramolecular inversion of ring-D,[3,4] to produce uro'gen-III (**3**). Complementary experiments are now outlined on a set of synthetic bilanes which differ from the unrearranged bilane (**2**) by having one ring 'inverted' [see (**4**)—(**7**)] or more than one [see (**8**)]. Enzymic experiments with bilanes have the clear advantage over those with pyrromethanes or tripyrroles that addition of PBG (**1**) is unnecessary, allowing a single substrate process to be observed.



† The bilane may be covalently bound to the enzyme with NH_2 being replaced by a nucleophilic group of the protein.



(2)

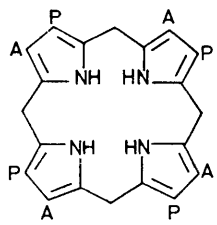
P A A P A P A P (4)

A P P A A P A P (5)

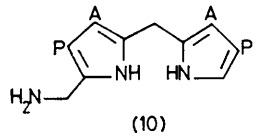
A P A P P A A P (6)

A P A P A P P A (7)

P A P A P A A P (8)



(9)



(10)

A = CH_2CO_2H

P = $CH_2CH_2CO_2H$

was no significant enhancement by the enzyme system of the ring closure of bilanes (**4**) and (**8**). In fact, (**8**) inhibited the action of deaminase–cosynthetase on PBG.

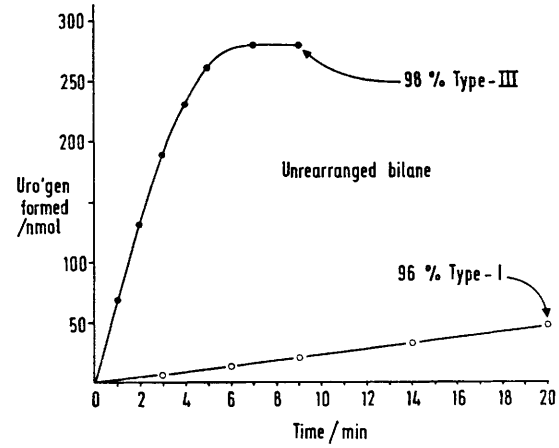


FIGURE. Rates of chemical (○) and enzymic (●) conversion of 21 μM unrearranged bilane (**2**) into uro'gen macrocycles at pH 7·75; 70,000 units of deaminase–cosynthetase (from *Euglena gracilis*) in total vol. of 30 ml (1 unit = 1 nmol porphyrinogen produced per hour from PBG).

Synthesis of the bilanes (**4**)—(**8**) followed the earlier strategy[2] for bilane (**2**) and the penultimate lactam heptamethyl esters (*cf.* ref. 2) were characterised by n.m.r. and accurate mass spectroscopy. Hydrolysis then afforded the aminomethylbilanes (**2**) and (**4**)—(**8**) for immediate incubation with deaminase–cosynthetase.‡

Isomer analyses as earlier[2] on the products from the foregoing work (Table) showed that there is an essentially quantitative switch from chemical formation of uro'gen-I (**9**) to enzymic production of uro'gen-III (**3**) from the regular bilane (**2**) under the best conditions; these gave uro'gen which is 98% type-III (**3**) and enzymic yields of up

TABLE. Interaction of aminomethylbilanes with deaminase–cosynthetase from *Euglena gracilis*[a]

Substrate	V_{max}[b]	$K_m/\mu M$[c]	Uro'gen isomers produced/% Chemical I	II	III	IV	Enzymic I	II	III	IV
Unrearranged bilane (**2**)	64±1	5·1±0·2	95	0	4	1	8	0	90	2
Reversed ring A (**4**)	—	—	3·5	0	94·5	2	3·5	0	91·5	5
Reversed ring B (**5**)	25±1	72±3	0	5·5	94·5		0	8·5	90	1·5
Reversed ring C (**6**)	—	—	0	2	94·5	3·5	0	2·5	90	7·5
Reversed ring D (**7**)	7·7±0·2	11±1	9·5	0	88	2·5	22	0	76	2
Reversed rings ABC (**8**)	—	—	2·5	0	95·5	2	2·5	0	94·5	3
PBG (**1**)	1000	104±7								

[a] Uro'gen production at pH 7·75 monitored at 406 nm after oxidation to porphyrins; chemical production of uro'gens was determined independently and subtracted from values found in enzymic runs to give true initial enzymic rates. Kinetic constants were obtained from double reciprocal plots using regression analysis. [b] V_{max} is arbitrarily set at 1000 for PBG and the rest are referred to this. [c] Concentration of bilane was calculated from weight of lactam heptmethyl ester hydrolysed; this gives maximum values for K_m.

It was found (see Table and Figure) that the unrearranged bilane§ (**2**) was the best substrate for deaminase–cosynthetase (highest V_{max} and lowest K_m) but ring-closure of bilanes (**5**) and (**7**) was also enzymically accelerated. In contrast, the enzymic acceleration for bilane (**6**) was only just detectable above the spontaneous (chemical) cyclization whilst there to 70%.¶ For the bilane (**7**), which binds relatively well, the α-free terminal ring is also inverted to some extent to give uro'gen-I (**9**), thus emphasising the role of deaminase–cosynthetase as an enzyme system designed for rearrangement at the tetrapyrrole level. There are indications (Table) that some enzymic reversal of the α-free terminal

‡ Extracted from *Euglena gracilis* and partially purified by $(NH_4)_2SO_4$ precipitation, gel filtration, and ion-exchange chromatography; it converted PBG into >98% uro'gen-III (**3**).

§ There are several explanations of the faster rate of uro'gen-III (**3**) production from PBG than from bilane (**2**), *e.g.* the bilane (**2**) built from PBG is correctly located on the binding sites whereas for the large mobile bilane in solution, many abortive or partial bindings are possible in competition with that leading to catalysis.

¶ Related work by K. E. Gustafson-Potter (Cambridge) has shown that deaminase alone handles the bilane (**2**) as efficiently as does deaminase–cosynthetase and it accelerates the cyclization to give essentially pure uro'gen-I (**9**).

ring may also occur for isomers (**4**), (**5**), and (**6**) but further work is needed.

The foregoing results add strong evidence against the view[5,6] that the biosynthesis of uro'gen-III (**3**) occurs *via* the rearranged pyrromethane (**10**), PA·AP, since the bilanes thought to be derived[5] from PA·AP are isomers (**6**) and (**8**) which are shown above not to be significant substrates for deaminase–cosynthetase. Rather, the new findings support our conclusion[2,3] that the biosynthesis of natural porphyrins involves three head-to-tail reactions of PBG (**1**) to produce the regular bilane (**2**) which undergoes rearrangement to give uro'gen-III (**3**) very probably[3] *via* a spirocyclic intermediate.

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