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The Mannose Transporter Complex: an Open Door for the Macromolecular Invasion of Bacteria

Bernhard Erni*

Department of Chemistry and Biochemistry, University of Bern, Bern, Switzerland

In this issue of the *Journal of Bacteriology*, Bieler et al. demonstrate (i) that the toxic activity of microcin E492 is independent of the "route of administration," viz. whether it is added from the outside (as in nature) or expressed endogenously in the cytoplasm, and (ii) that microcin E492 requires the transporter for mannose to deploy its ion channel-forming activity. This is the strongest and most direct evidence to date that the mannose transporter is involved in microcin activity in gram-negative enterobacteria.

Bacteriocins, colicins, and microcins are proteins produced by gram-positive and gram-negative bacteria to ward off closely related competitors (5). Compared with the other means of bacterial defense, viz. secondary metabolites with antibiotic properties, hydrolytic enzymes, and exotoxins, the microcins have high specificity and potency. "Communication" through bacteriocins may establish a balance of power between bacterial communities and thus maintain the astounding diversity of microbes in nature (2, 14). Bacteriocins are a heterogeneous family of proteins that vary in size from heptapeptides containing modified amino acids (lantibiotics) to proteins of up to 40 kDa. They inhibit the growth of bacteria by forming ion channels in the cytoplasmic membrane, degrading DNA, blocking protein synthesis, or inhibiting peptidoglycan synthesis (24). They are encoded as precursors in operons or gene clusters together with proteases and modifying enzymes for precursor processing, immunity proteins that protect the producing cell against self-killing by the bacteriocin, and dedicated ABC transporters for bacteriocins that are exported rather than released by lysis of the producer cell.

Bieler et al. demonstrate that at least 11 C-terminal residues of microcin E492 (15) which are essential for penetration across the outer membrane probably are not essential for penetration into the inner membrane, and definitely are not essential for activity from inside. On the other hand, all modifications that favor membrane insertion from the inside increase the toxic efficacy of the cytoplasmically expressed microcin E492, viz. a *cis*-active N-terminal leader/targeting sequence or a *trans*-active mutant secretion system that can secrete proteins without a signal sequence. Bieler et al. further present convincing evidence that microcin E492 can insert directly from the cytoplasm into the inner membrane without the detour of export and reinsertion from the periplasmic face.

The susceptibility of the target cells to bacteriocins is contingent first on receptors in the outer membrane and second on a number of outer membrane, periplasmic, and inner membrane proteins, such as the Ton and Tol pathways (4, 16, 27). These proteins, which normally mediate the uptake of iron chelates and vitamins, are utilized as carriers for the translocation of the bacteriocin to its molecular target. However, there are some inner membrane proteins that not only are "parasitized" transiently as carriers but also act as "cofactors" of bacteriocin action.

Bieler et al. demonstrate that the mannose transporter of the phosphoenolpyruvate:sugar phosphotransferase system (17, 20, 26) is absolutely essential and directly involved in the toxic effect, viz. membrane depolarization by microcin E492. This transporter consists of three subunits: $IIAB^{Man}$ (ManX), IIC^{Man} (ManY), and IID^{Man} (ManZ) (8, 28). $IIAB^{Man}$ (35 kDa) is a two-domain hydrophilic subunit with two phosphorylation sites that are necessary for the transport and phosphorylation of mannose. IIC^{Man} (29 kDa) and IID^{Man} (31 kDa) are membrane-embedded subunits that span the membrane six and three (or five) times, respectively (11, 13) (Fig. 1). IIC and IID are necessary and sufficient for microcin action, and, importantly, no mutations in other genes were found to confer resistance against internally produced microcin.

This is not for the first time that the membrane subunits of the mannose transporter appear to "chaperone" foreign macromolecules in or across the inner membrane: class IIa bacteriocins and bacteriophage lambda DNA also rely on these proteins. Class IIa bacteriocins are produced by gram-positive bacteria (5). They are unrelated to microcin E492 as far as size and sequence are concerned. Resistance of *Listeria* spp. to IIa bacteriocins was correlated with the following phenotypes: (i) absence of $IIAB^{Man}$ in the proteomes of resistant bacteria (however, only the soluble IIAB and not the membrane-inserted IIC and IID can be detected on standard two-dimensional gels) (22), (ii) mutations in the σ^{54} (*rpoN*) factor and the σ^{54}-dependent transcription activator ManR of the *Listeria mpt* operon (homologous to the *Escherichia coli man* operon) (3, 10, 29), (iii) a (polar?) mutation in the promoter proximal *mptA* (IIA) cistron (22), and (iv) in-frame deletions in the *mptD* (IID) gene (which may have compromised the folding and stability of IID and IIC) (3). The interaction with bacteriophage lambda was discovered by Scandella and Arber (25) while they were looking for host-specific restriction properties. They found *E. coli* mutants that were resistant to low doses of bacteriophage lambda. The adsorption of phage lambda to these mutants was normal, but the phage DNA was not injected. This so called Pel^- phenotype (*pe*netration of *l*ambda [6]) is caused by the loss of the IIC^{Man} and/or IID^{Man} subunit. The *pel* defect is phage specific, conferring resistance to phages λ, 434, Hy-2, and 82 but not to the closely related phage ϕ80 or to the unrelated phages T4, P1, and M13. Resistance was not absolute but leaky (small plaques and 10^5-fold reduced effi-

