ATP INHIBITION OF AMINOLEVULINATE (ALA) SYNTHETASE ACTIVITY IN Rhodopseudomonas spheroides Y.

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SUMMARY

ATP can affect the first stage of bacteriochlorophyll synthesis i.e. at the enzymatic level of ALA synthetase. This enzyme purified 300 fold is inhibited about 75% by 10^{-3}M ATP. This result is consistent with a regulatory function involving ATP in tetrapyrrole biosynthesis.

INTRODUCTION

An inverse relation has been observed between bacteriochlorophyll biosynthesis and the intracellular ATP level during the growth of several bacteria, Rps. spheroides strain Y (1_a - 1_b), Chromatium vinosum (2) and R. rubrum (3). These results have been confirmed by studies (4) of Bchl and ATP levels during transitions induced by changes in light intensities.

There are strong indications that inhibition by ATP of porphyrin synthesis occurs before or at the level of ALA synthesis and also acts at the same place in the case of bacteriochlorophyll (4).

ALA synthetase activity has been tested by FERRETI and GRAY (5) on Rps. spheroides during transitions following changes in light intensity. They showed that a high rate of chlorophyll synthesis corresponded to a high level of ALA synthetase

Abbreviations :
ATP : Adenosine 5' triphosphate; ADP : Adenosine 5' diphosphate; AMP : Adenosine 5' monophosphate; ALA : S amino levulinic acid; Rps spheroides Y. : Rhodopseudomonas spheroides Y.; TCA : trichloracetic acid; DTNB 5-5' dithiobis - 2 nitrobenzoic acid; CoA : Coenzyme A, Bchl : bacteriochlorophyll.

activity and vice-versa. This result suggests that ATP may act at the enzymatic level of ALA synthetase.

MATERIALS AND METHODS.

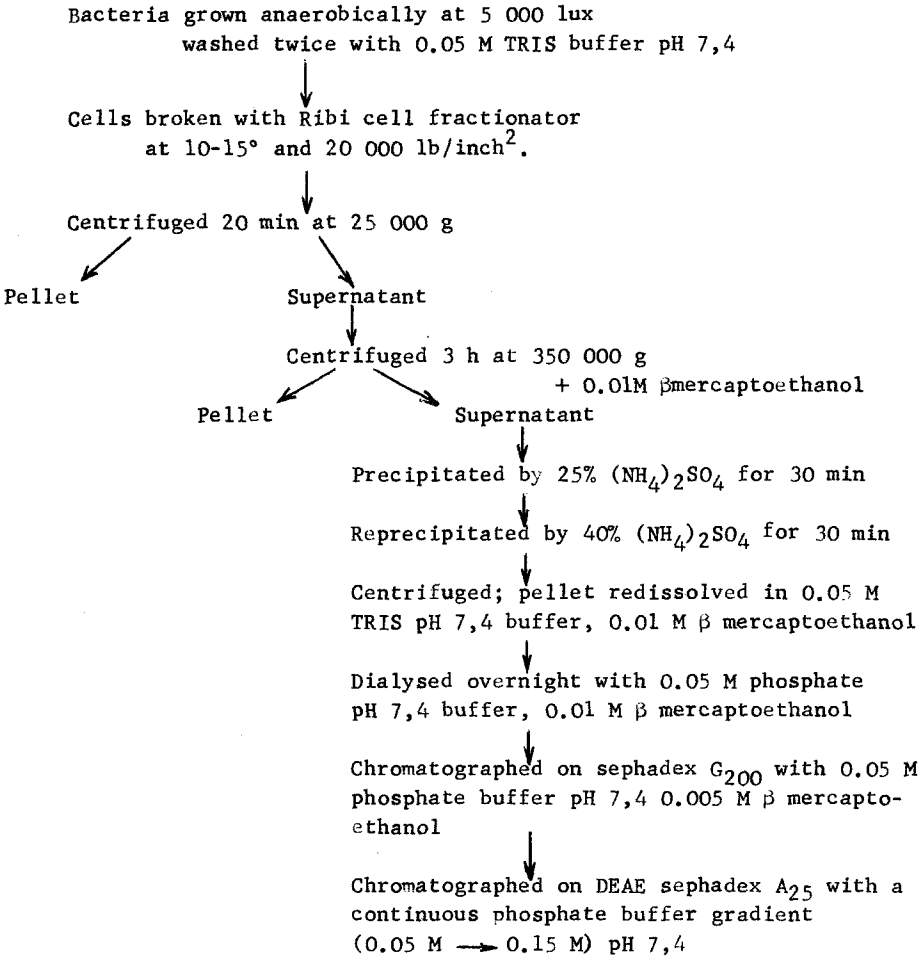
Cell culture :

