EFFECTS OF ADENOSINE 3',5'-CYCLIC MONOPHOSPHORIC ACID ON CERTAIN LIGHT-INDUCED REACTIONS AND ON ATPASE ACTIVITY OF ISOLATED CHROMATOPHORES FROM RHODOSPIRILLUM RUBRUM.*

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Summary

The effects of adenosine 3',5'-cyclic monophosphoric acid (cAMP) on a number of reactions catalyzed by isolated chromatophores of Rhodospirillum rubrum were examined and compared with those produced by adenosine 5'-monophosphoric acid (AMP). Photophosphorylation and dark ATPase are inhibited about 50% by 10 mM cAMP, while 10 mM AMP has no inhibitory effect. NAD photoreduction and energy-linked transhydrogenation are more strongly inhibited by cAMP than by AMP, thus demonstrating a marked inhibitory effect of a naturally occuring nucleotide on energy-linked reactions in isolated chromatophores. Cyclic AMP phosphodiesterase can be demonstrated as a soluble enzyme which is not associated with the chromatophore fraction.

Introduction

The regulatory role of adenosine 3',5'-cyclic monophosphoric acid (cAMP; cyclic AMP) in animal metabolism is well established. It affects the activity of several enzymes or enzyme systems like phosphofructokinase, phosphorylase, glycogen synthetase (1) and protein kinase (2) which catalyze reactions involving "high energy" phosphate.

Chromatophores isolated from Rhodospirillum rubrum can carry out several light induced reactions. They can catalyze the photoreduction of NAD to NADH (3), energy-linked transhydrogenation from NADH to NADP (4,5), and photophosphorylation of

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ADP to ATP (6). The chromatophores also contain an ATPase (7).

NAD photoreduction is thought to involve a "high energy" intermediate common with photophosphorylation and energy-linked transhydrogenation (8). This reaction was shown to be partially inhibited by adenosine 5'-monophosphoric acid (AMP) (9). Hence it was of interest to study the effects of cyclic AMP and of AMP on the energy-linked reactions carried out by Rhodospirillum rubrum chromatophores. A preliminary report of this investigation has been presented (10).

Methods

Rhodospirillum rubrum strain-I was grown as reported previously (11). Actively growing 36-42 hour cells were washed once and suspended in 0.1 M Tris. (pH 8.0) containing 10% sucrose. They were used immediately or frozen at -20°C until such time as chromatophores were prepared from these cells. Chromatophores were prepared by sonic disruption of cells followed by differential centrifugation (12). The high speed sediment (54,000 x g for 1 hour) was resuspended in the buffer, recentrifuged once under the same conditions and stored for up to three days at -2°C. The bacteriochlorophyll was determined according to the method of Clayton (13).

Energy-linked transhydrogenation and NAD photoreduction were assayed spectrophotometrically using a Cary model 14 spectrophotometer with actinic illumination of the reaction mixture, through a Corning CS7-69 deep red filter, at right angle to the measuring beam. The reactions were carried out at 26°C. Photophosphorylation and ATPase assays were carried out in a water bath at 30°C and were followed by analyzing

changes in the level of orthophosphate (14). All illuminations were at light saturation.

Cyclic AMP phosphodiesterase activity was assayed according to Cheung (15). The reaction was carried out at 30°C and terminated by heating the reaction mixture in boiling water for three minutes. The mixture was cooled to 30°C and incubated with 0.1 mg of Crotalus atrox venom (for 5'-nucleosidase activity) for 10 minutes. The nucleosidase reaction was stopped by placing the reaction mixture in boiling water for three minutes. Pi was determined on an aliquot after removal of the precipitated protein (14).

cAMP, AMP, NAD, NADP, Tris., succinic acid, alcohol dehydrogenase and snake venom were obtained from Sigma Chemical Company; ATP and ADP were purchased from Calbiochem.

Results

NAD Photoreduction: Fig. 1 illustrates the time course of NAD photoreduction in presence of succinate and the effects of cAMP and AMP on the time course of this reaction. The percent inhibitions calculated from the initial rates of figure 1 are presented as a function of the concentrations of cAMP and of AMP (Fig. 2 and Table I), indicating that the inhibition produced by cAMP is considerably greater than that produced by AMP. Our data confirms the findings of Horio et al (9) for the extent of inhibition produced by AMP (See Fig. 2).