* Corresponding author. Mailing address: Department of Chemistry and Biochemistry, University of Bern, Freiestr. 3, CH-3012 Bern, Switzerland. Phone: 41 31 631 4346. Fax: 41 31 631 4887. E-mail: erni@ibc.unibe.ch.

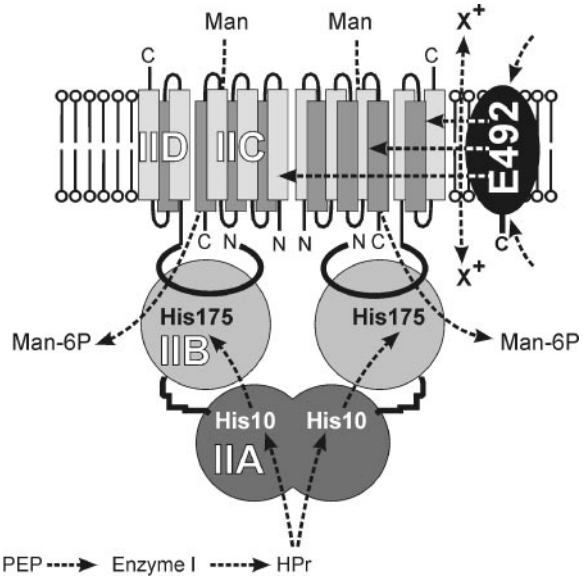

FIG. 1. Topology model of the mannose transporter complex. His-10 and His-175 of $IIAB^{Man}$ (ManX) transfer phosphate from phospho-HPr (PtsH) to the sugar that is translocated by the IIC/IID (ManY/ManZ) complex. The highly specific interaction between microcin E492 and the membrane-spanning IIC and/or IID subunit causes inner membrane depolarization. HPr is phosphorylated with phosphoenolpyruvate (PEP) by enzyme I (PtsI) of the phosphotransferase system. The model is predicted according to reference 18 with the constraints from references 11 and 13.

ciency of plating), and it was more pronounced against phages with smaller-than-wild-type genomes (7, 12).

It is not clear which properties make the IIC/IID complex a vulnerable spot for macromolecular invasion. The fact that microcin E492 can penetrate into the membrane from both sides could indicate that the IIC/IID complex is symmetrically oriented in the membrane. Indeed, a functional symmetry of the IIC/IID complex has been proposed by Beneski et al. based on their observation that the complex could phosphorylate mannose on the inside as well as outside of membrane vesicles, while the functionally related but structurally different glucose transporter phosphorylated glucose only on the inside (1). This symmetry, however, has not been confirmed. Although symmetrical, dual-topology membrane proteins exist (19, 23), they are much smaller than the IIC/IID complex.

It is also not known with which of the two subunits (i.e., IIC or IID) microcin E492, IIa bacteriocins, and the tail proteins of bacteriophage lambda interact. IIC and IID cannot be expressed independent of each other, at least not in biochemical amounts, and they also cannot not be separated under nondenaturing conditions (9). There are two apparently conflicting reports, one indicating that a mutation in *mptD* (IID) suffices to confer resistance (3) and the other indicating that heterologous expression of *Listeria monocytogenes mptC* (IIC) confers sensitivity to a resistant *Lactococcus lactis* strain (21). The conflict may be resolved by assuming that *Listeria* IIC is unstable without IID but that it can be stabilized by heterologous *L. lactis* IID. A chimeric complex between IIC of *E. coli* and IID of *Klebsiella pneumoniae* supports lambda penetration, while the inverse combination is inactive (9). Taken together, these results suggest that the interaction between IIC and IID may be important for stability but that it is most probably IIC that determines the high specificity for microcin E492 and class IIa bacteriocins.

The molecular determinants of IIC and/or IID for interaction with microcin E492 could be identified with mutations that confer microcin resistance but continue to allow the transportation and phosphorylation of mannose. Such mutants could be selected with mannose as the only carbon source and with microcin E492 as the counterselectant.

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