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The influence of maltoporin affinity on the transport of maltose and maltohexaose into *Escherichia coli*

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The kinetics of maltose transport and its inhibition by maltohexaose were investigated using *Escherichia coli* strains with engineered modifications of maltoporin. The permeation of lactose through maltoporin was also measured, as well as its inhibition by maltohexaose. Based on these results, the role of the maltoporin binding site in transport was evaluated.

Maltose and maltodextrins are transported into *Escherichia coli* using a complex, five-component transport system [1]. One of these components, maltoporin (LamB protein), is found in the outer membrane and is required for maltose transport at low concentrations [2] as well as for the transport of all maltodextrins larger than maltotriose [3]. This protein forms a maltooligosaccharide-selective pore in the outer membrane [4–6] and possesses a maltodextrin binding site [7]. In recent genetic studies, we have isolated strains carrying single-amino acid substitutions in maltoporin with specific changes in maltodextrin binding [8–11]; both mutants with reduced and increased binding affinities for maltose or large maltodextrins are available. The pore-forming properties of five of these variant proteins have been recently described [12]. This communication describes the influence of the binding site changes on the transport of substrates through maltoporin, and aims to evaluate the significance of the binding site affinity to transport into whole bacteria.

The strains of bacteria used in these experiments and the affinity of the maltoporin variants is shown in Table I. The pore-forming properties of maltoporin from each of the variants towards maltohexaose is also shown in Table I. Using these strains, the kinetics of transport into maltose-grown bacteria were assayed with [^{14}C]maltose as substrate as previously described [13]. As shown in Table I, the transport affinity (K_s) for maltose was not greatly affected in strains expressing maltoporin with a wide range of binding site modifications; only BW1040, with greatly decreased binding affinity, showed a minor reduction in transport affinity for maltose. The simplest explanation of this result was that maltoporin was not rate-limiting even if the binding site/pore was sub-optimal for maltodextrins. These results were consistent with the conclusions of Wandersman and Schwartz [14], who suggested that the high levels of expression of *lamB* present in fully induced bacteria masked transport defects in maltoporin.

The affinity of the transport system for maltohexaose was tested by measuring its ability to inhibit maltose transport since labelled maltohexaose was not available. As previously found, and as confirmed in preliminary experiments, maltohexaose was a competitive inhibitor of trans-

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TABLE I

INFLUENCE OF MALTOPORIN AFFINITIES ON MALTOSE AND MALTODEXTRIN TRANSPORT INTO *ESCHERICHIA COLI*

Strain	Maltoporin affinity		Maltohexaose permeation [b]	Transport affinity for	
	maltose [a]	starch [a]		maltose (K_s, μM) [c]	maltohexaose (K_i, μM) [c]
BW1022 (W.T.)	+	+ +	+ +	2.2	0.9
BW1040	n.a.	−	−	4.5	23.3
BW1101	(+)	+	+	2.9	8.5
BW1400	+ +	+ + + +	+ + + +	1.1	2.5
BW1500	+	+ + + +	+ + +	3.1	2.5

[a] The isolation and starch/maltose-binding properties of the mutants were described in Refs. 8 and 10. The relative affinities were assayed by binding to starch-Sepharose columns and by the ability of maltose to elute bound bacteria. n.a. denotes not assayable.

[b] The relative rates of permeation of maltohexaose through reconstituted maltoporin pores made by *lamB* mutants shown were as described in Ref. 12.

[c] The kinetics of maltose transport and its inhibition by maltohexaose were measured as previously described [13].

port [13]. The K_i for maltohexaose was increased by an order of magnitude in BW1040. The K_i in BW1101 was also not higher, suggesting that low binding affinity in maltoporin resulted in lower access of maltohexaose to the transport system. However, the mutants expressing maltoporin with higher binding affinities were not inhibited with a higher affinity (lower K_i) than wild-type bacteria. This was also consistent with the notion that maltoporin affinities do not determine the affinity of transport unless the changes make maltoporin rate-limiting; in the case of BW1040 and to a lesser extent BW1101, reconstitution studies showed a marked reduction in maltohexaose permeation in vitro through maltoporin pores [12].

