Uptake of Cyclic AMP by Natural Populations of Marine Bacteria

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The major objective of this study was to describe the mechanism(s) of cyclic AMP uptake by natural populations of marine bacteria. A second objective was to determine whether this uptake could contribute to the intracellular regulatory pool of cyclic AMP. Using high-specific-activity 32P-labeled cyclic AMP, we found several high-affinity uptake systems. The highest-affinity system had a half-saturation constant of <10 nM. This system was extremely specific for cyclic nucleotides, particularly cyclic AMP. It appeared to meet the criteria for active transport. Uptake of cyclic AMP over a wide concentration range (up to 2 μM) showed multiphasic kinetics, with half-saturation constants of 1 nM and greater. These lower-affinity systems were much less specific for cyclic nucleotides. Although much of the labeled cyclic AMP taken up by the high-affinity systems was metabolized, some remained as intact cyclic AMP within the cells during 1 h of incubation. This suggests that at least some of the bacteria use cyclic AMP dissolved in seawater to augment their intracellular pools.

We have recently reported the occurrence of dissolved cyclic AMP (cAMP) in seawater (1). Its concentration averages about 10 nM, but shows significant temporal and spatial variability. We also reported that natural populations of marine plankton take up cAMP from seawater; size fractionation shows that bacteria are largely responsible (1). The purpose of the present study was to examine the mechanism(s) of cAMP transport in natural populations of marine bacteria and its ability to contribute to the intracellular regulatory pool of cAMP.

cAMP is an important regulatory compound in both procaryotes (20) and eucaryotes (19). In Escherichia coli and other bacteria, cAMP is intimately involved with sugar catabolism. Exogenous cAMP can overcome catabolite repression, permitting synthesis of inducible catabolic enzymes for other sugars (lactose, arabinose, etc.) in the presence of glucose (18, 20). cAMP binds to its receptor protein (CRP), and together they attach to a specific site on the promoter adjacent to the RNA polymerase binding site. cAMP-CRP binding apparently facilitates polymerase binding by unwinding the DNA and thus promotes transcription of the operon (15, 18). The transcription of the chemotactic and flagellar genes in E. coli (20) and the synthesis of the luminescence system in the marine bacterium Beneckea harveyi (16) are subject to similar control.

Recent evidence suggests that cAMP may play another important role in the regulation of bacterial gene expression (27). cAMP markedly decreases the polarity (or premature transcription termination of genes distal to the promoter) of the lactose operon. The galactose operon may be subject to similar regulation (10), though some believe that its regulation is best explained by the action of two promoters, one activated by cAMP-CRP and the other inhibited by it (24).

Bacteria produce cAMP from ATP with the enzyme adenylyl cyclase, which is apparently associated with the cell membrane (18, 20). Intracellular cAMP levels are lowered through hydrolysis by cAMP phosphodiesterase, and in some cases cAMP may be excreted by an energy-dependent transport system (22). Although exogenous cAMP can overcome catabolite repression in bacterial cells (see above), we are aware of only two studies of cAMP uptake in bacterial cells or membrane vesicles (8, 12). Both of these used high (millimolar) cAMP levels, and the more detailed study (8) reports a K of 10 mM. (K is the designation used in aquatic microbiology for the half-saturation constant for transport.) Bacteria would be unlikely to increases their intracellular cAMP concentration via this uptake system except at high extracellular cAMP concentrations.

In this study, uptake of cAMP by natural populations of marine bacteria and in several
Phase; West activity of isolation of marine isolates was investigated with $^3$H- and $^{32}$P-labeled cAMP. Studies with natural populations were essential because of the great selectivity of isolation media and the changed physiological state of cells in highly enriched (relative to seawater) cultures (1a). We found that natural populations of marine bacteria, in contrast to marine isolates and the studies mentioned above, take up cAMP via high-affinity, high-specificity, active transport systems. This uptake probably contributes to the regulatory pool of cAMP within the cells.

