CELLULAR COMPARTMENTATION OF TWO SPECIES OF δ-AMINOLEVULINIC ACID SYNTHETASE IN A FACULTATIVE PHOTOHETERO-TROPHIC BACTERIUM (Rps. spheroides Y.)

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SUMMARY. Both species of δ-aminolevulinic acid (ALA) synthetase appear compartmentalized in Rps. spheroides Y. The ALA synthetase, "F_I", activity is correlated with dark respiratory metabolism and is a cytoplasmic "soluble" enzyme. The other enzyme, "F_{II}", induced only in light, is in chromatophores and its activity is correlated with photometabolism.

INTRODUCTION

In a previous report we have considered the role of ALA synthetase in the regulation of porphyrin and bacteriochlorophyll synthesis (1). In particular we have isolated and purified two species of ALA synthetase in Rps. spheroides Y (2-3). These two enzymes share many properties in common : same molecular weight, same content of pyridoxal 5'phosphate and same type of inhibition by ATP. They only differ in their content of -SH groups (2-3).

Previously (2), we have observed the same total inhibition by 10^{-4}M protoheme for both enzymes. Because of their very similar properties, the hypothesis of Lascelles (4) that there may be one enzyme for chlorophyll metabolism and another one for heme synthesis seems untenable.

Abbreviations : ALA : δ-aminolevulinic acid. Rps. spheroides : Rhodopseudomonas spheroides. PLP : pyridoxal 5' phosphate. ATP : adenosine triphosphate. DTNB : 5-5' di-thio bis-2-nitrobenzoic acid.

It is possible to assume that two enzymes have the same role in porphyrin synthesis, but, as has been observed in yeast (5) there is a compartmentation of the two ALA synthetases in Rps. spheroides Y.

In this paper, we attempt to demonstrate that ALA synthetase F_I is associated with dark metabolism and is in the cytoplasm of the cells and to show that ALA synthetase F_{II} is induced by light and located in the chromatophores.

MATERIAL AND METHODS

Growth of cells.

Pigmented cells : Rps. spheroides Y was grown photosynthetically in medium "$O_2$1 + Fe" as described previously (1) harvested during late exponential phase, resuspended and washed twice in 0.05 M Tris buffer pH = 7.5.

Depigmented cells : Cells were grown in the same medium (1). The inocula which were depigmented under aerobic conditions in light, were then either transferred :

a) to a 10 l. cylinder under high aerobic light conditions (10 000 lux), or

b) into a fermenter under high aerobic dark conditions.

In both cases, the yield was very low : 2 g/l.

Spheroplast preparation.

Spheroplasts were obtained following the method described by Kaback (6) (Table I) :

The yield of spheroplasts was about 80 %.

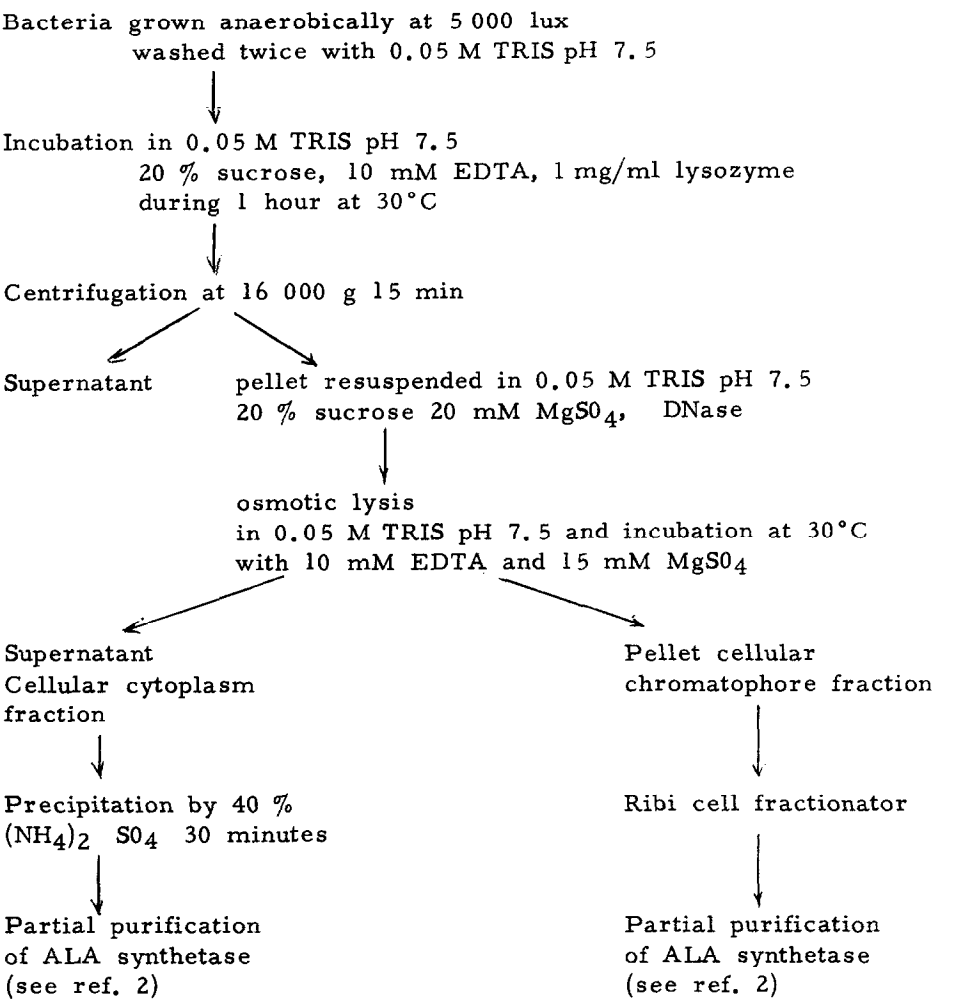
Assays.

