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Minireview

Maltose transport system of *Escherichia coli*: an ABC-type transporter

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Abstract

The maltose transport system of *E. coli* is composed of a periplasmic maltose-binding protein (MBP), the presumed transmembrane channel made up of MalF and MalG proteins, and two copies of the ATPase subunit, MalK. The membrane-associated transporter complex was purified in a functional form both from the wild-type strain and from mutants that do not require MBP for transport, and was reconstituted into proteoliposomes. A major function of MBP is to send a transmembrane signal, in the presence of ligands, to the ATPase subunits on the inner side of the membrane. In addition, MBP performs a special function in the translocation of the larger ligands, maltodextrins, perhaps by aligning them for entry into the channel.

Key words: Maltodextrin; ATPase; Chemotaxis; Binding protein; Channel; Active transport

1. Introduction

Many membrane transporters in bacteria, yeast, and animals belong to a large family, the ABC (ATP-binding cassette) transporters [1]. An ABC transporter, which is also called 'traffic ATPase', is composed of a membrane-associated complex which typically contains four domains. Two of these are transmembrane proteins, which presumably form a transport channel, and each of which most frequently appears to contain six membrane-spanning α-helices. Each of the other two domains contain a nucleotide-binding site, and these domains show significant homology among different members of the ABC transporter family. The various domains frequently exist as independent subunits, especially in bacteria, but in animal cells they are often parts of a larger, multi-domain protein. An ABC transporter may function either in nutrient uptake or in the export of drugs, peptides, or proteins. This superfamily thus includes not only the transporters functioning in the uptake of small molecules, but also the multi-drug resistance protein (MDR) pumping out anticancer drugs from resistant tumor cells, the presumed chloride channel altered in cystic fibrosis patients (CFTR), and the transporter functioning in the presentation of antigenic peptides on T-cell surface [1]. Bacterial ABC transporters functioning in nutrient uptake characteristically contain an additional hydrophilic subunit, the binding protein, which resides in the periplasm in Gram-negative bacteria.

An extensively studied example of ABC transporters of *Escherichia coli* is the maltose transport system, which accumulates maltose and maltodextrins from the external medium. The influx of these ligands through the outer membrane is facilitated by a specific outer membrane channel, LamB [2,3]. Once maltose and maltodextrins traverse the outer membrane, they are bound by maltose-binding protein (MBP), the product of the *malE* gene [4]. The liganded MBP then interacts with the membrane-associated transporter (Fig. 1) which is made up of one copy each of MalF and MalG, the putative membrane-spanning units, and two copies of MalK, the ATP-binding subunit [5], and then the ligands are transported across the cytoplasmic membrane concomitant with the hydrolysis of ATP [6,7]. This review will describe recent progress in the study of this system. Because of space limitation, some papers could not be cited. Nor can we discuss other ABC transporters, such as the histidine transporter that has been studied extensively [8,9].

2. Maltose-binding protein

The binding proteins must have conferred significant improvement to the uptake systems during evolution. Clearly, the most important benefit must have been to increase the affinity of the transport system. When the affinity of an enzyme to its substrate is increased, the catalytic efficiency usually decreases. The addition of binding proteins to the transport systems, however, allows cells to accumulate substrates with a high affinity and still at a high rate. The maltose system, for example, can transport the substrate with a K_m of around 1 μM [10]. In contrast, proton symporter systems, which do not use binding proteins, show much lower affinity to their substrates, with K_m values in the range of tens-to-hundreds of μM [11].

