5-AMINO-4-HYDROXYVALERIC ACID: A NEW INTERMEDIATE IN 5-AMINOLEVULINATE METABOLISM OF RHODOSPIRILLUM RUBRUM

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SUMMARY:
When [4-^{14}C]-5-aminolevulinate was incubated with intact cells of Rhodospirillum rubrum under illumination, a large part of aminolevulinate was rapidly converted to an unknown metabolite. It was identified as 5-amino-4-hydroxyvalerate, based on its unique properties as exhibited in the behavior on paper electrophoresis, in the reactivity with ninhydrin and with $NaIO_4$, and in the capability to be transformed into lactone- as well as lactam derivative with ease. The metabolic significance of this compound was discussed with a special reference to its possible role in the control of tetrapyrrole biosynthesis.

5-Aminolevulinate is well known as a metabolic precursor of tetrapyrroles (1, 2), but the possible involvement of this compound in other pathways has also been suggested (3). According to Shemin and Russell (1), 5-aminolevulinate was reported to give rise to a compound of one-carbon unit after conversion to dioxovalerate via the succinate-glycine cycle (3, 4). As to the subsequent metabolism of dioxovalerate, another series of reactions which lead to the formation of 2-ketoglutarate as an intermediate was implicated (1). Recently, much attention has been paid to these metabolic routes with respect to their possible contribution to the regulation of tetrapyrrole biosynthesis (5, 6).

In the course of studies on the metabolic role of 5-aminolevulinate in a photosynthetic bacterium, Rhodospirillum rubrum, we discovered an additional, new type of conversion of this compound to a metabolite which has previously been unknown. This communication deals with the isolation and identification of the newly found metabolite.

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METHODS

R. rubrum was grown anaerobically on malate-$(NH_4)_2SO_4$ medium (7) under illumination for 40 hrs at 30° C.

[4-^{14}C]-5-Aminolevulinate (1.0×10^5 cpm/μmole) was incubated with intact cells in 5 ml of 0.1 M potassium phosphate buffer, pH 7.0, containing 1 mM $MgCl_2$ in a 50 ml conical flask at 30° C for indicated period aerobically under illumination with a 300 W tungsten lamp. The reaction was stopped by heating the mixture. After removal of cell debris by centrifugation, the supernatant solution was applied to a column (0.8 × 5 cm) of Dowex 2x8, acetate form. The effluent contained non-acidic compounds including unassimilated 5-aminolevulinate and was designated as the neutral amino acid fraction. The components adsorbed on the column were eluted from it with 2 N HCOOH and were designated as the acidic fraction.

The former fraction was reacted with acetylacetone (0.2 ml/10 ml of the fraction) at 100° C for 10 min to convert the unassimilated 5-aminolevulinate to an acidic pyrrole derivative, 2-methyl-3-acetylpyrrole-5-propionic acid (8), and then the mixture was submitted to paper electrophoresis on Toyo Roshi No. 514 paper at 50 v/cm using 1 M pyridine-acetate buffer, pH 6.0. The radioactivity in these fraction was determined with an end-window gas flow counter. 5-Aminolevulinate was estimated by the method of Mauzerall and Granick (8).

Periodate oxidation of the isolated product was performed by reacting it with 2 μmoles of $NaIO_4$ in 1 ml of 0.1 M $NaHCO_3$ at 30° C for 10 min (9). The amount of $NaIO_4$ consumed was estimated by the decrease of absorbance at 240 mμ. Succinic semialdehyde formed in the above reaction was converted to its 2,4-dinitrophenylhydrazone. The derivative was separated on bicarbonate-treated paper (Toyo Roshi No. 50) using butanol-ethanol-0.1 M $NaHCO_3$ (3:1:3, upper phase) according to the method of Katsuki et al. (10).

Authentic 5-amino-4-hydroxyvalerate was synthesized by reducing 5-aminolevulinate with $NaBH_4$ in 0.1 M $NaHCO_3$ by a modification of the method described by Gallop et al. (11).

