Growth of the marine luminous bacterium *Vibrio fischeri* on 3':5'-cyclic AMP: correlation with a periplasmic 3':5'-cyclic AMP phosphodiesterase

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The marine luminous bacterium *Vibrio fischeri*, the species-specific light-organ symbiont of monocentrid (pinecone) fish, grew on 3':5'-cyclic adenosine monophosphate (cAMP) as a sole source of carbon and energy, a capability not previously described in bacteria. In a minimal, chemically defined medium containing cAMP as the sole source of carbon and energy, *V. fischeri* cells grew more slowly than on glucose or ribose, but as quickly as on 5'-AMP. Expression of luminescence, which is dependent on cAMP in *V. fischeri*, was stimulated in cells grown on cAMP compared to cells grown on glucose or ribose. All strains of *V. fischeri* tested (MJ-1, B-61, ATCC 7744, MJ-A1, CG-A1) grew on cAMP, as did strains of two other marine luminous bacteria, *V. logei* and *Photobacterium phosphoreum*, and strains of the terrestrial enteric bacterium *Serratia marcescens*. Other tested species of marine luminous bacteria (*P. leiognathi*, *Shewanella* [*Alteromonas*] *hanedai*, *V. harveyi*, *V. orientalis*, *V. splendidus*) and terrestrial enteric bacteria (*Escherichia coli*, *Salmonella typhimurium*), which grew on 5'-AMP or ribose, did not grow on cAMP. Assays on intact cells and periplasmic extracts revealed that *V. fischeri* MJ-1 produced a periplasmic 3':5'-cAMP phosphodiesterase (cAMP phosphodiesterase) of very high specific activity [$9.0 \mu\text{mol phosphate released (mg protein)}^{-1} \text{ min}^{-1}$] and narrow substrate specificity (3':5'-cAMP and 3':5'-cGMP were attacked). The novel periplasmic location and unusually high activity of cAMP phosphodiesterase appear to account for the ability of this species to grow on cAMP. The periplasmic cAMP phosphodiesterase of *V. fischeri* might play a role in degrading free cAMP in seawater or in a cAMP-mediated aspect of the light organ symbiosis with monocentrid fish.

Introduction

3':5'-Cyclic AMP (cAMP) is a regulatory molecule found in a wide variety of prokaryotes and eukaryotes; it is involved in transcriptional control of certain genes in some bacteria, and as a second messenger in several hormonally regulated processes in animal cells (Robison *et al.*, 1971; Botsford, 1981; Francko, 1983; Ullman & Danchin, 1983; Devroetes, 1989). Cyclic AMP is synthesized from ATP by adenylate cyclase (EC 4.6.1.1) and is degraded to 5'-AMP (AMP) by cAMP phosphodiesterase (EC 3.1.4.17). In bacteria, cellular cAMP

levels are apparently regulated by the activities of these two cytoplasmic enzymes, and by excretion of excess cAMP into the growth medium. However, the role of cAMP phosphodiesterase in this regulation has not been completely resolved (Monard *et al.*, 1969; Buettner *et al.*, 1973; Alper & Ames, 1975; Saier *et al.*, 1975; Botsford, 1981, 1984).

Bacterial degradation of, and growth on, phosphorylated compounds such as ATP and AMP has been extensively documented (e.g. Neu, 1967; Unemoto *et al.*, 1978; Azam & Hodson, 1977; Bengis-Garber & Kushner, 1982; Ammerman & Azam, 1985; Bengis-Garber, 1985; Sakai *et al.*, 1987; Itami *et al.*, 1989; Tamminen, 1989). However, little is known about bacterial degradation of cAMP (and other cyclic nucleotides) in the environment. In aquatic environments, levels of dissolved cAMP are pM to nM (Ammerman & Azam, 1981; Francko, 1983). Nonetheless, certain components of natural marine bacterial populations take up cAMP at these levels using high-affinity transport systems specific for cyclic nucleotides,

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Table 1. Growth of marine and terrestrial bacteria on 5'-AMP and 3':5'-cAMP as sole carbon and energy sources

