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NOTES

An ATP Transport System in the Intracellular Bacterium, *Bdellovibrio bacteriovorus* 109J

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The intracellularly growing bacterium *Bdellovibrio bacteriovorus* 109J transports intact ATP by a specific, energy-requiring process. ATP transport does not involve either an ADP-ATP or an AMP-ATP exchange mechanism but, instead, has characteristics of an active transport permease. Kinetically distinct systems for ATP transport are expressed by the two developmental stages of the bdellovibrio life cycle.

Bacteria typically hydrolyze extracellular phosphorylated organic compounds (2), transporting the organic portion and taking up the phosphate moiety separately as P_i. Thus, while other bacteria have only limited abilities to transport intact phosphorylated compounds (4, 21, 23), *Bdellovibrio bacteriovorus* 109J is unusual in that it possesses the capacity to accumulate nucleoside monophosphates (NMPs) unaltered (15, 19). It has been suggested that this transport capability is an adaptation to the natural growth environment of these predatory bacteria: the intracellular milieu of other gram-negative bacteria (13). The breakdown of ribosomes and nucleic acids of the prey bacterium yields NMPs, which serve as biosynthetic precursors for growth and proliferation (5). Because transport of NMPs intact requires only a single step rather than two separate steps, and there is no need to expend a high-energy equivalent to resynthesize the phosphate bond prior to biosynthetic utilization, this capability has been linked to the high growth efficiency of bdellovibrios (14, 15).

The intracellular parasites of eucaryotic cells, such as *Rickettsia prowazeki* (1, 24) and *Chlamydia psittaci* (3), as well as the parasitic *Mycoplasma mycoides* (10), also transport phosphorylated forms of nucleosides. While mycoplasmas appear to transport NMPs in a manner analogous to bdellovibrios, rickettsiae and chlamydiae transport only phosphorylated adenylates by a specific, non-energy-requiring exchange mechanism, which is consistent with their use of the host cell as a source of ATP in an energy parasitism (9). Because *B. bacteriovorus* inhabits a metabolically inactive prey cell, it is not believed to be an energy parasite (16). However, because bdellovibrios transport NMPs and live within the confines of another cell, we wished to determine whether they can also transport ATP intact and, if so, by what mechanism and in which stages of their developmental cycle.

To determine whether the ATP molecule is transported intact, cells were incubated with [γ-^{32}P]ATP, and the accumulation of label was measured. Attack-phase *B. bacteriovorus* 109J was suspended to 4×10^{10} cells per ml in HM buffer (19) containing 1 mM glutamate as an energy source. As previously reported (19), incubations were performed under conditions that maintained aerobic metabolism, even at these high cell densities. Where stated, cells were preincubated for 3 min at 30°C with 5 mM NaCN. Transport assays in cell suspension were initiated by the addition of 10 nmol of either [γ-^{32}P]ATP (4,500 Ci/mmol; 9.0 μCi per ml) or [2,8-^{3}H]ATP (30 Ci/mmol; 5 μCi per ml) per ml. At regular intervals 100 μl of cell suspension was passed through a 0.2-μm-pore-sized membrane filter and washed with HM buffer at room temperature (19). The radioactivity retained on the filters was determined by liquid scintillation counting.

In cell suspensions to which γ-labeled ATP was added there was a roughly linear uptake of ^{32}P for 1 or 2 min that was not influenced by the addition of 0.1 mM potassium phosphate to the suspension (Fig. 1a). This indicated that the ^{32}P label was not being transported after hydrolysis to P_i. In addition, the observed rate of [γ-^{32}P]ATP accumulation (148 pmol/min per mg of protein) was almost identical to the rate of accumulation of ATP that was labeled with ^{3}H in the adenine ring (164 pmol/min per mg of protein) (Fig. 1b). These two results are consistent with the conclusion that bdellovibrio cells transport ATP as an intact molecule. The accumulation was a temperature-dependent process and was sensitive to respiratory inhibitors such as cyanide (Fig. 1a), azide, and the proton gradient uncoupler carbonyl cyanide *m*-chlorophenyl hydrazone (data not shown).

