Biochimica et Biophysica Acta 860 (1986) 44–50
Elsevier

BBA 73152

Substrate specificity of the *Escherichia coli* maltodextrin transport system and its component proteins

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(Received March 14th, 1986)

Key words: Sugar transport; Maltodextrin transport; Substrate specificity; Transport protein; (*E. coli*)

Maltooligosaccharides up to maltoheptaose are transported by the maltodextrin transport system of *Escherichia coli*. The overall substrate specificity of the transport system was investigated by using 15 maltodextrin analogues with various modifications at the reducing end of the oligosaccharides as competing substrates. The binding interaction of the analogues with maltoporin in the outer membrane and the periplasmic maltose-binding protein, the two protein components of the transport system with known specificity for maltodextrins, was also investigated. All analogues containing several α,1 → 4-glucosyl linkages were bound with high affinity by maltoporin and maltose-binding protein, regardless of *O*-methyl, *O*-nitrophenyl, β-glucosyl or β-fructosyl substitutions at the reducing end of the dextrins. Introduction of a negative charge or lack of a ring structure at the reducing end were also ineffective in abolishing binding by these two proteins. These results suggest that the structure of the reducing glucose is not important in the binding specificity of maltoporin or maltose-binding protein. However, the high affinity of these proteins for analogues was not in itself sufficient for recognition by the transport system overall. Maltohexaitol, 4-nitrophenyl α-maltotetraoside and 4-β-D-maltopentaosyl-D-glucopyranose were bound with the same affinity as comparable maltodextrins by both maltoporin and maltose-binding protein but were poorly recognized by the transport system. These results suggest that another, yet uninvestigated component of the transport system has a more restricted specificity towards changes at the reducing end of the maltodextrin molecule.

Introduction

The maltose/maltodextrin transport system of *Escherichia coli* consists of five known proteins: maltoporin in the outer membrane [1–4], the periplasmic maltose-binding protein [5,6], the MalF and MalG proteins in the cytoplasmic membrane [7,8] and the MalK protein on the cytoplasmic side of the cytoplasmic membrane [9]. Only maltoporin and maltose-binding protein have known binding sites for maltodextrins, although there is some genetic evidence that MalF and MalG proteins may also constitute a binding site [10]. It is an open question how the overall specificity of the transport system is determined by the individual specificities of the component proteins. Indeed, in the case of the maltodextrin transport system, the sugar specificity of the intact transport system has hardly been investigated. The purpose of the present study was to test the specificity of the transport system using maltodextrin analogues and to compare these specificities with the binding properties of maltoporin and maltose-binding protein. Such a comparison

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should decide whether the substrate specificity of the transport system was indeed determined by these two proteins.

Substrates of the transport system are known to include linear maltodextrins up to maltoheptaose [11]. Isomaltose or cyclodextrins are not recognized [12]. The only analogues known to be transported are 5-thiomaltose and methyl α-maltoside [13]. Since these analogues were effectively recognized by the transport system and maltose-binding protein with high affinity, this study tests whether more extensive modifications of the molecule affect transport.

A means of generating a large number of maltodextrin analogues is through the use of the extracellular 'amylase' preparations from *Bacillus macerans*, which transfers linear, α,1 → 4-linked glucosyl units from cyclodextrins onto acceptor molecules related to glucose and which have a free 4-position [13,14]. As a number of mono- and disaccharides can be used as acceptor molecules, several maltodextrin analogues could be synthesized using this approach. Another advantage was that the analogues generated in this way included the whole range of oligomers containing from two to over ten glucose unit equivalents. Fractionation of these molecules on the basis of size then permitted the isolation of, for example, maltose and maltohexaose analogues. This allowed us to test whether recognition of a substrate molecule by the maltose/maltodextrin transport system was also influenced by the number of α,1 → 4 linkages in the analogue.

Materials and Methods

Sugars and sugar analogues

Methyl β-D-glucopyranoside, α-cyclodextrin and methyl α-D-xylopyranoside were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A.; maltotetraose, maltohexaose, 4-nitrophenyl α-D-maltoside, 4-nitrophenyl α-D-maltotetraoside and analytical enzymes were from Boehringer Mannheim, F.R.G.; maltose (pure biochemical grade) was from Merck Chemical Co., Darmstadt, F.R.G.; cellobiose was from Calbiochem-Behring, La Jolla, CA, U.S.A. Other chemicals were pure grades from commercial sources.