RESULTS

Production of unknown metabolite(s) from 5-aminolevulinate by intact cells When [4-^{14}C]-5-aminolevulinate (8.0 μmoles) was incubated with intact cells of R. rubrum under illumination, a rapid decrease of this compound estimated by the method of Mauzerall and Granick, to an extent as low as 10 % of the amount added in less than 30 min, was observed as shown in Fig. 1. On the other hand, the radioactivity in the neutral amino acid fraction decreased much less rap-

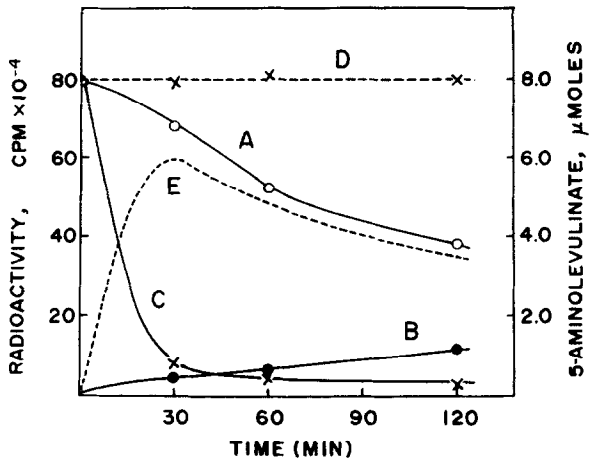

Fig. 1 Assimilation of [4-^{14}C]-5-aminolevulinate in R. rubrum. [4-^{14}C]-5-Aminolevulinate (8.0 μmoles) was incubated with intact cells of R. rubrum (100 mg in dry wt) as described in the text. Aliquots (0.5 ml) were taken at indicated time intervals and were analyzed for their radioactivity and the content of 5-aminolevulinate after separation into the neutral amino acid fraction and the acidic fraction. The results were expressed as the amount per 5 ml of the original reaction mixture:

Curve A; radioactivity in the neutral amino acid fraction
Curve B; radioactivity in the acidic fraction
Curve C; amount of 5-aminolevulinate in the neutral amino acid fraction
Curve D; amount of 5-aminolevulinate in the boiled cell suspension
Curve E; formation of unidentified product(s), showing the difference between Curves A and C.

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idly. It decreased to 87 % of the initial total radioactivity after 30 min and reached to 48 % after 120 min. This large discrepancy between the content of 5-aminolevulinate and that of radioactivity in the fraction implied a production of some unidentified radioactive intermediate(s).

Fig. 1 also indicates that a small but significant amount of radioactivity was incorporated into the acidic fraction. Intermediates and their derivatives involved in the succinate-glycine cycle, such as 4,5-dioxovalerate, 2-ketoglutarate, succinate and glutamate (1), were demonstrated in this fraction. Details of the data will be described elsewhere.

Isolation and identification The neutral amino acid fraction (4.9×10^5 cpm) containing unassimilated ^{14}C-5-aminolevulinate, which was prepared as described in the legend of Fig. 2, was reacted with acetylacetone and it was applied to a

