Stereochemistry of Hydrogen Elimination in the Enzymic Formation of the C-2—C-3 Double Bond of Porphobilinogen

By MUAYAD M. ABBOUD and MUHAMMAD AKHTAR

(*Department of Physiology and Biochemistry, University of Southampton, Southampton* SO9 3TU)

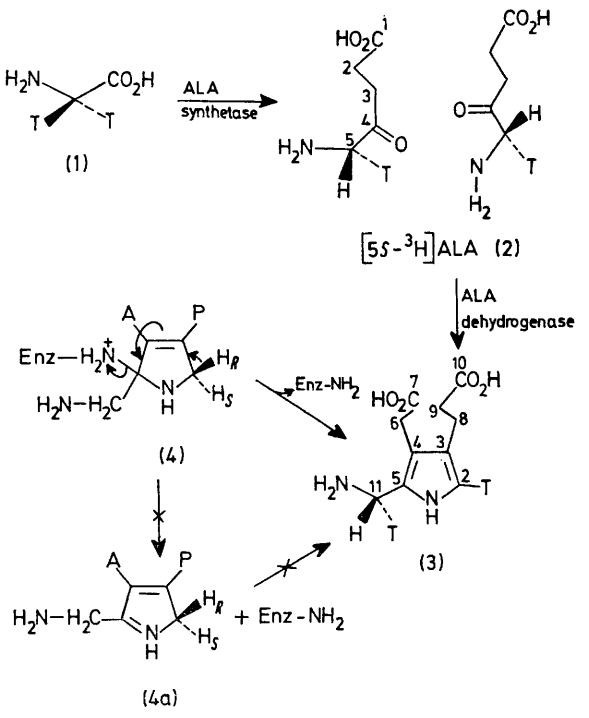
Summary Using 5-aminolaevulinate dehydratase from *Rhodopseudomonas spheroides* it is shown that the tritium located in the *pro-S* position at C–5 of 5-aminolaevulinate (**2**) is retained at C–2 of porphobilinogen (**3**).

5-AMINOLAEVULINATE DEHYDRATASE from *Rhodopseudomonas spheroides* catalyses the condensation of two molecules of 5-aminolaevulinic acid (ALA; **2**, T = H) to produce porphobilinogen (PBG; **3**, T = H) which is a committed intermediate in the biosynthesis of porphyrins and related natural products. We have recently shown that when [2*RS*-$^3H_2$]glycine (**1**) was converted into ALA and then PBG through the co-operation of two purified enzymes, 5-aminolaevulinate synthetase and 5-aminolaevulinate dehydratase, the C–5 of ALA and the amino methyl group of PBG contained tritium in the *pro-S* position.[1] Since the C–2 of PBG is derived from C–5 of one of the molecules of ALA, the preceding approach could, in principle, be used to determine the stereochemistry of hydrogen elimination in the formation of the C–2–C–3 double bond of PBG. This objective has hitherto not been realized, primarily owing to the fact that under the conditions of ion exchange chromatography normally used for the isolation and purification of biosynthetic PBG its C–2 hydrogen atom exchanges with the protons of the medium, thus making the result of an isotopic experiment uninterpretable This difficulty has now been overcome by our discovery that PBG may be isolated without disturbing the C–2 hydrogen atom by cellulose t.l.c. using n-butanol–acetic acid–water (4 : 1 : 1, v/v) as the solvent.

[2*RS*-$^3H_2$]Glycine was incubated with ALA-synthetase and ALA-dehydratase, both purified from *Rhodopseudomonas spheroides*, for 15 min.† The incubation mixture after the addition of $NaBH_4$ was chromatographed on cellulose t.l.c. which allowed an initial separation of glycine (R_f 0·3), dihydro-5-aminolaevulinic acid (R_f 0·55), and PBG (R_f 0·7). The PBG band was eluted with water and the sample rapidly freeze-dried and rechromatographed to give a single Ehrlich positive radioactive band which ran with authentic PBG. The material from the band was removed and used for the determination of radioactivity. To ensure that the radioactivity incorporated in the R_f 0·7 band was, in fact, due to PBG biosynthesis, two types of control experiments were also carried out in