Species	Strain (source or reference)	Growth on*†	
		5'-AMP	3':5'-cAMP
<i>Vibrio fischeri</i>	MJ-1 (E. G. Ruby; Ruby & Nealson, 1976)	+	+
	ATCC 7744 (ATCC‡)	+	+
	B-61 (K. H. Nealson; Nealson, 1977)	+	+
	MJ-A1 (light organ of <i>Monocentris japonicus</i>)	+	+
	CG-A1 (light organ of <i>Cleiodopus gloriamaris</i>)	+	+
<i>V. harveyi</i>	B378 (P. Baumann; Reichelt & Bauman, 1973)	+	—
	B392 (P. Baumann; Reichelt & Bauman, 1973)	+	—
	FLB-17 (Fukasawa <i>et al.</i> , 1987)	+	—
<i>V. logei</i>	ATCC 29985 (ATCC)	+	+§
<i>V. orientalis</i>	ATCC 33934 (ATCC)	+	—
<i>V. splendidus</i>	ATCC 33125 (ATCC)	+	—
<i>Photobacterium leiognathi</i>	LN-1a (Dunlap, 1985)	+	—
	ATCC 25521 (ATCC)	+	—
<i>P. phosphoreum</i>	HE-1a (light organ of <i>Paratrachichthys prosthemi</i>)	+	+§
	NZ-11D (E. G. Ruby; Ruby & Morin, 1978)	+	+§
	B423 (E. F. DeLong; Reichelt & Baumann, 1973)	+	+§
	B425 (E. F. DeLong; Reichelt & Baumann, 1973)	+	+§
<i>Shewanella hanedai</i>	ATCC 33224 (ATCC)	+	—
<i>Escherichia coli</i>	LS 340 (CGSC¶)	+	—
	DH-1 (CGSC)	+	—
<i>Salmonella typhimurium</i>	LT-2 (B. Ames)	—	—
<i>Serratia marcescens</i>	ATCC 13880 (M. Gallo)	+	+
	ATCC 25419 (M. Gallo)	+	+

* Minimal, chemically defined media (see Materials) with 5 mM of each carbon and energy source.

† *V. fischeri* MJ-1 also grew on the following compounds: adenosine, 3'-AMP, ADP, ATP, 2':3'-cAMP, 5'-GMP, 3':5'-cGMP, and guanosine. Growth was not observed on adenine or guanine.

‡ ATCC, American Type Culture Collection, Rockville, MD, USA.

§ Slow growth on cAMP compared to *V. fischeri*.

|| Eleven newly isolated strains of luminous bacteria (P. V. Dunlap, unpublished) also were able to grow slowly (like *V. logei* ATCC 29985) on cAMP. Like *V. logei*, each strain produced a yellow cell-associated pigment, fermented glucose, grew and produced luminescence at 4 °C and 15 °C, and grew but produced little or no visible luminescence at room temperature. Based on these preliminary criteria, these strains are tentatively identified as *V. logei*.

¶ CGSC, *E. coli* Genetic Stock Center, Yale University, New Haven, CT, USA.

thereby supplementing the cellular regulatory pool of this nucleotide (Ammerman & Azam, 1982, 1987). Environmental levels of cAMP, however, are apparently too low to support bacterial growth, and the bacterial cytoplasmic membrane is in most cases highly impermeable to nucleotides (Neuhard & Nygaard, 1987). For these reasons, cAMP has not been considered to be a substrate for bacterial growth.

In this report we describe the novel ability of the marine luminous bacterium *V. fischeri*, the species-specific light-organ symbiont of monocentrid (pinecone) fish, to utilize cAMP as a sole source of carbon and energy for growth. This ability correlates in *V. fischeri* with an unusually high level of cAMP phosphodiesterase and with a novel location for this enzyme, the periplasmic space.

