Lysine as the Substrate Binding Site of Porphobilinogen Synthase of *Rhodopseudomonas spheroides*

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Borohydride Reduction, Labelled Protein, Hydrolysis. Lysine, Active Site

The ^{14}C labelled inactive protein obtained by sodium borohydride reduction of the enzyme, porphobilinogen synthase of *Rhodopseudomonas spheroides*, in the presence of [4-^{14}C]5-aminolevulinic acid, gave on acid hydrolysis and subsequent electrophoresis or two-dimensional chromatography a major radioactive spot which was confirmed to be N-ε-[4-(-5aminovaleric acid)]lysine (ALA-lysine) by comparing its co-chromatographic and electrophoretic behaviour with the chemically synthesized ALA-lysine. An ε-NH_2 group of lysine residue of porphobilinogen synthase, is thus the binding site of the substrate, 5-aminolevulinic acid.

It was suggested by us in our previous report on the mechanism of porphobilinogen synthesis by the enzyme, porphobilinogen synthase (EC 4.2.1.24) of *Rhodopseudomonas spheroides*, that the linkage of the substrate to the enzyme should be through Schiff base formation with one of its amino groups at the active site [1]. On reduction with borohydride the Schiff base intermediate would be converted to a stable secondary amine. We have now isolated the amine from the acid hydrolysate of the reduced enzyme complex and have evidence for the presence of lysine at the active site.

Experimental

The enzyme, porphobilinogen synthase, (1 mg/ml) purified by improved techniques [2, 3], was reduced in the presence of [4-^{14}C]5-aminolevulinic acid(ALA) by $NaBH_4$, dialyzed and then hydrolyzed with 6 N HCl. Aliquots of the acid hydrolysate was subjected to high voltage (2000 V) electrophoresis on Whatman 3 MM paper (28 × 57 cm) in 0.025 M acetate buffer, pH 5.2 for $1\frac{1}{2}$ hours in Savant's water cooled apparatus. The major ^{14}C labelled residue was obtained by elution from the paper with water. The acid hydrolysate was also analyzed by two-dimensional chromatography [4]. For thin-layer chromatography on silica gel, a solvent system was used which was made by gradual addition of 15% NH_3 to a mixture of 30 ml of chloroform and 30 ml of methanol to a very slight turbidity. N-ε-[4-(5-aminovaleric acid)]lysine (ALA-lysine) was synthesized by the reductive alkylation of N-acetyl ALA with lysine whose primary amino group was blocked. This was obtained as a gift from Dr. D. Gurne of our laboratory.

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Results and Discussion

The enzyme, porphobilinogen synthase of *Rhodopseudomonas spheroides*, became labelled when reduced by $NaBH_4$ in the presence of [4-^{14}C]ALA with the concomitant loss of enzymatic activity. The material on acid hydrolysis and subsequent two-dimensional chromatography showed a major radioactive spot (Fig. 1) which, in the electrophoretic run, ran close to the dibasic amino acids and far away from the neutral amino acids including ALA, while the minor hot spot seemed to move to the position of ALA (partly shown in Fig. 2). Upon oxidation with 0.02 M $NaIO_4$ [5] in a stoppered tube for 24 hours at pH 8.5 in a 50 °C water bath, the major radioactive material gave hot succinic

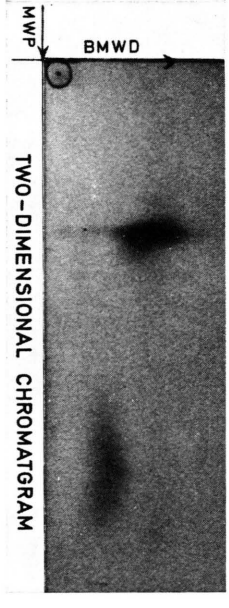

Fig. 1. Radioautograph of an acid hydrolysate of the enzyme, porphobilinogen synthase, reduced by $NaBH_4$ in the presence of [4-^{14}C]-ALA following two-dimensional paper chromatography. O, the origin of the sample. For details see "Methods".