† The incubation mixture in a final 1-ml volume contained [2*RS*-$^3H_2$: 2-^{14}C]glycine (^{3}H : ^{14}C ratio 7·7 : 1) (4 μmol), succinyl CoA (2·5 μmol), potassium phosphate buffer at pH 7·0, 0·1M, pyridoxal phosphate 5×10^{-3} (μmol); 2-mercaptoethanol, 0·2 (μmol); ALA synthetase (8 units); 1000–1500 fold purified) (G. R. Warnick and B. F. Burnham, *J. Biol. Chem.*, 1971, **246**, 6880) and ALA dehydratase (12 units; 500 fold purified) (D. L. Nandi, K. F. B. Cohen, and D. Shemin, *J. Biol. Chem.*, 1968, **243**, 1224). The incorporation of glycine into PBG in a 15-min incubation was about 2%.

parallel. In the first a mixture identical to the one used above was prepared and subjected to chromatography without incubation, while in the second, the incubation was allowed to proceed in the absence of ALA-dehydratase. With both samples no radioactivity was detected in the PBG region of the chromatograms. In four consecutive experiments the relative $^3H:^{14}C$ ratios of the parent as well as the recovered glycine, of the C–5 of 5-aminolaevulinic acid isolated as the formaldehyde dimedone derivative, and of PBG were found to be 1·0, 0·43, and 0·48, respectively. Thus the tritium content of PBG was exactly half of that originally present in glycine but similar to that in ALA. That the $^3H:^{14}C$ ratio of ALA was slightly lower than that of the PBG merely emphasises the extreme instability of the hydrogen atoms attached to C–5 of ALA and reflects the loss of ^3H which occurs during the full 15 min incubation.‡ The molecules of ALA which commit themselves to PBG during the incubation are protected from this loss.



SCHEME. (**4**) and (**4a**) are the intermediates in the mechanism of ALA-dehydratase proposed by Nandi and Shemin (ref. 3); –A and –P are acetic and propionic side chains, respectively.

These results showing the stereospecific retention of the C–5 *pro-S* hydrogen atom of ALA in the aromatization process suggest that the removal of the *pro-R*-proton from an intermediate of the type (**4**) occurs while the species is enzyme-bound and not after its release into the medium (**4**→**4a**→**3**) as is implicated in the otherwise excellent mechanistic sequence proposed by Nandi & Shemin.[2]

This approach has subsequently been extended by Dr P. M. Jordan and Mr A. G. Chaudry to ALA-dehydratase from bovine liver[3] and it was found that, like the bacterial enzyme, the bovine enzyme also gave PBG which contains half the amount of tritium present in the precursor [2*RS*-^3H]glycine. Furthermore the biosynthetic sample of PBG lost nearly 50% of the tritium on being treated with HCl.

(*Received*, 16*th August* 1976; *Com.* 944).

‡ The exactly 50% loss of tritium in the conversion of [2*RS*-$^3H_2$]glycine into ALA previously reported (Z. Zaman, P. M. Jordan, and M. Akhtar, *Biochem. J.*, **1973**, **135**, 257) was when the incubation was performed in Tris buffer. The exchange of the C–5 hydrogen atom of ALA in the latter buffer is lower than that in phosphate buffer. However, the knowledge that a five-fold improvement in the yield of ALA from glycine is observed in phosphate buffer necessitated the use of the latter buffer in the present work.

[1] M. M. Abboud, P. M. Jordan, and M. Akhtar, *J. C. S. Chem. Comm.*, 1974, 643; M. Akhtar, M. M. Abboud, G. Barnard, P. M. Jordan, and Z. Zaman, *Phil. Trans. B*, 1976, **273**, 117.
[2] D. L. Nandi and D. Shemin, *J. Biol. Chem.*, 1968, **243**, 1236.
[3] A. G. Chaudhry and P. M. Jordan, *Biochem. Soc. Trans.*, 1976, in the press.