A TRAP Transporter for Pyruvate and Other Monocarboxylate 2-Oxoacids in the Cyanobacterium *Anabaena* sp. Strain PCC 7120

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Received 19 August 2010/Accepted 3 September 2010

In the cyanobacterium *Anabaena* sp. strain PCC 7120, open reading frames (ORFs) alr3026, alr3027, and all3028 encode a tripartite ATP-independent periplasmic transporter (TRAP-T). Wild-type filaments showed significant uptake of [14C]pyruvate, which was impaired in the alr3027 and all3028 mutants and was inhibited by several monocarboxylate 2-oxoacids, identifying this TRAP-T system as a pyruvate/monocarboxylic acid 2-oxoacid transporter.

The tripartite ATP-independent periplasmic transporter (TRAP-T) family of proteins (family 2.A.56 in the transporter classification database [19]) comprises transporters that consist of three components: a small membrane protein usually bearing 4 transmembrane segments (TMSS), a large membrane protein usually bearing 12 TMSSs that is the membrane translocator, and a periplasmic substrate binding protein (10). The TRAP transporters use the energy of an electrochemical ion gradient to drive uphill substrate transport (7, 14). TRAP-T family members are widely present in bacteria and archaea, but only a few substrates, including different types of carboxylates, have been identified for them (20).

Cyanobacteria are a morphologically diverse group of phototrophic bacteria that includes unicellular and multicellular (filamentous) organisms (18). Most cyanobacteria can use autotrophic bacteria that includes unicellular and multicellular organisms (18). Most cyanobacteria can use ammonia or nitrate ions as nitrogen sources, and some can use 4 transmembrane segments (TMSS), a large membrane protein usually bearing 12 TMSSs that is the membrane translocator, and a periplasmic substrate binding protein (10). The TRAP transporters use the energy of an electrochemical ion gradient to drive uphill substrate transport (7, 14). TRAP-T family members are widely present in bacteria and archaea, but only a few substrates, including different types of carboxylates, have been identified for them (20).

In vitro binding analyses with the periplasmic solute binding proteins RRC01191 from *Rhodobacter capsulatus* (20) and TakP from *Rhodobacter sphaeroides* (8) have shown that they bind monocarboxylate 2-oxoacids, including pyruvate. Additionally, pyruvate induces the TRAP-T periplasmic solute binding protein SMb21353 in *Sinorhizobium meliloti* strain 1021 (13). It was of interest, therefore, to determine the substrate(s) for this *Anabaena* transporter, which we approached by mutation and transport analysis.

**Methods.** *Anabaena* sp. strain PCC 7120 was grown in medium BG11 (nitrate-containing medium [18]) or BG11o (BG11 medium lacking NaNO₃) at 30°C in 25 μE · m⁻² · s⁻¹ of light in a shaker. For the mutants, the medium was supplemented with 10 to 40 μg of Nm · ml⁻¹ or 2 to 5 μg of Sp · ml⁻¹ and 2 to 5 μg of Sm · ml⁻¹. For plating, the medium was solidified with 1% separately sterilized agar. Cyanobacterial cell masses were estimated by measuring the concentrations of chlorophyll *a* (Chl) in the cultures (12). *Escherichia coli* strain DH5α was used for plasmid constructions and was grown in LB medium supplemented when appropriate with antibiotics at standard concentrations (1), as were strains HB101 and ED8654, which were used for conjugations with *Anabaena* sp.

To inactivate alr3027, an internal 565-bp fragment was amplified by PCR using primers alr3027-7120-1 and alr3027-7120-2, which contain BamHI restriction sites in their 5’ ends (Table 1), and as a template, strain PCC 7120 genomic DNA was used (2). The amplified fragment was cloned into vector pGEM-T and transferred to BamHI-digested pCSV3 (16), producing pCSR11 (Sm’ Sp’), or to BamHI-digested pRL424 (4), producing pCSR18 (Nm’). To inactivate all3028, an internal 470-bp fragment was amplified by PCR using primers all3028-7120-1 and all3028-7120-2, which contain BamHI restriction sites in their 5’ ends; strain PCC 7120 genomic DNA was used as a template. The amplified fragment was cloned into vector pGEM-T Easy and transferred to BamHI-digested pGEM-T Easy.
TABLE 1. Oligodeoxynucleotide primers used in this worka