To demonstrate that a compound is accumulated by active transport, it must be shown both that a radioactive label is concentrated intracellularly and that the label is in a chemically unaltered form. Over 94% of the total radioactivity taken up by bdellovibrios exposed to [^{3}H]ATP for 1 min was present in the trichloroacetic acid-soluble fraction of the cell contents. Chromatography of this fraction by reverse-phase high-performance liquid chromatography (19) revealed that essentially all of the label comigrated with authentic ATP (Fig. 2). By using an internal volume of 1 μl/10^{10} cells (19), it is possible to calculate that the internal concentration of labeled ATP after 1 min of accumulation was 5.5 times the concentration of ATP in the suspending medium. Thus bdellovibrios have the capacity to internally concentrate intact ATP against a gradient.

During the course of their unique developmental life cycle bdellovibrios exist in two differentiated forms: an intracellular growth-phase cell and an extracellular attack-phase cell

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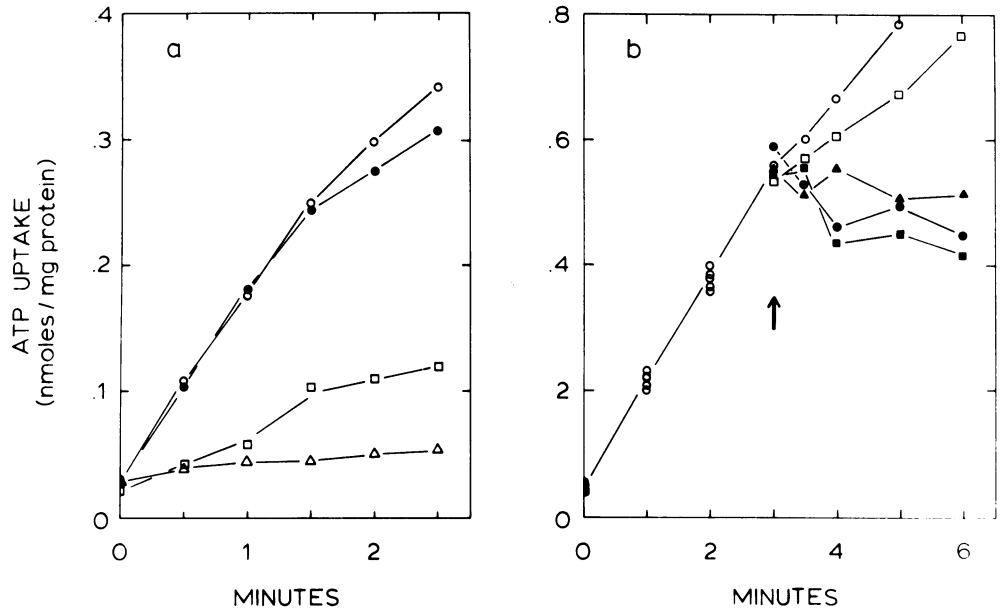

FIG. 1. Uptake of ATP by *B. bacteriovorus*. (a) Uptake of 10 μM [γ-^{32}P]ATP by cells suspended in HM buffer was determined at 30°C in the absence (●) or presence of either 0.1 mM potassium phosphate (○) or 5 mM NaCN (□). Uptake by a cell suspension held at 0°C (△) was negligible. (b) Uptake of 10 μM [2,8-^{3}H]ATP by cells suspended in HM buffer was determined at 30°C (○). Three minutes after the addition of label (arrow) a 40-fold molar excess of unlabeled ATP (●), ADP (■), AMP (▲), or adenosine (□) was added to the suspension.