Previous studies have shown that other saccharides such as lactose can penetrate LamB pores, though not as well as a comparable maltodextrin (4,12]. The transport of lactose can be made LamB-rate-limiting by reducing the expression of other outer membrane pore proteins, which can be achieved with an *ompB* mutation [15]. Hence the uptake of [^{14}C]lactose was measured into strains which had an $ompB_7$ mutation as well as the altered maltoporins [12]. The ability of maltohexaose to block the uptake of lactose was also investigated.

There was a considerable difference in the rate of lactose permeation through different variant proteins. As shown in Fig. 1, lactose transport into BW1042 was only a quarter of the rate of wild-type whereas the two high affinity maltoporin variants transported lactose up to 2-fold faster than wild-type. This showed that the transport of lactose under these conditions was indeed dependent on the nature of maltoporin. The differences in lactose transport rates were due either to a change in the size of the maltoporin pore or a change in affinity towards lactose. Interestingly, neither such change was detected in in vitro pore assays of maltoporin [12]; there was no obvious reason for the discrepancy, but it may be the result of different properties of the protein after reconstitution.

The influence of maltodextrin binding affinities were clearly observed in the ability of maltohexaose to block the permeation of lactose through maltoporin. In bacteria with wild-type maltoporin (BW1027), maltohexaose at 10 μM or 100 μM resulted in 21% and 40% inhibitions of lactose uptake, as shown in Fig. 1. In the PB1042 mutant, with no hexaose affinity, maltohexaose was unable to inhibit lactose uptake under these conditions. Mutants with minor changes in affinity showed neither altered lactose transport rates nor altered maltohexaose inhibitions (Fig. 1). In contrast, the ability of hexaose to inhibit lactose transport was greater in the high-affinity variants: for example, BW1402 lactose transport was 50% inhibited by 10 μM and 76% inhibited by 100 μM maltohexaose.

In summary, these results confirm that maltoporin is not a rate-limiting component for

maltoporin is not a rate-limiting component for maltose transport into bacteria, and its binding affinity is not crucial to maltose transport under normal conditions. However, for maltohexaose, the binding affinity of maltoporin influences recognition by the transport system. This was particularly noticeable under conditions in which maltoporin function is rate-limiting, as in the