Rps spheroides Y was grown as described by F. REISS-HUSSON et al (6) in "L + 17μM Fe" medium and harvested at the end of the exponential phase.

Assays :

Proteins were determined by the method of LOWRY et al (7). The protein concentration in the eluates of the column chromatography was measured by absorbance at 280 nm.

TABLE I



Purification of ALA synthetase was effected following the method used by TUBOI et al (8) with minor variations : Table I.

Assay of ALA synthetase activity :

Two methods were employed.

a) During the purification steps, ALA synthetase activity was assayed by determining the amount of ALA formed at 22°C after 15 min in a reaction mixture containing :TRIS buffer pH 7,8 : 400 μ moles, succinyl CoA : 600 mμ moles, glycine 200 μ moles, pyridoxal phosphate : 0,2 μ moles and enzymatic extract in a final volume of 1 ml.

The reaction was terminated by 1 ml of TCA 10%. The ALA formed was determined by reaction with modified EHRLICH reagent (9).

b) For determination of the enzyme activity after the sephadex steps, ALA synthetase activity was assayed in the same reaction mixture (a) containing 0,2 μmoles of DTNB. CoASH reacts with DTNB (Ellman's reagent) to give a mercaptide ion with maximum absorbance at 412 nm (ε_M = 13 600), (10). Thus, the rate of CoASH formation can be followed at 412 nm with a CARY 14 spectrophotometer after removal of essentially all the β mercaptoethanol by dialysis.

This assay is more convenient for kinetic experiments of the enzyme. Under the condition of the assay, the change in absorbance at 412 nm is proportional to the enzyme concentration.

When using both methods on the same enzymatic preparation, no difference in the specific activity was noticed.

RESULTS AND DISCUSSION.

A.- Purification of ALA synthetase.

The results of the purification of ALA synthetase of Rps. spheroides Y are summarized in Table II and Fig. 1 and 2. Two fractions were obtained. Both were purified approximately 300 fold. β mercaptoethanol was required because of the instability of the enzyme (8, 11).

These values are identical to those obtained by TUBOI et al. (8) when the bacteria grew in the solution "L-low Fe".

TABLE II

Fraction	volume	protein concentration mg/ml	total proteins (mg)	specific activity units mg prot.	total units	purification factor
Crude extract (Spinco)	280	2.4	672	1.8	1 200	1
Dialysed fraction	25	4,2	105	8	800	4
Sephadex G_{200} fraction	60	1,4	84	17,7	1 512	100
DEAE Sephadex Fraction I						
Exp. I	60	0,2	12	570	5 700	310
Exp. II	50	0.15	7.5	490	3 675	266
DEAE Sephadex Fraction II						
Exp. I	50	0.2	10	420	4 200	233
Exp. II	50	0.15	7,5	410	3 075	233
1 unit : 1 n mole ALA or CoA SH formed per hour.						