(13). Both developmental forms have the capacity to accumulate ATP from the external environment, but by kinetically distinct, saturable processes. Measurement of transport rates as a function of external ATP concentration revealed that ATP transport by attact-phase cells had an apparent substrate affinity constant (K_t) of 51 μM and a V_{max} of 1.0 nmol/min per mg of protein. However, results of studies of the kinetics of ATP transport by isolated growth-

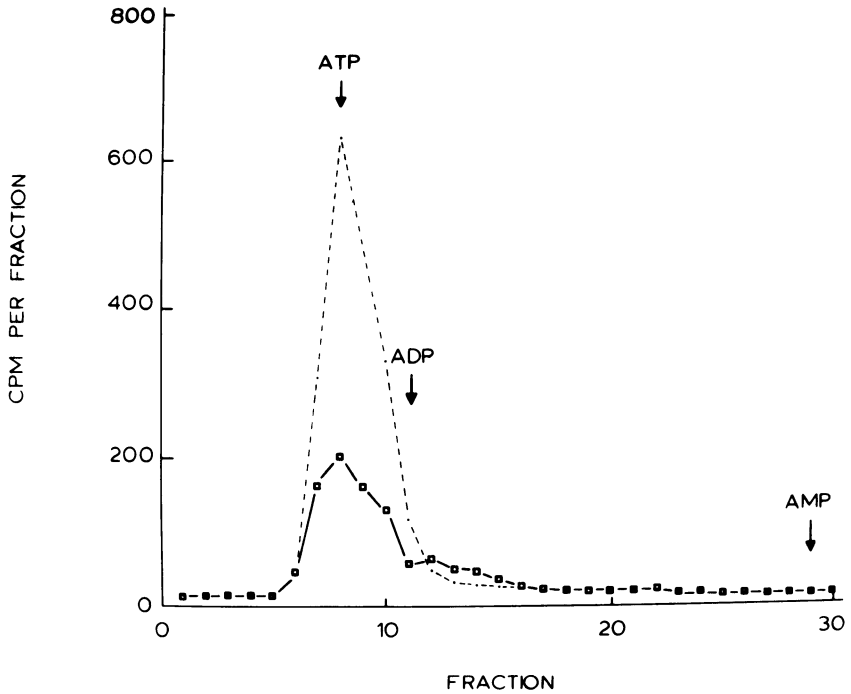

FIG. 2. Identity of soluble, intracellular radioactivity after exposure of bdellovibrio cells to extracellular, labeled ATP. One minute after the addition of [2,8-^{3}H]ATP to a suspension of bdellovibrios in HM buffer, the cells were collected on a filter and washed; and the intracellularly accumulated, trichloroacetic acid-soluble label was extracted. This material was applied to a reverse-phase high-performance liquid chromatography column (19), and the radioactivity of the eluent fractions was determined (●). The elution profile of [2,8-^{3}H]ATP (□) and the relative elution positions of adenylate standards (arrows) are indicated.

