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Synthesis and Release of Cyclic Adenosine 3′:5′-Monophosphate by *Ochromonas malhamensis*[1]

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ABSTRACT

The chrysophycean alga, *Ochromonas malhamensis* Pringsheim, was shown to synthesize cyclic adenosine 3′:5′-monophosphate (cAMP) and to release it into the culture medium. Cells contained 3 to 3,000 picomoles per gram fresh weight; medium contained up to 20 times the amount in the cells. Putative [^{32}P]cAMP was purified from cultures supplied [^{32}P]phosphate. The compound was identified as [^{32}P]cAMP by co-chromatography with authentic cAMP through 10 serial steps; by chemical deamination at the same rate as authentic cAMP, to a ^{32}P compound with the chromatographic behavior of cIMP; and by its conversion through the action of cyclic nucleotide phosphodiesterase to a ^{32}P compound with the chromatographic behavior of 5′-AMP. A two-step procedure involving chromatography on alumina and on Dowex 50 purified the unlabeled compound from cells or medium sufficiently for it to be assayable by competitive inhibition of binding of [^{3}H]cAMP to cAMP-binding protein (Gilman assay) or by stimulation of cAMP-dependent protein kinase. The activity was destroyed by cyclic nucleotide phosphodiesterase with the same kinetics as authentic cAMP, provided that an endogenous inhibitor of the phosphodiesterase was first removed by an additional purification step.

In bacteria and animals, cAMP[4] is often involved in the response of the organism to a change in the supply of a nutrient, particularly the carbon source (18). For example, the level of glucose is elevated in mammals by cAMP-mediated activation of phosphorylase, which generates glucose via phosphorolysis of glycogen. In *Escherichia coli* and related bacteria, a decline in glucose supply leads to elevation of cAMP, which in turn promotes the synthesis of enzymes that catalyze the utilization of other carbon sources. Because photoautotrophic members of the plant kingdom normally synthesize glucose from CO_2, rather than absorb glucose from the environment, the question arises: do these photoautotrophs have need of such a cAMP system?

Despite numerous studies on the possible presence and functions of cAMP in photosynthetic organisms, the extent and circumstances of occurrence of this nucleotide in such organisms in the plant kingdom remain uncertain and controversial (1, 12). A large part of the confusion is attributable to incomplete characterization of the substances measured. Thorough characterization is necessary because of the occurrence in plants of substances which interfere in analytical methods which are adequate for cAMP extracted from other types of organisms; in some cases the interfering substances mimic cAMP sufficiently to produce positive but erroneous assay results (3, 4, 7).

Strong evidence has been presented for the occurrence of low concentrations of cAMP in sterile cultures of the angiosperm *Lolium multiflorum* (ryegrass) (3), the moss *Funaria hygrometrica* (7), and the green alga *Chlamydomonas reinhardtii* (2). The evidence reported here established the presence of high concentrations of cAMP in the chrysophycean alga, *Ochromonas malhamensis* and in the culture medium in which the cells have grown.

MATERIALS AND METHODS

Growth of *Ochromonas*. *O. malhamensis* Pringsheim (recently renamed *Poterioochromonas* (19), UTEX strain 1297, was grown in medium described by Kauss and Quader (9) on reciprocal shakers at 28 C under either continuous illumination from fluorescent lamps (0.5–3 mw cm^{-2}), or the same illumination for 12 h, followed by 12 h of darkness. Stationary phase cells were used for all experiments. One-liter flasks containing 500 ml of medium were inoculated with 0.5 ml from a stationary phase culture, and were grown 10–14 days at which time they were in stationary phase (8–18 g fresh weight l^{-1}).

Chromatography Solvents. The following solvent systems were used for TLC: A: methanol-ethyl acetate-concentrated NH_4OH-1-butanol (3:4:4:7, v/v); B: methanol-1 M ammonium acetate (7:3, v/v); C: isopropyl alcohol-NH_4OH-H_2O (7:1:2, v/v); D: 1 M LiCl; E: isobutyric acid-concentrated-NH_4OH-H_2O (57:4:39, v/v); F: isopropyl alcohol-formic acid-H_2O (7:2:1, v/v); G: isopropyl alcohol-methanol-concentrated NH_4OH-H_2O (11:1:6:2, v/v); H: ethanol-100 mM sodium borate (pH 9.0) (63:32, v/v); I: 1-butanol-acetic acid-H_2O (7:2:1, v/v).

Measurement of Radioactivity. Radioactivity was measured with a Beckman LS-133 liquid scintillation spectrometer. To determine radioactivity, aqueous aliquots were placed in a dioxane-based scintillation fluid, but cellulose or silica gel from thin layer strips were placed into vials containing toluene based scintillation fluid (4). Those ^{32}P compounds and the [^{3}H]cAMP which absorbed to the silica gel or cellulose were recovered after determination of radioactivity by elution with water after several rinses with toluene to remove PPO and POPOP.