The contemporary binding proteins may perform

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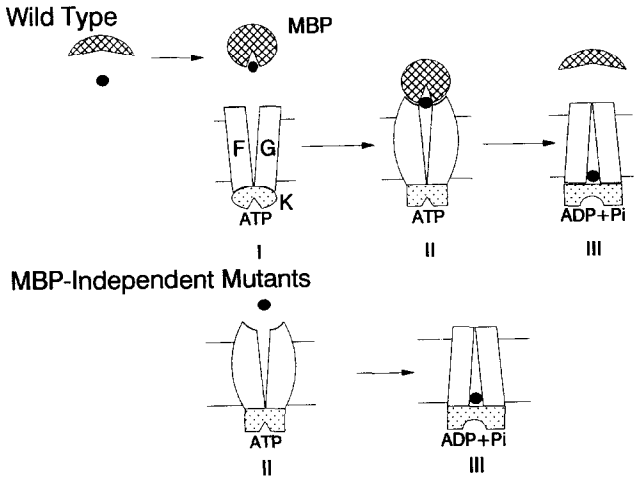

Fig. 1. Interaction between the liganded MBP and the membrane-associated maltose transporter complex. In the wild-type, the ATPase subunits, K subunits (here shown as a monomeric entity for clarity), are in an inactive conformation (I), but the binding of the liganded MBP to the outside produces conformational changes that are transmitted via F and G subunits and activate the ATPase (II and III). In the binding protein-independent mutants, the altered F and G subunits are already in the 'activated' conformation, and therefore the ATPase is also in the active form. ATP is thus constantly hydrolyzed regardless of the presence or absence of liganded MBP. Note also that the external face of the mutant FG channel is expected to have a high affinity for the liganded MBP (shown as a curved surface complementary to the surface of liganded MBP, at lower left). Modified from [22].

other functions as well. One such function of MBP is its signaling function in chemotaxis, performed through its interaction with the sensor protein, Tar. The structure of the MBP, determined by X-ray crystallography [12], shows that the face of MBP that interacts with Tar is quite different from the face that interacts with the membrane-associated transporter complex.

Another contemporary function of MBP is related to the fact that the maltose system transports both the disaccharide maltose and higher oligosaccharides, maltodextrins. When Treptow and Shuman [13] isolated mutants of *E. coli* that transported maltose in the absence of MBP, they were found not to transport maltodextrins. This result suggested to us that longer maltodextrins may have to be presented to the transporter complex by MBP in a correctly aligned position, a requirement which is not necessary for the compact maltose molecule.

In what way are the maltodextrins presented by the MBP? Although MBP binds both maltose and maltodextrins with high affinity, the binding of maltoheptaose produces a blue shift in the fluorescence emission spectrum of MBP, whereas the binding of maltose produces a marked red shift [14]. The molecular basis of this observation was elucidated by the ^{3}H NMR and UV spectroscopic studies of the ligand binding to MBP [15,16]. The ligands bind to MBP in one of two alternative ways. In the 'end-on' binding, the reducing end glucose interacts strongly with the binding pocket. This binding mode shows a strong preference for α-anomers of the ligands. Maltose binds mostly in this manner, and this is apparently the binding mode found in the X-ray crystallographic study of the maltose–MBP complex [12]. In contrast, longer maltodextrins also use 'middle binding'. In this mode, the oligosaccharide molecule relies more on its internal glucose residues for binding and there is no preference for either of the anomeric forms. The contribution of this binding mode explains how the binding of maltose and higher maltodextrins produces different NMR signals, fluorescence emission spectra, and UV absorption spectra.

Our recent study suggests that there are functional differences in the two binding modes. Earlier, Ferenci and co-workers studied the transport of maltodextrin derivatives into *E. coli* [17]. When the C-1 of the reducing terminal glucose residue of maltodextrins had been reduced, oxidized, or substituted, such derivatives could not be transported, although most of them could still bind tightly to MBP. We found, from fluorescence emission spectra and UV absorption spectra, that reduced and oxidized maltodextrin derivatives bind to MBP essentially through the middle binding (J.A. Hall and H. Nikaido, unpublished results). Thus it may be that the middle binding is a mode that does not result in transport, and that the maltodextrins and their derivatives must be bound end-on in order to be delivered properly into the transporter channel.