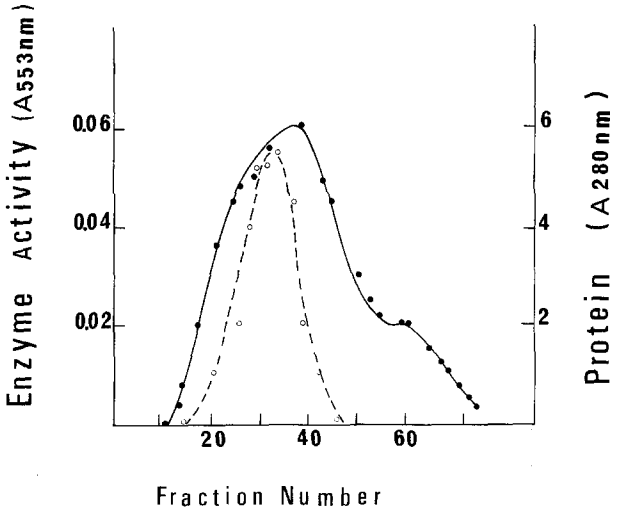


Fig. 1 - Fractionation of ammonium sulfate fraction (25-40% saturation) by séphadex G_{200} column chromatography. Protein fraction was applied on sephadex G_{200} column, eluted by 0,05 M phosphate buffer (pH 7,4) containing 0,005 M mercaptoethanol and collected in 5 ml fractions. (●—●) protein (○—○) ALA synthetase activity.

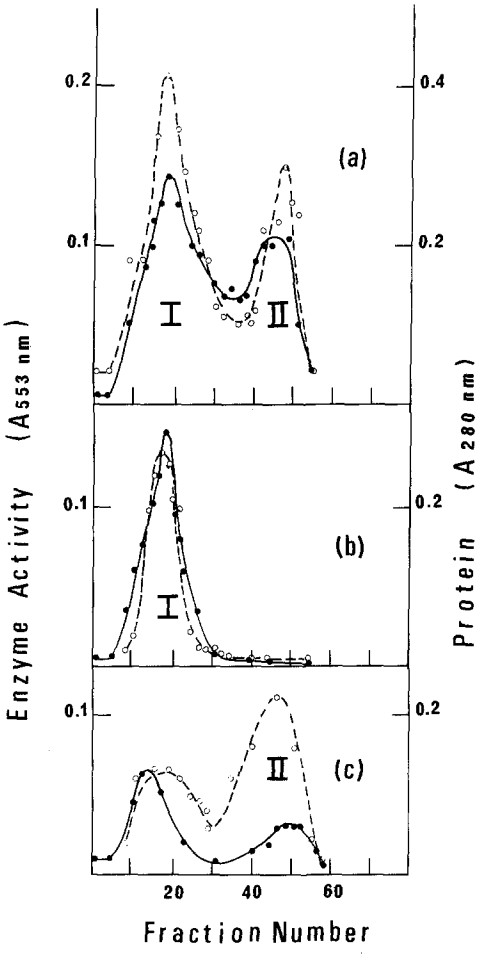


Fig. 2 - Fractionation of enzymatically active eluates from sephadex G_{200} column by DEAE sephadex column chromatography. Fractions(25-41) after gel filtration were combined and applied on a DEAE sephadex A_{25} column. Proteins were eluted with 200 ml of phosphate buffer pH 7,4) containing β mercaptoethanol 0,005 M, using a continuous linear gradient between 0,05 M and 0,15 M. 5 ml fractions were collected. (⟵●) protein ($A_{280\ nm}$); (o⟵o) ALA synthetase activity.

a) ALA synthetase fraction obtained by sephadex G_{200} treatment was chromatographed on the first DEAE sephadex column
b) Fraction (I) (12-25) was rechromatographed.
c) Fraction (II) (43-53) was rechromatographed.

Table III : Influence of culture medium on ALA synthetase activity.

Culture medium	ALA synthetase activity
L + 17 μM Fe	1.8 n mole/hr/mg protein
L - low Fe	40 n mole/hr/mg protein

B.- In vitro study of adenine nucleotides on the ALA synthetase activity.

