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Modulation of Adenylate Energy Charge During the Swarmer Cycle of *Hyphomicrobium neptunium*

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Adenylate energy charge was measured in the budding bacterium *Hyphomicrobium neptunium* through the course of the swarmer cycle. The energy charge was modulated, being low in swarm cells (0.64) and in cells initiating bud formation (0.57), an event which coincides with a round of DNA replication.

Morphogenesis of the budding bacteria, particularly of *Hyphomicrobium neptunium* (31), *Hyphomicrobium* sp. strain B-522 (26), and *Rhodomicrobium vannielii* (33) is well documented. All share elements of biphasic life cycles (12). In the swarmer cycle segment, small spherical to pear-shaped cells termed swarm cells remain motile for an extended period, followed by outgrowths of prosthecae. The prosthecae elongate until buds emerge from the distal tips. The buds eventually mature, produce flagella, and separate to complete the cycle (23).

Of all the features of this relatively complex life cycle, the properties of swarm cells, generally characterized as being motile and unable to reproduce, are quite intriguing. It has been reported that although swarm cells are motile, they are relatively inactive in DNA replication, transcription, and translation (8, 31). This has led to the suggestion that they maintain a depressed metabolic state which enables them to delay development in suboptimal conditions so as to enhance survival in inadequate environments (8).

Adenylate energy charge (EC_A), defined as EC_A = (ATP + 0.5 ADP)/(ATP + ADP + AMP), appears to be a useful parameter when the metabolic state of the cell is being probed (2, 21), although agreement as to the significance of EC_A or its validity as an indicator of physiological well-being (21) is not unanimous. Growing cells are reported to maintain an EC_A of 0.7 to 0.9 (21), although in some instances it is as low as 0.6 (10, 19, 34). Myxospores are reported to have an EC_A of 0.73 (29), and resting cells such as *Bacillus* endospores have an EC_A of 0.1 (28) to 0.3 (16).

EC_A parameters are reported to fluctuate in response to substrate availability (4, 10, 24, 30, 34) and growth phase (25, 27) and during morphological transition (16, 29). *Coxiella burnetii* regulates EC_A possibly to conserve metabolic energy so as to enhance survival in harsh environments (11).

EC_A has rarely been examined in time course studies of synchronous cultures (9, 16, 17, 29). For those systems in which it has been studied, little, if any, fluctuation of energy charge is detected in *Myxococcus xanthus* (29), but it is detected in *Bacillus subtilis* (16) and in eucaryotic trypanosomatids (9).

We probed EC_A in synchronous cultures of *H. neptunium* to determine whether this parameter of metaboism reflects observations of low anabolic activity of swarm cells. We present evidence that it does and that EC_A is modulated during the swarmer cycle of this budding bacterium.

H. neptunium has been placed in the genus *Hyphomonas* (14) because it produces a single nonfilamentous prostheca (23) and metablizes exogenous amino acids (13). In these experiments, however, it was cultivated on semisynthetic estuarine agar (32). Based on our observations that swarm cells were developmentally retarded 10-fold more than other *H. neptunium* cell types at 4°C, synchronous populations were initiated by cold enrichment and size sorting procedures (31).

Nucleotides were most reliably extracted from cells in synchronous culture in Tris-hydrochloride buffer (0.02 M, pH 7.75) at 100°C for 5 min followed by rapid cooling at 0°C (15). Internal standards of ATP, ADP, and AMP (Sigma Chemical Co., St. Louis, Mo.) were included in extraction mixtures to enable correction for experimental substrate degradation.

Adenine nucleotide pools were analyzed by the firefly bioluminescence assay (integrating photometer, model 3000; SAI Technology Co., San Diego, Calif.) and high-grade luciferin-luciferase reagents (E. I. Du Pont de Nemours & Co., Inc., Wilmington Del.). The methodology

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TABLE 1. ATP content of *H. neptunium* in synchronous culture

Time of swarm cell cycle (T_0 + x min)	Developmental process and morphological transition[a]	Avg vol × 10^8 $\mu m^3/ml$[b]	pmol of ATP × 10^8 per cell[c]	pmol of ATP × 10^8 per vol[d]
0	Swarm maturation	2.9	1.0	4.5
15		2.8	1.1	5.1
30		3.2	1.1	4.7
45		3.3	1.4	5.8
60		5.0	1.4	5.1
75		5.6	1.4	4.3
90	Hyphal outgrowth	5.9	2.1	6.4
98	Hyphal growth	ND[e]	2.0	ND
105		6.2	1.6	4.5
113		ND	1.7	ND
120		6.9	2.1	5.4
128		ND	1.4	ND
135		7.2	1.8	4.3
143		ND	1.6	ND
150		7.7	1.1	2.5
158		ND	2.2	ND
165		7.9	1.0	2.3
173		ND	1.3	ND
180	Bud formation	8.3	1.5	3.2
188	Bud maturation	ND	1.5	ND
195		8.6	1.8	3.8
210		8.7	2.1	4.3
225		8.7	2.2	4.5
240		10.7	1.9	3.7
255		9.6	1.6	3.5
270	Bud detachment	11.0	1.4	3.5

[a] In agreement with Wali (31).

[b] Calculated with the following average volumes: swarm cells (*S*), 0.19 μm^3/cell; hyphal cells (*H*), 0.55 μm^3/cell; budding cells (*B*), 0.68 μm^3/cell. Diversity indices (DI) have been described previously (31). Viable cells per milliliter (*C*) were equated with CFU. Average volume = $C[(S_{vol} \times S_{DI}) + (H_{vol} \times H_{DI}) + (B_{vol} \times B_{DI})]$.