Processing of *Ochromonas* Cells and Medium after Growth in the Presence of [^{32}P]Pi. *Ochromonas* cells were cultured as described above in 0.5 liter of medium except that the Pi concentration was reduced 10-fold to 0.22 mM and the medium also contained either 2.8 or 6 mCi of [^{32}P]Pi (carrier-free). The cells were harvested after 10 days of growth by centrifugation in a Sorval GSA rotor at 2000 rpm (650 g) for 10 min at 22 C. The resulting pellets were then suspended, 3 ml/g fresh weight in ice-cold 0.5 N $HClO_4$ containing [^{3}H]cAMP to serve as internal standard. This

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[4] Abbreviations: cAMP: cyclic adenosine 3′:5′-monophosphate; PDE: cyclic 3′:5′-nucleotide phosphodiesterase; MIX: 1-methyl 3-isobutyl xanthine; cIMP: cyclic inosine 3′:5′-monophosphate.

cell suspension was frozen, thawed, and centrifuged to remove debris. The supernatant was brought to pH 7.0 by the slow addition of ice-cold 10 N KOH. After removal of $KClO_4$ by centrifugation (10,000*g* for 10 min) the supernatant was brought to 50 mM Tris-HCl by the addition of 1.0 M Tris-HCl (pH 6.8).

To the cell-free medium was added washed (7) activated charcoal (3 mg/ml) and [^{3}H]cAMP. The resulting suspension was stirred overnight at 4 C. The cAMP was eluted from the charcoal with 50% ethanol (v:v) to which was added 3% concentrated NH_4OH (v/v) and the charcoal was collected on Whatman No. 4 paper by vacuum filtration. This eluate was dried in an air stream and the residue was redissolved in 50 mM Tris-HCl (pH 6.8). The putative cAMP in the buffered neutralized cell extract or that from the medium, which is in the buffered eluate from charcoal, was then purified by a series of chromatographic steps. Each successive chromatographic separation was performed on the material that co-chromatographed with the [^{3}H]cAMP during the previous step. Both ^{3}H and ^{32}P which was present in aliquots from column chromatograph fractions and strips of thin layer chromatograms were measured by liquid scintillation spectrometry.

Processing of *Ochromonas* Cells for cAMP Assay by the Gilman Procedure. Cells grown in medium containing 2.2 mM phosphate and no ^{32}P were harvested, extracted, and the putative cAMP purified as described above. Cyclic AMP was eluted from the alumina and Dowex 50 columns as described by Ashton and Polya (3). The amounts of cAMP present in eluate fractions from chromatographic columns or eluates of thin layer chromatogram strips were determined with the Gilman assay (6), and the amount of [^{3}H]cAMP present was measured by liquid scintillation spectrometry before performing the next chromatographic separation.

Gilman Assay and Protein Kinase Stimulation Assay. Beef heart protein kinase was isolated as described by Kuo and Greengard (11) with modification of the DEAE-column elution (4). Peak I of the elution was used for the Gilman assay (6) which was modified slightly (4). Peak II was used for the protein kinase stimulation assay which was performed according to the procedure of Kuo and Greengard (11). The [γ-^{32}P]ATP was synthesized as described by Post and Sen (15). Standards of cAMP, 0.5 to 50 pmol, were included in each set of determinations. Both procedures could easily detect 0.5 pmol of cAMP.

Treatment with PDE. Beef heart PDE from Sigma was used for the enzymic hydrolysis of cAMP. Standards and unknowns in 50 μl of H_2O were incubated in a water bath at 30 C with 5 μg of the enzyme for various time periods, after which the reactions were stopped by boiling for 3 min. Samples purified from cultures containing ^{32}P were then mixed with 50 nmol each of unlabeled cAMP and 5'-AMP in water and chromatographed on silica gel in solvent A. Regions containing cAMP and 5'-AMP were detected with an UV lamp and removed for the measurement of ^{32}P and ^{3}H. Samples purified from cultures lacking ^{32}P, and containing a known amount of [^{3}H]cAMP-displacing activity as measured with the Gilman assay were again assayed after PDE treatment to determine the amount of displacing activity remaining. MIX, 0.5 mM, was used to inhibit PDE in some incubations.

Deamination. Putative [^{32}P]cAMP was eluted with H_2O from the silica gel (Table I, step 5 of medium purification) and a portion of the eluate was subjected to deamination treatment as described by Ownby *et al.* (14). After incubation, the reaction mixture was treated with activated charcoal (3 mg/ml). The charcoal was eluted with 2 ml of 50% ethanol containing 3% NH_4OH. After drying, 50 nmol each of cIMP and cAMP in 50 μl of H_2O was used to take up the residue, and it was chromatographed on silica gel in solvent A. Part of the silica gel eluate from step 5 which had not been subjected to deamination was chromatographed as a control on one side of the same silica gel plate. The chromatographic lanes of the control and treated samples were divided into 1-cm strips and the ^{32}P and the ^{3}H were measured in each strip.

Chemicals. [8-^{3}H]cAMP (25–40 Ci/mmol) was obtained from New England Nuclear; [^{32}P]Pi (carrier-free) was obtained from ICN Tracerlab. Beef heart PDE and MIX were purchased from Sigma Chemical Co. All TLC plates were precoated and were obtained from Brinkmann Instruments Inc. Baker chromatographic grade alumina was used for alumina columns.

