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Regulatory Role of Phosphate and Other Anions in Transport of ADP and ATP by *Rickettsia prowazekii*

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ADP and ATP were transported in *Rickettsia prowazekii* by an obligate exchange system without prior hydrolysis. The uptake of ATP and ADP by the obligate exchange system in *R. prowazekii* was dependent upon the anionic composition of the medium. The rate of transport of ATP was about three times greater than that of ADP in the absence of anions, and the rates of transport of both were about doubled by a variety of anions. However, phosphate anions were able to stimulate greatly the uptake of ADP so that in the presence of these anions, the uptake of ATP and that of ADP were about equal. Millimolar concentrations of anions were required to elicit the stimulation of ADP and ATP transport. The ADP-dependent efflux of ADP and ATP was also greatly stimulated by phosphate anions. The stimulation of ADP and ATP transport required that the anions be present in the external medium, as preincubation of the rickettsiae with phosphate anions was neither necessary nor sufficient. The competitive inhibition of ATP uptake by ADP required phosphate anions, indicating that phosphate anions increased the affinity of ADP for the transport system. The role of phosphate in the regulation of ATP and ADP exchange and its significance are discussed.

Rickettsia prowazekii, the etiological agent of epidemic typhus, is an obligate, intracellular, parasitic bacterium of eucaryotic cells. These organisms, unlike other intracellular bacteria, grow in the cytoplasm of their host cell unbounded by any parasitophorous vacuole and, hence, have access to a rich and unusual source of metabolites. These rickettsiae do not have the phosphatases required to hydrolyze external adenine nucleotides. Radioactive nucleotides added to a medium containing rickettsiae remain nucleotides, and formation of nucleosides and bases is not observed. *R. prowazekii* was shown to have an obligate exchange transport system that is specific for ADP and ATP (11). This system enables the rickettsiae to exchange either ADP or ATP in the cytoplasm of the host cell with either ADP or ATP in the rickettsial cytoplasm. In the simplest model, one without controls, this exchange would maintain the same ratio of ATP to ADP in both cytoplasmic compartments. The rickettsiae do not have to rely solely on this exchange system to obtain ATP as an energy source. They have a proton ATPase, can generate a proton motive force, and are capable of phosphorylating ADP to ATP (1, 9, 10, 13, 14).

In this study, we show that the anionic composition of the medium cna influence the exchange of adenine nucleotides in *R. prowazekii* and that phosphate anions in the medium are required for a high level of influx of ADP but not ATP. In the absence of inorganic anions, very little ADP and only moderate amounts of ATP can be transported, and in the absence of phosphate and the presence of the other anions tested, the rickettsiae take up high levels of ATP but not ADP.

MATERIALS AND METHODS

Rickettsial growth and preparation. *R. prowazekii*, Madrid E strain, was propagated in 6-day-old, embryonated, antibiotic-free hen eggs by inoculation with a dilution of a seed pool (yolk sac passage no. 280). Rickettsial suspensions were prepared from infected yolk sacs that were harvested 8 days postinoculation, and further purification was done at 4°C in standard sucrose-phosphate-glutamate buffer (2) as previously described (3, 7, 12). Only fresh (unfrozen) material was used in these investigations.

Transport assay. The anion-free buffer used in this study was SHTG, consisting of sucrose (0.218 M), glutamic acid (5 mM), and *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) (10 mM), adjusted to pH 7 with Tris base. The assay was initiated by adding 1 volume of rickettsiae (at a concentration of ca. 3 to 5 mg of protein per ml) in SHTG to 10 volumes of SHTG containing [^{3}H]ATP (6 μM; 1 to 2 μCi/ml) or [^{14}C]ADP (6 μM; 0.25 μCi/ml) or both plus or minus effectors. A double-label experiment could be done without competition for the carrier taking place, as the substrate concentrations were lower than the K_m of the transport system (11). Each assay system contained atractyloside (80 μg/ml) to inhibit any contaminating mitochondria (11). At appropriate times, 0.1-ml portions were filtered onto prewetted membrane filters (HAWP025; Millipore Corp., Bedford, Mass.) which were then washed with 5 ml of SHTG. Filters were then dissolved in 5 ml of Filter-Count solution (Packard Instruments, Inc., Downer's Grove, Ill.) and counted by liquid scintillation. Efflux experiments were carried out by diluting a portion of rickettsial suspension preloaded with radioactive ATP and ADP 2,500-fold into SHTG plus or minus any nonradioactive effectors and filtering 25 ml of this diluted suspension onto prewetted membrane filters as described above. All assays were performed at room temperature.