Synthetic analogues in which α,1 → 4-linked glucosyl units from α-cyclodextrin were transferred to methyl β-D-glucoside, methyl α-D-xylopyranoside, cellobiose or sucrose as acceptor molecules were synthesized according to the previously described methods using *B. macerans* extract [13]. In each case, synthesis of analogues started with 0.5 g acceptor sugar and 1 g α-cyclodextrin and was carried out as described [13]. The size fractionation and analysis of sugars using thin-layer chromatography were also according to published methods [13]. The structures of the analogues isolated are shown in Fig. 1. Evidence for these structures was obtained from sugar analyses, assay of reducing groups and analysis of products formed on hydrolysis with α-glucosidase and/or glucoamylase (results not shown).

The glucose at the reducing end of maltose, maltotetraose and maltohexaose was also modified by borohydride reduction to the corresponding glucitol derivatives and bromine oxidation to the corresponding gluconic acid derivatives. Both types of reaction were carried out by standard methods [15,16]. Both modifications were taken to completion, as judged by the lack of maltooligosaccharides in the products, using thin-layer chromatography as above. The structures of these analogues is also shown in Fig. 1.

Bacterial strains and growth experiments

The two strains of *Escherichia coli* K12 used in these experiments were HFrG6, which is wild-type with respect to the maltose transport system, and pop3325, which is wild type for the transport system but carries a $malT^c$ mutation resulting in constitutive expression of the system [17].

Growth experiments were carried out in minimal medium A [18] with the carbon sources specified in Table I; pop3325 was used in these experiments in case an analogue was a substrate but not an inducer of the maltose system. Each sugar was present at 0.5–1 mM concentration as carbon source. For testing the inhibitory effects of analogues, the carbon source was 0.2% glycerol and the analogues were also at 0.5–1 mM concentration. Growth was monitored by measuring the absorbance of the culture at 580 nm.