RESULTS

Chromatography of ^{32}P-labeled Products from *Ochromonas* Medium. Out of 110×10^8 cpm, over 6×10^8 cpm of ^{32}P remained in the medium after removal of the cells. After adsorption to charcoal, less than 20% of this radioactivity could be eluted with the [^{3}H]cAMP by ethanolic ammonia (Table I). More than 99.7% of the ^{32}P contaminants were removed from the cAMP fraction by the alumina column fractionation. A large amount of additional ^{32}P not in cAMP eluted from alumina both before and after cAMP (Fig. 1A). Following TLC of the [^{3}H]cAMP-containing fractions eluted from alumina, only two major ^{32}P peaks remained, one which moved ahead of the [^{3}H]cAMP and another which migrated with [^{3}H]cAMP (Fig. 1B). This co-migrating ^{32}P continued to co-chromatograph exactly with [^{3}H]cAMP through eight more chromatographic steps performed in series (Fig. 1, C–J). The ratio of ^{32}P/^{3}H remained constant through the eight purification steps (Table I).

PDE Treatment of Putative [^{32}P]cAMP. Part of the ^{32}P material which eluted from the [^{3}H]cAMP-containing region of the first silica gel thin layer chromatograph of medium (Table I) and cell extract (Table II) was treated with PDE for various times. Only one ^{32}P degradation product was found by chromatography on cellulose in solvent B (Fig. 2). The product migrated to the position of 5'-AMP in this chromatography system. To determine the kinetics of the breakdown of the ^{32}P compound, the enzymic products and substrates were separated on silica gel in solvent A and the logarithm of the fraction of initial cAMP remaining was plotted as a function of time (Fig. 3). The ^{32}P compound and authentic [^{3}H]cAMP were degraded at the same rate. Furthermore, the degradation of both compounds was completely blocked by

Table I. *Purification of ^{32}P Compound from Ochromonas Medium*

Cells were grown 10 days in 500 ml medium containing [^{32}P]Pi at a specific radioactivity of 20 cpm/pmol. The yield was 9.2 g fresh weight. ^{32}P cpm are corrected for decay, and all ^{3}H cpm are corrected for ^{32}P spillover and differences in ^{3}H efficiency on various chromatographic plates compared to [^{3}H]cAMP in scintillation fluid alone (32%). Efficiencies of ^{3}H present on different chromatograms were determined by measuring the radioactivity from known amounts of ^{3}H cAMP placed on the various chromatograms.

Purification Step	^{32}P	[^{3}H]cAMP	[^{3}H]cAMP Recovery	^{32}P/^{3}H
	cpm		*%*	*ratio*
Decanted medium	626,410,000	700,000	100	894.87
Charcoal eluate	122,725,000	...	...	...
Alumina column	1,472,000	458,300	65.5	3.2
Cellulose solvent B	276,680	440,250	62.9	0.63
Silica gel solvent A	257,514	436,464	62.4	0.59
PEI cellulose solvent D	145,748	214,186	30.6[a]	0.68
Silica gel solvent E	124,893	199,644	28.5	0.63
Cellulose solvent C	71,677	128,640	18.4	0.56
Cellulose solvent F	44,888	89,413	12.8	0.50
Silica gel solvent G	36,650	66,676	9.5	0.55
Silica gel solvent H	3,865	6,732	1.0[a]	0.57
Silica gel solvent I	3,817[b]	6,772	1.0	0.56

[a] Part of the sample was removed for other analyses before this step.

[b] Corresponds to 2,074 pmol cAMP/g fresh weight, including correction for recovery.

