DISSOLVED CYCLIC ADENOSINE 3':5'-MONOPHOSPHATE (cAMP) IN A EUTROPHIC RESERVOIR LAKE

David A. Francko

Department of Botany and Microbiology, Oklahoma State University, Stillwater, OK 74078

Dissolved cAMP was found in significant quantities (0.17 - 50.76 nmol liter^{-1}) in the waters of a eutrophic reservoir lake typical of many such systems found in the Great Plains. Seasonal changes in cAMP concentrations found in Sangre Isle Lake, OK, were generally similar to those reported in trophically dissimilar hardwater lakes in Michigan. However, on several sampling dates, cAMP was found in concentrations approximately one order of magnitude higher than previously reported. Collectively, the data support the view that cAMP is a dynamic rather than a conservative molecule in a wide variety of lakes.

INTRODUCTION

Cyclic adenosine 3':5'-monophosphate (cAMP) is a ubiquitous regulatory molecule in animal and bacterial cells (1). In the former organisms, cAMP functions as a primary and secondary messenger in intra-and intercellular regulation of homeostatic responses. In bacteria, cAMP modulates aspects of carbon, phosphorus, and nitrogen metabolism.

Aquatic photoautotrophs, including algae and aquatic macrophytes, contain cAMP in their tissue and release cAMP extracellularly into the dissolved phase (2-4, 8, 9, 14). In lake water (3, 7) and nearshore ocean water (6), dissolved cAMP (cAMP capable of passing through 0.45-μm pore-size filters) has been isolated in quantities (<0.01 -ca. 5 nmol l^{-1}) approaching those in human plasma (1); the levels in water vary greatly on seasonal and diurnal bases and between lakes of differing trophic status.

Recent investigations have begun to address the physiological ecology of dissolved cAMP in aquatic photoautotrophs (3, 7-11). However, as only a few published accounts of cAMP levels in lake water exist, it could be argued that these reports represent site-specific phenomena. More importantly, more precise knowledge of the range of naturally occurring dissolved cAMP concentrations in a variety of lakes is essential to further physiological and ecological research on putative cAMP-regulated systems in aquatic communities. Here, I present data on the seasonal dynamics of dissolved cAMP in a small eutrophic reservoir lake in northcentral Oklahoma.

MATERIALS AND METHODS

Water samples were collected from the 0.1-m and 3-m strata of Sangre Isle Lake, a small (ca. 3.2 ha.), shallow (maximum depth ca. 3.0 m) eutrophic reservoir system located in Payne County (R2E, T18N, S17), northcentral Oklahoma. The lake, impounded before 1920, is located in a small, sheltered and wooded residential watershed so that it has taken on many of the characteristics of a natural lake, including a neutral to slightly alkaline solution (pH ca. 7.0 - 8.2), low alkalinity and conductivity (ca. 1.0 meq l^{-1} and 250 µmho cm^{-1}, respectively), and moderate nonbiogenic turbidity (suspended clay). Hypolimnetic waters of this lake (below ca. 2 m) quickly become anoxic upon thermal stratification (early May to late September in 1982).

An extensive review of cAMP analytical methodologies in lakewater systems may be found in Francko and Wetzel (3). Water samples (50 - 100 m*l*) were filtered prior to analysis with 0.45-µm membrane filters (Millipore type HA; 0.5 atm. vacuum differential). Filtrates were then amended with 80×10^3 DPM (1.0 pmol) of [^{3}H]-cAMP (Amersham) which served as an internal standard for calculating percentage recoveries of filtrate cAMP. Dissolved nucleotides were then adsorbed to purified activated charcoal (Norit A) and recovered by elution with 6% NH_4OH in 50% EtOH (w:v). Eluates; were air-dried and residues were redissolved in 40 ml of 50 mM Tris-HCl, pH 6.8, purified by neutral alumina and cation exchange chromatography (Dowex 50), lyophilized, redissolved in deionized water, and assayed for cAMP by a modified protein binding assay (12) to a precision of ca. ±10 percent (SD; N = 2). Recoveries of [^{3}H] -cAMP internal standard generally ranged from 3 - 10 percent, a range consistent with previous investigations (3, 7). Sample-specific recoveries of [^{3}H]-cAMP internal standard were used to correct data for cAMP lost during sample preparations.

The dissolved organic phosphorus content of some filtered (0.45-μm membrane) lakewater samples (2.5-m*l* aliquots) used for cAMP analyses was determined by the nitric acid hydrolysis method of Francko and Heath (13). Absorbance of the resultant molybdenum blue complex at 690 nm was determined in 1-cm quartz cuvettes. Organic phosphorus concentrations (μg l^{-1} as orthophosphate) were used to calculate the percentage of organic P present as cAMP in each lakewater sample.