**MATERIALS AND METHODS**

**Sampling locations.** Surface (0- to 2-m) samples were collected with sterile containers from the end of the pier at Scripps Institution of Oceanography (32°53'N, 117°15'W).

**Radiochemicals and inhibitors.** [2,8-$^3$H]cAMP (37 Ci/ mmol) was obtained from Amersham Corp., Arlington Heights, Ill., and [32P]cAMP (2,200 Ci/mmol) was from New England Nuclear Corp., Boston, Mass. (production discontinued August 1981). The purity of both stock solutions was checked frequently by thin-layer chromatography (TLC). Sodium arsenate, carbonyl cyanide $m$-chlorophenylhydrazone (CCCP), and valinomycin were obtained from Sigma Chemical Co., St. Louis, Mo. All other chemicals were reagent grade.

**Uptake experiments.** Experiments were initiated by addition of 1 µCi or less of $^3$H- or [32P]cAMP to a 10-ml seawater sample. Samples were incubated at near-ambient temperature (16 to 18°C) for 1 h and then filtered onto 0.45-µm membrane filters (type HA, Millipore Corp., Bedford, Mass.) at a vacuum of 200 mmHg (ca. 26 kPa) or less and rinsed twice with cold artificial seawater (0°C). Filters were dissolved in scintillation vials with 1 ml of ethyl acetate, which was then mixed with 10 ml of scintillation fluid (Beta Phase; West Chem, San Diego, Calif.). Radioactivity was counted by liquid scintillation spectrometry. In experiments with metabolic inhibitors, the samples were preincubated with the inhibitor for 10 min before the addition of label. Competitive inhibitors, in contrast, were added simultaneously with the label.

TLC. Samples (2 to 5 µl) were spotted on polyethyleneimine cellulose plates (E. Merck AG, Darmstadt, West Germany) with a micropipette. The chromatogram was developed by methods similar to those of Tao (25), using 1.0 M ammonium acetate–95% ethanol (30:75, vol/vol). Each lane was cut into 1- by 2-cm pieces which were placed in scintillation vials. One milliliter of 2 M lithium chloride was added, and the samples were shaken for several hours before the addition of scintillation fluid. One lane was spotted with standard unlabeled nucleotides, which were visualized with short-wavelength UV illumination. The location of these nucleotides was then compared with the radioactivity in the extracts.

**RESULTS**

Time course of cAMP uptake. With the exception of the first few minutes, uptake of [32P]cAMP was linear for at least 1 h (Fig. 1). The initial rapid uptake may represent nonspecific binding or binding to a receptor in the outer membrane as found for vitamin B$_{12}$ uptake in E. coli (11). The substrate turnover rate represented by the linear portion of the curve in Fig. 1 was 1.43% h$^{-1}$. Uptake of [32P]cAMP by an HgCl$_2$-killed control (0.7 µM) in a comparable experiment was only 6% of the rate of uptake of an untreated sample. A similar experiment (data not shown) demonstrated that the linear uptake lasted at least 8 h and the rate had declined only 27% after 24 h. This suggests that cAMP itself and not a minor contaminant or breakdown product was taken up by the cells.