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5'→3')</th>
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<tr>
<td>all3028-7120-1</td>
<td>GGA TCC CTG TGG AAT GTG GTC ATA C</td>
</tr>
<tr>
<td>all3028-7120-2</td>
<td>GGA TCC GTT AGC TTC TAC AGT CGC CG</td>
</tr>
<tr>
<td>all3028-7120-3</td>
<td>GGT GCA GAA ACC GTT GCC</td>
</tr>
<tr>
<td>all3028-7120-4</td>
<td>CAT TAA CCC GAT TCC AGG CG</td>
</tr>
<tr>
<td>alr3027-7120-1</td>
<td>GAC ACT GCC TTA TGA ATG G</td>
</tr>
<tr>
<td>alr3027-7120-2</td>
<td>GGA TCC</td>
</tr>
<tr>
<td>alr3027-7120-3</td>
<td>GGT AGT ATC GCA GAC TTG GC</td>
</tr>
<tr>
<td>all3028-7120-1</td>
<td>GAC ACT GCC TTA TGA ATG G</td>
</tr>
<tr>
<td>all3028-7120-2</td>
<td>GGA TCC</td>
</tr>
<tr>
<td>all3028-7120-3</td>
<td>GGT AGT ATC GCA GAC TTG GC</td>
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aIntroduced restriction enzyme cutting sites are in boldface type.

pCSV3, producing pCSR56 (Sm' Sp'). Conjugation of Anabaena sp. strain PCC 7120 with E. coli HB101 carrying pCSR11, pCSR18, or pCSR56 with helper and methylation plasmid pRL623 was effected by the conjugal plasmid pRL443, carried in E. coli ED8654, and was performed as previously prepared (3), with selection for resistance to Sm and Sp or Nm. The genetic structures of selected clones were studied by PCR with DNA from the clones and primer pairs alr3027-7120-3/alr3027-7120-4 or all3028-7120-3/all3028-7120-4 for the alr3027::pCSV3 and all3028::pCSV3 mutants, respectively, or by Southern analysis performed by standard procedures (1), with EcoRV-digested DNA from the wild type and the alr3027::pRL424 mutant and a probe corresponding to the DNA fragment amplified for inactivation of the gene (see above). Anabaena strains homozygous for the mutant chromosom- es were obtained and named CSR7a (alr3027::pCSV3), CSR7b (alr3027::pRL424), and CSR13a (all3028::pCSV3), respectively.

For uptake assays, filaments of the wild-type and mutant strains were grown in BG11 or BG110 medium (supplemented with antibiotics for the mutants), harvested at room temperature, washed, and suspended in 25 mM N-tris(hydroxymethyl)-methylglycine (tricine)-NaOH buffer (pH 8.1) to give a cell density corresponding to 3 to 10 × 10^6 cells ml^-1. After a preincubation at 30°C in 200 mM NaCl, the suspensions of cells were mixed with different concentrations of [1-14C]pyruvate (0.44 to 85 μM), and the uptake rates were studied pyruvate uptake. Linear uptake was observed in the wild type for at least 10 min with different concentrations of [1-14C]pyruvate (0.44 to 85 μM), and the uptake rates were similar for BG11- or BG110-grown filaments. The uptake of [1-14C]pyruvate was scarcely affected in the mutants compared to that of the wild type for substrate concentrations below 15 μM, but it was affected significantly and to a similar extent for the three mutants at substrate concentrations from 30 to 90 μM (Fig. 2). These results suggest that there is a high-affinity mechanism for the uptake of pyruvate that is not inactivated in the mutants. However, the fact that a similar impairment in

Impaired transport of pyruvate. Two strains with mutations in alr3027, strains CSR7a and CSR7b, and one with a mutation in all3028, strain CSR13a, were generated as described above (Fig. 1) and used in transport assays that were performed in the presence of 10 mM Na^+ ions. Because of the homology of the membrane proteins Alr3026 and Alr3027 to proteins of the Synechocystis Gtr system, the uptake of a set of amino acids, including glutamate (16), was tested in BG11-grown filaments of CSR7b and compared to that of the wild type, but no consistent impairment was observed (data not shown). Because the periplasmic solute binding protein, All3028, is annotated in some databases as an element of a mannitol trans- porter, we also tested the uptake of [1-14C]mannitol (88 μM), but we found a very low level of uptake activity in wild-type filaments either grown in BG11 medium or incubated for 24 h in BG110 medium and no indication of impairment in mutant strain CSR7b.