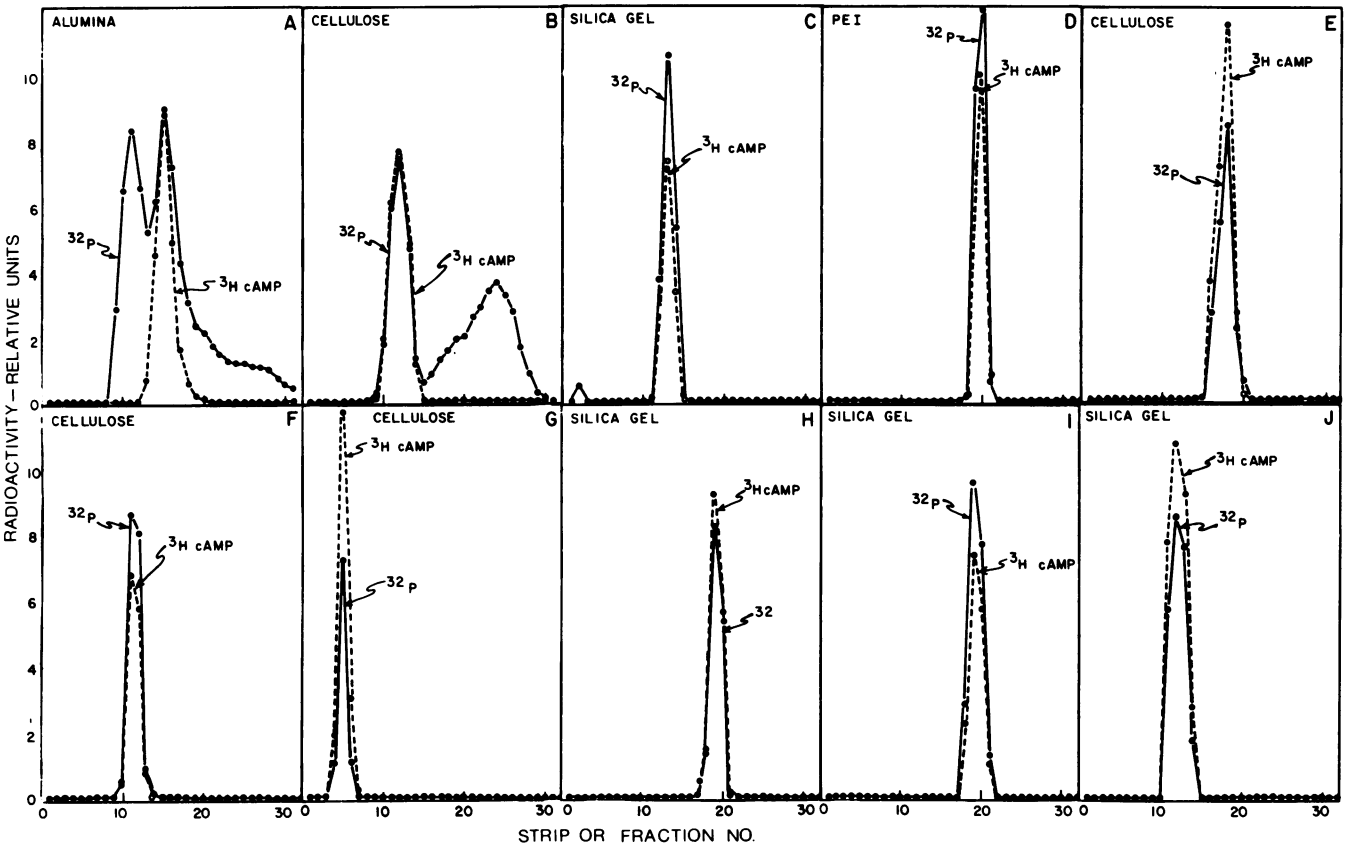

FIG. 1. Purification of ^{32}P compound from *Ochromonas* medium. The charcoal eluate was applied to an alumina column (1.2 × 15 cm) and 5-ml aqueous fractions were collected. The ^{3}H and ^{32}P present in each fraction were measured by liquid scintillation spectrometry, and the results are given in A. Each of the subsequent TLC purifications was performed in series, using only the portions of the previous separation which contained [^{3}H]cAMP. The purifications were as follows: B: cellulose with solvent B; C: silica gel with solvent A; D: polyethyleneimine (PEI) cellulose with solvent D; E: silica gel with solvent E; F: cellulose with solvent C; G: cellulose with solvent F; H: silica gel with solvent G; I: silica gel with solvent H; J: silica gel with solvent I. The absolute amounts of radioactivity (cpm × 10^{-3}, efficiency 32%) present in [^{32}P]cAMP putative and [^{3}H]cAMP marker in the peak fraction of each profile were as follows: $^{32}P/^{3}H$ = A: 350/182; B: 91/153; C: 136/232; D: 78/115; E: 56/92; F: 33/62; G: 31/62; H: 16/30; I: 2.1/3.9; J: 1.4/2.4.

Table II. *Purification of ^{32}P Compound from Ochromonas Cells*

Ochromonas was grown for 9 days in medium containing 2.8 mCi of [^{32}P]Pi at a specific radioactivity of 56 cpm/pmol. The yield was 8 g fresh weight. Calculations of radioactivity were handled as in Table I.

Purification Step	^{32}P	[^{3}H]cAMP	[^{3}H]-cAMP Recovery	$^{32}P/^{3}H$
	cpm		*%*	*ratio*
Alumina column	1,304,200	579,400	97.0	16.89
Paper solvent B	5,254	463,500	77.3	0.0110
Silica gel solvent A	523	206,666	34.4	0.0025
Silica gel solvent E	276	120,000	20.0	0.0023
PEI cellulose solvent D	233[a]	99,300	16.6	0.0023

[a] Corresponds to 3.13 pmol/g fresh weight, including correction for recovery.

MIX, a specific inhibitor of PDE, at 0.5 mM, indicating that added PDE was responsible for the enzymic degradation of both compounds (Fig. 3). Similar results were obtained using ^{32}P compound from either the medium or the cells (data not shown).