We used the methods described previously (1-3) to purify the two fractions to the DEAE Sephadex step and for enzymic assays.

RESULTS AND DISCUSSION

A. Regulation of the synthesis of the two fractions F_I and F_{II} ALA synthetase of Rps. spheroides Y.

TABLE I



Results of a comparative study on pigmented cells and depigmented cells are shown in Table II.

It is seen that in aerobic dark cells, there is no ALA synthetase F_{II} whereas ALAsynthetase F_I is present, suggesting that light is obligatory for induction of fraction F_{II}. Results of experiments under light aerobic and light anaerobic conditions (Table II) are consistent with this hypothesis. TUBOI et al (7) have indicated there is also probably a different effect of oxygen on the two enzymes.

TABLE II

Balance of two forms of ALA synthetase : comparative study on pigmented and depigmented cells. (These values are those obtained at DEAE Sephadex A 25 step ; vide, ref. (2))

		Volume ml	Prot. conc. mg/ml	Total proteins	Specific activity units/mg prot.	Total units
Light pigmented anaerobic cells	F I	60	0.2	12	570	6840
	F II	50	0.2	10	420	4200
Aerobic light depigmented cells	F I	35	0.2	7	1800	12600
	F II	25	0.25	3.7	680	2500
Aerobic dark depigmented cells	F I	40	0.15	6.5	390	2500
	F II	-	-	-	-	-

One unit = one mole of ALA formed per hour.

We propose tentatively, different physiological roles for the two fractions. Thus, F_I may be assigned a function in relation with respiratory and dark metabolism, and F_{II} a function with photometabolism. As pigment synthesis in light metabolism is associated with the formation of chromatophores, we can suggest further different locations for these two enzymes, that is, F_I in the cytoplasm and F_{II} in chromatophores.

B. Compartmentation of the two enzymes of Rps. spheroides Y.

To test the hypothesis of compartmentation, we have studied the distribution of the two enzymes in cellular cytoplasmic fraction and in cellular chromatophores fractions (see Table I) from whole light anaerobic pigmented cells.

In whole cells, grown in anaerobic light conditions, we obtained two isoenzymes whose specific activities are very similar. Cellular cytoplasmic fraction contains only soluble ALA synthetase F_I. Cellular chromatophore fraction contains mostly ALA synthetase F_{II}. The presence of a small amount of F_I arises from the 20 % non-lysed whole cells which contaminate this fraction. The yield of spheroplasts is about 80 %. It may be supposed that enzyme F_{II} is loosely bound to chromatophore membranes, from which it may be easily dissociated by a Ribi cell fractionator as is found for hepatic ALA synthetase (8) which occurs in only one form.

In conclusion, both ALA synthetases of a photosynthetic bacteria Rps. spheroides Y, are compartmentalized, as is the case for several isofunctional enzymes, e.g. : malate dehydrogenase (9), alanine amino transferase (10) aspartate transaminase (11), and yeast ALA synthetase (5). Furthermore, the location of ALA synthetase (F_I) (probably a constitutive enzyme) in the cytoplasm is consistent with its function in respiratory metabolism, whereas that of ALA synthetase F_{II} in chromatophores correlates with its function in photometabolism as a light-inducible enzyme.

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