BIOCHEMISTRY
including biophysical chemistry & molecular biology

Rapid Report

pubs.acs.org/biochemistry

Competition between Different S-Components for the Shared Energy Coupling Factor Module in Energy Coupling Factor Transporters

Maria Majsnerowska,[†,‡] Josy ter Beek,[†,‡,§] Weronika K. Stanek,[†] Ria H. Duurkens,[†] and Dirk J. Slotboom*[,†,‡]

[†]Department of Biochemistry, University of Groningen, Groningen Biomolecular Science and Biotechnology Institute, Nijenborg 4, 9747 AG Groningen, The Netherlands

[‡]University of Groningen, Zernike Institute for Advanced Materials, Nijenborg 4, 9747 AG Groningen, The Netherlands

Supporting Information

ABSTRACT: Energy coupling factor (ECF) transporters take up micronutrients in Bacteria and Archaea. They consist of a membrane-embedded S-component that provides substrate specificity and a three-subunit ECF module that couples ATP hydrolysis to transport. The S-components ThiT (for thiamin) and NiaX (for niacin) from *Lactococcus lactis* form complexes with the same ECF module. Here, we assayed the uptake of thiamin and niacin in *Escherichia coli* cells expressing the transporter genes. We demonstrate that the two different S-components compete for the ECF module, and that competition is more efficient in the presence of the transported substrate. The data suggest that binding and release of the S-components is a step in the transport cycle.

In a series of publications in the late 1970s, Gary Henderson et al. described the folate, thiamin, and biotin transport systems from *Lactobacillus casei*.[1−4] Uptake experiments in *L. casei* cells revealed the existence of specific binding proteins for each of the vitamins. These proteins were small integral membrane proteins with high affinity for their substrates and are now named S-components.[1−4] Henderson et al. also showed that transport of one vitamin was inhibited by the presence of another.[5] To explain this observation, they proposed that individual vitamin binding proteins competed for a common partner that would provide energy for substrate translocation. The shared component was named energy coupling factor (ECF) and was shown to use ATP hydrolysis as an energy source.[6] However, the genes encoding proteins involved in vitamin uptake were not identified at that time.

Recently, the molecular identity of the vitamin transporters was discovered.[7−9] These transporters constitute a new type of ATP binding cassette (ABC) importer, specific for vitamins and trace elements, and were named ECF transporters. They contain two identical or similar ATPases [EcfA and EcfA′, homologues of the classical ABC transporter ATPases or nucleotide binding domains (NBDs)], which associate with the integral membrane protein EcfT to form the ECF module.[7−9] A second, unrelated membrane protein is responsible for substrate binding (the S-component). Bioinformatic analysis of bacterial and archaeal genomes revealed the existence of two groups of ECF transporters. In group I ECF transporters, the genes for S-components and the ECF module are clustered in the same operon, and the proteins form a dedicated complex. For group II transporters, only the genes encoding the ATPases and EcfT are encoded in an operon (often *ecfAA′T*). In this case, there are usually several genes encoding different S-components found elsewhere in the genome. Each S-component can form a complex with the same ECF module as Henderson et al. had proposed.[9] ECF transporters with shared ECF modules are very abundant in Firmicutes.

To study the competition between different S-components for the same ECF module, we expressed the genes encoding the ECF module from *Lactococcus lactis* in *Escherichia coli* cells and coproduced either ThiT (the S-components specific for thiamin), NiaX (the S-component specific for niacin[10]), or both. Figure 1A provides an overview of the constructs used in this study. We chose *E. coli* for expression because the organism lacks endogenous ECF transport systems, and in previous work, we showed that thiamin uptake via the ECF transport system (EcfAA′T-ThiT) can be assayed well in *E. coli*.[11] Here we also show that niacin uptake by ECF-NiaX can be assayed in *E. coli* cells that heterologously express the *ecfAA′T-niaX* genes (Figure 1B). The presence of both NiaX and the ECF module was required for niacin uptake. Cells overexpressing only the genes for the ECF module did not show association of [^{3}H]niacin with the cells. When only NiaX was produced, we observed rapid association of a small amount of [^{3}H]niacin with the cells, indicative of binding rather than transport.