Methods

Bacterial strains and culture conditions. The bacterial strains used in this study and their sources are listed in Table 1. The marine bacteria were grown at room temperature (22–25 °C), as described previously (Dunlap & Greenberg, 1985), except that cultures for cAMP phosphodiesterase assays were grown at 28 °C. The complete medium for marine bacteria was based on SWC of Nealson (1978) and contained artificial seawater (ASW; 17.55 g NaCl, 0.75 g KCl, 12.35 g MgSO₄·7H₂O, and 1.45 g CaCl₂·2H₂O per litre distilled water), 5 g tryptone, 3 g yeast extract, 50 mM-HEPES (pH 7.5), 40 mM-glycerol (unless otherwise indicated), and for plates, 15 g agar. The minimal medium for marine bacteria was based on that of Haygood & Nealson (1985) and contained ASW, ferric ammonium citrate (FAC, 20 mg l⁻¹), 19 mM-NH₄Cl, 0.3 mM- α -glycerophosphate, and 50 mM-HEPES (pH 7.5), except that for strains of *Photobacterium*, 0.33 mM-KH₂PO₄ and 25 mM-Tris/HCl replaced the α -glycerophosphate and HEPES. The terrestrial bacteria were grown at 30 °C, as described previously (Dunlap & Greenberg, 1985). The complete medium for terrestrial

bacteria (Luria-Bertani, [LB], Silhavy *et al.*, 1984) contained (per litre of distilled water): 10 g tryptone, 5 g yeast extract, 86 mM-NaCl, and 50 mM-Tris/HCl (pH 7.5). The minimal medium for terrestrial bacteria was either M9 (Davis *et al.*, 1980) or one containing 1 mM- K_2HPO_4 , 1.5 mM- $(NH_4)_2SO_4$, 0.08 mM- $MgCl_2$, FAC (20 mg l⁻¹), and 25 mM-Tris/HCl (pH 7.5). For *E. coli* strains, the minimal media were supplemented with the appropriate amino acids and vitamins (Davis *et al.*, 1980) necessary for growth. The minimal and complete media were supplemented with carbon and energy sources as indicated.

Growth controls and stability of cAMP. *V. fischeri* was unable to grow in the minimal medium (broth or plates) without an added source of carbon and energy regardless of source of inoculum (cells grown on complete, minimal-glucose, or minimal-cAMP medium), the presence or type of buffer used (Tris, HEPES, phosphate, or none), or the type of agar used (regular agar or agar noble). No visible digestion of the agar was observed.

PEI-cellulose thin-layer chromatography plates containing a fluorescent indicator were used to determine if stock solutions of cAMP broke down during storage. No breakdown of cAMP was detected, even with heavy loading of the plates (2 µl 10 mM solution); the cAMP ran as a single spot well separated from its constituent compounds (R_F : AMP, 0.06; cAMP, 0.28; adenine, 0.41; adenosine, 0.54). Minimal medium plates containing cAMP (5 mM) were allowed to incubate at room temperature and at 37 °C to determine whether cAMP incorporated into media broke down during incubation. No breakdown was detected; after two days the plates were inoculated and they supported the growth of *V. fischeri* but did not support the growth of bacteria able to grow on AMP and on ribose (*P. leiognathi* and *E. coli*).

Determination of cellular luminescence and luciferase activity. The light-measuring equipment and standard to calibrate the equipment were as described previously (Dunlap & Greenberg, 1985), as were the procedures for measuring luminescence of broth cultures and luciferase activity in cell extracts (Dunlap & Greenberg, 1985).

Determination of cAMP phosphodiesterase activity. Assays for cAMP phosphodiesterase activity were carried out essentially as described by Cheung (1971) using snake venom (from *Crotalus adamanteus*) as the source of 5'-nucleotidase. Phosphate released was determined by the method of Fiske & SubbaRow (1925). For whole cell assays, cells grown to $OD_{660} = 1.0$ – 1.2 were diluted in ASW containing 50 mM-Tris/HCl (pH 7.5) (buffered ASW) to $OD_{660} = 0.52$ – 0.55 , and 0.5 ml of this cell suspension was washed twice by centrifugation and resuspended in 0.15 ml of buffered ASW. For the terrestrial bacteria, 0.9% NaCl was used to dilute and wash cells. In general, 10 µl of this cell suspension was used for phosphodiesterase activity assays. The reaction mixture (0.5 ml) contained 10–20 µl of cell suspension (or crude cell lysate, see below), 40 mM-Tris/HCl (pH 8.0), 0.1 mM- $MnCl_2$, and 2 mM-cAMP, addition of which initiated the reaction. After 20 min at room temperature, the reaction was stopped by boiling for 2 min. After thermal equilibration of the reaction mixture to room temperature, 50 µl snake venom (1 mg ml⁻¹) was added, with incubation for a further 10 min. The reaction was stopped by adding 50 µl 55% (w/v) trichloroacetic acid, followed by 750 µl water and 150 µl 2.5% ammonium molybdate in 2.5 M- H_2SO_4 . Cellular debris was removed by microcentrifugation (12000 g, 3 min), and the clear supernatant was added to tubes containing 50 µl Fiske-SubbaRow reducer. Absorbance of the blue colour which developed was measured at 660 nm.

