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The Nucleotide Transporter of Caedibacter caryophilus Exhibits an Extended Substrate Spectrum Compared to the Analogous ATP/ADP Translocase of Rickettsia prowazekii

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The two obligate intracellular alphaproteobacteria Rickettsia prowazekii and Caedibacter caryophilus, a human pathogen and a paramaecium endosymbiont, respectively, possess transport systems to facilitate ATP uptake from the host cell cytosol. These transport proteins, which have 65% identity at the amino acid level, were heterologously expressed in Escherichia coli, and their properties were compared. The results presented here demonstrate that the caedibacter transporter had a broader substrate than the more selective rickettsial transporter. ATP analogs with modified sugar moieties, dATP and dADP, inhibited the transport of ATP by the caedibacter transporter but not by the rickettsial transporter. Both transporters were specific for di- and trinucleotides with an adenine base in that adenosine tetraphosphate, AMP, UTP, CTP, and GTP were not competitive inhibitors. Furthermore, the antiporter nature of both transport systems was shown by the dependence of the efflux of [α-32P]ATP on the influx of substrate (ATP but not dATP for rickettsiae, ATP or dATP for caedibacter).

The ability to exchange ATP and ADP across a biological membrane as a mechanism of energy transport is a hallmark of obligate intracellular bacterial parasites ranging from human pathogens such as rickettsiae and chlamydiae to the endosymbionts of protists such as Caedibacter caryophilus, a member of the Rickettsiales (6). This transport ability is also inherent in eukaryotic organelles whose ancestors were bacteria, such as plastids and mitochondria (13). The ATP/ADP transport systems of obligate intracellular organisms and plastids are analogous to, but evolutionarily distinct from, that of mitochondria (1). In addition, the mitochondria altruistically supply the host with energy by importing host cell ADP in exchange for mitochondrial ATP, whereas the nonmitochondrial transporters are used to scavenge energy from the host cell. Plasma or cell membrane-located ATP/ADP transport systems have not been found in free-living organisms. The lack of these transport systems seems appropriate, since ATP would not be readily available in the environments of these organisms. However, an explanation for the apparent absence of such a translocase from facultative intracellular bacteria is not as obvious. Perhaps the chance that a malfunction in the obligate exchange nature of these antiporters could provide a pathway for the loss of ATP when the facultative bacteria are extracellular is too great a risk.

The nonmitochondrial ATP/ADP translocases are at present the sole members of a distinct antiporter transport protein family in the classification scheme of Paulsen et al. (8). The first ATP/ADP translocase to be characterized was that of Rickettsia prowazekii, the etiological agent of epidemic typhus (11). Host cell cytosolic ATP that enters the bacteria is exchanged in a one-for-one manner for rickettsial ADP, so that the net effect is the uptake of a high-energy phosphate (11). This transporter (Tlc1) was subsequently cloned and expressed in Escherichia coli (5).

In this study, we extended our characterization of a novel translocase found in C. caryophilus, an endosymbiont of Paramaecium spp. (9), and compared it with the well-characterized ATP/ADP transport system of R. prowazekii. The translocase of C. caryophilus can be examined only by heterologous expression in E. coli because methods to obtain isolated and functional C. caryophilus are not available. To facilitate our comparison of these two translocases, they were both cloned into pET vectors and expressed in the C41 strain of E. coli (a BL21[DE3] derivative) (7). With this approach, the uptake of ATP can be readily measured in a bacterium that is easily cultured in the laboratory and lacks any inherent ability to transport nucleotides. When appropriate, data for transport in native rickettsiae are also presented to confirm observations obtained when rickettsial Tlc1 was expressed and assayed in E. coli. The transport of [α-32P]ATP or [α-32P]dATP was routinely measured by membrane filtration at the Km substrate concentration unless otherwise indicated (10).

The rickettsial and caedibacter ATP/ADP transport systems share 65% identity and 78% similarity at the amino acid level (6). Interestingly, the 65% identity in amino acid sequences calculated for these two translocase homologs is the highest intergeneric identity yet found in the translocase homologs. Both R. prowazekii and C. caryophilus are obligate intracellular alphaproteobacteria with 84% similarity in their 16S rRNA
sequences. Although the genome of *C. caryophilus* has not been sequenced, an examination of the sequence of the *tlc* gene suggests that *C. caryophilus* is a low-G+C organism with *R. prowazekii*-like codon usage (i.e., wobble positions are almost exclusively A or T) (14). Accordingly, the rickettsial and caedibacter translocase nucleotide sequences are 68% identical.

The caedibacter translocase recognizes ATP and dATP as substrates, whereas the rickettsial translocase does not recognize dATP. Both transporters are specific for substrates with adenine as the nucleobase and two or three phosphates. Neither transporter recognizes AMP, CXP, GXP, UXP (where X represents mono-, di-, or triphosphates), dCTP, dTTP, dGTP, or adenosine tetraphosphate (A4P) as a substrate (reference 11 and data not shown) (see Fig. 2). However, the transporter of *C. caryophilus* will transport dATP, whereas the rickettsial transporter is specific for the ribose moiety (Fig. 1). This finding came as a surprise considering the high degree of identity shared between the two translocases. In the case of *C. caryophilus* Tlc, ATP transport is inhibited by both ATP and dATP and the transport of dATP is inhibited by both ATP and dATP (Fig. 1). The *K*ₐₜ values for ATP and dATP transport by the caedibacter system expressed in *E. coli* are 180 μM and 1 mM, respectively. The *K*ₐₜ for rickettsial ATP transport are 75 μM for the native organism and 100 μM when such transport is heterologously expressed in *E. coli* (4, 11). Again, the inability to isolate functional *C. caryophilus* has precluded the measurement of transport activity under native conditions.

