Biosynthesis of 5-Aminolevulinic Acid: Involvement of a Retention–Inversion Mechanism

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Summary It has previously been shown that in the biosynthesis of 5-aminolevulinic acid the *pro*-2*R*-hydrogen atom of glycine is removed; the present work shows that the *pro*-2*S*-hydrogen atom of the precursor glycine occupies the *S*-configuration at C-5 of 5-aminolevulinic acid.

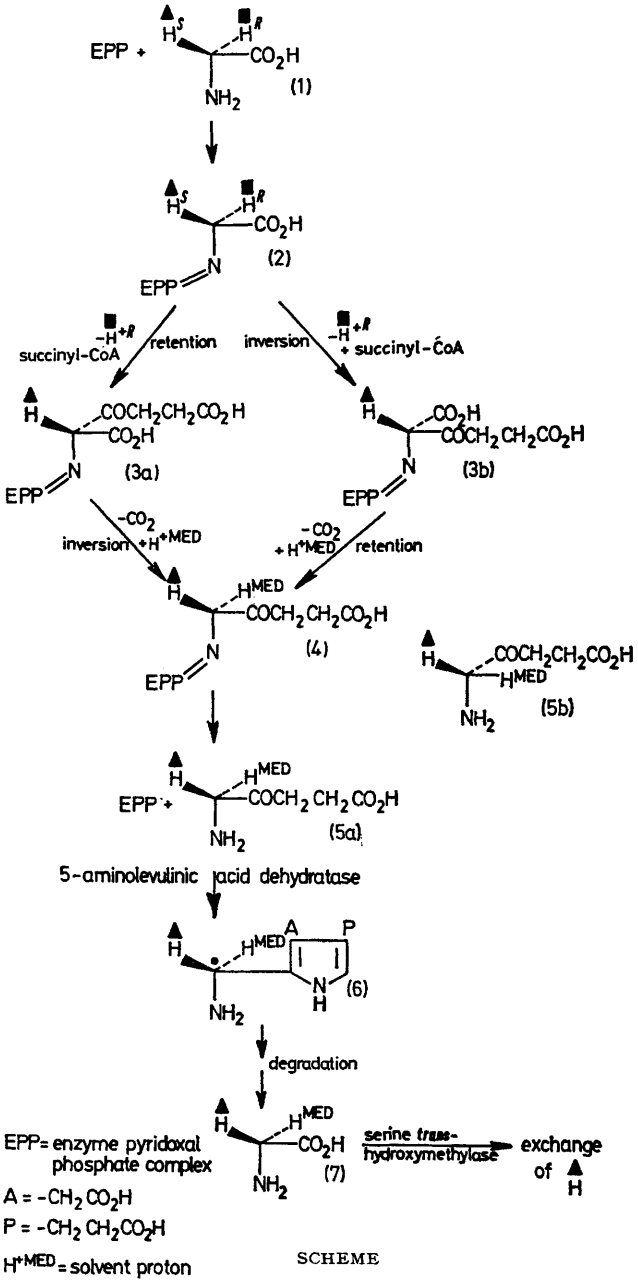
5-AMINOLEVULINIC ACID synthetase catalyses the condensation between glycine and succinyl CoA and requires the participation of pyridoxal phosphate as a cofactor.[1]

$$NH_2CH_2CO_2H + CoASCOCH_2CH_2CO_2H \rightarrow NH_2CH_2COCH_2CH_2CO_2H + CoASH + CO_2$$

Previous work in our laboratories[2,3] using glycines variously tritiated in the 2-position showed that during the synthesis of 5-aminolevulinic acid by 5-aminolevulinic acid synthetase from *Rhodopseudomonas sphereoides*, the *pro-R* hydrogen atom of glycine is removed. The *pro-S* hydrogen atom however, is retained and becomes incorporated into the 5-position of 5-aminolevulinic acid. We now describe further experiments pertinent to the elucidation of the stereochemical events occuring at position 5 during the conversion of glycine into 5-aminolevulinic acid.

Because of the relatively unstable nature of 5-aminolevulinic acid (**5**) particularly with respect to the hydrogen atoms at the 5-position, 5-aminolevulinic acid itself is unsuitable for a detailed stereochemical study. We, therefore, developed a system whereby 5-aminolevulinic acid, as soon as it was produced by 5-aminolevulinic acid synthetase,[4] was enzymically converted into porphobilinogen (**6**) by a highly purified preparation of 5-aminolevulinic acid dehydratase,[5] the next enzyme in the porphyrin biosynthetic pathway. The hydrogen atoms in question are now essentially in a stable position (● in 6 in the Scheme). The biosynthetic porphobilinogen (PBG) was then *N*-acetylated and oxidized with permanganate to yield *N*-acetylglycine which was hydrolysed to glycine (**7**). The stereochemistry of the tritium present in the glycine (**7**) so produced was assessed by an exchange reaction using highly purified serine transhydroxymethylase (SHM)[6] as previously described.[7] It is well documented[7] that this enzyme totally exchanges the *S* hydrogen atom of glycine in 1 h. When tritiated glycine obtained from the degradation of PBG together with 2-^{14}C glycine (initial $^3H:^{14}C$ ratio 3·7:1) was incubated with SHM for 2 h, the final $^3H:^{14}C$ ratio was 0·49:1 showing a loss of 87% of the tritium label. This clearly demonstrates that almost all the tritium present in the glycine occupies the *pro S* configuration. As a control when 2*RS*-$^3H_2$:2-^{14}C glycine (initial $^3H:^{14}C$ ratio 3·65:1) was incubated with SHM, the final $^3H:^{14}C$ ratio of 1·82:1 showed the expected loss of 50% of the tritium label.

The cumulative evidence presented in this communication and elsewhere[2,3] shows that in the biosynthesis of 5-aminolevulinic acid the *pro R* hydrogen atom of the precursor glycine ■ is removed as a proton and the *pro S* hydrogen atom (▲) is retained and occupies the *pro S* configuration at the 5-position of 5-aminolevulinic acid (**5a**, Scheme). This information allows the deduction to be made that of the two crucial bond-forming events (**2**) → (**3**) and (**3**) → (**4**) one of them proceeds by an inversion and the other by a retention of configuration, either by the route



SCHEME

(**2**) → (**3a**) → (**4**) or (**2**) → (**3b**) → (**4**). Had these reactions proceeded by either two inversions or two retentions the species of 5-aminolevulinic acid (**5b**) with the opposite configuration to (**5a**) would have resulted. Furthermore, the formation of a chiral centre at the 5-position of 5-aminolevulinic acid (**5a**) eliminates the participation of a possible pathway involving the hydrolysis of the intermediate (**3**) to α-amino-β-ketoadipic acid with subsequent non-enzymic decarboxylation to 5-aminolevulinic acid.

It is interesting to note that the stereochemistry of the *R* hydrogen atom (■) originally removed in the reaction (**2**) → (**3**) has the same stereochemistry as the proton H^{MED} which is added in (**3**) → (**4**), thus suggesting that the same group on the enzyme may be involved in both processes.

Similar mechanistic conclusions have been drawn from independent work carried out at E.T.H. Zurich, but using a different approach. We are most grateful to Professor D. Arigoni for informing us of his results.

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