Transport assays and inhibition studies

Transport rates were obtained by measuring

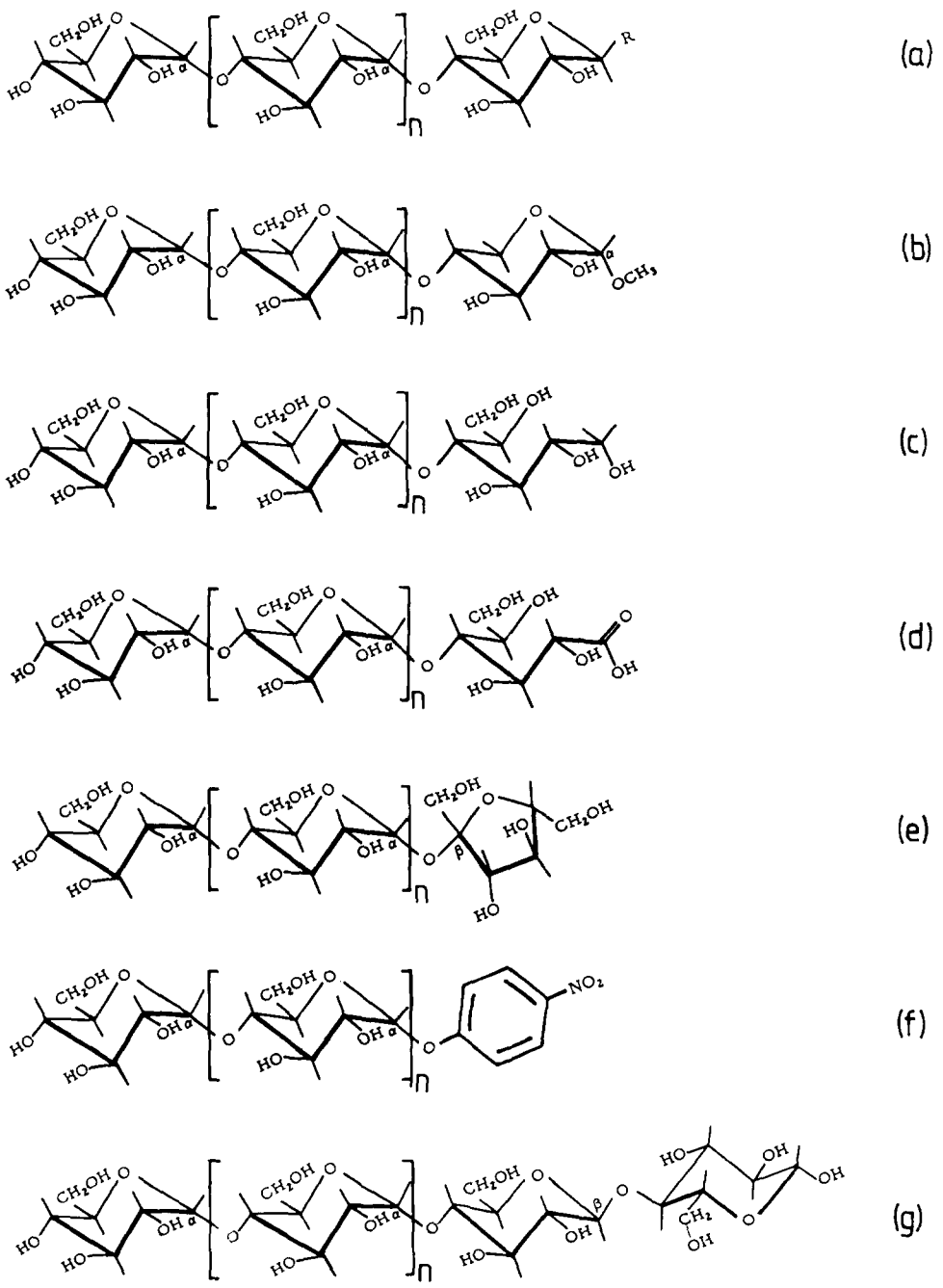

Fig. 1. Structures of the substrates and analogues used in this study. {1} maltose: (a), $n = 0$, R = OH; {2} maltotetraose: (a), $n = 2$, R = OH; {3} maltohexaose: (a), $n = 4$, R = OH; {4} methyl β-maltoside: (a), $n = 0$, R = OCH_3; {5} methyl β-maltotetraoside: (a), $n = 2$, R = OCH_3; {6} methyl α-D-xylopyranosyl-(1 → 4)-α-D-glucopyranoside: (b), $n = 0$; {7} methyl α-D-xylopyranosyl-(1 → 4)-α-maltoside: (b), $n = 1$; {8} maltitol: (c), $n = 0$; {9} maltotetraitol: (c), $n = 2$; {10} maltohexaitol: (c), $n = 4$; {11} maltobionic acid: (d), $n = 0$; {12} maltotetraonic acid: (d), $n = 2$; {13} maltohexaonic acid: (d), $n = 4$; {14} β-D-fructofuranosyl-(1 → 4)-α-maltopentaoside; (e), $n = 4$; {15} 4-nitrophenyl α-D-maltoside: (f), $n = 1$; {16} 4-nitrophenyl α-D-maltotetraoside: (f), $n = 3$; {17} 4-β-D-maltosyl-D-glucopyranose: (g), $n = 0$; {18} 4-β-D-maltopentaosyl-D-glucopyranose: (g) $n = 3$.

the uptake of [U-^{14}C]maltose as previously described [11]. Maltose was present at 1 μM in the assay and analogues were present at 10 or 100 μM. The uninhibited rate of maltose transport in the inhibition studies was 1.33 nmol maltose taken up per min per 10^8 bacteria, using maltose-grown G6 cells.

Binding affinities of maltoporin and maltose-binding protein

The affinities of analogues for maltoporin in intact bacteria were determined from plots of concentration dependence of inhibition of binding of fluorescein-labelled amylopectin as previously described [2]. Maltose-binding protein was purified by affinity chromatography on cross-linked amylose and elution by maltose [19]. The purified protein formed a single band after gel electrophoresis in the presence of sodium dodecyl sulphate. The protein was dialyzed using four changes of 500 volumes of 10 mM Tris-HCl buffer (pH 7.2) before binding assays. The affinities of maltose-binding protein were determined from the concentration dependence of protein fluorescence quenching by the analogues as previously described [19].

Results

A preliminary study with all the analogues was to test whether any were transported or hydrolyzed by bacteria at a sufficient rate to support growth. None of the maltose/maltodextrin analogues listed in Table I was an effective carbon source of *E. coli*. The only analogues supporting slow growth were {5}, {14} and {18}, which were oligomers containing at least four glucose units. It was possible that this slow growth was due to the hydrolysis of these oligosaccharides by the periplasmic α-amylase [20] and not to transport of intact molecules. It was also tested whether any of the analogues were growth-inhibitory; when tested with glycerol as carbon source, none of the analogues gave rise to a toxic level or type of molecule which could have resulted in growth stasis. The only exception was β-methyl maltoside, which caused a minor reduction in growth rate on glycerol.

The lack of growth on the above analogues did not necessarily reflect lack of recognition by the transport system, since the analogues may not be substrates of amylomaltase or maltodextrin phosphorylase, the enzymes necessary for maltodextrin metabolism [21,22]. Therefore, each of the analogues in Table I was tested directly as a potential inhibitor of maltose transport. The analogues were tested at 10-fold and 100-fold excess over the maltose concentration in the transport assay. As shown in Table I, only methyl β-maltoside{4} and methyl β-tetraoside{5} were able to give over 50% inhibition at a 10-fold excess of analogue. These two analogues gave near-total inhibition at a 100-fold excess; lesser inhibitions were also found with {8–10} and {13–18}. Each of these analogues gave over 50% inhibition of maltose transport but only at a 100-fold molar excess. At this molar excess, possible inhibition by low-level contamination by maltodextrins could not be ruled out.