The requirement of both the S-component and the ECF module for vitamin uptake is consistent with what had been shown before for thiamin uptake by EcfAA′T-ThiT.[11] The same dependency has been shown using various assays for several ECF transporters from different organisms.[9,12−16]

It is noteworthy that the rates of uptake of niacin by cells that produced the EcfAA′T-NiaX complex were approximately 100-fold higher than the rates of uptake of thiamin by cells producing EcfAA′T-ThiT (compare ref 11 and Figure 1B). The difference in rates was not caused by differences in expression levels, because it was observed even when we compared niacin transport in cells with low levels of EcfAA′T-NiaX (uninduced expression from a leaky rhamnose promoter) with thiamin transport in cells strongly induced for expression of the genes

Received: June 3, 2015
Revised: July 28, 2015
Published: July 28, 2015

DOI: 10.1021/acs.biochem.5b00609
Biochemistry 2015, 54, 4763−4766

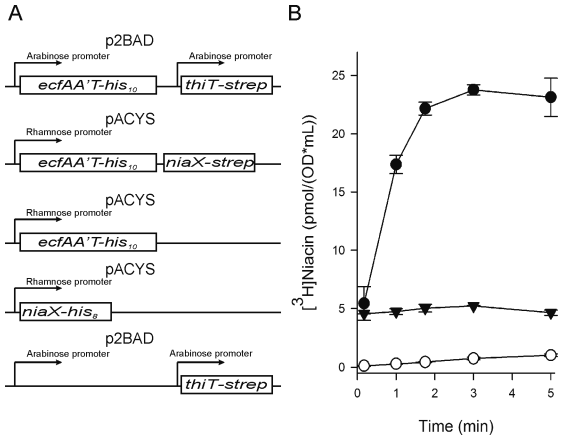

Figure 1. (A) Schematic representation of the expression plasmids used in this study. From top to bottom: (i) the p2BAD vector with the *ecfAA′T* operon under the control of the first arabinose promoter and the gene for thiamin specific S-component *thiT* under the control of the second arabinose promoter, (ii) the pACYS vector with the *ecfAA′T* operon and the *niaX* gene in an engineered operon under the control of the rhamnose promoter, (iii) the pACYS vector with the *ecfAA′T* operon under the control of the rhamnose promoter, (iv) the pACYS vector with the *niaX* gene under the control of the rhamnose promoter, and (v) the p2BAD vector with the first multiple cloning site empty and the gene for the thiamin specific S-component *thiT* under the control of the second arabinose promoter. (B) Uptake of niacin by recombinant *E. coli* cells. Niacin transport was measured in cells producing EcfAA′T-NiaX (●), only EcfAA′T (○), or only NiaX (▼). Error bars indicate the range of values from two measurements.

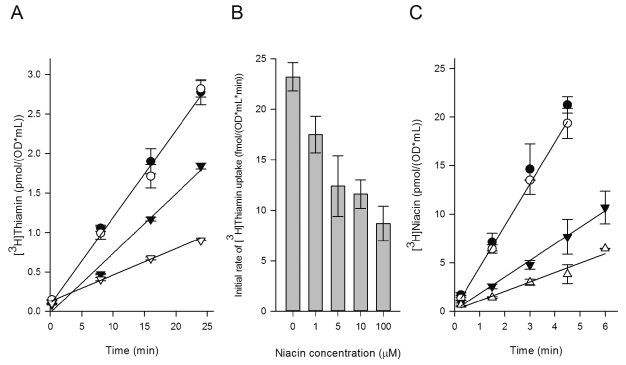

Figure 2. Competition between the S-components NiaX and ThiT for the ECF module. (A) Expression of genes encoding EcfAA′T and ThiT was induced with 10^{-3}% arabinose (*ecfAA′T* operon and *thiT* both downstream of an arabinose promoter), while *niaX* expression (under a rhamnose promoter) was varied: no rhamnose added (● and ○) and 250 μM rhamnose added (▼ and ▽). Black symbols represent data for cells to which no niacin was added, while white symbols represent data for the same cells to which 100 μM niacin was added during the transport assay. (B) Expression of the genes encoding EcfAA′T-ThiT was induced with 10^{-3}% arabinose, and *niaX* expression was induced with 250 μM rhamnose. Bars represent initial rates of uptake of thiamin in the presence of increasing niacin concentrations. Error bars represent the range of two independent measurements. (C) The genes encoding EcfAA′T-NiaX were expressed from the leaky rhamnose promoter, and *thiT* expression was varied: no arabinose added (● and ○) and expression induced with 3×10^{-3}% arabinose (▼ and ▽). Black symbols represent data for cells to which no thiamin was added, while white symbols represent data for the same cells to which 100 μM thiamin was added during the transport assay. In panels A and C, linear fits are shown as solid black lines. Error bars represent the range of two independent measurements.