Deamination. Part of the same eluate used for PDE treatments was subjected to deamination. After the deamination treatment

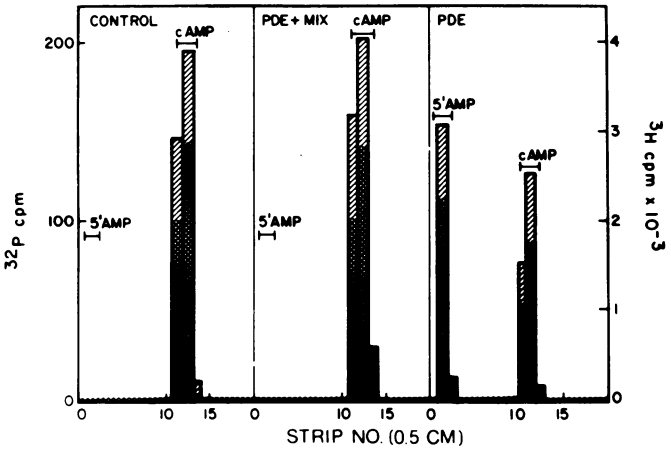

FIG. 2. Chromatography of PDE products. Putative [^{32}P]cAMP purified from medium through silica gel, solvent A (Table I, step 5) and authentic [^{3}H]cAMP were mixed, treated with beef heart PDE (5 μg) for 30 min, in the presence or absence of 0.5 mM MIX. The reaction products were mixed with 50 nmol each of cAMP and 5'-AMP and chromatographed on silica gel in solvent B. One-half-cm strips were removed and ^{32}P and ^{3}H were determined in each strip. The relative migration of unlabeled cAMP and 5'-AMP standards is indicated by bars. The cross-hatched areas represent ^{32}P; the full areas of the bars represent ^{3}H.

only one product could be identified by chromatography on silica gel in solvent A, and this product co-chromatographed with authentic cIMP (Fig. 4). Deamination was done only on the ^{32}P

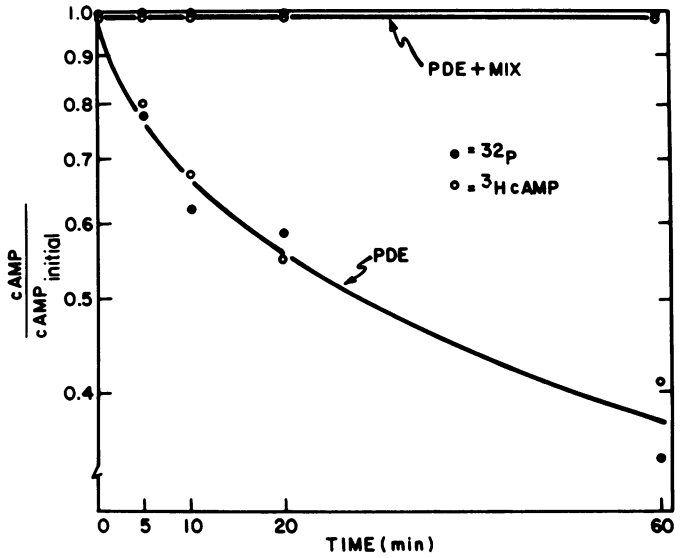

FIG. 3. Kinetics of putative [^{32}P]cAMP and authentic [^{3}H]cAMP degradation by PDE. [^{32}P]cAMP was purified as described in Figure 2. Reactions and analyses were performed as described in Figure 2. The fraction of radioactivity in cAMP *versus* incubation time is given.

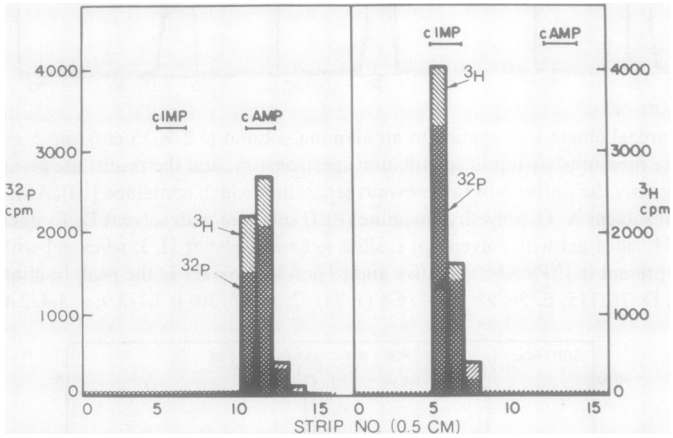

FIG. 4. Deamination of putative [^{32}P]cAMP (purified from medium as described in Fig. 2) and authentic [^{3}H]cAMP. After deamination treatment, the reaction mixture was desalted by absorption to, and elution from charcoal, 50 nmol each of cAMP and cIMP were added to the charcoal eluate before silica gel chromatography in solvent A. A: untreated control; B: deaminated. Chromatograms were run and analyzed as described in Figure 2. Positions of the cAMP and cIMP standards are shown as bars.

compound from the medium.

Chromatography of ^{32}P Products from *Ochromonas* Cells. Over 95% of the [^{32}P]Pi in the medium was absorbed by the cells during 10 days of growth. The buffered-neutralized cell extract was also chromatographed through a series of different steps (Table II). The individual profiles are not shown as they were similar to those given for ^{32}P recovered from the medium (Fig. 1). Loss of ^{32}P through decay prevented further purification past the final step in Table II. Nevertheless, the ratio of ^{32}P/^{3}H remained constant through the last three steps (Table II), strongly indicating that the ^{32}P migrating with the [^{3}H]cAMP in these steps is in cAMP.