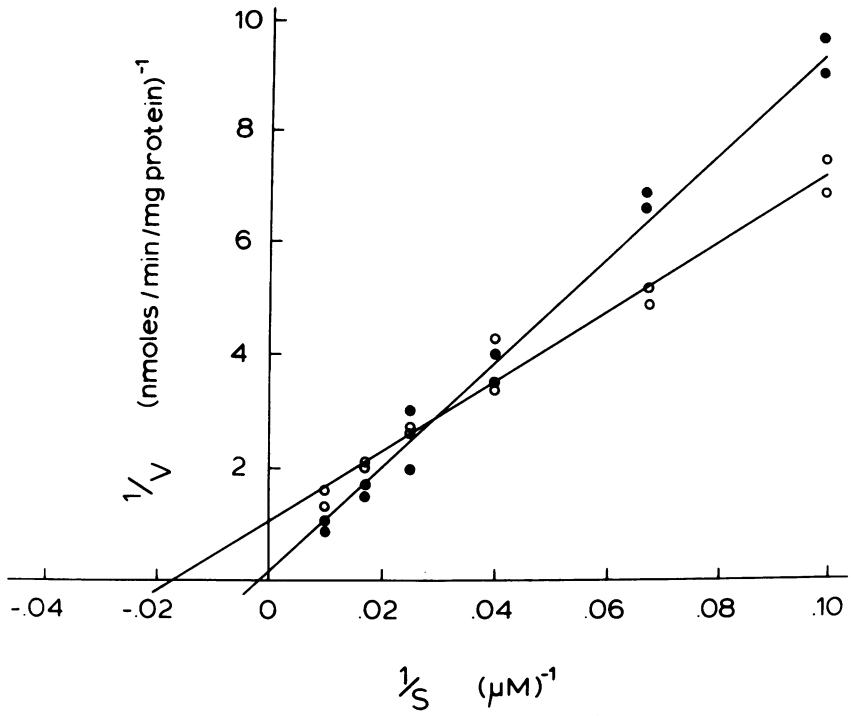

FIG. 3. Lineweaver-Burk transformations of concentration-dependent ATP transport velocities. Rates of accumulation of [2,8-^{3}H]ATP at substrate concentrations (S) between 10 and 100 μM were determined for attack-phase (○) and growth-phase (●) bdellovibrios suspended in HM buffer at 30°C. K_t and V_{max} values were calculated from linear regression of the data points.

phase cells (prepared as described previously [20]) indicated the presence of a different uptake system with an apparent K_t of 1,800 μM and a V_{max} of 19 nmol/min per mg of protein (Fig. 3). Thus the intracellular stage of bdellovibrios expresses a considerably greater capacity for ATP transport. This may reflect (i) the higher ambient concentration of ATP encountered by the intracellular form, (ii) the need for phosphorylated nucleosides as biosynthetic as well as respiratory substrates during the growth phase of bdellovibrio (5, 6), or both (i) and (ii).

Competition experiments with a 10-fold addition of related compounds revealed that transport is relatively specific for nucleoside triphosphates (Table 1). ADP was considerably less effective than ATP as a competitor, while AMP and adenosine had little inhibition on ATP transport. Note that the nonhydrolyzable ATP analog β,γ-imido-ATP was as effective a competitor for [γ-^{32}P]ATP uptake as authentic unlabeled ATP. This further substantiated the conclusion that ATP is not hydrolyzed before transport. AMP (and to a lesser extent adenosine) exhibited a more complete inhibition of ATP uptake when the ratio of competitor to labeled ATP was increased to 175:1.

While it is not yet possible to describe the mechanism by which bdellovibrio cells transport ATP, the process is fundamentally different from the only other mechanism reported in bacterial cells or mitochondria (7): an exchange of a molecule of ATP for a molecule of either ADP or AMP. This exchange process has three characteristics: (i) a specificity for adenylates, (ii) a free exchangeability across the cell membrane, and (iii) an independence from the availability of metabolic energy.

In contrast to the specificity of rickettsiae and chlamydiae for phosphates of adenosine, but not guanosine or the pyrimidines (1, 3, 25), bdellovibrios transport the pyrimidine triphosphates dTTP and UTP at rates less than, but comparable to, ATP transport (70 and 47 pmol/min per mg of protein, respectively, at a substrate concentration of 10 μM). Thus bdellovibrios appear to be prepared to utilize the variety of nucleoside triphosphates they may encounter during intercellular predation. Whether there are several transport systems for the different triphosphates or, as suggested by the competition data in Table 1, the ATP transport permease can serve to transport a variety of nucleotides must await more detailed biochemical and genetic studies.

Experiments designed to determine whether internal, labeled ATP exchanged with a 40-fold excess of external

TABLE 1. Competition for uptake of labeled ATP by related compounds[a]

Competitor	Inhibitory effect (% relative to ATP) at the following competitor ratios:	
	10:1[b]	175:1[c]
ATP	100	100
Imido-ATP	107	98 ± 3[d]
GTP	101	102 ± 6
UTP	95	96 ± 4
ADP	55	92 ± 6
AMP	1	103 ± 7
Adenosine	6	31 ± 7
Phosphate	7	2 ± 3

[a] Initial rates of substrate transport by a suspension of attack-phase *B. bacteriovorus* 109J were determined by previously described methods (19).

[b] Uptake of [γ-^{32}P]ATP at a concentration of 10 μM was measured in the presence of competitors at a concentration of 100 μM.

[c] Uptake of [2,8-^{3}H]ATP at a concentration of 5 μM was measured in the presence of competitors at a concentration of 875 μM.

[d] Mean value ± 1 standard deviation.