This hypothesis is also supported by the phenotype of 'maltodextrin-negative' mutants of MBP [18]. These mutants can transport maltose but not longer maltodextrins. Yet, the mutant MBPs show only a marginally lower affinity to maltodextrins. Earlier, this phenotype was explained by assuming that the physical interaction between MBP and the LamB protein was necessary to bring maltodextrins (but not maltose) across the outer membrane, and that the mutant MBP was defective in this interaction. However, the influx rate of *p*-nitrophenylmaltohexaoside was shown to be independent of the presence or absence of MBP [19], and the diffusion of maltodextrins across the outer membrane is unlikely to require the participation of MBP. Alternatively, the mutant MBP may be incapable of binding the ligands in the end-on manner, as suggested by Gehring et al. [16]. Our preliminary results indeed suggest that this hypothesis is correct (J.A. Hall and H. Nikaido, unpublished results). In conclusion, we assume that ligands bind to the wild-type MBP either via the end-on mode or the middle mode. Even free maltose molecules can enter the channel, as shown by the behavior of the binding protein-independent mutants (see below). However, maltodextrins must be presented to the transporter in a correct orientation, and this can apparently occur only with the end-on binding mode. Finally, we emphasize that the 'end-on' and 'middle' nomenclature is purely opera-

tional, and should not be taken literally. X-ray crystallography showed that three glucose residues in the cyclic maltodextrin occupy roughly the same sites occupied by the residues in linear maltotriose [20], and this suggests the differences in these binding modes can actually be quite subtle.

3. The membrane-associated transporter

The folding patterns of MalF and MalG were studied using the alkaline phosphatase fusion method [21,22]. They were found to cross the membrane 8 and 6 times, respectively, a finding consistent with the notion that they form the transmembrane channel. We have been able to purify the intact maltose transporter complex to about 90% purity from over-producing strains [5]. To our knowledge this represents the only reported case of a successful purification of an ABC transporter complex. The transporter is fully active when reconstituted into proteoliposomes, allowing the accumulation of maltose inside the vesicles, driven by hydrolysis of ATP, when the liganded MBP is added from the outside.

MalK contains a characteristic sequence found in ATPases [1]. Indeed ATP binds to MalK in the absence of MalF and MalG (H. Nikaido, unpublished results), and purified MalK is reported to hydrolyze ATP [23]. The rate of hydrolysis, however, is less than one-tenth of the rate observed with the full complex containing MalF and MalG [24].

The maltose transporter complex in wild-type cells absolutely requires the presence of MBP for transport [10]. Treptow and Shuman [13] made a most important contribution by isolating *E. coli* mutants that did not require MBP any longer for the uptake of maltose. These mutants are altered in MalF and MalG subunits and transport maltose with a much lower affinity (with a typical K_m of 1 mM rather than 1 μM in the wild-type cells) actively and specifically. We discovered the reason why MBP was no longer required by studying the mutant transporter complex reconstituted into proteoliposomes. These mutant transporters hydrolyzed ATP constitutively without the addition of maltose or MBP, whereas ATP hydrolysis by the wild-type transporters occurred at the full rate only in the presence of both maltose and MBP [24]. This unexpected finding suggested the following (Fig. 1). It would be wasteful to hydrolyze ATP in the absence of the ligand molecules to be transported. Because the ABC transporters have their ATPase subunits, such as MalK, on the inner side of the membrane (see [25]), the ATPase subunits need to be informed of the presence of ligand molecules on the other side of the membrane so that they can become activated. We believe that this transmembrane signaling function is fulfilled by the liganded MBP. The system uses liganded MBP, rather than free maltose, as the signaling molecule because at a low external maltose concentration practically all of the maltose molecules in the periplasm would exist as a maltose–MBP complex.

ATP is constitutively hydrolyzed by the mutant $MalFGK_2$ complex, whereas the hydrolysis by the wild-type complex requires the binding of liganded MBP [24]. The conformation of the mutant complex, therefore, may already resemble that of the wild-type complex to which the liganded MBP has become bound (Fig. 1). This prediction was borne out by measuring the affinity of liganded MBP to membrane vesicles isolated from the wild-type strain and the mutant strains: the apparent dissociation constant (measured as K_m) with the wild-type vesicles was 20–50 μM, whereas that with the mutant was only 2.7 μM [26]. This extremely high affinity may cause significant 'substrate inhibition' of transport in intact cells, in which the periplasmic MBP concentration may reach 1 mM. This is probably the reason why maltose transport becomes inhibited in cells containing the wild-type MBP and the mutant transport complex [26].