**Kinetics.** Uptake of [32P]cAMP was readily measurable from added tracer concentrations of 1 pM or less. Figure 2 shows two similar Wright-Hobbie plots (modified Woolf linear transformation of the Michaelis-Menten equation [28, 29]) of the kinetics of cAMP uptake by natural populations of marine bacteria on successive days. This type of plot is based on the equation $t/f = A/V_{max} + (K_s + S_o)/V_{max}$, and it yields values for $V_{max}$ (maximal rate of uptake) and $(K_s + S_o)$, where $S_o$ is the ambient substrate concentration. The ordinate is the turnover time ($t/f$; $t$ is time, and $f$ is fraction of substrate taken up) and the abscissa is the concentration of added substrate (A). This transformation of the Michaelis-Menten equation was necessary because $S_o$ was not measured in these experiments. These plots both show families of "multiphasic" uptake curves (1a), as would be expected in natural populations containing many species. Other experiments used [32P]- or [3H]cAMP over a much greater concentration range (up to 2 µM). These yielded a similar group of curves up to the highest concentration used (data not shown).
The values for \((K_r + S_a)\) and \(V_{\text{max}}\) determined from Fig. 2 are shown in Table 1. The x-intercept is \((K_r + S_a)\), and \(V_{\text{max}}\) is the reciprocal of the slope (28, 29). The lowest \((K_r + S_a)\) values for the two experiments shown in Fig. 2 were both below 10 pM (Table 1), suggesting very high-affinity uptake systems for cAMP. The \(V_{\text{max}}\) values (<4 pmol h\(^{-1}\) liter\(^{-1}\); Table 1) also indicate that these were low-capacity uptake systems for cAMP. cAMP transport systems with lower affinities but higher capacities were found at the higher substrate concentrations (data not shown). The highest \((K_r + S_a)\) values for these systems approached 1 \(\mu\)M, with \(V_{\text{max}}\) values of 1 nmol h\(^{-1}\) liter\(^{-1}\). Throughout the rest of this paper, the cAMP transport systems with picomolar \((K_r + S_a)\) values (Fig. 2, Table 1) will be referred to as high-affinity systems, and those with nanomolar and higher \((K_r + S_a)\) values will be referred to as low-affinity systems.

### Table 2. cAMP uptake (at two different substrate concentrations) in the presence of structurally related competitors

<table>
<thead>
<tr>
<th>Competitor</th>
<th>Concn ((\mu)M)</th>
<th>Uptake (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>2 nM ([3H]cAMP)</td>
<td>100 (91–108)(^a)</td>
</tr>
<tr>
<td>P</td>
<td>20</td>
<td>100 (95–106)(^b)</td>
</tr>
<tr>
<td>Adenine</td>
<td>2</td>
<td>96 (95–96)</td>
</tr>
<tr>
<td>Adenosine</td>
<td>2</td>
<td>63 (55–76)</td>
</tr>
<tr>
<td>3',5'-AMP</td>
<td>2</td>
<td>42 (41–44)</td>
</tr>
<tr>
<td>3',5'-cAMP</td>
<td>2</td>
<td>62 (59–65)</td>
</tr>
<tr>
<td>3',5'-cGMP</td>
<td>2</td>
<td>48 (43–52)</td>
</tr>
</tbody>
</table>

\(^a\) Mean and range (in parentheses) shown for each sample. For controls, \(n = 4\); for experimental samples, \(n = 3\).

\(^b\) Control turnover rate, 0.31% h\(^{-1}\).

\(^c\) Control turnover rate, 1.16% h\(^{-1}\).

Substrate specificity. Table 2 shows the results of competitive uptake experiments, using either 10 pM \([32P]cAMP\) or 2 nM \([3H]cAMP\) in the presence of large excesses (up to 2 \(\times\) 10\(^6\)) of various structurally related organic compounds or inorganic phosphate. Note that with 10 pM labeled cAMP only the three cyclic nucleotide competitors inhibited uptake of label, whereas at the higher cAMP concentration several other compounds were nearly as effective (e.g., adenosine). This dramatically illustrates the much greater specificity of the high-affinity transport systems for cyclic nucleotides. Because of this great specificity and the fact that seawater cAMP concentrations probably never exceed 50 pM (1) (except possibly in enriched microzones), we have concentrated on the high-affinity systems.