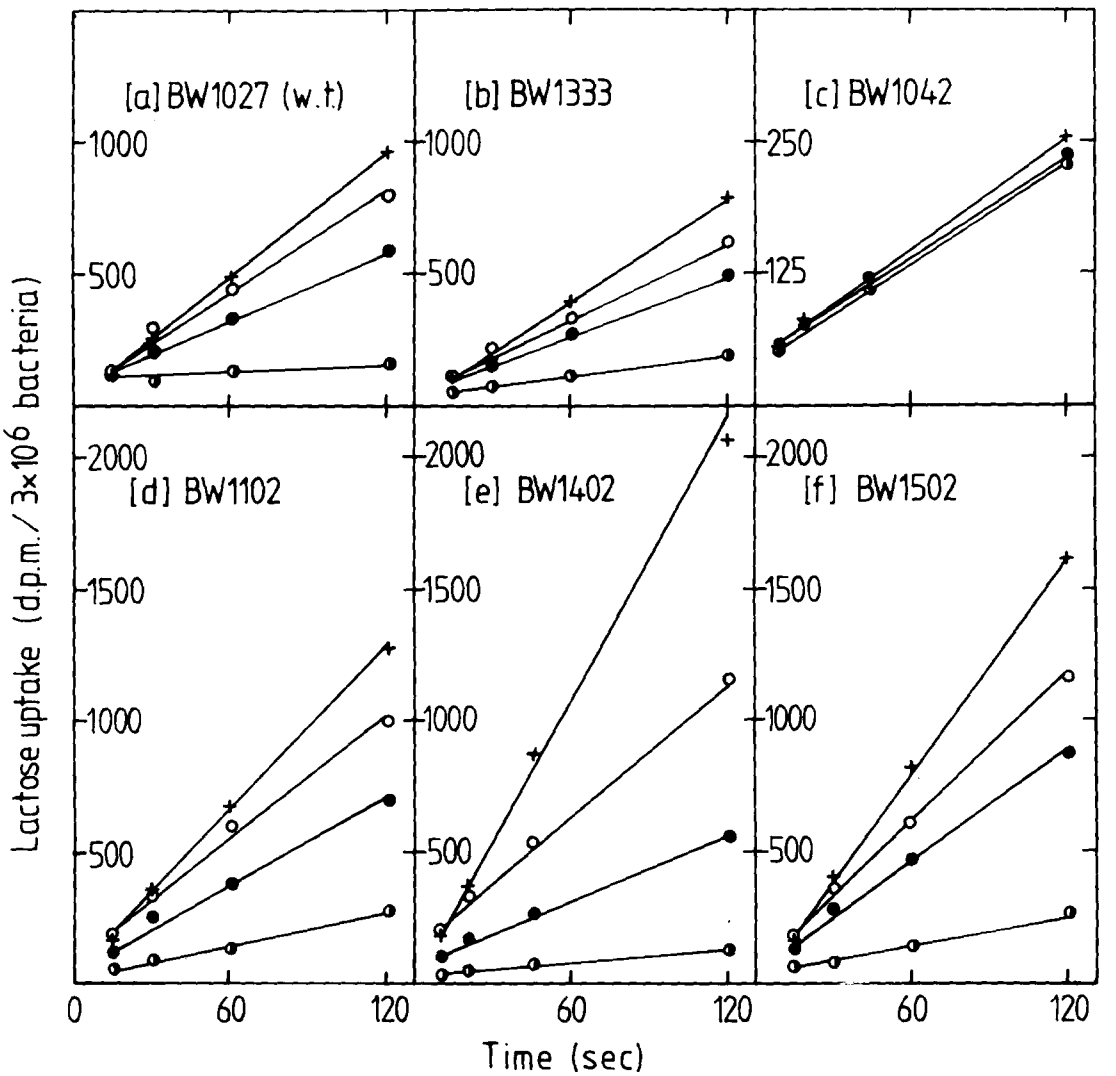

Fig. 1. Lactose transport into *Escherichia coli* and its inhibition by maltohexaose. The strains used in these experiments were constructed as described in Ref. 12 and all contained an $ompB_7$ mutation derived from pop128 [16]. The LamB protein in the outer membrane was present in equivalent amounts in all strains, as shown by electrophoresis of outer membrane preparations [12] BW1027 (a) had no additional *lamB* mutation whereas BW1333 (b) contained *lamB* from BW1330 [12]; BW1042 (c) contained *lamB* from BW1040; BW1102 (d) contained *lamB* from BW1101; BW1402 (e) contained *lamB* from BW1400; and BW1502 (f) contained *lamB* from BW1500. Bacteria were grown on minimal medium containing 0.2% w/v maltose plus 1 mM isopropylthio-β-galactoside to induce both maltose and lactose transport systems. The transport experiments were carried out essentially as described in Ref. 13 except that 50 μM [^{14}C]lactose was used as substrate instead of maltose. At this concentration of lactose, the uptake of lactose is limited by maltoporin-mediated transport across the outer membrane [15]. The assays contained $1.5 \cdot 10^8$/ml bacteria. Transport rates were measured in the absence of maltohexaose (+) and in the presence of 10^{-5} M (○), 10^{-4} M (●), and 10^{-3} M (◑) maltohexaose, respectively.

lactose transport experiments. The binding affinity for maltohexaose does determine the effectiveness of maltodextrins in blocking the maltoporin pore, as would be expected if the maltodextrin binding site was indeed part of the pore.

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