RESULTS

Role of inorganic anions in the uptake of ADP and ATP. The uptake of ATP and especially ADP by the obligate exchange system in *R. prowazekii* required anions in the medium (Table 1). A buffer solution (SHTG) containing sucrose, glutamic acid, and HEPES and adjusted to pH 7 with Tris was chosen as the standard anion-free medium; various combinations of the components of SHTG, namely, sucrose-glutamic acid, sucrose-Tris, and sucrose-HEPES, supported the same very low level of ADP uptake and moderate levels of ATP uptake as SHTG itself (data not

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TABLE 1. Effect of various anions on the uptake of ATP and ADP

Anion added (cation)[a]	Ratio of uptake in the presence of anions to that in the control of[b]:	
	ADP	ATP
Cl (X)	1.7 ± 0.5 (10) [<0.001]	1.8 ± 0.2 (10) [<0.001]
PO_4 (X)	4.7 ± 1.9 (15) [<0.001]	1.6 ± 0.5 (15) [<0.001]
AsO_4 (Na)	2.6 ± 0.1 (3) [0.02]	1.9 ± 0.4 (3) [0.02]
SO_4 (K)	1.7 ± 1.0 (4) [0.16]	2.6 ± 0.9 (4) [0.08]
MoO_4 (NH_4)	1.1 ± 0.4 (3) [0.75]	0.4 ± 0.3 (3) [0.13]
AsO_2 (Na)	1.1 ± 0.2 (2) [0.65]	1.2 ± 0.2 (2) [0.52]

[a] Salts were added to SHTG at a final concentration of 12.5 mM. X indicates that Na, K, and NH_4 were used without any significant differences.

[b] The mean control uptake for 1 min in 15 experiments was 36 ± 20 pmol/mg of protein for ADP and 106 ± 28 pmol/mg of protein for ATP. Ratios are given as means ± standard deviations. The number of independent experiments shown in parentheses. The *P* value based on the Student *t* test (comparing the control and experimental values) is shown in brackets.

shown). A wide variety of anions were tested for their ability to stimulate ADP and ATP uptake (Table 1). Whereas a variety of anions stimulated the uptake of ATP to approximately the same extent, phosphate and arsenate were markedly more effective than other anions in stimulating ADP uptake. The active transport of lysine was measured as an index of any deleterious effects of these compounds on the metabolic state of the rickettsiae (8). Of the anions tested, only arsenite and molybdate inhibited lysine uptake (data not shown). Figure 1 shows the effect of various concentrations of chloride and phosphate anions on the uptake of ADP and ATP. Millimolar concentrations, rather than trace amounts, of these anions were required for stimulation.

This selectivity for phosphate could also be seen in an efflux experiment (Fig. 2). In this experiment, the rickettsiae were loaded in the presence of phosphate for 15 min with radioactive ADP and ATP and then diluted into a solution containing nonradioactive ADP. During this time period, the intracellular radioactive material remained as adenine nucleotides. Only when phosphate was present did an exchange of the labeled intracellular pool with the extracellular ADP occur. In similar experiments with nonradioactive ATP in the medium, there was a low level of exchange in the absence of inorganic anions which was markedly stimulated by either chloride or phosphate.