To investigate whether the Anabaena TRAP system could be involved in the transport of monocarboxylate 2-oxoacids, we studied pyruvate uptake. Linear uptake was observed in the wild type for at least 10 min with different concentrations of [1-14C]pyruvate (0.44 to 85 μM), and the uptake rates were similar for BG11- or BG110-grown filaments. The uptake of [1-14C]pyruvate was scarcely affected in the mutants compared to that of the wild type for substrate concentrations below 15 μM, but it was affected significantly and to a similar extent for the three mutants at substrate concentrations from 30 to 90 μM (Fig. 2). These results suggest that there is a high-affinity mechanism for the uptake of pyruvate that is not inactivated in the mutants. However, the fact that a similar impairment in

FIG. 2. Effect of inactivation of ORFs alr3027 and all3028 on pyruvate uptake. Filaments of strains PCC 7120 (closed circles), CSR13a (all3028::pCSV3; triangles), CSR7a (alr3027::pCSV3; squares), and CSR7b (alr3027::pRL424; open circles) grown in BG11 medium were used in 10-min assays of uptake of [1-14C]pyruvate supplied at the indicated concentrations.
shows that different monocarboxylate 2-oxoacids, tested at 500 

2-oxobutyrate 

H11022 

type than in the mutant strains CSR7b and CSR13a. The different 

oxovalerate 

2-oxovalerate (2-OV), 2-oxoisocaproate (2-OIC), or 2-oxo-3-methy-

lylvalerate (2-OMV). The bars represent the percentages of activity in 

the presence versus the absence of competitor. Black bars, strain PCC 

7120; gray bars, strain CSR7b (alr3027::pRL424); white bars, strain CSR13a (all3028::pCSV3).

Inhibition by monocarboxylate 2-oxoacids. To investigate whether the TRAP transporter could recognize other monocarboxylate 2-oxoacids, the effects of several monocarboxylate 2-oxoacids on the uptake of [1-14C]pyruvate were tested. Figure 3 shows that different monocarboxylate 2-oxoacids, tested at 500 

mM, inhibited the uptake of pyruvate (50 

mM) more in the wild 

type than in the mutant strains CSR7b and CSR13a. The different 

monocarboxylate 2-oxoacids tested showed different degrees of 

inhibition, in the following order (from more to less inhibitory): 

2-oxobutyrate>2-oxovalerate>2-oxoisovalerate>2-oxoisocaproate>2-oxo-3-methylvalerate. These results indicate that the 

system missing in the mutants is sensitive to the tested monocarboxylate 2-oxoacids and, therefore, that the Anabaena TRAP 

transporter recognizes monocarboxylate 2-oxoacids other than pyruvate.

Concluding remarks. The Anabaena sp. strain PCC 7120 

alr3026-alr3027-all3028 gene cluster encodes a TRAP trans-

porter for pyruvate and possibly more generally for monocarboxylate 2-oxoacids. Although this transporter is annotated in some databases as a mannitol transporter, neither our results nor those of other authors studying homologues from Rhodobacter spp. support such an assignment (8, 20). Whereas the Anabaena TRAP transporter shows a relatively low affinity for pyruvate, the dissociation constants of pyruvate binding to the corresponding Rhodobacter binding proteins are very low, suggesting high affinity (8, 20). It is possible that other steps of the transport process, such as translocation through the mem-

brane, affect the apparent affinity of uptake. Our results also 

show the important role that the solute binding protein has in determining the substrate selectivity of the transporter (20). The Synechocystis and Anabaena TRAP-T membrane proteins are highly similar. However, whereas the Synechocystis system includes a binding protein that, among characterized proteins, is most similar to glutamate binding proteins such as the Campylobacter jejuni PEB1a protein (11) and mediates the uptake of glutamate (17), the Anabaena system includes a binding protein that is most similar to monocarboxylate 2-oxoacid binding proteins such as Rhodobacter sphaeroides TakP (8) and mediates the uptake of pyruvate. In filaments of Anabaena sp. strain PCC 7120 incubated with [1-14C]pyruvate, a wide distribution of labeling among metabolic products was observed (15), which indicates that pyruvate is a usable sub-

strate, thus rationalizing the presence of pyruvate transport-

er(s) in this organism.

This work was supported by grant BFU2008-03811 from the Minis-
terio de Ciencia y Tecnología (Madrid, Spain), cofinanced by FEDER.

REFERENCES