acid as one of the products of degradation and this confirmed the presence of ALA in the labelled residue. The nature of the amino acid residue of the enzyme bound to ALA was confirmed by showing the identification of the major radioactive residue of the reduced protein hydrolysate as N-ε-[4-(5-aminovaleric acid)]lysine and this was achieved by subjecting the labelled protein hydrolysate to high voltage electrophoresis and the isolated labelled residue to thin – layer chromatography, together with the authentic sample. They moved to the identical position away from ALA and L-lysine during electrophoresis (Fig. 2) and also co-chromatographed during thin-layer chromatography (Fig. 3). It can thus be concluded that the isolated labelled residue has the same structure as that of N-ε-[4-(5-aminovaleric acid)]lysine and ε-aminolysyl group is indeed the active site of the enzyme.

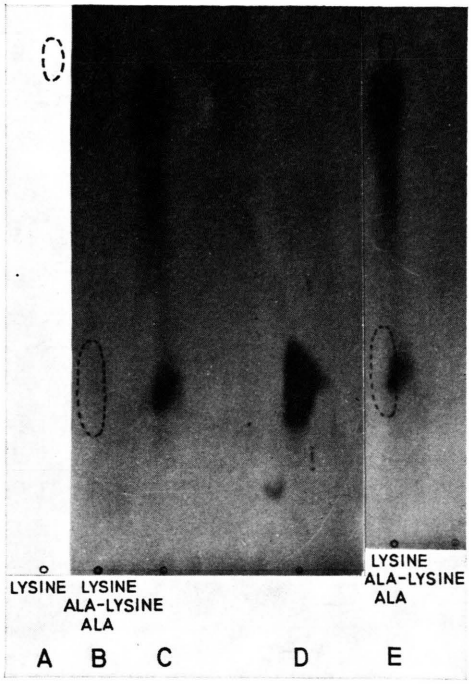

Fig. 2. High voltage electrophoresis of the labelled protein hydrolysate along with L-lysine, a mixture of L-lysine, ALA-lysine and ALA, and [4-^{14}C]ALA. The paper was sprayed with ninhydrin to locate the positions of the amino acids and was also radioautographed. A, L-lysine; B, L-lysine (top), ALA-lysine (middle) and ALA (bottom); C, radioautograph of the protein hydrolysate; D, [4-^{14}C]ALA; E, C superimposed on B showing identical electrophoretic mobility of the labelled residue and ALA-lysine.

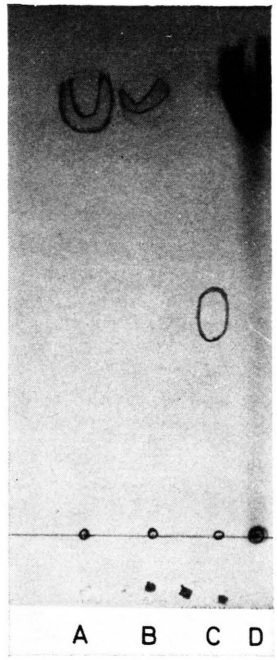

Fig. 3. Thin-layer chromatography on a silica gel plate showing identity of the major labelled residue with ALA-lysine. The labelled residue was run along with ALA-lysine and lysine on the same plate. The amino acids were located by spraying with ninhydrin and the radioactivity by autoradiography. A, a mixture of ALA-lysine and the labelled residue; B, ALA-lysine; C, L-lysine; D, radioautograph of A showing co-chromatographic behaviour of the labelled residue with the chemically synthesized ALA-lysine.

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[1] D. L. Nandi and D. Shemin, J. Biol. Chem. **243,** 1236 (1968).
[2] S. V. Heningen and D. Shemin, Biochemistry **10,** 4676 (1971).
[3] D. L. Nandi and D. Shemin, Arch. Biochem. Biophys. **158,** 305 (1973).
[4] R. R. Redfield, Biochim. Biophys. Acta **10,** 344 (1953).
[5] B. L. Horecker, P. T. Rowley, E. Grazi, T. Cheng, and O. Tchola, Biochims. Z. **338,** 36 (1963).