Protein content was determined on 10–20 µl cell suspension or crude cell lysate, according to the method of Bradford (1976). Periplasmic proteins were released from cells using the chloroform shock treatment of Ames *et al.* (1984). For total cell lysates and lysates of cell pellets following chloroform shock, cells were disrupted by sonication. The procedure for ammonium sulphate precipitation of proteins released by

Fig. 1. Growth of *V. fischeri* MJ-1 on 3':5'-cAMP as a sole carbon and energy source. Symbols (open, glucose; closed, 3':5'-cAMP): ○, 5 mM-substrate; △, 3 mM; ▽, 1 mM.

chloroform shock followed that of Okabayashi & Ide (1970a). Precipitated proteins were resolubilized in 10 mM-Tris/HCl, pH 8.0.

Chemicals. Fiske-SubbaRow reducer, HEPES, phosphorylated substrates, salts, snake venom, and Tris were purchased from Sigma.

Results

Growth and luminescence of V. fischeri on cAMP as a sole carbon and energy source

During studies of the role of 3':5'-cAMP in sugar catabolism and luminescence in *V. fischeri* MJ-1 (Dunlap, 1989), we noticed that this strain grew on minimal medium plates that lacked an added sugar but contained cAMP (5 mM). The possibilities that growth could be accounted for by a carry-over of nutrients from complete medium cultures, by components of the minimal medium other than cAMP, or by the spontaneous breakdown of cAMP to AMP, adenosine or ribose were tested and excluded (see Methods). Because the use of cAMP as a growth substrate has not been previously described for bacteria, we examined this ability in more detail.

V. fischeri MJ-1 consistently grew rapidly in minimal medium broth containing cAMP (1–5 mM) as the sole carbon and energy source (Fig. 1). Compared to cAMP,

Fig. 2. Luminescence and growth of *V. fischeri* MJ-1 on different sole carbon and energy sources (each 10 mM). Symbols: ○, glucose; △, 3':5'-cAMP; □, ribose.

growth was faster on glucose (Fig. 1) and ribose, slower on adenosine, and similar on 5'-AMP. Little or no growth occurred on adenine. Similar results were obtained for cells pre-grown on glucose, glycerol or cAMP, and cells could be subcultured repeatedly in minimal-cAMP broth.

As a regulatory molecule, cAMP is required for autoinduction of the *V. fischeri* luminescence system. The type of carbon source provided for growth also influences luminescence (Ruby & Nealson, 1976; Friedrich & Greenberg, 1983; Dunlap & Greenberg, 1985, 1988, 1991; Dunlap & Ray, 1989; Dunlap, 1989). Therefore, to examine the luminescence behaviour of *V. fischeri* MJ-1 growing on cAMP, cells were inoculated into minimal medium broth containing cAMP, glucose, or ribose (10 mM each), and growth and luminescence were monitored. Luminescence of each culture exhibited a decline and recovery during growth (Fig. 2) that is characteristic of the autoinducible nature of luminescence in *V. fischeri* (Eberhard, 1972; Nealson, 1977). In the cAMP-grown culture, however, luminescence was substantially higher than in the glucose- or ribose-grown cultures (Fig. 2).

Utilization of cAMP as a growth substrate by other marine luminous and terrestrial bacteria

All tested strains of *V. fischeri* grew on cAMP (Table 1). Two other species of marine luminous bacteria, *V. logei* and *P. phosphoreum*, also grew on cAMP (Table 1), but did so more slowly than *V. fischeri*. No other marine luminous bacteria, of those species tested, were found to have this ability, although all were able to grow on 5'-AMP (Table 1). Of the terrestrial enteric bacteria examined, only the strains of *Serratia marcescens* were able to grow on cAMP. The ability to utilize cAMP as a sole carbon and energy source apparently is uncommon in marine luminous bacteria and it might be rare among Gram-negative bacteria in general.