Effect of phosphate location and anions on competitive inhibition. To determine whether intracellular or extracellular anions were responsible for the stimulation, rickettsiae were preincubated in SHTG plus phosphate and then diluted 20-fold (which lowers the concentration of phosphate in the medium below its effective level) into SHTG without and with phosphate or chloride (Fig. 3). Despite the preincubation with phosphate, only those rickettsiae diluted into medium containing the appropriate extracellular anion had a high level of uptake of ADP or ATP. The uptake at 1 min was never higher than that at 2 min, as would be expected if the internal anion pool were the determinant.

In the simplest model of a transport system, stimulation of uptake could be caused by an increase in the affinity of the substrate for the carrier, a more rapid translocation of the carrier-substrate complex across the membrane, or an increase in the energy coupling so that the ratio of influx to efflux would increase. The last does not apply to the stimulation of ADP uptake by phosphate because initial rates were measured and because this transport system is an obligate exchange system wherein the total intracellular pool of ADP plus ATP does not change (11).

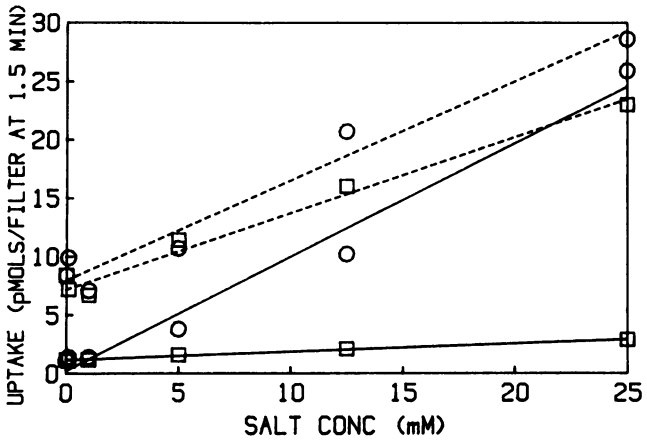

FIG. 1. Effect of chloride and phosphate concentrations (CONC) on the uptake of ADP and ATP. ADP (solid lines) and ATP (broken lines) transport was measured in SHTG buffer plus the indicated concentrations of potassium phosphate (○) or potassium chloride (□). Portions containing ca. 50 μg of rickettsial protein were sampled at 1 and 2 min and averaged to give the 1.5-min rate.

The first two mechanisms can be distinguished by examining the effect of phosphate not on ADP uptake but on ADP as a competitive inhibitor of ATP uptake. If phosphate were to affect the rate of translocation of ADP but not the affinity of ADP for the carrier, then ADP would be as effective an inhibitor of ATP uptake in the absence of phosphate as in its presence. Figure 4 shows that whereas nonradioactive ATP could inhibit the uptake of radioactive ATP equally well in SHTG plus either phosphate or chloride, the competitive inhibition of ATP uptake by ADP required phosphate. Although these data indicate that the stimulation of ADP uptake by phosphate was caused at least in part by a greater affinity of ADP for the transport system in the presence of phosphate, they did not eliminate the possibility that the rate of translocation had increased as well.

