Physical Parameters and Possible Regulation of ζ-Aminolevulinic Acid Synthetase

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Physical measurements were made on the ζ-aminolevulinic acid synthetase from *Rhodopseudomonas spheroides*. These include a Stokes radius of 3.8 nm, determined by gel filtration, and sedimentation coefficient of 5.46 S by sucrose density gradient centrifugation. From these measurements and the value of partial specific volume of 0.732 ml/g determined from the amino acid composition, the following physical constants were calculated: molecular weight, 88000; diffusion coefficient, 5.65×10^{-7} cm^2 s^{-1}; frictional ratio, 1.30; axial ratio, 5.0. The enzyme is inhibited by more than 50% by glyceraldehyde 3-phosphate (1 mM), pyruvate (0.02 M), α-ketoglutarate (0.02 M), urea (0.8 M), $CoCl_2$ (0.2 mM) and hemin (5 μM). The effect of these inhibitors on the possible regulation of this enzymic activity is discussed.

ζ-Aminolevulinic acid synthesized from glycine and succinyl CoA by ζ-aminolevulinic acid synthetase (EC 2.3.1.37) is the precursor of heme, cytochromes, chlorophyll and vitamin B-12 in bacteria, plants and animal tissues. Iron deficient cultures seem to help the induction of this enzyme in *Rh. spheroides* [1]. There is also an induction of this enzyme in liver and liver cells in the presence of phenobarbital, porphyrogenic drugs and various environmental toxic substances [2–4]. A detailed study of this enzyme in terms of its molecular structure and the mechanism of its regulation in the cell are obviously of great interest. I report in this communication some physical parameters of this enzyme and its inhibition by some metabolites which may influence the level of ζ-aminolevulinic acid in the cell and consequently the porphyrinogenesis.

Experimental Procedure

ζ-Aminolevulinic acid (ALA) synthetase was prepared from *Rhodopseudomonas spheroides* by a method modified from those used by others [5–7]. The enzymatic activity was assayed by a method similar to that described by Tuboi et al. [5]. Protein contents were estimated by the method of Lowry *et al.* [8]. The amino acid composition of the enzyme was determined by analyzing aliquots of protein samples hydrolyzed in 6 N HCl at 110 °C for 24, 48 and 72 hours, using Durrum Amino Acid Analyzer. The partial specific volume was estimated according to the method of Cohn *et al.* [9] from the amino acid composition. The sedimentation coefficient was determined by the sucrose gradient centrifugation method of Martin and Ames [10] using bovine serum albumin, hemoglobin and liver alcohol dehydrogenase as markers. The Stokes radius (α) was determined by chromatography on Sephadex G-200 with catalase, yeast alcohol dehydrogenase and α-chymotrypsinogen as standards with known Stokes radii [11, 12]. From the plot of K_d versus Stokes radius for the protein markers the Stokes radius of the enzyme was obtained. From the Stokes radius the apparent diffusion coefficient ($D_{20,w}$) was calculated using the equation $\alpha = \varkappa T/6\pi\eta D$ where $\varkappa$ is Boltzmann's constant ($\varkappa = 1.380004\times10^{-16}$ ergs per degree). The apparent molecular weight of the native enzyme based on the enzymatic activity was obtained by combining the sedimentation coefficient and Stokes radius using the relationship $M = 6\pi\eta N\alpha s/1\text{-}\bar{\nu}\varrho$ [11]. The frictional ratio (f/f_0) was calculated by the equation $f/f_0 = \alpha/(3\bar{\nu}M/4\pi N)^{1/3}$ [11]. The axial ratio was determined from Perrin's rule from the calculated value of f/f_0 [13].

Results and Discussion

The amino acid composition of ζ-aminolevulinic acid synthetase of *Rhodopseudomonas* spheroides is shown in Table I. The partial specific volume of the protein determined from the amino acid composition is found to be 0.732 ml/g. Other physical measurements and calculated physical constants are summarized in Table II. These indicate that ALA synthetase is a globular protein. It may be pointed out that ALA synthetase appears as slightly elongated particles under electron microscope (Fig. 1) and the molecular weight calculated from the electron photomicrograph with 68 Å and 48 Å as the length and breadth of the particles, agrees fairly well with the value in Table II. Table III shows the

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Table I. Amino acid composition of ALA synthetase.