Periplasmic 3':5'-cAMP phosphodiesterase in *V. fischeri*

The ability of *V. fischeri* to grow on cAMP suggested that its 3':5'-cAMP phosphodiesterase (EC 3.1.4.17) might be unusual in its level of activity and cellular location. Because *V. fischeri* cells can grow on AMP as a sole carbon and energy source, cAMP phosphodiesterase, by degrading cAMP to AMP, could account for the ability to grow on cAMP. The cAMP phosphodiesterase of enteric bacteria, however, occurs in the cytoplasm (Brana & Chytil, 1966; Alper & Ames, 1975; Calcott & Calvert, 1981; Catanese *et al.*, 1989). Given that the bacterial cytoplasmic membrane is highly impermeable to nucleotides (Neuhard & Nygaard, 1987) (except in certain intracellular pathogens; Winkler, 1976; Hatch *et al.*, 1982; Ruby *et al.*, 1985), it is unlikely that cAMP can enter the cytoplasm of *V. fischeri* in amounts sufficient to support rapid growth. Bengis-Garber (1985), however, reported preliminary data consistent with a periplasmic location for cAMP phosphodiesterase in *V. fischeri* and *P. phosphoreum*. Therefore, we examined *V. fischeri* MJ-1 for the presence and cellular location of this enzyme.

cAMP phosphodiesterase activity was readily detected in *V. fischeri* MJ-1 (Tables 2–4). The activity occurred predominantly in the periplasmic space as determined both by the chloroform-shock method for releasing periplasmic proteins (Ames *et al.*, 1984) (Table 2) and by whole (intact) cell assays (Beacham, 1979) (Tables 3 and 4). The conclusion that the cAMP phosphodiesterase is periplasmic was further supported by the results of assays for luciferase, a known cytoplasmic enzyme (Nealson, 1978). In contrast to cAMP phosphodiesterase activity, luciferase activity was found predominantly in the cytoplasmic fraction of cells fractionated by the chloroform-shock method (Table 2). Consistent with these results, cAMP phosphodiesterase activity in *E. coli* and *S. typhimurium*, which is known to be cytoplasmic (Alper & Ames, 1975), was not detected in the intact cell

Table 2. Cyclic AMP phosphodiesterase and luciferase activities of *V. fischeri* MJ-1

	cAMP Phosphodiesterase activity [$\mu\text{mol phosphate released (mg protein)}^{-1} \text{ min}^{-1}$]	$10^5 \times$ Luciferase activity
Cytoplasmic fraction*	0.29	1.50
Periplasmic fraction†	6.80	0.02

* Cells, grown in minimal medium containing glycerol (20 mM), were pelleted after chloroform shock, resuspended and sonicated (see Methods). Data are from a representative experiment (variation of replicate assays $< \pm 20\%$).

† Supernatant of chloroform-shock treated cells (see Methods).

Table 3. Cyclic AMP phosphodiesterase activities of different bacterial species

Species	Medium	cAMP phosphodiesterase activity*† [$\mu\text{mol phosphate released (mg protein)}^{-1} \text{ min}^{-1}$]
<i>V. fischeri</i> MJ-1	complete	0.97 ± 0.28 (7)
	minimal‡	6.40 ± 0.62 (8)
<i>P. phosphoreum</i> NZ-11D	complete	0 (2)
	minimal	0.35 ± 0.11 (4)
<i>P. leiognathi</i> LN-1a	complete	0 (1)
	minimal	0 (2)
<i>E. coli</i> AB-257	complete	ND
	minimal	0 (2)
		0.02 (1)§
<i>S. typhimurium</i> LT-2	complete	ND
	minimal	0.01 (1)§
<i>S. marcescens</i> ATCC 25919	complete	2.29 ± 0.59 (5)
	minimal	0.18 (1)

ND, not determined.

* Whole cell assays except as noted (see Methods).

† Mean value, variation between experiments, and number of separate experiments.