Gilman Assay of cAMP from *Ochromonas*. To determine whether routine assays of cAMP from *Ochromonas* could be done accurately with the Gilman procedure, a study was made of the chromatographic properties of material from *Ochromonas* which reacted in the Gilman assay. Also, measurements of cAMP per-

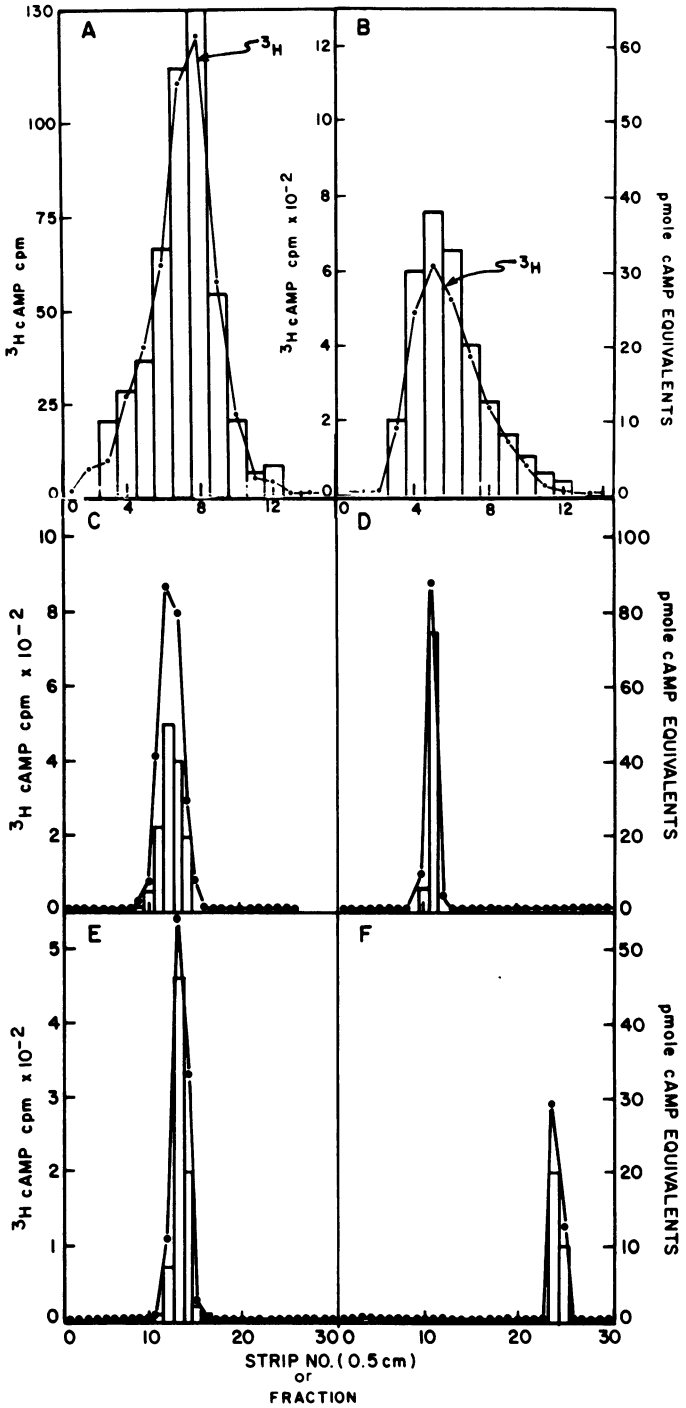

FIG. 5. Purification of Gilman assay activity from *Ochromonas* cells. Neutralized cell extract containing [^{3}H]cAMP was fractionated by alumina column chromatography (A). Each fraction was tested with the Gilman assay, and the amount of [^{3}H]cAMP present was determined. Fractions containing [^{3}H]cAMP were rechromatographed on Dowex 50, and again each fraction was assayed for Gilman activity and [^{3}H]cAMP (B). This process was repeated at each step, taking only the fractions containing [^{3}H]cAMP to the next step. C: descending paper chromatography in solvent B; D: silica gel in solvent A; E: cellulose in solvent C; F: cellulose in solvent E. Strips from chromatograms were eluted to give fractions for [^{3}H]cAMP and for Gilman activity testing. Height of bars represent cAMP equivalents measured by the Gilman assay. The amount of [^{3}H]cAMP is indicated by solid lines. Aliquots (μl) used for ^{3}H measurement: cAMP pmol equivalent measurement were as follows (μl): A: 5:40; B: 50:50; C: 50:50; D: 50:50; E: 50:50; F:50:50. Fraction sizes in ml, from which the aliquots were taken were A:4; B:5; C:2; D:2; E:1; F:1.