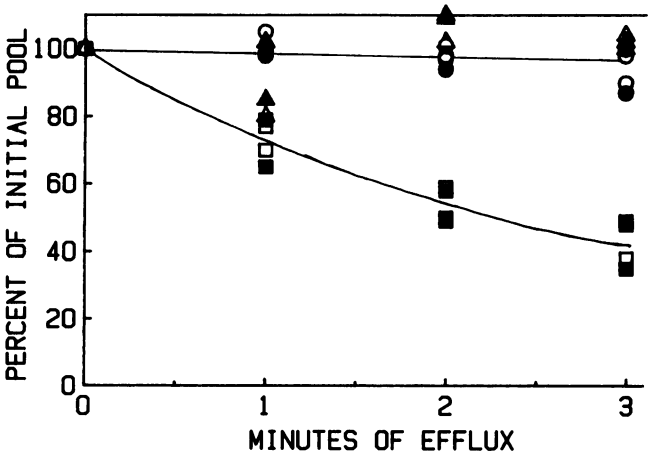

FIG. 2. Effect of anions on the efflux of radiolabeled ATP and ADP in exchange for ADP. Rickettsiae were loaded with [^{3}H]ATP (solid symbols) and [^{14}C]ADP (open symbols) in the presence of phosphate anions and then diluted 2,500-fold into SHGT with 1 mM ADP plus nothing (△ and ▲), potassium chloride (25 mM; ○ and ●), or potassium phosphate (12.5 mM; □ and ■). At the initiation of efflux, the rickettsiae contained ca. 400 pmol of ADP and ATP per mg of protein.

DISCUSSION

Four species of bacteria have now been shown to share with mitochondria the property of being able to transport intact molecules of ATP and ADP. These bacteria are rickettsiae, intracellular parasites that grow in the cytoplasm of their host cell (11), chlamydiae, intracellular parasites that grow in a vacuole within their host cell (5), the archebacterium *Methanobacterium thermoautotrophicum* (4), and *Rhodopseudomonas capsulata*, a photosynthetic free-living organism (6). In the case of rickettsiae, it is clear that these organisms are in an environment in which it would be to the advantage of the parasite to be able to equilibrate the ATP/ADP ratio in its cytoplasm with that of a healthy host cell. However, the host cell is not a constant environment, and the rickettsiae have some ability to regulate their metabolism as their external milieu changes (7). As the metabolic capacity of the host cell is compromised by the growth of the parasite, this exchange of nucleotide would most likely become disadvantageous to the parasite. For example, the rickettsiae, as they generate their own ATP through oxidative phosphorylation, could supply the moribund host cell with ATP in exchange for the ADP of the host cell. In other words, the rickettsiae would be playing the role of mitochondria. However, the ability to phosphorylate ADP to ATP is a definite advantage to the rickettsiae in a host cell that has a low energy charge and during their passage through the extracellular environment between host cells. This presents the rickettsiae with paradox which may be resolved by either sequestration of the ATP produced from oxidative phosphorylation, a difficult feat in a procaryotic organism without organelles, or control of the ATP and ADP transport system such that when it is disadvantageous to the rickettsiae to engage in this exchange, the transport system can be down regulated. In the present study, we have begun an investigation of the regulation of this transport system in purified *R. prowazekii*.

A high level of transport of ATP could take place in the presence of almost any inorganic anion. However, the transport of ADP required the presence of inorganic phosphate in the medium. It must be supposed that any biological environment would have an appreciable concentration of some suitable inorganic anion, so that under all conditions, the transport of ATP would proceed maximally and the rickettsiae would be able to take advantage of a high ATP/ADP ratio in the host cell. However, the absence of inorganic phosphate would mean that the rickettsiae could not phosphorylate ADP even if they could transport it. In fact, in the absence of phosphate anions, the rickettsiae were unable to take up appreciable amounts of ADP. Moreover, in the absence of phosphate anions, ADP was unable to inhibit the transport of ATP even when ATP was at a much lower concentration than ADP. It would seem evolutionarily advantageous to demand the presence of inorganic phosphate to transport ADP into the rickettsiae. It would be to the advantage of the rickettsiae in the absence of inorganic phosphate to essentially ignore ADP and wait for a molecule of ATP to come along. Indeed, the normal inorganic phosphate concentration in the cytosol of the host cell may be low enough to insure that the transport of ATP is greatly favored over that of ADP. The transport and phosphorylation of ADP may only occur when the rickettsiae are in the extracellular milieu or in a damaged cell, where the inorganic phosphate concentration is higher. The variation in the inorganic phosphate concentration in the cytoplasm of an infected cell as an infection progresses is currently under study.