Energy-linked Transhydrogenation from NADH to NADP: On illumination this reaction was linear up to three minutes and showed an initial rate of 36 μM NADP reduced / μM bacteriochlorophyll / hour. The effects of cAMP and AMP on this reaction (Fig. 3 and Table I) are similar to the effects

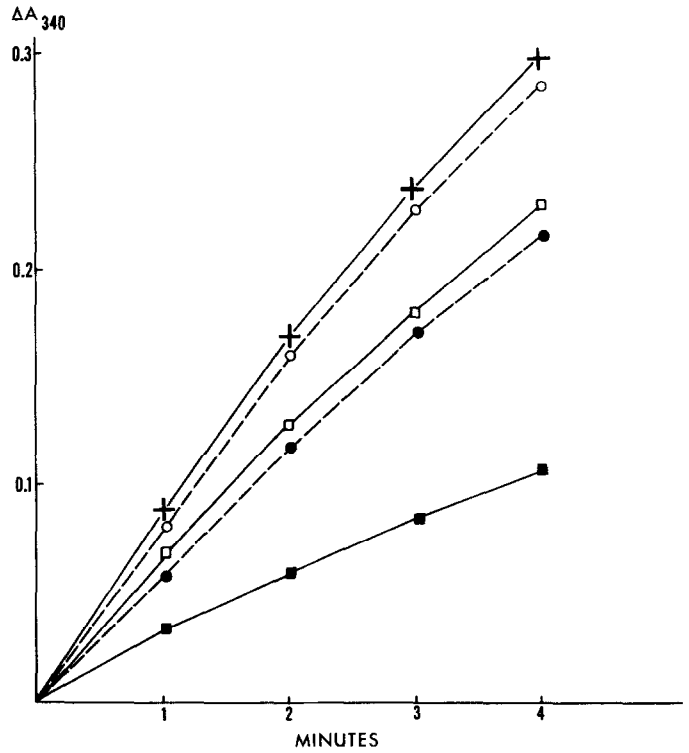

Fig. 1 -- Effects of cAMP and of AMP on the time course of NAD photoreduction. The reaction mixture contained: 50 mM Tris. (pH 8.0); 3.3 mM NAD; 0.3 mM NADH; 5.0 mM $MgCl_2$; 6.6 mM sodium succinate; 26 μM bacteriochlorophyll; and 1.0 mg bovine serum albumin / ml. Final volume: 3.0 ml. —+—+—+ control. Additions as follows (final contrations) —□—□—□ 3.0 mM cAMP; —■—■—■— 10 mM cAMP; ---o---o---o-- 3.0 mM AMP; --●---●---● 10 mM AMP. The reaction was carried out under anaerobic conditions in Thunberg tubes which were evacuated and flushed three times with Argon. The mixture was preincubated for three minutes in the dark before illumination. The intensity of illumination was 1.5×10^{-5} erg. cm^{-2}. sec^{-1}. t=26°C.

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of these nucleotides on NAD photoreduction, the inhibition by cAMP being markedly greater than that produced by AMP. Keister and Yike (16), using thioNADP instead of NADP to assay transhydrogenase activity reported an 18% inhibition of this reaction by 5 mM AMP which is in line with our results.

Photophosphorylation and dark ATPase: The rates based on 10 minute reaction times were 1.38 ± 0.15 μM Pi esterfied /