formed on cell extracts with the Gilman assay were compared with measurements based on protein kinase-stimulating activity. The serial purification from cell extracts of [^{3}H]cAMP-displacing activity clearly shows that the substance which reacts in this assay co-chromatographs with authentic [^{3}H]cAMP through six successive steps (Fig. 5). The specific radioactivity ratio of pmol cAMP equivalents to pmol [^{3}H]cAMP remains constant after purification over alumina (Table III). The purified displacing activity can be destroyed by treatment with PDE, and its destruction by PDE is blocked by MIX, a specific inhibitor of PDE (Figs. 6 and 7). The difference in kinetics of destruction of pure authentic cAMP and of the factor from *Ochromonas* indicates the presence of an endogenous PDE inhibitor activity in the cell extract. The inhibitor is not separated from cAMP by alumina or Dowex 50 chromatography (Fig. 6). However, it is removed by another purification step, TLC on silica gel in solvent A (Fig. 7). PDE destroyed both authentic cAMP and the *Ochromonas* factor with first order kinetics until 96 to 98% of the cAMP had been consumed. For some unknown reason, further destruction proceeded at a substantially reduced first order rate.

Another assay for cAMP is based on stimulation of protein kinase activity (11). The protein kinase assay is considered more stringent than the more convenient Gilman assay because interfering substances, if present, cause underestimates of cAMP, rather than the overestimates characteristic of the Gilman assay. Measurements made with the protein kinase assay, of cAMP in cell extracts purified through the alumina and Dowex 50 steps agreed with those obtained with the Gilman assay (Table IV).

DISCUSSION

It appears from all of the evidence presented here that cAMP is synthesized by *O. malhamensis* and that cAMP can be found in the medium in which the cells have grown. The evidence for the occurrence of cAMP in this organism consists of: (*a*) incorporation of [^{32}P]Pi into a compound which co-purifies from cell extracts or culture medium with authentic [^{3}H]cAMP; (*b*) conversion by chemical deamination to a compound which behaves identically with cIMP; (*c*) conversion by the action of PDE in the absence of MIX to a compound which behaves identically with 5'-AMP; (*d*) co-purification from cell extracts, or culture medium, with authentic [^{3}H]cAMP of a compound which competes with [^{3}H]cAMP in the Gilman assay, and which activates cAMP-dependent protein kinase; (*e*) destruction of the [^{3}H]cAMP-displacing activity during incubation with PDE, but not during incubation with PDE plus MIX; (*f*) identity of the kinetics of destruction of authentic cAMP and the compound from *Ochromonas*.

Table III. *Purification of Unlabeled Putative cAMP from Ochromonas Cells*

The $HClO_4$ extract of 25 g fresh weight was used. Calculations of radioactivity were handled as in Table I. The specific radioactivity of [^{3}H]cAMP was 17,500 cpm/pmol. Radioactivity from [^{3}H]cAMP marker present in assay samples was negligible compared to [^{3}H]cAMP added for assay.

Purification Step	[^{3}H]cAMP	[^{3}H]-cAMP Recovery	Gilman Assay cAMP Equivalents	pmol cAMP Equivalents/pmol [^{3}H]cAMP
	cpm	*%*	*pmol*	*ratio*
Neutralized extract	969,700	98.9	168,736	3,045
Alumina	360,000	36.7	24,000	1,167
Dowex 50	266,500	27.2	19,300	1,267
Paper solvent B	90,868	9.3	5,680	1,094
Silica gel solvent A	42,600	4.3	3,323	1,365
Cellulose solvent C	20,000	2.1	1,540	1,348
Cellulose solvent E	8,400	0.9	600[a]	1,250

[a] Corresponds to 2,667 pmol/g fresh weight, including correction for recovery.