encoding EcfAA′T-ThiT (from the arabinose promoter). Figure 1 of the Supporting Information shows Western blot analysis of the amounts of protein. The different rates could indicate that the interaction between NiaX and the ECF module differs from the ThiT interaction, leading to higher rates of transport of niacin. However, other factors may also affect the rates, for instance, differences in affinities of ThiT and NiaX for their respective substrates.

To test whether competition between the S-components NiaX and ThiT for the same ECF module could take place, we used *E. coli* cells expressing *ecfAA′T* together with both *thiT* and *niaX*. To detect competition, it was important that the amount of ECF module was limiting. Additionally, the ability to independently vary the S-component levels was necessary because NiaX and ThiT appeared to have different affinities for the ECF module. To modulate the levels of the S-components and the ECF module, we used two different expression vectors (Figure 1A). The p2BAD vector contains two arabinose-inducible promoters (PBAD) and an ampicillin resistance marker.[17] The pACYS-derived vector called pLEMO contains a rhamnose-inducible promoter and a chloramphenicol resistance marker.[18] Protein levels as detected by Western blot analysis are shown in Figure 1 of the Supporting Information.

First, we tested if thiamin uptake by the EcfAA′T-ThiT complex was affected by coproduction of NiaX. [^{3}H]Thiamin transport was measured in the cells in which the genes for the EcfAA′T-ThiT complex were expressed from the arabinose promoter (with 10^{-3}% L-arabinose) and the *niaX* gene was under the control of the rhamnose promoter (Figure 2A). Cells in which *niaX* expression was induced with a high rhamnose concentration of 250 μM showed a rate of uptake of [^{3}H]thiamin that was lower than that of cells in which *niaX* expression was not induced (black triangles compared to black circles in Figure 2A). It is possible that the reduced rate was caused by binding of NiaX to the ECF module. The formation of a complex between the ECF module and a substrate-free S-component is in agreement with the recent crystal structures.[16,19] Alternatively, nonspecific stress caused by the coproduction of multiple membrane proteins could affect the uptake rates. However, the latter explanation is less likely, because the coproduction of an unrelated membrane protein (the aspartate transporter Glt_{Ph}) did not affect thiamin uptake (Figure 2 of the Supporting Information).

Second, we tested whether the presence of niacin (not radiolabeled) affected the uptake of radiolabeled thiamin. Addition of an excess of unlabeled niacin (100 μM) had no effect on uptake of [^{3}H]thiamin by the cells that were not induced for NiaX production (white circles compared to black circles in Figure 2A) but reduced [^{3}H]thiamin uptake activity in cells with coproduced NiaX (white triangles compared to black triangles). These results indicate that substrate-bound NiaX competes more effectively for the ECF module than substrate-free NiaX. The effect of unlabeled niacin on the [^{3}H]thiamin transport was dependent on the concentration of added niacin (Figure 2B). The apparent inhibition constant (K_i) was in the low micromolar range (between 1 and 5 μM).

In a reciprocal experiment, the transport of radiolabeled niacin by the EcfAA′T-NiaX complex in the presence and absence of ThiT and unlabeled thiamin was assayed. Overproduction of ThiT reduced the [^{3}H]niacin uptake rate, which again indicates competition by apo-ThiT. The addition of unlabeled thiamin (100 μM) affected [^{3}H]niacin uptake only

DOI: 10.1021/acs.biochem.5b00609
Biochemistry 2015, 54, 4763−4766

when ThiT was present (Figure 2C). Therefore, the results from the reciprocal experiment also indicate that the substrate-bound S-component competes more effectively for the ECF module than the substrate-free molecule. The effect of unlabeled thiamin on the [^{3}H]niacin transport was dependent on the concentration of added thiamin with the apparent inhibition constant (K_i) between 5 and 20 μM (not shown).