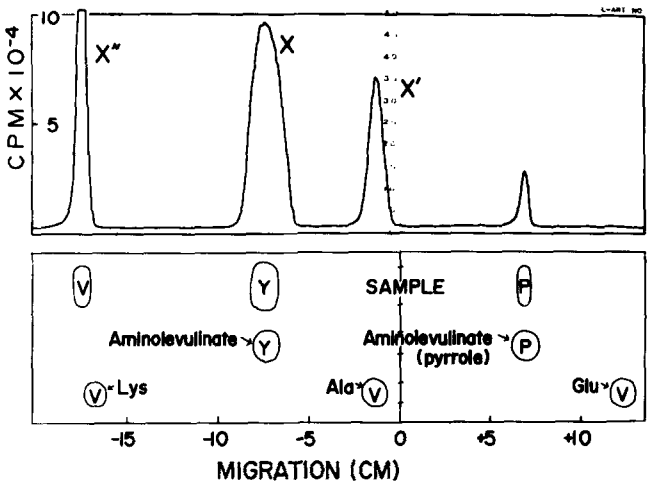

Fig. 2 Paper electrophoresis of the neutral amino acid fraction. The neutral amino acid fraction containing 4.9×10^5 cpm of radioactivity was prepared as described under METHODS except that the incubation time was 60 min and that the whole reaction mixture was used for the analysis. The sample was subjected to paper electrophoresis at pH 6.0 after pretreatment with acetylacetone and passing through a column of Dowex 50, H^+ form, as described in the text. A part of the chromatogram was cut out in a thin strip and scanned for radioactivity in a radiochromatogram scanner (Actigraph II, Nuclear Chicago Co.). Then, it was sprayed with 0.1 % (w/v) ninhydrin in acetone to detect amino compounds and subsequently, with the Ehrlich-perchloric acid reagent (8) to detect the pyrrole derivative of 5-aminolevulinate. The total radioactivities in cpm $\times 10^{-3}$ recovered from the corresponding radioactive areas were: Compound X, 315; Compound X', 82.0; Compound X", 90.0; 5-aminolevulinate (pyrrole), 8.9. Roman letters marked in the spots indicate developed colors: V, violet; Y, yellow; P, pink.

column (0.8 × 5 cm) of Dowex 50x8, H^+ form. All of the radioactive substances including the pyrrole derivative of 5-aminolevulinate, which were retained on the column, were eluted from it with 2 N NH_4OH. After concentration at low temperature in vacuo, the eluate was subjected to paper electrophoresis at pH 6.0. As shown in Fig. 2, three unidentified radioactive peaks (X, X', X") were observed in the cathodic area in addition to a minor one, which corresponded to the pyrrole derivative of 5-aminolevulinate, in the anodic area. On treatment with ninhydrin, X and X" produced yellow and purple colors, respectively but X' showed no color. Each of these compounds was eluted from the chromatogram with water and was submitted to various analyses.

Compound X resembled 5-aminolevulinate in the behavior on paper chromato-

graphy as well as on paper electrophoresis (Fig. 2), but slightly differed from the latter in the reaction with ninhydrin: They produced greyish yellow and bright yellow colors, respectively. The colors turned to purple after a long standing. From these results, 5-amino-4-hydroxyvalerate was speculated for the structure of compound X. The validity of this structure was verified by

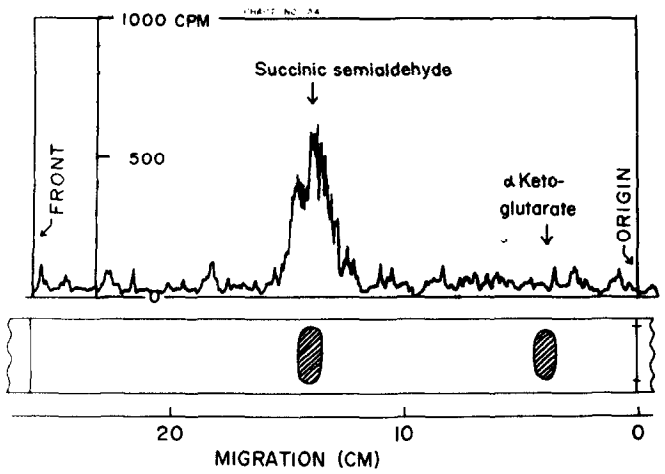

Fig. 3 Formation of succinic semialdehyde from compound X by oxidation with $NaIO_4$. A sample of compound X (56.4×10^3 cpm), which was obtained in the experiment presented in Fig. 2, was reacted with $NaIO_4$ as described under METHODS. Subsequently, the reaction mixture was brought to 0.3 N with regard to HCl and incubated with 10 μmoles of 2,4-dinitrophenylhydrazine at 30° C for 30 min. The mixture was shaken with ethylacetate. The upper layer containing 36.6×10^3 cpm of radioactivity was concentrated and subjected to paper chromatography. The radioactivity in the recovered 2,4-dinitrophenylhydrazone was 33.9×10^3 cpm.