In closing, it must be emphasized that although much has been learned of the maltose transport system of *E. coli*, we know almost nothing about the central processes involved in solute translocation, including the manner by which ATP hydrolysis drives the solute movement. It is hoped that attempts to crystallize the transporter complex, currently in progress in our laboratory, may one day contribute significantly to progress in this field.

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References

[1] Higgins, C.F. (1992) Annu. Rev. Cell Biol. 8, 67–113.
[2] Luckey, M. and Nikaido, H. (1980) Proc. Natl. Acad. Sci. USA 77, 167–171.
[3] Gehring, K., Cheng, C.S., Nikaido, H. and Jap, B.K. (1991) J. Bacteriol. 173, 1873–1878.
[4] Duplay, P., Bedouelle, H., Fowler, A., Zabin, I., Saurin, W. and Hofnung, M. (1984) J. Biol. Chem. 259, 10606–10613.
[5] Davidson, A.L. and Nikaido, H. (1991) J. Biol. Chem. 266, 8946–8951.
[6] Dean, D.A., Davidson, A.L. and Nikaido, H. (1989) Proc. Natl. Acad. Sci. USA 86, 9134–9138.
[7] Davidson, A.L. and Nikaido, H. (1990) J. Biol. Chem. 265, 4254–4260.
[8] Petronilli, V. and Ames, G.F.-L. (1991) J. Biol. Chem. 266, 16293–16296.
[9] Baichwal, V. and Liu, D. and Ames, G.F.-L. (1993) Proc. Natl. Acad. Sci. USA 90, 620–624.
[10] Hengge, R. and Boos, W. (1983) Biochim. Biophys. Acta 737, 443–478.
[11] Henderson, P.J.F. (1990) J. Bioenerg. Biomembr. 22, 525–569.
[12] Spurlino, J.C., Lu, G.-Y. and Quiocho, F.A. (1991) J. Biol. Chem. 266, 5202–5219.

[13] Treptow, N.A. and Shuman, H.A. (1985) J. Bacteriol. 163, 654–660.
[14] Szmelcman, S., Schwartz, M., Silhavy, T.J. and Boos, W. (1976) Eur. J. Biochem. 65, 13–19.
[15] Gehring, K., Williams, P.G., Pelton, J.G., Morimoto, H. and Wemmer, D.E. (1991) Biochemistry 30, 5524–5531.
[16] Gehring, K., Bao, K. and Nikaido, H. (1992) FEBS Lett. 300, 33–38.
[17] Ferenci, T., Muir, M., Lee, K.-S. and Maris, D. (1986) Biochim. Biophys. Acta 860, 44–50.
[18] Wandersman, C., Schwartz, M. and Ferenci, T. (1979) J. Bacteriol. 140, 1–13.
[19] Freundlieb, S., Ehmann, U. and Boos, W. (1988) J. Biol. Chem. 263, 314–320.
[20] Sharff, A.J., Rodseth, L.E. and Quiocho, F.A. (1993) Biochemistry 32, 10553–10559.
[21] Froshauer, S., Green, G.N., Boyd, D., McGovern, K. and Beckwith, J. (1988) J. Mol. Biol. 200, 501–511.
[22] Dassa, E. and Muir, S. (1993) Mol. Microbiol. 7, 29–38.
[23] Walter, C., Honer zu Bentrup, K. and Schneider, E. (1992) J. Biol. Chem. 267, 8863–8869.
[24] Davidson, A.L., Shuman, H.A. and Nikaido, H. (1992) Proc. Natl. Acad. Sci. USA 89, 2360–2364.
[25] Shuman, H.A. and Silhavy, T.J. (1981) J. Biol. Chem. 256, 560–562.
[26] Dean, D.A., Hor, L.I., Shuman, H.A. and Nikaido, H. (1992) Mol. Microbiol. 6, 2033–2040.