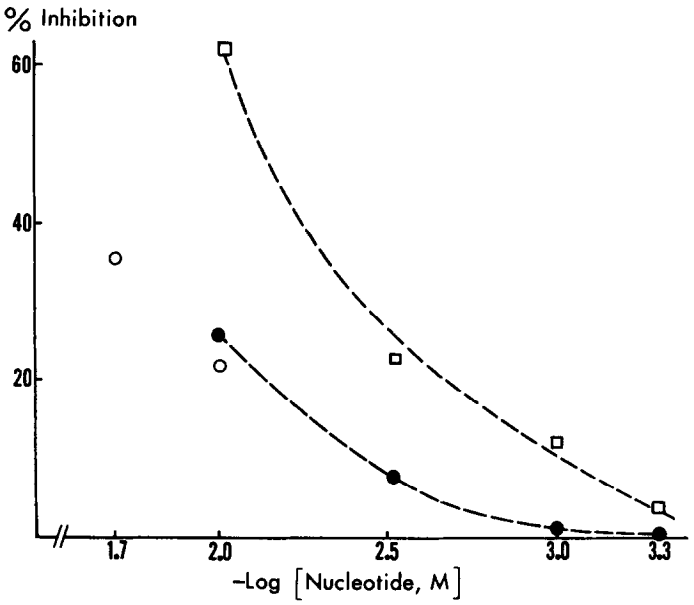

Fig. 2 -- Effects of cAMP and of AMP on NAD Photoreduction. Inhibition effects calculated on basis of initial rates of Fig. 1, presented as a function of nucleotide concentration. --□--□--□-cAMP; --●--●--●-AMP; o Inhibitions reported by Horio et al. (1963).

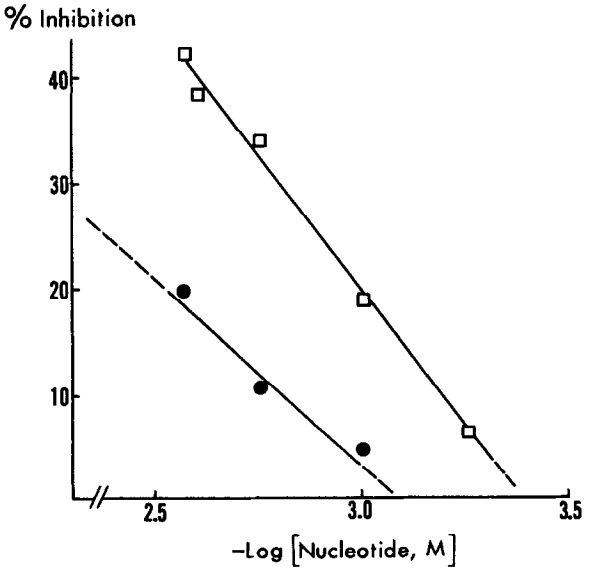

Fig. 3 -- Effects of cAMP and of AMP on energy linked transhydrogenase activity observed on illumination. The reaction mixture contained: 50 mM Tris. (pH 8.0); 1.0 mM $MgCl_2$; 0.16 mM NADP; 0.1 mM NADH; 15 μM bacteriochlorophyll; 0.33 M ethanol, and 100 μg alcohol dehydrogenase / ml. Final volume 3.0 ml. Additions as follows: —□—□—□— cAMP; —●—●—●— AMP. The reaction was carried out under aerobic conditions. The mixture was preincubated for three minutes in the dark before illumination. Intensity of illumination and temperature the same as for Fig. 1.

TABLE I

EFFECTS OF cAMP AND OF AMP ON ENERGY-LINKED REACTIONS IN RHODOSPIRILLUM RUBRUM CHROMATOPHORES

REACTION	CONC. OF NUCLEOTIDE mM	PERCENT INHIBITION	
		cAMP	AMP
a) ATPase	5	13	0
	10	44	5
b) PHOTO-PHOSPHORYLATION	3	12	-2
	10	42	-6
c) TRANSHYDROGENASE	1	19	5
	2	33	11
	3	38	20
d) NAD PHOTO-REDUCTION	1	12	5
	3	22	5
	10	62	29

a). The reaction mixture for assay of dark ATPase contained: 50 mM Tris. (pH 8.0); 5.0 mM $MgCl_2$; 60 μM bacteriochlorophyll; 10 mM ATP; cAMP or AMP as indicated. The reaction was carried out under aerobic conditions at 30°C for 10 minutes and terminated with an equal volume of cold 10% TCA. Pi was determined on an aliquot after removal of protein by centrifugation.

b). The reaction mixture, assay conditions, and reaction time were the same as for (a) above except that ATP was omitted and additions were made as follows (final concentrations): 2.5 mM ADP; 2.5 mM Pi and 50 mM Na succinate. The intensity of illumination was 2.5×10^5 erg. cm^{-2}. sec^{-1}.

c). Conditions the same as in legend of Fig. 3.

d). Conditions the same as in legend of Fig. 1.