In Table 2 the effects of the three different cyclic nucleotides on uptake of \([32P]cAMP\) (via the high-affinity system) are indistinguishable. Table 3 shows that at lower concentrations of these unlabeled competitors, cAMP was an

### Table 3. Uptake of \([32P]cAMP\) (11 pM) in the presence of three different concentrations of unlabeled cyclic nucleotides

<table>
<thead>
<tr>
<th>Competitor</th>
<th>Uptake (% of control)(^a) at given competitor concn</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100 pM</td>
</tr>
<tr>
<td>2',3',5'-cAMP</td>
<td>95 (91–99)</td>
</tr>
<tr>
<td>3',5'-cAMP</td>
<td>95 (91–100)</td>
</tr>
<tr>
<td>3',5'-cGMP</td>
<td>92 (84–96)</td>
</tr>
</tbody>
</table>

\(^a\) Mean and range (in parentheses) shown for each sample \((n = 3)\). Control turnover rate was 3.03% h\(^{-1}\) (range, 2.90 to 3.24% h\(^{-1}\); \(n = 6\)).
FIG. 3. Effect of temperature on uptake of cAMP by natural marine bacterial populations. All samples were preincubated at their respective assay temperatures for at least 30 min before inoculation. Uptake from 10 pM $^{32}$P (●) and uptake from 2 nM $^3$H (○); mean and range ($n = 3$) are shown for both.

Metabolism of cAMP. To determine the fraction of transported radioactive cAMP remaining in the soluble cellular pool, freshly filtered cells were extracted with ice-cold 5% trichloroacetic acid for 5 min and rinsed twice with the same. Radioactivity remaining on the filter (trichloroacetic acid insoluble) was assumed to be in macromolecules, probably RNA. The results of a 1-h time course experiment are shown in Fig. 1; the proportion of label that was trichloroacetic acid soluble decreased from 80 to 50% during that time period.

To determine the distribution of radioactivity

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Conc</th>
<th>Uptake (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Expt 1 (11 pM)</td>
</tr>
<tr>
<td>None</td>
<td></td>
<td>100 (94-107)</td>
</tr>
<tr>
<td>Ethanol (5 μl)</td>
<td></td>
<td>104 (95-114)</td>
</tr>
<tr>
<td>Arsenate</td>
<td>5 mM</td>
<td>53 (31-64)</td>
</tr>
<tr>
<td>CCP</td>
<td>1 μM</td>
<td>67 (64-70)</td>
</tr>
<tr>
<td>CCP</td>
<td>10 μM</td>
<td>21 (17-26)</td>
</tr>
<tr>
<td>Valinomycin</td>
<td>1 μM</td>
<td>42 (39-44)</td>
</tr>
<tr>
<td>Valinomycin</td>
<td>10 μM</td>
<td>31 (30-32)</td>
</tr>
</tbody>
</table>

* Mean and range (in parentheses) shown for each sample. For controls and ethanol controls, $n = 4$ or 5; for experimental samples, $n = 3$.
* Control turnover rate, 2.65% h$^{-1}$.
* Control turnover rate, 1.71% h$^{-1}$.
* Rates shown are percentage of ethanol control.

equal or better competitor than cyclic adenosine 2',3'- or cyclic monophosphate guanosine 3',5'-monophosphate.

Effect of temperature. Uptake of $[^{32}$P]cAMP (10 pM) at six temperatures from 0 to 50°C is shown in Fig. 3. cAMP uptake was highly temperature dependent, increasing linearly to about 24°C and decreasing at higher temperatures. $Q_{10}$ values calculated from Fig. 3 (top curve) were 2.1 (0 to 10°C) and 1.5 (10 to 20°C). Uptake of $[^3$H]cAMP (2 nM) showed the same pattern (Fig. 3), suggesting a similar effect of temperature on both low- and high-affinity systems.

Effect of metabolic inhibitors. Arsenate partially inhibited uptake of cAMP (Table 4). CCCP, an uncoupler or proton ionophore, strongly inhibited cAMP uptake when used at concentrations of 10 μM. Valinomycin, a potassium ionophore, was a less effective inhibitor than CCCP at a 10 μM concentration, but at 1 μM it was more effective than CCCP (Table 4).

