The role of the periplasmic maltose-binding protein and the outer-membrane phage λ receptor in maltodextrin transport of Escherichia coli

THOMAS FERENCI, JOHANNES BRASS and WINFRIED BOOS
Department of Biology, University of Konstanz, P.O. Box 5560, D-7750 Konstanz, Federal Republic of Germany

Introduction

The cell envelope of the Gram-negative Escherichia coli consists of two membranes separated from each other by the periplasmic space. The properties of these two membranes are very different. The inner, cytoplasmic membrane establishes the osmotic barrier of the cell and the location of the periplasmic space. The function and properties of these two membranes are very different. The inner, cytoplasmic membrane represents essentially a molecular sieve towards the outside medium. Due to the presence of pore proteins (porins) the outer membrane is largely unspecifically permeable to polar molecules up to a mol.wt. of 600-700 (Nikaido, 1975). The periplasmic space seen in the electron microscope as an electron-dense gap between inner and outer membrane is thought to contain the 'periplasmic' proteins that are released from the cell envelope by the classical cold osmotic-shock procedure of Neu & Heppel (1965). Among the various types of transport systems recognized in E. coli one can be characterized by the essential participation of periplasmic substrate-binding proteins (Silhavy et al., 1978). The most thoroughly studied system of this type is the maltose/maltodextrin transport system in E. coli. It is coded for by five genes that are located in the malB region (Raibaud et al., 1979; Silhavy et al., 1979a).

The products of two of these genes are well characterized proteins. One is the periplasmic maltose-binding protein (product of the malF and malK gene; Kellenberger & Szmelcman, 1974), the other is the receptor for phage λ (product of the lamB gene; Randall-Hazelbauer & Schwartz, 1973) located transmembranally in the outer membrane. The remaining genes are so far only identified as intrinsic membrane proteins located within the cytoplasmic membrane (the malF and malK gene products; Shuman et al., 1980), while the gene products of malG, the last identified gene of the system (Silhavy et al., 1979a), is not known at present.

Even though the complete reaction sequence of active transport through the complex cell envelope catalyzed by these five proteins is not understood, the first part of the sequence has become accessible to experimental approach. This review deals with the recognition of substrate on the cell surface and its permeation through the outer membrane. Responsible for this process is the successful interaction of the λ receptor with the maltose-binding protein.

The maltose-binding protein

It is a typical water-soluble monomeric protein of mol.wt. 40000 and carries a single binding site for maltose and maltodextrins that is in the micromolar range (Schwartz et al., 1976). Binding of substrate results in a conformational change that can be monitored by the alteration of the intrinsic fluorescence of the protein (Szmelcman et al., 1976). Its presence in the cell envelope is obligatory for maltose and maltodextrin transport, since mutants that carry non-polar deletions in the malE gene and synthesize the remaining malB genes constitutively are unable to transport maltose and cannot grow even on high concentrations (greater than millimolar) of maltose as sole source of carbon (H. Shuman, personal communication). The biosynthesis of maltose-binding protein as well as of the λ receptor occurs as precursor with an N-terminal signal peptide on membrane-bound ribosomes (Randall et al., 1978). With these two proteins the technique of gene fusion has been exploited in detail to study membrane protein localization and biosynthesis (Silhavy et al., 1979b).

The λ receptor

The λ receptor is an intrinsic membrane protein with a molecular weight of 47000-55000 per polypeptide chain (Randall-Hazelbauer & Schwartz, 1973; Enderman et al., 1978; Sanderman et al., 1978). It is exclusively found in the outer membrane as a trimeric structure that is non-covalently linked to the peptidoglycan network (Palva & Westerman, 1979). Since it is a phage receptor it has to have access to the external medium and must therefore span the outer membrane. Fully induced cells contain about 100000 copies of λ receptor polypeptides (Braun & Krieger-Brauer, 1977) and 25000 copies of maltose-binding protein (Dietzel et al., 1978). The synthesis of both proteins is linked to the cell cycle of the cell (Ryter et al., 1975; Dietzel et al., 1978).

Kinetics of maltose transport

Using 14C-labelled maltodextrins, the transport affinities for maltose, maltotetraose and maltohexaose in wild-type cells have been determined. They are similar for all substrates (0.8-1.6μM) (Ferenci, 1980) and reflect the affinity of the maltose-binding protein towards these compounds. However, despite the similar transport affinity the maximal rate of transport declines with increasing chain length. Maltodextrins longer than maltotetraose cannot be transported nor can they serve as a source of carbon (Wandersman et al., 1979; Ferenci, 1980). It is, however, an important finding that longer maltodextrins (even amylpectin) are still able to interact with the binding protein in vivo as they are able to inhibit maltose transport with a K, that is comparable with that of the affinity of maltose-binding protein as measured in vitro towards them (about micromolar) (Ferenci, 1980).

Since the maltose-binding protein is not accessible to antibodies or proteinases from the outside, one would have to conclude that the outer membrane of a cell fully induced for the maltose transport system is quite permeable to maltose and maltodextrins, even though some of these compounds exceed the usual size limit of mol.wt. 600-700. Studies with mutants lacking the λ receptor have revealed that this protein is responsible for the high permeability of the outer membrane for maltodextrins (Szmelcman & Hofnung, 1975). Such a mutant can still grow on high concentrations (greater than millimolar) of maltose even though the Km for maltose transport is increased 100-fold (Szmelcman et al., 1976). Despite the presence of unspecified porins maltotetraose (mol.wt. 500) and longer maltodextrins are no longer transported and cannot be utilized as carbon sources (Wandersman et al., 1979). Treatment of these cells with Ca2+ renders the outer membrane permeable for rather large molecules and allows again transport of maltotetraose.

The porin property of the λ receptor

When purified λ receptor is incorporated in black lipid membranes, the electrical conductivity across the membrane is dramatically increased. At very low protein concentrations the conductivity increase can be observed in single steps that are due to pores formed by the λ receptor (Boehler-Kohler et al., 1979). By studying the permeability properties of liposomes in which λ receptor was incorporated other authors also came to the conclusion that λ receptor forms unsppecific pores for sugars other than maltodextrins that have similar size limits as pores (Nakae, 1979). However, maltodextrins that exceed this size limit were also found to diffuse through the λ receptor pore (Tokunaga et al., 1979; Luckey & Nikaido, 1980). This would
The transport of organic substances is well investigated for a number of bacterial species and mammalian tissues, whereas transport systems of plants are rarely described. One reason might be that the necessity for e.g. sugar transport does not seem so obvious for plant cells as for heterotrophic cells such as bacteria, since plant cells are well known to be capable of photosynthetic sugar production from CO₂. But in reality only the light-exposed green cells benefit from photosynthesis and the majority of cells in shoots and roots depend on external supply of organic carbon, mostly as sugar. Special tissues in higher plants are devised only for the purpose of long-distance sugar uptake.

**Glucose uptake by Chlorella vulgaris: the coupling of protonmotive potential difference to glucose transport**

**EWALD KOMOR**

*Institut für Botanik, Fakultät Biologie und Vorklinische Medizin, Universität Regensburg, Universitätsstrasse 31, 8400 Regensburg, Federal Republic of Germany*

The transport of organic substances is well investigated for a series of bacterial species and mammalian tissues, whereas transport systems of plants are rarely described. One reason