μM bacteriochlorophyll / hour for photophosphorylation, and 0.73 ± 0.08 μM Pi released / μM bacteriochlorophyll / hour for the dark ATPase reaction. Both photophosphorylation and dark ATPase are inhibited approximately 50% by 10 mM cAMP,

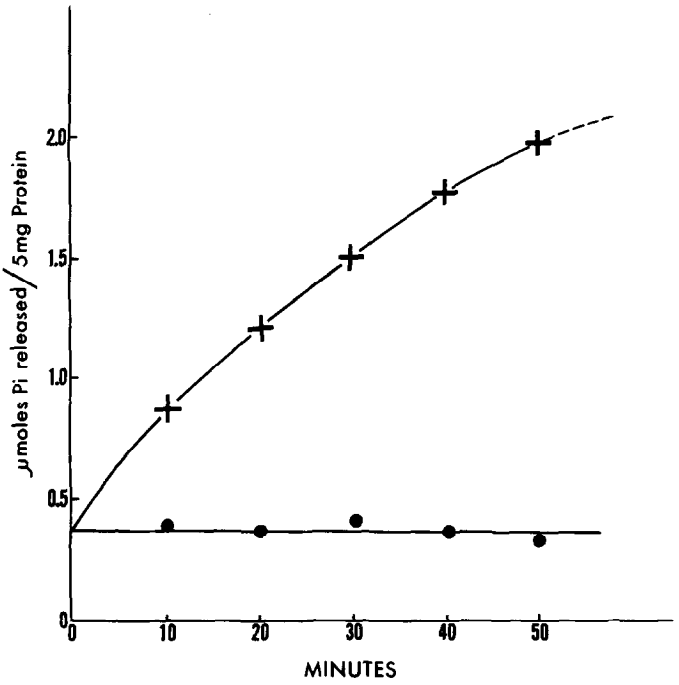

Fig. 4 -- cAMP phosphodiesterase activity in extracts of Rhodospirillum rubrum. The reaction mixture contained: 40 mM Tris. (pH 8.0); 1.8 mM $MgCl_2$; 2.0 mM cAMP, and 0.3 ml of supernatant (containing 5.0 mg protein) obtained by high speed separation (54,000 x g) of chromatophores. Assay was performed as indicated in Methods section. —+—+—+— reaction mixture; —●—●—●— reaction mixture without cAMP. t=30°C.

while 10 mM AMP has no inhibitory effect. Photophosphorylation is slightly stimulated by AMP (Table I) as reported already in an earlier publication (6).

Demonstration of cyclic AMP phosphodiesterase: Appreciable cyclic phosphodiesterase activity (Fig. 4) was observed in the supernatant obtained by high speed sedimentation (54,000 x g for 1 hr.) of chromatophores. On the basis of the assay procedure employed (14), the enzyme appears to hydrolyze 3',5'-cAMP to 5'-AMP. No phosphodiesterase activity was detected in the washed chromatophores.

Discussion

All the reactions of isolated chromatophores of Rhodospirillum rubrum, studied here, are inhibited to a significantly greater extent by cAMP than by AMP. These reactions may be subdivided further into two groups with regard to their relative response to these nucleotides. NAD photoreduction and the energy-linked transhydrogenase reaction are inhibited by cAMP as well as by AMP, while photophosphorylation and dark ATPase are inhibited by cAMP but not by AMP at the levels of the nucleotides used here.

Cyclic AMP has been reported to perform a regulatory role in the energy transforming reactions in non-photosynthetic organisms (1,2). Thus far we do not have any direct evidence for the "in vivo" involvement of cAMP in bacterial photometabolism; the indirect evidence reported here relating to the effects of cAMP on energy-linked reactions and the presence of a cyclic phosphodiesterase point to a possible regulatory role of cyclic nucleotides in the light and dark metabolism of Rhodospirillum rubrum.

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