Efflux. Figure 4 shows the results of an experiment designed to look for efflux of cAMP. After 4 min of uptake from 10 pM $[^{32}$P]cAMP, the sample was split, and 10 μM (final concentration) unlabeled cAMP was added to one subsample. Uptake was monitored in both subsamples for another 25 min. Addition of unlabeled cAMP stopped uptake of label but caused little apparent efflux of the labeled cAMP previously taken up by the cells.
in the soluble pool, cells exposed to 20 pM [³²P]cAMP for various time periods up to 1 h were filtered, rinsed as above, and extracted in boiling water for 10 min. (Boiling water and cold trichloroacetic acid extracted the soluble pools from the cells with comparable efficiency.) The extracts were then refiltered, and the filtrate was evaporated to a small volume for spotting on a TLC plate. Figure 5 shows that most of the label (at all time points) was in the region on the TLC plate which includes Pi, AMP, ADP, and ATP. These four compounds were not separated by TLC with this solvent system. More important, however, was the persistence, throughout the experiment, of a peak of ³²P which comigrated with authentic cAMP. This peak initially accounted for 10 to 15% of the label but dropped to 3 to 5% after 60 min. Due to the large increase in total ³²P in the soluble pool over this time (Fig. 1), however, the intracellular pool of [³²P]cAMP was probably constant or increasing.

In a similar experiment, cells which had been incubated with [³²P]cAMP for 15 min were extracted, and the extract was split into two fractions. One fraction was treated with 50 μg of bovine heart cyclic 3',5'-nucleotide phosphodiesterase (EC 3.1.4.17) for 2 h, under conditions similar to those used by Butcher (3). After TLC, only the untreated fraction retained the peak of label which migrated with cAMP (data not shown), suggesting that the peak was authentic cAMP taken up by the cells.

**Culture studies.** Several marine isolates grown in C-P medium (4) slowly took up added [³H]cAMP from relatively high concentrations (2 to 4 nM; data not shown). This uptake was not specific for cAMP, however; adenine and adenosine were better competitors than unlabeled cAMP. An isolate also took up [³²P]cAMP (10 pM added) but the rate per cell was much less than natural populations and AMP was a better competitor than unlabeled cAMP.

Natural populations of marine bacteria which are diluted to 10⁵ ml⁻¹ in sterile filtered seawater...
usually grow back to normal seawater concentrations (=10^6 ml^{-1}) in 3 to 7 days. These "seawater cultures" did not take up 10 pM \[^{32}\text{P}\]cAMP (data not shown). This suggests that the bacteria with the high-affinity cAMP uptake systems either did not grow or did not synthesize these uptake systems in seawater cultures.

**DISCUSSION**

Our results clearly show that there are bacteria in seawater which have highly specific and very high-affinity transport systems for cAMP. This is in sharp contrast with both cultured marine isolates and seawater cultures. In a natural bacterial assemblage, however, all of the transport parameters will represent overlapping responses attributable to the various bacterial species present. (We have previously shown that algal uptake of cAMP is negligible [11].) The measurements of kinetics, substrate specificity, temperature response, and energy coupling will represent the combined characteristics of many species. Fortunately, however, a mixed assemblage generally yields interpretable results, possibly because all of the bacteria are adapted to, and were studied in, the same environment. The kinetics and temperature characteristics of glucose uptake by natural marine bacterial assemblages have previously been studied with unambiguous results (1a, 23).

The cAMP concentration in seawater averages only 10 pM (1), a level which is much less (about 100 times) than the concentration of dissolved AMP, ADP, and ATP (14), and probably adenine and adenosine as well. Therefore, to effectively take up cAMP from seawater, a cAMP uptake system not only would require a very low \(K_r\) but also must be immune to competition from the compounds mentioned above. Our lowest \((K_r + S_n)\) values were below 10 pM, and since \(S_n\) alone is in this range (1), \(K_r\) may be even lower. Only cyclic nucleotides competed with cAMP for uptake by the high-affinity systems, even at competitor concentrations 2 \times 10^7 times greater than the substrate. \(K_r\) values calculated from Table 2 (last column) were 100 times higher for the noncyclic adenine derivatives tested than for the three cyclic nucleotides, regardless of the value assumed for \(K_r\). Further experiments with lower concentrations of competitors (Table 3) suggested that cAMP was bound by the transport system with a higher affinity than the other two cyclic nucleotides.