‡ Containing either 20 mM-glycerol or 10 mM-glucose.

§ Assays on cell sonicates (see Methods).

Table 4. Effect of carbon and energy source on cAMP phosphodiesterase activity in *V. fischeri* MJ-1

Carbon source added	cAMP phosphodiesterase activity* [$\mu\text{mol phosphate (mg protein)}^{-1} \text{ min}^{-1}$]	
	Minimal medium†	Complete medium†
Glucose (10 mM)	7.4 ± 1.15 (8)	0.77 ± 0.17 (3)
Glycerol (20 mM)	6.4 ± 0.62 (8)	0.97 ± 0.28 (7)
Ribose (20 mM)	5.0 ± 1.43 (3)	0.78 – (1)
3':5'-cAMP (10 mM)	1.6 ± 0.45 (4)	0.74 ± 0.05 (2)
5'-AMP (10 mM)	ND‡	0.77 – (1)
Adenosine (10 mM)	9.0 ± 2.74 (4)	0.77 – (1)
Succinate (20 mM)	3.9 ± 0.41 (7)	0.61 ± 0.10 (4)

ND, Not determined.

* Whole cell assays (see Methods).

† Mean value, variation between experiments, and number of separate experiments.

assay but was detected in cell sonicates (Table 3). Furthermore, the periplasmic location of cAMP phosphodiesterase is consistent with the preliminary results of Bengis-Garber (1985), who also used intact cell assays.

The level of periplasmic cAMP phosphodiesterase activity in *V. fischeri* was 300–600 fold higher than in *E. coli* and *S. typhimurium* (Tables 2 and 3). With intact cell assays, high levels of cAMP phosphodiesterase activity were also detected in *P. phosphoreum* and *S. marcescens*, but not in *P. leiognathi* or *E. coli* (Table 3). These results are consistent with the ability of *V. fischeri*, *P. phosphoreum* and *S. marcescens* to grow on cAMP and with the lack of this ability in *P. leiognathi* and *E. coli*. These results suggest that *P. phosphoreum* and *S. marcescens* also synthesize a periplasmic cAMP phosphodiesterase.

Table 5. Substrate specificity of 3':5'-cAMP phosphodiesterase of *V. fischeri* MJ-1*

Substrate†	% Activity
3':5'-cAMP	100‡
5'-AMP	—
3'-AMP	0.3
2':3'-cAMP	—
ADP	2.3
ATP	1.8
3':5'-cGMP	49
5'-GMP	0.4
Glucose-6-phosphate	—
Pyrophosphate	2.2
β -Glycerophosphate	0.3
bis- <i>p</i> -Nitrophenylphosphate	—

—, No activity detected.

* Assays were conducted on ammonium sulphate-precipitated (40–75% fraction) supernatants of chloroform-shock treated cells (see Methods). Assays were conducted two or three times for each substrate, with variations similar to that described in Table 4 for a single substrate.

† Substrates present at 5 mM.

‡ 100%, 31 μ mol phosphate released (mg protein⁻¹) min⁻¹.

To examine the substrate range of the *V. fischeri* periplasmic cAMP phosphodiesterase, the enzyme was partially purified using chloroform shock to release periplasmic proteins (Ames *et al.*, 1984) followed by ammonium sulphate precipitation. Most of the periplasmic cAMP phosphodiesterase activity occurred in the fraction precipitated by 40–75% ammonium sulphate; therefore this fraction was used for substrate assays. Of the substrates tested, only 3':5'-cAMP and 3':5'-cyclic guanosine monophosphate (cGMP) were attacked significantly (Table 5). The activity against cGMP was approximately half of that against cAMP.