TABLE 2. Alkaline phosphatase activity and P_i uptake in *E. coli* ML35 and *B. bacteriovorus* 109J

Species	Alkaline phosphatase (U/mg of protein)	External ATP loss (% lost in 15 min)	Phosphate uptake (nmol/min per mg of protein)
E. coli	16	58	48
B. bacteriovorus	0.01	1	1.7

unlabeled ATP or related compounds provided no evidence for such a hypothesis (Fig. 1b). Instead, the internal concentration of label that had accumulated for as little as 3 min was only slightly diminished by such additions. This indicates that the mechanism of ATP transport does not catalyze a significant bidirectional movement of ATP in healthy cells. Coupled with its inhibition by cyanide (Fig. 1a), the differences in specificity, the concentrative ability, and the lack of exchangeability suggest that the bdellovibrios employ a more typical active transport-linked permease(s) (26) for ATP transport, rather than the facilitated exchange processes of rickettsiae and chlamydiae.

To utilize intact the phosphorylated compounds present in their extracellular milieu, bdellovibrios not only must have a transport mechanism but they also must diminish the likelihood of enzymatic dephosphorylation of the substrate by periplasmic alkaline phosphatase (8). We compared the alkaline phosphatase acivity of permeabilized (11) cells of either freshly released *B. bacteriovorus* (20) or log-phase *Escherichia coli* as described by Torriani (22). Not surprisingly, bdellovibrios have, as previously noted (12), greatly reduced levels of alkaline phosphatase activity relative to those of *E. coli* (Table 2), a bacterium that takes up phosphorylated nucleosides in the more typical manner as the dephosphorylated nucleoside and P_i (27). While the prey cell itself may contain a periplasmic alkaline phophatase that would interfere with the availability of unhydrolyzed NMPs to the invading bdellovibrio, Odelson et al. (12) have reported that this enzyme is inactivated in *E. coli* prey before the onset of bdellovibrio growth.

When ATP was added externally to a suspension of metabolically inactivated bdellovibrios, practically no appreciable hydrolysis occurred for several hours. Suspensions of *B. bacteriovorus* (2×10^{10}/ml) or *E. coli* (4.4×10^9/ml) in HM buffer were preincubated for 60 min in 10 mM NaN_3 to deplete the cells of energy for transport of ATP. After preincubation in inhibitor, 100 pmol of ATP per ml was added to the cell suspension at 30°C. At 15-min intervals 1 ml of suspension was removed and centrifuged to remove cells. The concentration of unhydrolyzed ATP remaining in the cell-free supernatant fluid was determined by the firefly luciferase reaction (19). The considerably greater stability of ATP in suspensions of bdellovibrios indicates an absence of alkaline phosphatase activity in unpermeabilized cells as well (Table 2).

Approximately 80% of the phosphorus demand of a growing bacterium is for the biosynthesis of nucleic acids and membrane phospholipids (17). Because bdellovibrios obtain nucleic acid precursors, and perhaps fatty acids as well (15), in the phosphorylated state, they may have little or no need for P_i. The capacity for phosphate accumulation was assayed in cell suspensions of *E. coli* (7×10^9/ml) or *B. bacteriovorus* (4×10^{10}/ml) in 1 mM potassium phosphate buffer (pH 7.4). The assay was initiated by the addition of filtered (18) ^{32}P-labeled P_i (21 Ci/mmol; 2.1 μCi/ml). At 0.5 min intervals fractions of the cell suspension were passed through membrane filters and washed with phosphate buffer at room temperature. Retained radioactivity was determined by liquid scintillation counting. The relatively low phosphate transport capacity of bdellovibrios (Table 2) may indicate an independence from extracellular P_i and supports the suggestion that bdellovibrios may, in fact, be net excreters of organically derived P_i (6).

Taken together, the data in Table 2 indicate that there is a unique phosphate metabolism in the bdellovibrios that is an adaptation to the presence of a high concentration of organic forms of phosphate in the prey cell, their natural growth environment. The ability of bdellovibrios to transport intact ATP, NMPs, and perhaps other phosphate esters present in the prey cell reduces their dependency on both P_i and the means to accumulate it. To what extent these adaptations may have developed in other predatory and parasitic microbes is not yet clear.

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