The effect of the ATP concentration on the activity of the ALA synthetase is shown in Table IV; at 10^{-3}M, the inhibition is almost complete.

Table IV :Effect of ATP concentration on ALA synthetase activity*

ATP (final concentration)	ALA synthetase activity (n mole/hr/mg protein)	% inhibition
0	82	0
10^{-4} M	25	70%
10^{-3} M	10	88%
10^{-2} M	0	100%
10^{-1} M	0	100%

The action of adenine nucleotides has also been studied on each of the separated fractions.

Table V indicates that both fractions exhibit similar behaviour in presence of ATP.

Table V: Effect of adenine nucleotides on ALA synthetase activity (n mole/h/mg protein).

Fraction	control	ATP	% inhibition ATP 10^{-3}M	ADP 10-3 M	% inhibition ADP 10^{-3}M	AMP 10^{-3}M	% inhibition AMP 10^{-3}M
I	340	66	80	310	10	340	0
II	263	75.3	72	226	14	260	0

In accordance with previous in vivo results(1), ATP alone significantly inhibits ALA synthetase activity while ADP and AMP have no action.

In vivo, it has been noted that 10^{-3}M exogenous ATP inhibits Bchl synthesis $\backsim$50%.

These results are consistent with the suggestion of a regulatory function of ATP in the synthesis of tetrapyrrolic groups. Also, other enzymes active later in the biosynthesis chain seem to be inhibited at the same ATP concentration. NANDI et al (12) showed that ALA deshydratase of wheat leaves is inhibited by 3×10^{-3}M ATP. Avian erythrocyte porphobilinogen desaminase-uroporphyrinogen III

* : These measures were obtained with G_{200} fraction.

cosynthetase (13) and rabbit reticulocytes heme synthetase (14) were also inhibited by ATP. Thus this inhibition by ATP could be general for much of the tetrapyrrolic pathway and not peculiar to ALA synthetase.

REFERENCES

1a. FANICA-GAIGNIER, M. - Thèse Doctorat 3° Cycle, Orsay, (1969).
1b. FANICA-GAIGNIER, M., CLEMENT-METRAL, J. and KAMEN, M.D. - Biochim. Biophys. Acta, 226, 135-143 (1971).
2. SCHMIDT, G.L. and KAMEN, M.D. - Archiv. Mikrobiol., 76, 51-64 (1971).
3. OELZE, L. and KAMEN M.D. - Biochim. Biophys.Acta,234,131-143 (1971).
4. FANICA-GAIGNIER, M.- 2nd Int.Congress Photosynthesis,Stresa (1971).
5. FERRETI, J.J. and GRAY, E.D.- Biochem.Biophys.Res.Comm.,29,501-507(1967)
6. REISS-HUSSON, F.,de KLERK, H.,JOLCHINE, G.,JAUNEAU, E. and KAMEN, M.D. - Biochim. Biophys. Acta, 234, 73-82 (1971).
7. LOWRY, O.H.,ROSEBROUGH, N.J.,FARR, A.L. and RANDALL, R.J. - J. Biol. Chem., 193, 265, (1951).
8. TUBOI, S., KIM, H.J. and KIKUCHI, G. - Archiv. Biochem. Biophys., 138, 147-154 (1970).
9. URATA, G., GRANICK,S. - J. Biol. Chem., 238, 811 (1963).
10. ELLMAN, G.L., Archiv. Biochem. Biophys., 82, 70-77 (1959).
11. BURNHAM, B.F. and LASCELLES, J. - Biochem. J., 87, 462 (1963).
12. NANDI, D.L. and WAY GOOD, E.R. - Canad. J. Biochem., 45, 327-336, 1967.
13. LIAMBIAS, E.B.C. and Del C. BATTLE, A.M. - Biochim. Biophys.Acta,227, 180-191 (1971).
14. PALMA-CARLOS,A.G. - Nouv. Rev. Fr. Hem.,8, 339-346 (1968).