The composition of the growth medium influenced the level of periplasmic cAMP phosphodiesterase in *V. fischeri*. The activity (intact cell assays) was 2- to 10-fold higher for cells grown in minimal medium compared to complete medium, depending on the carbon source, and for cells grown in minimal medium it varied with the carbon source by approximately 5-fold (Table 4). The stage of growth, however, apparently did not influence the level of this enzyme, at least in complete medium. Cells grown to mid-exponential phase (OD 0.5–0.6) had a similar level of periplasmic cAMP phosphodiesterase activity as cells grown to stationary phase (OD > 1.5) [glycerol, 1.2 compared to 1.4; glucose, 0.8 compared to 0.7; succinate, 0.6 compared to 0.7, all μ mol phosphate released (mg protein⁻¹) min⁻¹, respectively]. Furthermore, like the cAMP phosphodiesterases of other bacteria (Nielsen *et al.*, 1973; Lee, 1978; Calcott &

Calvert, 1981), activity of the *V. fischeri* periplasmic enzyme was inhibited by addition of EDTA to the assay buffer [no EDTA added, 21.4; with EDTA added to 0.15 mM, 10.9; with EDTA added to 5 mM, 4.6; all μ mol phosphate released (mg protein⁻¹) min⁻¹].

Discussion

We have reported here the novel ability of the marine luminous bacterium *V. fischeri*, and certain other bacteria, to use cAMP as a sole source of carbon and energy for growth. The use of cyclic nucleotides as growth substrates by bacteria (or other organisms) has not been described previously to our knowledge. Growth of *V. fischeri* on cAMP (Fig. 1) correlated with the presence of a cAMP phosphodiesterase of very high specific activity in the periplasm of this species (Tables 2–4), a novel cellular location for this enzyme. This constitutes, to our knowledge, the first report of a periplasmic enzyme in this species. The ability to cleave 3':5'-cAMP to 5'-AMP in the periplasm may be the only requirement for growth on cAMP, since many bacteria can grow on 5'-AMP through the activity of 5'-nucleotidase and adenosine transport systems (Table 1; Neu, 1967; Yagil & Beacham, 1975; Beacham, 1979; Bengis-Garber & Kushner, 1982; Neuhaard & Nygaard, 1987; Sakai *et al.*, 1987). The ability of *V. fischeri*, along with *V. logei* and *P. phosphoreum*, to grow on cAMP (Table 1) provides an additional phenotypic trait (Baumann & Baumann, 1981) for identification of these species among marine luminous bacteria.

The cAMP phosphodiesterase of *V. fischeri* differs from that of previously described bacterial 3':5'-cAMP phosphodiesterases in its periplasmic location, its high level of activity, and its apparent regulation by conditions of the growth environment (Tables 2–4). In general, bacterial cAMP phosphodiesterase is found in the cytoplasm and exhibits a low and relatively invariant activity (< 0.03 μ mol phosphate released (mg protein⁻¹) min⁻¹ in crude cell extracts), although in different species it differs markedly in size, requirement for iron or other metal activators, effects of metal inhibitors, temperature optima, pH optima, effects of organic inhibitors (such as caffeine and its derivatives), and substrate specificity (e. g. Brana & Chytil, 1966; Ide *et al.*, 1967; Okabayashi & Ide, 1970*a, b*; Aboud & Burger, 1971; Nielsen *et al.*, 1973; Alper & Ames, 1975; Lee, 1978; Gulletta *et al.*, 1979; Rivera & Botsford, 1981; Calcott & Calvert, 1981; Makemson & Hastings, 1982; Botsford, 1984; Gerber *et al.*, 1987). Previously described exceptions to the general pattern of a cytoplasmic location and low activity are the presence of both a soluble and a membrane-associated cAMP phosphodi-

esterase in *Bradyrhizobium japonicum* (Catanese *et al.*, 1989) and a high cAMP phosphodiesterase activity in *S. marcescens* (2.0 μmol phosphate released (mg protein) $^{-1}$ min $^{-1}$ in crude cell extracts; Winkler *et al.*, 1975; Table 3). Bengis-Garber (1985) presented preliminary evidence consistent with a periplasmic location for cAMP phosphodiesterase in *V. fischeri* and *P. phosphoreum*. Our results (Tables 2–4) confirm and extend her observations. Furthermore, the substrate specificity of the *V. fischeri* enzyme (Table 5) clearly distinguishes it from periplasmic ribonucleoside 2':3'-cAMP phosphodiesterases produced by many bacteria (Anraku, 1964*a, b*; Neu, 1968; Beacham *et al.*, 1973, 1977; Beacham & Garrett, 1980; Uerkvitz & Beck, 1981; Anderson *et al.*, 1985). *V. fischeri* could produce a cytoplasmic cAMP phosphodiesterase similar in activity to that of other bacteria; this issue was not addressed in the present study.