The failure of a large excess of Pi (20 \(\mu\)M) or 3'- or 5'-AMP (2 \(\mu\)M) to inhibit uptake of \[^{32}\text{P}\]cAMP (10 pM) is strong evidence that cAMP crosses the cytoplasmic membrane intact and not as a breakdown product (1). Authentic \[^{32}\text{P}\]cAMP could also be extracted from the cells for at least 1 h after addition of \[^{32}\text{P}\]cAMP to the medium. The percentage of total intracellular \[^{32}\text{P}\] which remained as cAMP decreased with time, suggesting entry as cAMP and subsequent metabolism. We could not determine what portion of the bacterial assemblage metabolized exogenous cAMP.

cAMP uptake showed obvious saturation kinetics over a broad range of substrate concentrations (10 to 2,000 pM; data not shown). Over a small range of cAMP concentrations (1 to 20 pM), however, saturation was obscured by the multiphasic uptake pattern observed with natural populations. This multiphasic cAMP uptake was probably the result of many different bacterial species each with different kinetic parameters. One (or a few) species with multiple transport systems, or a system showing cooperativity (13), is an alternate (or additional) explanation.

This multiphasic pattern yielded numerous lower-affinity, higher-capacity cAMP transport systems in addition to the high-affinity systems described above. In these low-affinity systems adenine and its derivatives competed with cAMP uptake almost as effectively as the cyclic nucleotides (Table 2). Therefore, these low-affinity transport systems may not have transported intact cAMP, but rather adenine or another fragment instead. This was even more likely in the cultured marine isolates which took up \[^{3H}\text{cAMP}\]. The isolate which took up \[^{32}\text{P}\] from 10 pM \[^{32}\text{P}\]cAMP probably actually transported \(\text{Pi}\).

cAMP uptake by the high-affinity systems in natural bacterial assemblages can be considered active transport because it showed: (i) net flux against a concentration gradient, (ii) saturation kinetics, (iii) substrate specificity, and (iv) sensitivity to inhibitors of transport and energy metabolism. Saturation kinetics and substrate specificity were discussed above; the other characteristics are discussed below.

CAMP was transported from seawater, which has a cAMP concentration of 10 pM (1), into bacterial cells with an intracellular concentration of 1 \(\mu\)M, a concentration gradient of 10^5. The intracellular concentration was calculated assuming a value of 1 pmol of total cAMP in the plankton in a liter of seawater (1). We further assumed a bacterial cell volume of 0.1 \(\mu\)m^3 (6), a bacterial population of 10^9 cells liter^{-1}, and that the bacteria accounted for 10% of the biomass and cAMP (5). The calculated intracellular concentration (1 \(\mu\)M) is very similar to that reported for E. coli and other procaryotes (18).

CCCP and valinomycin were more effective than arsenate in inhibiting uptake of cAMP by the high-affinity systems (Table 4). CCCP dissipates both components of the proton motive force, the membrane potential and the pH gradient (21). (CCCP could also deplete ATP pools in
whole cells which vainly try to maintain a proton motive force [21].) Valinomycin makes the membrane permeable to potassium, allowing the membrane potential to reach a potassium equilibrium potential as determined by the Nernst equation (21). This could also cause an indirect change in the pH gradient (21). Arsenate depletes cellular pools of ATP and other high-energy phosphate compounds (21, 22). Therefore, the proton motive force (particularly the membrane potential) was apparently the driving force for cAMP transport, rather than high-energy phosphate compounds such as ATP. Although several energy-dependent cAMP efflux systems have been reported in different organisms (see below), this is the only cAMP uptake system reported to date which requires energy.