Amino acid	No. of residues per 88000 mol. wt.
Aspartic acid	78
Threonine	38
Serine	44
Glutamic acid	64
Proline	ND
Glycine	82
Alanine	74
Half-cysteine	ND
Valine	44
Methionine	16
Isoleucine	44
Leucine	62
Tyrosine	20
Phenylalanine	26
Histidine	26
Lysine	40
Arginine	40
Tryptophan	12

ND, not determined.

Table II. Measured and calculated physical values of ALA synthetase.

Physical constant	
Partial specific volume ($\bar{v}$)	0.732 ml/g
Sedimentation coefficient ($s_{20,w}$)	5.46 S
Stokes radius (a)	3.8 nm
Diffusion coefficient ($D_{20,w}$)	5.65×10^{-7} cm^2 s^{-1}
Molecular weight	88000
Frictional ratio (f/f_0)	1.30
Axial ratio (prolate ellipsoid)	5.0

Table III. Inhibition of ALA synthetase by cell metabolites. Conditions of incubation and assay are as described [5] except that inhibitors were also added to the indicated concentrations. A radioassay with [1-^{14}C]glycine was also used.

Addition	Per cent inhibition
Glyceraldehyde 3-phosphate (1 mM)	51
Pyruvate (0.02 M)	55
α-ketoglutarate (0.02 M)	50
Urea (1 M)	70
Guanidine hydrochloride (0.4 M)	95
Cobalt chloride (1 mM)	70
Hemin (10 μM)	55
ATP (0.01 M)	0
ALA (0.05 M)	53
Serine (0.05 M)	35
NaCN (0.1 mM)	93

Pyruvate and α-ketoglutarate are uncompetitive to succinyl CoA and PLP; pyruvate is uncompetitive to glycine and α-ketoglutarate is competitive to glycine; ALA and serine are both uncompetitive to succinyl CoA and competitive to glycine; glyceraldehyde 3-phosphate is linear noncompetitive to PLP; urea and guanidine hydrochloride are competitive to PLP.

inhibition of the enzyme by different cell metabolites or biological materials. Hemin has been recognized as a feedback inhibitor of this enzyme. It probably acts as a deadend complex, because it is noncompetitive to glycine and PLP, and uncompetitive to succinyl CoA. It does not act by causing a conformational change of the enzyme as no change in the sedimentation pattern of this enzyme has been observed in the presence of hemin (1 mM). The pool size of the metabolites such as glyceraldehyde 3-phosphate, pyruvate, α-ketoglutarate and other essential biological materials which appear to inhibit the ALA synthetase activity, may necessarily affect the level of ALA in the cell and consequently the porphyrinogenesis. The relation between diphosphoglycerate, glyceraldehyde 3-phosphate, 3-phosphoglyceric acid, glycine and porphobilinogen may be considered a fine mechanism of self-control of porphyrin biosynthesis. It is probable that this mechanism acts at the level of ALA formation from glycine and succinyl CoA by ALA synthetase which appears to be regulated in multiple ways [1, 14, 15].

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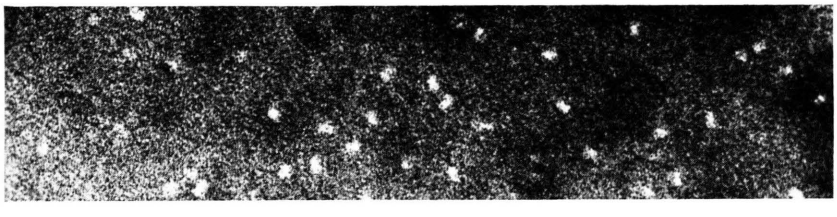

Fig. 1. Electron photomicrograph of ALA synthetase. The enzyme was negatively stained with sodium phosphotungstate, 280,000 X.

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