RESULTS AND DISCUSSION

Table 1 summarizes data on dissolved cAMP concentrations in surface (0.1 m) and hypolimnetic (3 m) waters of Sangre Isle Lake in the fall of 1981 and in 1982. Surface water cAMP concentrations were lowest during the fall months in both years and highest during midsummer. This general pattern was similar to that reported for Lawrence Lake and Wintergreen Lake in Michigan (3). On several dates (3 June, 22 July, 4 Aug, and 19 Aug, 1982), cAMP concentrations greatly exceeded the highest concentrations previously reported in lake water (Lawrence Lake, MI; ca. 6 nmol l^{-1}) (3). Similarly high levels of cAMP have been found in cultured phytoplankton (*Poterioochromonas*; up to 300 nmol l^{-1}) (8), but in such systems algal cell densities were generally several orders of magnitude greater than in Sangre Isle Lake. During the 22 July and 4 Aug, 1982, sampling periods, a massive surface bloom of bluegreen algae (*Anabaena*, *Microcystis*, and *Oscillatoria*) covered the surface of Sangre Isle Lake, so that algal cell densities in surface waters (ca. 1.5 - 4.7 × 10^5 cells ml^{-1}) approached those commonly found in laboratory culture systems. However, another surface bloom of *Anabaena* and the green alga *Closteriopsis longissima* covered the lake surface from mid-September to early October in 1982 and cAMP concentrations were much lower, arguing against algal cell density as the sole determinant of dissolved cAMP concentrations.

Dissolved cAMP accounted for between 0.03 and 8.0 percent of the dissolved organic phosphorus content of lakewater samples analyzed (Table 1). These data, similar to those reported in studies on Lawrence Lake, MI, underscore the relatively high concentrations of cAMP which can apparently occur in lake water.

Speculations on the factors controlling dissolved cAMP concentrations in this system are premature. Dissolved cAMP is released into the water column by algae (14, 15), bacteria (6), aquatic macrophytes (4), and zooplankton (5). Since all these organisms occurred in this lake, any or all could have produced some of the cAMP found *in situ.*

Nonetheless, the data show that seasonal concentrations of cAMP varied greatly during the season in this small, typical reservoir lake and that considerable vertical stratification may occur between cAMP in aerobic surface waters and anaerobic hypolimnetic waters. Additionally, large differences in cAMP concentrations were found

TABLE 1. *Dissolved cAMP concentrations, Sangre Isle Lake, 1981-1982. Values reported in nmol liter^{-1} ± SD (N = 2). All values from 0.1-m stratum unless otherwise indicated.*

Date	cAMP Concentration[a]
	1981
10 Oct	0.20 ± 0.02
30 Oct	0.21 ± 0.03
	1982
20 May	1.38 ± 0.08
1 June	0.96 ± 0.06
3 June	50.76 ± 1.5
3 June (3m)	1.08 ± 0.12
9 July	5.36 ± 1.0 (8.0)
22 July	18.48 ± 0.7
4 Aug	17.50 ± 3.0
5 Aug	1.12 ± 0.27 (1.9)
5 Aug (3m)	4.61 ± 0.92
19 Aug (3m)	18.00 ± 11.3 (2.6)
2 Sept	1.04 ± 0.33 (0.1)
16 Sept	0.23 ± 0.11 (0.06)
29 Sept	0.17 ± 0.01 (0.03)
4 Oct	0.43 ± 0.02 (1.5)
14 Oct	0.33 ± 0.11 (2.5)

[a]Parentheses indicate percentage of dissolved organic phosphorus present at cAMP.

in samples collected only days apart (e.g., 1 and 3 June and 4 and 5 Aug, 1982). These differences, both seasonal and short-term, did not appear to be due to patchiness and subsequent sampling anomalies (Francko, unpublished data). Rather, the collective data presented here and from previously cited studies on other lake systems suggest that the dynamic rather than conservative nature of cAMP in lake water may be a general characteristic of lake systems. This observation supports the belief that this molecule is of ecological significance in lacustrine systems.

ACKNOWLEDGMENTS

This research was supported by a grant from the National Science Foundation (DEB-8012164), the Water Research Center, and the College of Arts and Sciences, Oklahoma State University.

REFERENCES

1. G. A. ROBISON, R. W. BUTCHER, and E. W. SUTHERLAND (eds.), *Cyclic AMP*, Academic Press, New York, 1971.

2. D. A. FRANCKO in G. A. ROBISON and P. GREENGARD (eds.), *Advances in Cyclic Nucleotide Research,* Vol. 15, Raven Press, New York, 1983, p. 97-117.

3. D. A. FRANCKO and R. G. WETZEL, Limnol. Oceanogr. 27:27-38 (1982).

4. D. A. FRANCKO and R. G. WETZEL, Physiol. Plant. 52:33-36 (1981).

5. D. A. FRANCKO and R. G. WETZEL, J. Freshw. Ecol. 1:365-371 (1982).

6. J. W. AMMERMAN and F. Azum, Mar. Ecol. Prog. Ser. 5:85-80 (1981).

7. D. A. FRANCKO and R. G. WETZEL, Oecologia (in review).

8. A. K. HANDA, R. A. BRESSAN, H. QUADER, and P. FILNER, Plant Physiol. 68:460-463 (1981).

9. D. A. FRANCKO and R. G. WETZEL, J. Phycol. 17:129-234 (1981).

10. D. A. FRANCKO, Arch. Hydrobiol. (1984a, in review).

11. D. A. FRANCKO, Arch. Hydrobiol. (1984b, in review).

12. A. G. GILMAN, Adv. Cyclic Nucleotide Res. 2:9-24 (1972).

13. D. A. FRANCKO and R. T. HEATH, Limnol. Oceanogr. 24:463-473 (1979).

14. R. A. BRESSAN, A. K. HANDA, J. CHERNIAK, and P. FILNER, Phytochemistry 19:2089-2093 (1980).

15. D. A. FRANCKO and R. G. WETZEL, Physiol. Plant. 49:65-67 (1980).