It should be emphasized that this particular control system for the transport of adenine nucleotides fails to solve com-

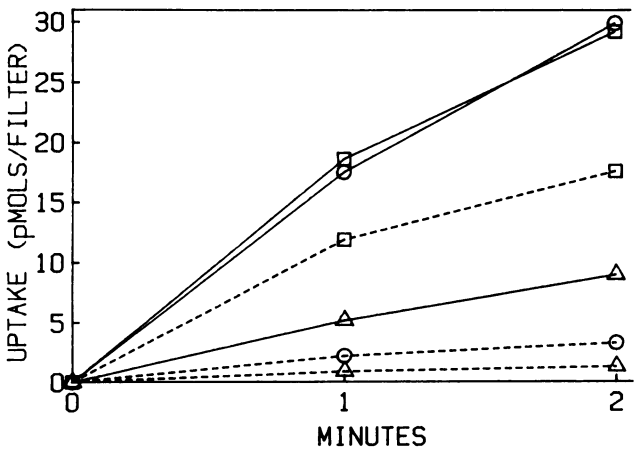

FIG. 3. Effect of preincubation with anions on the uptake of ADP and ATP after dilution. Rickettsiae in SHTG plus potassium phosphate (25 mM) were diluted into SHTG with [^{3}H]ATP (solid lines) and [^{14}C]ADP (broken lines) plus nothing (△), potassium chloride (25 mM; ○), or potassium phosphate (25 mM; □).

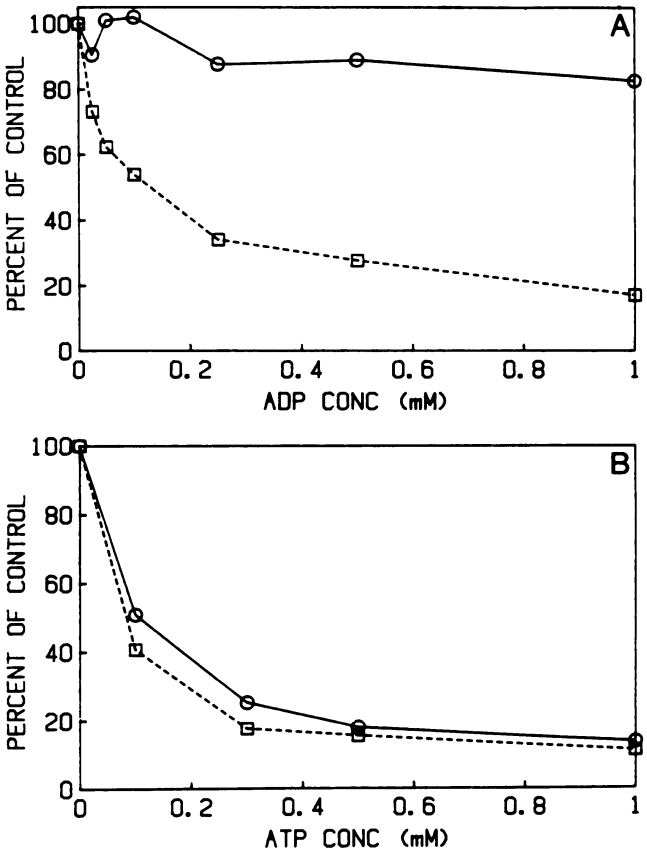

FIG. 4. Effect of phosphate and chloride on the inhibition of ATP uptake by ATP and ADP. The uptake of [^{3}H]ATP was measured at 1 and 2 min in SHTG buffer plus potassium phosphate (25 mM; □) or potassium chloride (25 mM; ○) and the indicated concentrations of ADP (A) or ATP (B). In the absence of unlabeled ADP, the rickettsiae transported ca. 130 pmol of ATP per min per mg of protein.

pletely the problem of the rickettsiae serving as mitochondria for the infected cell. If the concentrations of both ADP and inorganic phosphate were high, ADP would be transported and phosphorylated by the rickettsiae, and the ATP formed would be exchanged with the ADP of the host cell. Perhaps a yet undiscovered control system prevents this from happening.

ACKNOWLEDGMENT

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