Plant Physiol. (1968) 43, 1411–1414

δ-Aminolevulinic Acid Transaminase in Chlorella vulgaris

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Received May 27, 1968.

Abstract. **An enzyme catalyzing the formation of δ-aminolevulinic acid by transamination of γ,δ-dioxovaleric acid with L-α-alanine, L-glutamic acid, or L-phenylalanine has been detected in extracts of *Chlorella vulgaris*. The activity of this enzyme does not appear to parallel changes in chlorophyll content in a *Chlorella* mutant which requires light for chlorophyll production. The role of this enzyme in δ-aminolevulinic acid metabolism in plants is not clearly understood.**

In experiments with δ-aminolevulinic acid-5-^{14}C administered to rats, Shemin *et al.* (14) observed radioactivity in carbon dioxide, urinary formate, and the ureido group of uric acid. When γ,δ-dioxovaleric acid-5-^{14}C was given to pigeons and ducks, radioactivity was detected in formate and the ureido group of guanine, and, to a lesser degree, in heme (12). These workers proposed a series of reactions by which the C_2 carbon of glycine might be utilized for purine formation. After condensation of glycine with succinate to form δ-aminolevulinic acid (ALA), a deamination reaction was postulated in which ALA would be converted to γ,δ-dioxovaleric acid (DOVA). The δ-carbon atom of the keto aldehyde might then be removed and incorporated into purines.

An enzyme from *Rhodopseudomonas spheroides* has been described which catalyzes the formation of ALA from DOVA and L-α-alanine (5, 6, 13). The bacterial enzyme is specific for L-α-alanine and the reaction it catalyzes favors the formation of ALA. Crude extracts of *Corynebacterium diphtheriae* can catalyze a transamination of ALA with α-ketoglutarate (1). Transamination from ALA to pyruvate or α-ketoglutarate yielding alanine and glutamate, respectively, has been observed in mammalian liver homogenates (8, 9). We have examined extracts of *Chlorella vulgaris* and of strain C-10, which forms normal amounts of chlorophylls only upon illumination (3), for the presence of a transaminase which might participate in ALA synthesis in this organism. A preliminary report of this work has appeared (4).

Materials and Methods

Chlorella vulgaris, mutant C-10 (3) or the normal-green parent stain, was grown in a dextrose-supplemented inorganic salts medium (2), containing Fe EDTA instead of $FeCl_3$, in a continuous culture apparatus or in 15-liter carboys under fluorescent illumination. When dark-grown cells of the mutant were desired, cells were cultured in 3-liter Fernbach flasks, protected from light, on a rotary shaker. To obtain greening cells, the algae were harvested by centrifugation, resuspended in distilled water, and placed on the shaker under 500 foot-candles of white fluorescent illumination.

Crude extracts of *Chlorella* were prepared by suspending 1 weight of lyophilized cells in 10 volumes of distilled water, sonicating for 1 hour in a Raytheon 10-kc sonic oscillator, and centrifuging for 30 minutes at 23,000*g*. All steps were carried out at 2 to 4°.

Subsequent purifications involved precipitation of protein from the supernatant between 40 and 70 % of saturation with ammonium sulfate. The precipitated protein was resuspended in a small volume of 0.1 M $K\text{-}PO_4$ buffer, pH 7.4, and dialyzed overnight against distilled water. Kinetic studies were performed using this fraction. No significant losses in activity were detected when the enzyme was stored at —15° for as long as 6 months. Protein was determined by the biuret method (10) using bovine serum albumin as a standard.

γ,δ-Dioxovaleric acid (DOVA) was prepared according to Wolff (15, 16) and purified by the method of Gnuchev *et al.* (7).

The assay mixture contained: $K\text{-}PO_4$ buffer, 200 μmoles; DOVA, 10 μmoles; amino acid, as indicated; 5.2 to 14.8 mg of protein; and water all in 1.8 ml. Incubations were at 37° for 0.5 to 2 hours. The reaction was stopped by the addition of

[1] Supported in part by research grants from the National Science Foundation and the National Institutes of Health.

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Table I. *Amino Donors for ALA Transaminase*

The concentration of γ,δ-dioxovalevic acid was 0.01 M.