In the reciprocal experiment the competition between NiaX and ThiT for the ECF module could be observed only when the expression of the *thiT* gene from the arabinose promoter was strongly induced (with 10^{-3}% L-arabinose) and the EcfAA′T-NiaX complex was produced at low levels from the uninduced (leaky) rhamnose promoter (Figure 2C). Under these conditions, the ECF module was probably limiting and the ratio between ThiT and NiaX was sufficiently high to detect competition. At lower ThiT:NiaX ratios, competition could not be detected, likely because NiaX competed more efficiently for the ECF module than ThiT.

Our results show that ThiT and NiaX compete more effectively for the ECF module in the presence of their specific substrates than in their absence. However, niacin transport was not affected as much by the presence of thiamin as thiamin transport was affected by the addition of niacin. Similar differences in the effectiveness of competition were observed in the pioneering experiments in *L. casei*, in which transport of folate was more severely inhibited in the presence of the thiamin binding protein and thiamin than the other way around (~45 and ~25% reduction, respectively). Interestingly, biotin transport could be inhibited by both thiamin and folate, whereas neither of them was affected by biotin.[5] These results indicate that the ECF module can exchange one S-component for another and that some S-components might interact more tightly with the ECF module than others. Moreover, the data show that competition occurs in a substrate concentration-dependent manner, which additionally implies that S-components in the substrate-bound state have a higher affinity for the ECF module than in the apo state. Thus, we have reconstructed the observation from the 1970s that competition for the ECF module between two S-components is dependent on the presence of the vitamin substrates.

The data presented here show that the interaction between the ECF module and the S-components is dynamic and suggest that binding and release of the S-components is a step in the transport cycle. This result is consistent with recently published biochemical data.[20] This mechanism allows bacteria to make efficient use of the ECF module under the changing growth conditions. In the natural host cells, S-components are under the control of substrate-induced promoters or riboswitches. They are upregulated in response to substrate deficiency in the cytoplasm. When expression of more than one S-component is induced, they have to compete for a limiting amount of the ECF module. The observed effects on the uptake rates of one vitamin in response to the presence of another vitamin are the result of differences in expression levels of specific S-component genes as well as differences in the affinities of the (substrate-loaded) S-components for the ECF module.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.biochem.5b00609.

Methods and Figures (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: d.j.slotboom@rug.nl. Phone: 0031-50-3634187.

Present Address

§J.t.B.: Department of Biochemistry and Biophysics, The Arrhenius Laboratories for Natural Sciences, Stockholm University, SE-106 91 Stockholm, Sweden.

Author Contributions

M.M., J.t.B., and W.K.S. contributed equally to this work.

Funding

This work was funded by The Netherlands Organization for Scientific Research (NWO; NWO ECHO Grant 711.011.001 and NWO Vici Grant 865.11.001 to D.J.S. and TopTalent grant to J.t.B.) and the European Research Council (ERC; ERC Starting Grant 282083 to D.J.S.).

Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Henderson, G. B., and Zevely, E. M. (1978) Binding and transport of thiamine by Lactobacillus casei. *J. Bacteriol. 133*, 1190−1196.

(2) Henderson, G. B., Zevely, E. M., and Huennekens, F. M. (1976) Folate transport in *Lactobacillus casei*: Solubilization and general properties of the binding protein. *Biochem. Biophys. Res. Commun. 68*, 712−717.

(3) Henderson, G. B., Zevely, E. M., and Huennekens, F. M. (1977) Purification and properties of a membrane-associated, folate-binding protein from *Lactobacillus casei*. *J. Biol. Chem. 252*, 3760−3765.

(4) Henderson, G. B., Zevely, E. M., Kadner, R. J., and Huennekens, F. M. (1977) The folate and thiamine transport proteins of *Lactobacillus casei*. *J. Supramol. Struct. 6*, 239−247.

(5) Henderson, G. B., Zevely, E. M., and Huennekens, F. M. (1979) Mechanism of folate transport in *Lactobacillus casei*: evidence for a component shared with the thiamine and biotin transport systems. *J. Bacteriol. 137*, 1308−1314.

(6) Henderson, G. B., Zevely, E. M., and Huennekens, F. M. (1979) Coupling of energy to folate transport in *Lactobacillus casei*. *J. Bacteriol. 139*, 552−559.