degradation of compound X with $NaIO_4$. When compound X (56.4×10^3 cpm, corresponding to 0.56 μmole of [4-^{14}C]-5-aminolevulinate originally used) was reacted with $NaIO_4$, this reagent was consumed in a nearly stoichiometric amount (0.54 μmole)to the former. A formation of succinic semialdehyde as sole radioactive product in the reaction was demonstrated by converting it to 2,4-dinitrophenylhydrazone derivative followed by separation by paper chromatography (Fig. 3), just as expected from the following equation:

$$HOOCCH_2CH_2\overset{*}{C}H(OH)CH_2NH_2 + NaIO_4 \longrightarrow$$

$$HOOCCH_2CH_2\overset{*}{C}HO + HCHO + NH_3 + NaIO_4,$$

where the asterisk (*) indicates the labeled carbon atom derived from [4-^{14}C]-

5-aminolevulinate. Further proof for the identity was provided from comparison of compound X with authentic [4-^{14}C]-5-amino-4-hydroxyvalerate. The authentic compound showed identical behaviors on paper electrophoresis and paper chromatography as well as in the reaction with ninhydrin and with $NaIO_4$, as described in the foregoing. In addition, it has been known that 5-amino-4-hydroxyvalerate undergoes cyclization reactions to yield a lactam (5-hydroxypiperidone) and a lactone (5-amino-4-valerolactone) (12, 13). Conversions of compound X to these derivatives and vice versa were demonstrated as shown in Fig. 4. As a result of the experiment, compounds X' and X" were found to be the lactam and lactone derivatives of 5-amino-4-hydroxyvalerate, respectively. In view of the ease of the cyclization reactions, the occurrence of these two derivatives as observed on the chromatogram of the neutral amino acid fraction (Fig. 2) is presumably an artifact which arose during the isolation procedure.

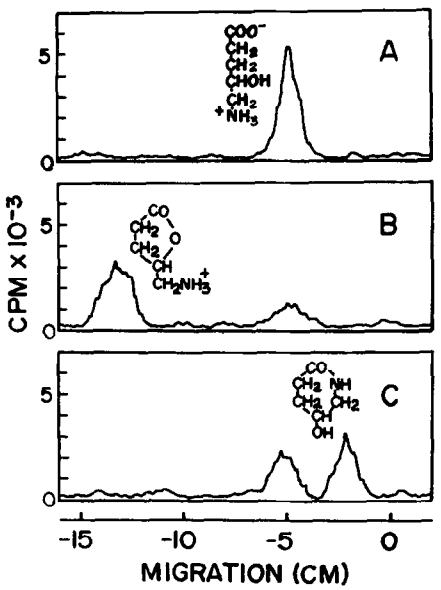

Fig. 4 Conversion of compound X to cyclized derivatives. Compound X was subjected to paper electrophoresis at pH 6.0 after various treatments: (A), no treatment; (B), exposing to 1 N HCl at room temperature for 1 hr; (C), boiling in 0.1 N NH_4OH for 1 hr. See the text for the assignment of chemical formula to each compound as illustrated in the figure.

DISCUSSION

This paper seems to be the first report on the natural occurrence of

5-amino-4-hydroxyvalerate as a metabolite of 5-aminolevulinate, though its chemical synthesis has already been accomplished by several investigators (12, 13). An analogous conversion of aminoacetone to aminopropanol has been reported and an enzyme, aminopropanol dehydrogenase, which catalyzes this reaction requiring NAD as cofactor, has been demonstrated (14). A similar dehydrogenase may be involved in the conversion of 5-aminolevulinate to 5-amino-4-hydroxyvalerate. The stereochemical configuration of this metabolite has not been determined yet.

At present, it is premature to assess the precise metabolic function of this aminohydroxy acid. But its importance in the over-all metabolism of 5-aminolevulinate was strongly suggested from the rapid rate of its production as indicated in Fig. 1. It is likely that such a rapid conversion of 5-aminolevulinate to the aminohydroxy acid may exert a profound influence on the availability of the former precursor for tetrapyrrole biosynthesis and thus, provide another regulatory mechanism for it in addition to the feed-back control at the level of 5-aminolevulinate synthesis as reported by Lascelles et al. (15, 16). As to the significance of 5-aminolevulinate metabolism in tetrapyrrole biosynthesis, the relation between the succinate-glycine cycle and the aminohydroxyvalerate pathway is obscure and remains to be solved.

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