Two other results deserve comment, the low \( Q_{10} \) values observed for cAMP uptake and the apparent lack of cAMP efflux. First, the \( Q_{10} \) values (2.1 [0 to 10°C] and 1.5 [10 to 20°C]) may seem low for an enzyme-catalyzed reaction, but similar low values have been reported for phosphotransferase-mediated glucose transport (above 15°C) in a marine isolate (9). Goldenbaum and Hall (8) argued that the low \( Q_{10} \) (above 20°C) they reported for cAMP uptake by E. coli membrane vesicles showed that a carrier was involved. Second, it is possible that a small efflux of \([^{32}P]cAMP\) occurred but was balanced by continued \([^{32}P]cAMP\) uptake. This possibility cannot be ruled out by our experiments.

As stated earlier, only a few studies have examined bacterial cAMP transport. In 1975, Saier et al. (22) described an energy-dependent cAMP efflux system in E. coli and Salmonella typhimurium strains which are deficient in cAMP phosphodiesterase. The addition of metabolizable sugars to a whole-cell suspension lowers intracellular cAMP levels, as does the addition of electron donors to membrane vesicles preloaded with cAMP (22). In both systems cAMP efflux is at least partially blocked by uncouplers (22). More recently, Goldenbaum and Hall (8) examined both cAMP uptake and efflux in E. coli membrane vesicles. They also described efflux as an energy-dependent, active transport process, with a very high \( K_c \) of 10 mM. Uptake, in contrast, occurs by facilitated diffusion and is inhibited by electron donors and stimulated by 2,4-dinitrophenol. The \( K_c \) is the same as for efflux, suggesting that the same carrier is involved. Since vesicles are used, there is little metabolism of the cAMP taken up. A separate brief study showed that synchronous cultures of Nocardia restricta and Arthrobacter globiformis take up cAMP during the DNA replication phase of the cell cycle (12). Substrate levels are high (1 to 5 mM), and 83% of the cAMP taken up in 30 min is extractable as cAMP.

There is little information on energy requiring cAMP uptake or excretion in other organisms. Aggregating Dictyostelium cells release cAMP (7), but we know of no studies on the energetics of this process. Dictyostelium cells also have specific cell surface receptors which bind but apparently do not transport cAMP (7). Human erythrocytes take up cAMP by a carrier-mediated facilitated diffusion system (26) which may act as an anion channel as well. Mammalian and avian erythrocytes also have energy-dependent cAMP efflux systems (2 and references therein).

We have previously shown (1) that marine bacteria can increase their intracellular cAMP pool by about 1 \( \mu \)M h\(^{-1} \) by uptake via the high-affinity, low-capacity uptake systems. The exceptionally high specificity of these cAMP transport systems will ensure that cAMP uptake is unaffected by the simultaneous presence of numerous organic compounds (including nucleotides) in seawater at much higher concentrations. If the low-affinity, high-capacity systems transport intact cAMP (see above), they would allow even faster cAMP accumulation by bacteria in cAMP-enriched microzones (such as around an algal cell).

Although the regulatory importance of cAMP in bacteria is undisputed (18, 20), the ecological significance of cAMP uptake by natural assemblages of marine bacteria is unclear. It is apparent, however, that the high-affinity uptake systems described in this study have among the lowest \( K_c \) values ever reported for any substrate transported by bacteria. Vitamin B\(_12\) uptake by natural populations of lake microplankton, including algae and cyanobacteria, is the only microbial system we are aware of with \( K_c \) values similar to those for cAMP uptake (17). In bacterial cultures, however, minimum \( K_c \) values for vitamin uptake are 1,000 times higher than reported here for cAMP (11). Coupled with the near-absolute specificity of these systems for cyclic nucleotides, this extremely high affinity implies that cAMP taken up from seawater plays a role in the metabolic regulation of marine bacteria.

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LITERATURE CITED