(7) Rodionov, D. A., Hebbeln, P., Gelfand, M. S., and Eitinger, T. (2006) Comparative and functional genomic analysis of prokaryotic nickel and cobalt uptake transporters: evidence for a novel group of ATP-binding cassette transporters. *J. Bacteriol. 188*, 317−327.

(8) Hebbeln, P., Rodionov, D. A., Alfandega, A., and Eitinger, T. (2007) Biotin uptake in prokaryotes by solute transporters with an optional ATP-binding cassette-containing module. *Proc. Natl. Acad. Sci. U. S. A. 104*, 2909−2914.

(9) Rodionov, D. A., Hebbeln, P., Eudes, A., ter Beek, J., Rodionova, I. A., Erkens, G. B., Slotboom, D. J., Gelfand, M. S., Osterman, A. L., Hanson, A. D., and Eitinger, T. (2009) A novel class of modular transporters for vitamins in prokaryotes. *J. Bacteriol. 191*, 42−51.

(10) ter Beek, J., Duurkens, R. H., Erkens, G. B., and Slotboom, D. J. (2011) Quaternary structure and functional unit of energy coupling factor (ECF)-type transporters. *J. Biol. Chem. 286*, 5471−5475.

(11) Erkens, G. B., Berntsson, R. P.-A., Fulyani, F., Majsnerowska, M., Vujičić-Žagar, A., ter Beek, J., Poolman, B., and Slotboom, D. J. (2011) The structural basis of modularity in ECF-type ABC transporters. *Nat. Struct. Mol. Biol. 18*, 755−760.

(12) Neubauer, O., Alfandega, A., Schoknecht, J., Sternberg, U., Pohlmann, A., and Eitinger, T. (2009) Two essential arginine residues in the T components of energy-coupling factor transporters. *J. Bacteriol. 191*, 6482−6488.

DOI: 10.1021/acs.biochem.5b00609
Biochemistry 2015, 54, 4763−4766

(13) Zhang, P., Wang, J., and Shi, Y. (2010) Structure and mechanism of the S component of a bacterial ECF transporter. *Nature 468*, 717−720.
(14) Zhang, M., Bao, Z., Zhao, Q., Guo, H., Xu, K., Wang, C., and Zhang, P. (2014) Structure of a pantothenate transporter and implications for ECF module sharing and energy coupling of group II ECF transporters. *Proc. Natl. Acad. Sci. U. S. A. 111*, 18560−18565.
(15) Karpowich, N. K., and Wang, D.-N. (2013) Assembly and mechanism of a group II ECF transporter. *Proc. Natl. Acad. Sci. U. S. A. 110*, 2534−2539.
(16) Xu, K., Zhang, M., Zhao, Q., Yu, F., Guo, H., Wang, C., He, F., Ding, J., and Zhang, P. (2013) Crystal structure of a folate energy-coupling factor transporter from *Lactobacillus brevis*. *Nature 497*, 268−271.
(17) Birkner, J. P., Poolman, B., and Koçer, A. (2012) Hydrophobic gating of mechanosensitive channel of large conductance evidenced by single-subunit resolution. *Proc. Natl. Acad. Sci. U. S. A. 109*, 12944−12949.
(18) Wagner, S., Klepsch, M. M., Schlegel, S., Appel, A., Draheim, R., Tarry, M., Högbom, M., van Wijk, K. J., Slotboom, D. J., Persson, J. O., and de Gier, J.-W. (2008) Tuning *Escherichia coli* for membrane protein overexpression. *Proc. Natl. Acad. Sci. U. S. A. 105*, 14371−14376.
(19) Wang, T., Fu, G., Pan, X., Wu, J., Gong, X., Wang, J., and Shi, Y. (2013) Structure of a bacterial energy-coupling factor transporter. *Nature 497*, 272−276.
(20) Karpowich, N. K., Song, J. M., Cocco, N., and Wang, D. N. (2015) ATP binding drives substrate capture in an ECF transporter by a release-and-catch mechanism. *Nat. Struct. Mol. Biol. 22*, 565−571.

DOI: 10.1021/acs.biochem.5b00609
Biochemistry 2015, 54, 4763−4766