[c] Picomoles of ATP from standard curve divided by viable cells per milliliter.

[d] Picomoles of ATP from standard curve divided by average volume.

[e] ND, No data.

used has been described previously (19). To establish ATP standard curves, ATP (Sigma Chemical Co.) was rehydrated in Tris-hydrochloride (0.02 M, pH 7.75; Sigma Chemical Co.) buffer and diluted to appropriate concentrations, including 1.0, 1.5, 2.5, 5.0, and 10.0 pmol. AMP and ADP were converted to ATP by using the myokinase and pyruvate kinase systems of Kimmich et al. (20). The ATP contents of the standards were determined with hexokinase (Sigma Chemical Co.) and glucose 6-phosphate dehydrogenase (22). ADP and AMP standards were quantitated with pyruvate kinase, myokinase, and lactic dehydrogenase (Sigma Chemical Co.) by the method of Adam (1).

We found that on a per cell basis, *H. neptunium*, which is relatively small, contained about 10% of the ATP reported for other bacteria, including *Escherichia coli* (6) and *Arthrobacter crystallopoietes* (24), a finding obtained in batch culture (data not shown) and generally throughout synchronous culture (Table 1). When considered on a cell volume basis, however, the ATP pool size of *H. neptunium* was commensurate with that reported for other bacteria (18). Fluctuations in the ATP pool size during synchrony were not considered to be significant, except possibly for the decrease during the end of hyphal growth.

Logarithmically growing batch cultures of *H. neptunium* maintained EC_A values of 0.72 to 0.76. In synchronous culture, the EC_A fluctuated from ca. 0.60 in swarm cells and those about to produce buds up to 0.76 during other times (Fig. 1). The first value is considered more than sufficient for survival, whereas the second is characteristic of growing cells (5, 34).

More consistent and interesting than the absolute values, which can vary with extraction technique and sample type (18), were the relative values which were reproducible in four distinct experiments and in duplicate samples of each (typical variation $_{N-1} = 0.003$). There were two definitive modulations of EC_A. The first was

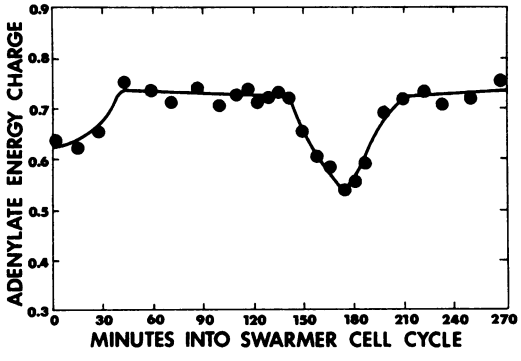

FIG. 1. EC_A in *H. neptunium* during synchronous growth in the swarmer cell cycle. Data are from a single representative experiment in which synchronous growth was initiated and maintained as described in the text. Cells were characterized morphologically (Table 1) by direct count and by CFU. Based on a 265-min swarmer cycle and the occurrence of bud detachment over a 40-min interval, the division index of synchrony was 85%. Samples for EC_A were taken at 15- to 30-min intervals and frozen (−70°C) until they were analyzed (see text).

observed in swarm cells as exemplified by the data from the experiment shown in Fig. 1 and Table 1. To determine whether the manipulations required to obtain synchronously growing populations also had an appreciable effect on EC_A, cold *H. neptunium* cells were size-sorted with the result that the pooled fractions had EC_A values (0.73) after 15 min in broth commensurate with unmanipulated batch populations. Thus, reduced EC_A was apparently characteristic of the *H. neptunium* swarm cell populations.

A pronounced decline in EC_A was recorded from 140 to 170 min into the swarmer cell cycle just before the initiation of bud formation (Fig. 1). The interval of depressed EC_A was also coincident with the first round of DNA replication in the swarmer cycle (5, 31).

In *E. coli*, dramatic fluctuations in nucleotide triphosphates over the cell division cycle have been reported (17). However, because these experiments were with cells with generation times such that the ends of the rounds of DNA replications and the initiation of DNA replication occurred simultaneously, it was postulated that no causal relationship between the initiation of DNA replication and fluctuation of nucleotide pools would be detected, and it was suggested that the experiments be repeated in cells with a temporal chromosome replication phase (S phase). In fact, in eucaryotes undergoing mitosis, ATP was depleted and the EC_A declined (7, 9), attributable to de novo synthesis of the ring systems of purines and pyrimidines, which may require a significant input of ATP with net accumulations of ADP and AMP. When RNA synthesis was specifically stimulated in *E. coli*, transient depletions of intracellular ribonucleotide triphosphates occurred (3). Our data suggest that S phase and heightened transcriptional activity may have the same consequences in *H. neptunium*, which transports some nucleotides poorly (B. McCardell, Ph.D. thesis, University of Maryland, College Park, Md., 1979). Alternatively, EC_A may be modulated by or may be regulating other cell cycle events (17).

The low EC_A recorded in swarm cells of *H. neptunium* implies that the marine bacterium may survive not only as a result of its tolerance of a variety of nutritional, thermal, and osmotic conditions, but also as a result of its relatively complex life cycle; a life cycle with one stage that is motile but growth static and another that is multiplicative. In this context, it may be significant that both increases in EC_A, from 0 to 45 min and from 170 to 200 min, correlate with ensuing increases in cell volume.

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