The periplasmic location and high level of activity of cAMP phosphodiesterase in *V. fischeri* indicate that this species could influence cAMP levels in the marine environment. Prokaryotic and eukaryotic micro-organisms are known to release cAMP, sometimes in substantial amounts (Ammerman & Azam, 1981; Matin & Matin, 1982; Francko, 1989), but levels of this nucleotide in seawater remain very low (1–35 pM, depending on distance from shore and depth; Ammerman & Azam, 1981). Rapid degradation of free cAMP in seawater by *V. fischeri* (and by other bacteria that can grow on cAMP; e. g., *P. phosphoreum* and *V. logei*, Table 1) might account for these low levels and for the rapid disappearance of cAMP added to seawater samples (Ammerman & Azam, 1981). We do not know how widespread the ability to consume extracellular cAMP is. However, we recently isolated from coastal and open ocean waters a few strains of luminous and non-luminous bacteria that can grow on cAMP as a sole source of carbon and energy (P. V. Dunlap, unpublished data).

Besides its role in the use of cAMP as a growth substrate, the periplasmic cAMP phosphodiesterase in *V. fischeri* might have other functions, such as protection against extracellular cAMP. This would contrast with a role in modulating the cellular level of cAMP, as described for other bacteria (Monard *et al.*, 1969; Buettner *et al.*, 1973; Alper & Ames, 1975; Botsford, 1981, 1984). Although the bacterial cytoplasmic membrane is relatively impermeable to nucleotides, extracellular cAMP present at mM levels can leak into the cell in amounts sufficient to complement mutations in adenylate cyclase (e. g. Pastan & Adhya, 1976; Botsford, 1981); it can alter normal gene expression and can exert growth-inhibitory and lethal effects in various Gram-negative bacteria (DeRobertis *et al.*, 1973; Judewicz *et al.*, 1974; Ackerman *et al.*, 1974; Alper & Ames, 1975; Iuchi *et al.*, 1975). Data consistent with a protective role for cAMP

phosphodiesterase were obtained for *S. typhimurium* (Botsford, 1984). These considerations, however, leave open the question of what environments would present *V. fischeri* with cAMP at levels sufficient for growth or at levels necessitating protection from the cAMP.

The light-organ symbiosis of *V. fischeri* with monocentrid (pinecone) fish is intriguing in this regard. *V. fischeri* cells are maintained in the fish light organs extracellularly at a high density (10^9 – 10^{10} cells ml $^{-1}$) within tubules composed of cuboidal epithelial cells (Ruby & Nealson, 1976; Tebo *et al.*, 1979). Little is presently known about the biochemical interactions between *V. fischeri* and its fish host (Nealson, 1979; Dunlap & Greenberg, 1991). Okada (1926), however, noted that the epithelial cells forming the light organ tubules lyse and are continually replaced by growth of underlying epithelial cells. The eukaryotic cell lysate might therefore be a source of nutrients for *V. fischeri* within the tubules. Furthermore, this cell lysate might contain a high level of cAMP; certain Gram-negative bacteria that associate with animals (*V. cholerae*, *E. coli* and *Bordetella pertussis*) release toxins that stimulate the production of host cAMP by interfering with the regulation of host adenylate cyclase (Middlebrook & Dorland, 1984). Possibly, *V. fischeri* secretes such a toxin or in some other way elicits host overproduction and release of cAMP. If so, cAMP could contribute to the specificity of the *V. fischeri*–monocentrid fish symbiosis, since many other marine luminous bacteria are not able to grow on cAMP (Table 1). Provision of cAMP as a growth substrate (compared to glucose; Nealson, 1979) also would benefit the fish host by stimulating bacterial light production while restricting the growth rate of the bacteria (Fig. 2). Alternatively, cells of the developing fish light organ might release cAMP in response to and as a defence against colonizing bacteria. The periplasmic cAMP phosphodiesterase of *V. fischeri* might permit *V. fischeri* to overcome this defence and selectively establish symbiosis with the fish. These ideas, although speculative, provide a basis for beginning to explore biochemical interactions between *V. fischeri* and monocentrid fish.

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