Donor (0.025 M)	Enzyme units/mg protein	
	Expt. 1	Expt. 2
L-Glutamate	23.9	23.4
L-Alanine	20.6	20.0
L-Phenylalanine	20.0	17.4
L-Valine	10.3	7.35
L-Leucine	7 94	1.15
L-Threonine	5.62	1.98
L-Serine	5.29	3.81
L-Lysine	4.17	2.11
L-Glutamine	4.32	10.4
L-Asparagine	3.36	4.50
Glycine	2.24	3.08
L-Aspartate	0 24	0

0.2 ml of 50 % trichloroacetic acid. After centrifugation, 0.5 ml of supernatant was removed for ALA analysis according to the method of Mauzerall and Granick (11).

One unit of activity is defined as the amount of enzyme preparation required to catalyze the formation of 1 mμmole ALA in 1 hour at 37°. The reaction rate was proportional to protein concentration in the range employed and was linear for up to 2 hours.

Results

The effectiveness of several amino acids as amino donors for the purified transaminase is shown in table I. L-Glutamate, L-α-alanine, and L-phenylalanine seem to be the most effective of those tested. Because these 3 amino acids are neither structural nor electronic analogs of one another, the possibility of 3 different transaminases was considered. Table II shows the results of adding 1 of these 3 amino acids to the reaction mixture in the presence of another. Since these concentrations are close to the Michaelis constant of alanine (fig 2), it is unlikely that the additions are further saturating 1 enzyme, especially by the activity changes noted. The peak of the pH-activity profile is very broad with a more-or-less flat region extending from about pH 6.7 to 7.6. The broad pH optimum curve could be suggestive of 2 functional groups with pK's in this region.

Table II. *Amino Donor Interactions With ALA Transaminase*

The concentration of γ,δ-dioxovalevic acid was 0.01 M.

Amino acid (0.025 M)	Addition (0.025 M)	Enzyme units/mg protein
L-Alanine	...	16.5
L-Alanine	L-Glutamate	48.5
L-Glutamate	...	36.5
L-Glutamate	L-Phenylalanine	42.6
L-Phenylalanine	...	10.9
L-Phenylalanine	L-Alanine	19.0

Lineweaver-Burk plots are shown in figure 1 for DOVA and in figure 2 for L-α-alanine. Substrate inhibition by DOVA is apparent at concentrations greater than 5 mM (see fig 1) when the alanine concentration is 0.1 M. The reciprocal plot for α-alanine (fig 2) suggests that the amino donor must exceed the acceptor by a certain order of magnitude to overcome substrate inhibition by the acceptor (*cf.* ref. 13 with regard to properties of the *Rhodopseudomonas spheroides* enzyme).

Kinetic constants of ALA transaminase are as follows: K*m*, DOVA = 2.62×10^{-3} M; K*m* alanine, = 2.86×10^{-2} M; Keq = 2.33×10^{-3}. The K*m* values were obtained from figures 1 and 2, while Keq was determined from 5 separate experiments in which various concentrations of both substrates were used. These K*m* values can only be approximate since DOVA is unstable in aqueous solution.

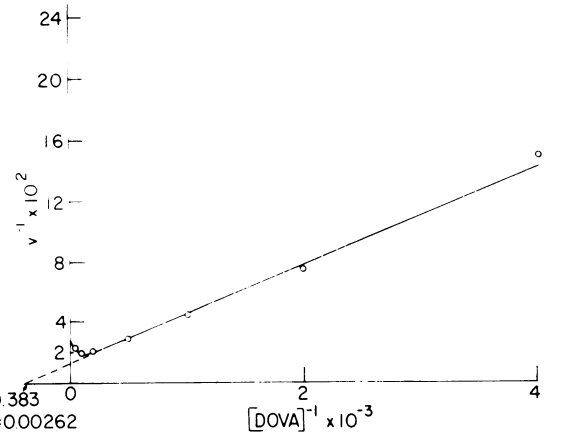

FIG. 1. Lineweaver-Burk plot for DOVA:ALA transaminase.