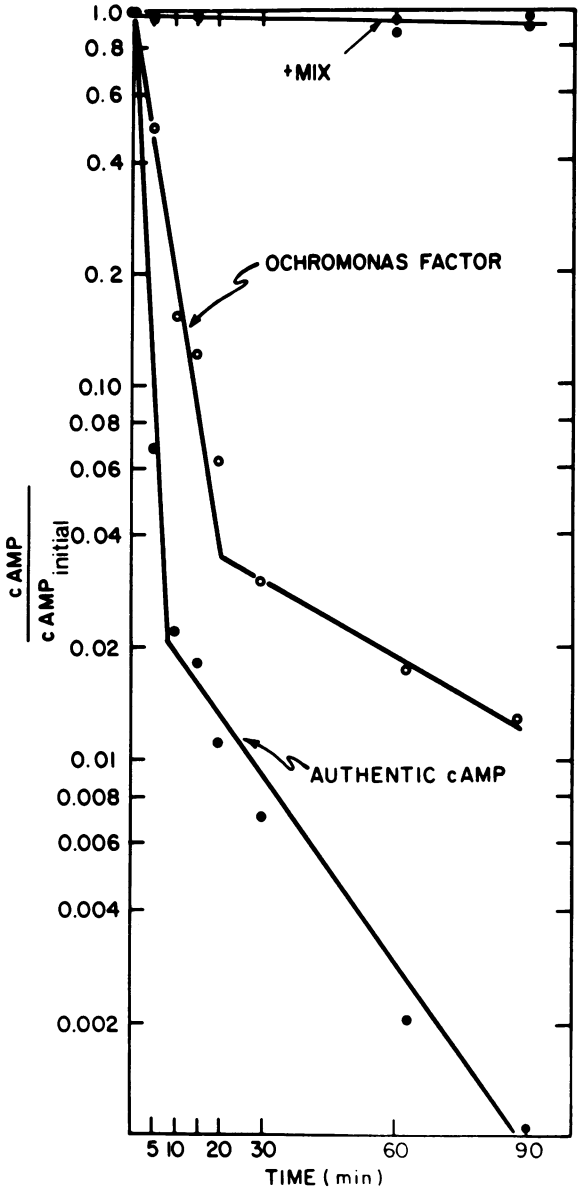

FIG. 6. PDE treatment of material which reacts in the Gilman assay. Samples known to give a positive response in the Gilman assay were taken from Dowex 50 eluates of cell extract purified on alumina and were incubated with PDE for the times indicated. After boiling for 3 min, cAMP in the reaction mixtures was determined by the Gilman assay. A set of control PDE reactions were run containing 0.5 mM MIX. Shown is the fraction of original cAMP remaining in the reaction mixture as a function of incubation time. Results obtained with authentic pure cAMP incubated without *Ochromonas* factor, with putative cAMP from *Ochromonas*, and with each of these plus MIX are given.

Accurate measurements of cAMP from this alga can be made routinely with the Gilman assay after a simple two-step purification using alumina and Dowex 50. The alumina step removes a large amount of interfering substances (Table III). The Dowex 50 step is necessary to remove the Tris used in the alumina step, but

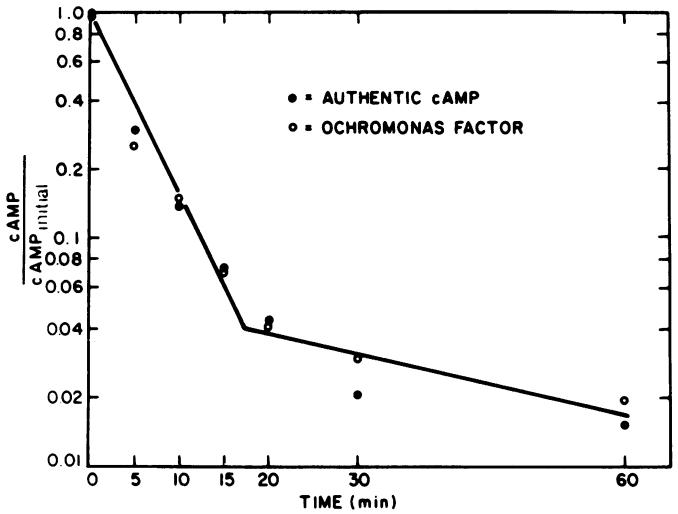

FIG. 7. Destruction of putative cAMP by PDE after removal of endogenous PDE inhibitor. Conditions were the same as Figure 6 except that the putative cAMP from *Ochromonas* was purified by an additional step, silica gel in solvent A.

Table IV. *Assay by Protein Kinase Activation and Gilman Assays of cAMP from Ochromonas Extracts after Partial Purification by Alumina and Dowex 50 Column Chromatography*

The data were corrected for losses of authentic [^{3}H]cAMP in the purification.

Experiment No.	cAMP Content	
	Protein kinase activation	Gilman assay
	pmol/g fresh wt	
1	190	150
2	270	300
3	2,250	2,300

it also removes some interfering substances in the extract (data not shown).

The levels of cAMP measured in cell extracts varied between 3 and 3,000 pmol/g fresh weight. The lower value is comparable to the cAMP levels found in ryegrass (3), *Funaria* (7), and *Chlamydomonas* (2). Part of the explanation for the extremely large fluctuation in intracellular cAMP concentration in *Ochromonas* may lie in the fact that our experimental conditions varied somewhat between experiments because of changes in the culturing equipment used. Light intensity, temperature, and cell density were affected by these changes. Physiologically determined alterations in cAMP level have also been encountered in *Ochromonas*, and will be reported separately.

Typically, more cAMP was found in the medium than in the cells. The medium contained as much as 20 times the amount in the cells. Release of cAMP into medium may be a general characteristic of algae. In studies being reported elsewhere, we have also observed release of cAMP into culture medium by *Chlamydomonas reinhardtii* and *Anabaena variabilis*. Ryegrass cells also release cAMP (3), and *Funaria* loses some cAMP when washed (7). Bacterial (17), fungal (13), and animal (5, 10) cells are known to release cAMP. These processes are known to have physiological roles in bacteria (17) and fungi (8). Further study of release of cAMP by algae is warranted. The possibility that such release may have an ecological function should not be overlooked.

The versatile nutritional characteristics of *Ochromonas* place it in a unique position between avid autotrophs and heterotrophs. Pringsheim (16) has described it as photo-sapro-phagotrophy. This organism grows heterotrophically on glucose even in the light, and does not develop its photosynthetic capability until starved for fixed carbon. Thus, *Ochromonas* provides an attractive opportunity to look for a role for cAMP in regulation of photosynthetic carbon metabolism.

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