The recognition of dATP by the *C. caryophilus* translocase prompted us to test ddATP as a putative competitive inhibitor of ATP uptake on both transporters when present at a concentration of 10 times the *K*ₐₜ concentration. *Rp*, *R. prowazekii* cells were purified from yolk sacs (11) and tested for ATP and dATP uptake as described above (10). Purified rickettsiae were assayed for ATP and dATP uptake by measuring the accumulation of [α³²P]ATP (filled squares) and [α³²P]dATP (open squares) at *K*ₐₜ concentrations by a standard filtration method (10). Unlabeled ATP (filled circles for ATP uptake, open circles for dATP uptake) or unlabeled dATP (filled triangles for ATP, open triangles for dATP uptake) was added as a competitive inhibitor at 10 times the *K*ₐₜ concentration. All data are expressed as percentages of the control values, which were 1.0 and 1.2 nmol mg of protein⁻¹ for ATP and dATP uptake, respectively. *Rp*.

The transporter is restricted to recognizing only the di- and triphosphates and not the mono- or tetraphosphates. In toto, these results indicate that the caedibacter transporter does discriminate based on the number of phosphates present on the molecule but has a more relaxed specificity for recognition of the sugar moiety than the rickettsial transporter. Both the rickettsial and the caedibacter translocases are obligate exchange antiporters. To verify the obligate exchange nature of these translocases, we performed efflux assays in which cells were preincubated for loading with [α³²P]ATP and then diluted 250-fold in buffer with or without substrate. The 1:1 exchange and the identities of the exchanged compounds were not measured but were assumed. Both the rickettsial and the caedibacter transporters showed no efflux of substrate from

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**FIG. 1.** The effects of excess ATP and dATP on ATP and dATP transport by rickettsial and caedibacter translocase. **Cc**, an *E. coli* C41 strain transformed with pET16b-Cc (encoding the caedibacter translocase) was grown in Luria-Bertani media containing ampicillin (100 μg/ml) to an optical density at nm of 0.6, and 1 mM IPTG (isopropyl-β-d-thiogalactopyranoside) was added to induce protein expression. After 1 h of induction, bacterial cells were collected by centrifugation and washed once in an equal volume of 50 mM potassium phosphate (KPi) buffer (pH 7.5). Cells were assayed for ATP and dATP uptake by measuring the accumulation of [α³²P]ATP (filled squares) and [α³²P]dATP (open squares) at *K*ₐₜ concentrations by a standard filtration method (10). Unlabeled ATP (filled circles for ATP uptake, open circles for dATP uptake) or unlabeled dATP (filled triangles for ATP, open triangles for dATP uptake) was added as a competitive inhibitor at 10 times the *K*ₐₜ concentration. All data are expressed as percentages of the control values, which were 1.0 and 1.2 nmol mg of protein⁻¹ for ATP and dATP uptake, respectively. **Rp**, *R. prowazekii* cells were purified from yolk sacs (11) and tested for ATP and dATP uptake as described above (10). Purified rickettsiae were assayed for ATP and dATP uptake by measuring the accumulation of [α³²P]ATP (filled squares) and [α³²P]dATP (open squares) at 100 μM. Other symbols are as described above, and those for the lowest curve have been offset for clarity. All data are expressed as percentages of the control value, which was 7.0 nmol mg of protein⁻¹ for ATP (dATP was not a substrate).
system. Thus, both the caedibacter and the rickettsial translo-
cases are antiprotectors that provide the cell with energy and do
not catalyze the net uptake of adenylates.

Remarks. Rickettsiae neither synthesize de novo nor trans-
port deoxyribonucleotides, but rather transport ribonucleoti-
des (12), and they use ribonucleotide reductase to form the
deoxyribonucleotides (3). Although the nucleotide metabolism
of caedibacter has not been characterized, it is questionable
whether natural selection for the transport of dATP has played
any role in the acquisition of this specificity in C. caryophilus.
An influx of dATP in exchange for ATP would provide the
caedibacter cell with a deoxynucleotide. However, this is an
unlikely function if caedibacters, like rickettsiae, have a func-
tional ribonucleotide reductase. Indeed, the exquisite regu-
lation retained by the ribonucleotide reductase of R. prowazekii
(3), an organism that possess several examples of genes that
have undergone “evolutionary meltdown” (2), reflects the ne-
cessity of having a balanced repertoire of dNTPs for DNA
synthesis. If dNTPs were transported into C. caryophilus in
exchange for ribonucleotides, the ratio of the four dNTPs in
the bacterial cytosol would reflect the properties of the various
transport systems as well as the ratio of dNTPs in its niche.
However, it seems likely that the transport of dATP would always
be inhibited by the higher concentration of ATP.

The alternative explanation is that in comparison to its rick-
ettsial cousin, the caedibacter ATP/ADP transport protein just
evolved to have more relaxed demands for substrate specificity
with a protein-substrate fit at the binding site that allows the
recognition of compounds with modifications of the ribose
moiety. Thus, the transport of dATP is insignificant for the
biology of the caedibacter but represents an exciting avenue by
which to approach an analysis of the molecular basis of sub-
strate specificity.

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