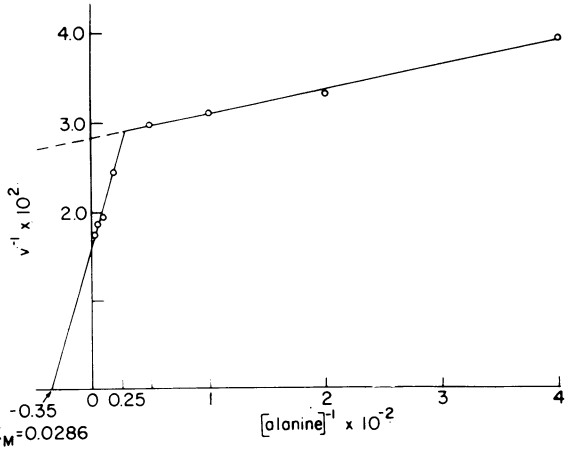

FIG. 2. Lineweaver-Burk plot for Alanine:ALA transaminase.

Table III. *Inhibition of* Chlorella *ALA Transaminase by Various Compounds*
The concentration of γ,δ-dioxovalevic acid was 0.01 M and alanine was 0.05 M.

Inhibitor	Activator	Inhibition
		%
. . .	Pyridoxal PO_4 (2.5×10^{-4} M)	6.7
NH_2OH (10^{-3} M)	. . .	79
NH_2OH (10^{-3} M)	Pyridoxal PO_4 (1.25×10^{-3} M)	70
NH_2NH_2 (10^{-3} M)	. . .	50
NH_2NH_2 (10^{-3} M)	Pyridoxal PO_4 (1.25×10^{-3} M)	50
Semicarbazide (10^{-3} M)	. . .	87
L-Cysteine (10^{-3} M)	. . .	54
NaCN (4×10^{-5} M)		50
p-CMB- (10^{-4} M)		16
$CuSO_4$ (10^{-3} M)		52
$FeSO_4$ (10^{-3} M)		27
$ZnSO_4$ (10^{-3} M)		14
$MnCl_2$ (10^{-3} M)		0

Activity was not enhanced by adding pyridoxal phosphate, a cofactor of mammalian transaminases, to the dialyzed extract (table III). Inhibitors of keto functions retarded the reaction but only at relatively high concentrations (table III). Pyridoxal phosphate had a negligible reversing effect and even was slightly inhibitory. Other inhibitors, such as *p*-chloromercuribenzoate (*p*-CMB) and some heavy metal ions, inhibited only slightly; azide did not inhibit at concentrations below 0.01 M. The greatest inhibition was by sodium cyanide: 50% inhibition at 4×10^{-5} M. Cyanide is known to form cyanohydrins with aldehydes and some ketones. Tris buffer was also inhibitory.

To see whether changes in ALA transaminase activity might be related to chlorophyll production, the enzyme activity was measured as the *Chlorella* mutant accumulated chlorophyll in response to illumination for 22 and for 44 hours. Cell disruption, in this experiment, was accomplished by 2 cycles of alternate freezing and thawing of freshly harvested cells. The ALA transaminase activity of illuminated and unilluminated cells was about equal. During the course of the experiment, the mutant attained a chlorophyll content approximately equal to that of the wild type. These results show that the activity of the enzyme is not correlated with the rate of chlorophyll formation. Therefore, it seems that the enzyme probably is not important in controlling the greening of this mutant.

Discussion

ALA transaminase in *Chlorella* seems to be a relatively stable enzyme and may actually be several enzymes characterized by different affinities for the amino donor. The significance of the enzyme in porphyrin metabolism is difficult to estimate because the equilibrium constant is relatively small. However, attempts to assay the reaction in the reverse direction met with only limited success. Glutamate and alanine could be detected with paper chromatography using ALA and α-ketoglutarate and pyruvate, respectively, as substrates. Furthermore, α-ketoglutarate and pyruvate inhibit the transamination of DOVA with alanine. The enzyme may be semi-specific; *i.e.*, recognizing a keto structure on one side of the reaction and an amino structure on the other. On the other hand, there may be several transaminases in the preparation.

The participation of the enzyme in the regulation of chlorophyll biosynthesis may not be important in higher plants or in the *Chlorella* mutant judging from the observation that ALA transaminase activity was about equal in dark-grown unilluminated and in rapidly greening or green cells. Its role in amino group metabolism remains to be elucidated.

Acknowledgments

Merrill Gassman was aided during the course of these studies by a fellowship and training grant from the National Aeronautics and Space Administration.

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