The affinity of interaction of each of the analogues was also tested with maltoporin, which has a known affinity for linear maltooligosaccharides [2]. As expected, the disaccharide analogues had the lowest affinities for maltoporin, as shown in Table I. Nevertheless, none of the analogues had a measurably lower affinity for maltoporin than did maltose itself. All other analogues bigger than disaccharides exhibited affinities comparable to the range of affinities exhibited by transported maltooligosaccharides.

The binding of each of the analogues to purified maltose-binding protein was also investigated using ligand-dependent fluorescence quenching techniques [19]. All the ligands showed a saturable quenching of binding protein fluorescence. However, the nitrophenyl derivatives also caused a linearly concentration-dependent decrease in fluorescence due to their absorption at 280 nm, which was the excitation wavelength for protein fluorescence. This apparent quenching could be estimated with protein whose binding-dependent decrease in fluorescence was already saturated with excess maltose (1 mM). The absorption-dependent decrease in fluorescence was corrected for in determining the binding-dependent quenching by nitrophenyl derivatives.

As previously found [12], all maltooligosaccharides had K_d values in the micromolar range; analogues {4,5,9,10,13–18} were also bound with

TABLE I

THE INTERACTION OF MALTODEXTRIN ANALOGUES WITH *ESCHERICHIA COLI* AND COMPONENTS OF THE MALTODEXTRIN TRANSPORT SYSTEM

Substrate/inhibitor	Bacterial growth [a]	Growth inhibition [b]	Maltose transport inhibition (%) [c]		LamB protein K_d (M)	MalE protein K_d (M)
			10 μM	100 μM		
{1} Maltose	+	−	95	99	$1.9 \cdot 10^{-2}$	$1.0 \cdot 10^{-6}$
{2} Maltotetraose	+	−	94	99	$3.0 \cdot 10^{-4}$	$1.6 \cdot 10^{-6}$
{3} Maltohexaose	+	−	96	99	$7.5 \cdot 10^{-5}$	$2.8 \cdot 10^{-6}$
{4} Methyl β-maltoside	−	±	52	94	$5.3 \cdot 10^{-3}$	$4.7 \cdot 10^{-6}$
{5} Methyl β-maltotetraoside	±	−	64	98	$4.8 \cdot 10^{-4}$	$3.3 \cdot 10^{-6}$
{6} Methyl α-xylosyl-(1 → 4)-α-D-glucopyranoside	−	−	< 8	26	$1.3 \cdot 10^{-2}$	$4.5 \cdot 10^{-5}$
{7} Methyl α-xylosyl-(1 → 4)-α-D-glucopyranosyl(1 → 4)-α-D-glucopyranoside	−	−	23	29	$3.1 \cdot 10^{-3}$	$2.8 \cdot 10^{-5}$
{8} Maltitol	−	−	10	57	$3.0 \cdot 10^{-3}$	$5.0 \cdot 10^{-5}$
{9} Maltotetraitol	−	−	16	56	$1.7 \cdot 10^{-4}$	$7.6 \cdot 10^{-6}$
{10} Maltohexaitol	−	−	14	73	$1.2 \cdot 10^{-4}$	$2.9 \cdot 10^{-6}$
{11} Maltobionic acid	−	−	0	0	$1.3 \cdot 10^{-2}$	$3.7 \cdot 10^{-3}$
{12} Maltotetraonic acid	−	−	16	26	$1.8 \cdot 10^{-3}$	$9.8 \cdot 10^{-5}$
{13} Maltohexaonic acid	−	−	12	57	$2.9 \cdot 10^{-4}$	$3.3 \cdot 10^{-6}$
{14} β-Fructofuranosyl tetra-[α-D-glucopyranosyl(1 → 4)]-α-D-glucopyranoside	±	−	31	75	$8.5 \cdot 10^{-5}$	$2.9 \cdot 10^{-6}$
{15} 4-Nitrophenyl α-D-maltoside	−	−	18	64	$2.2 \cdot 10^{-3}$	$7.0 \cdot 10^{-7}$
{16} 4-Nitrophenyl α-D-maltotetraoside	−	−	17	72	$2.4 \cdot 10^{-4}$	$4.7 \cdot 10^{-7}$
{17} 4-β-D-Maltosyl-D-glucopyranose	−	−	17	62	$1.9 \cdot 10^{-3}$	$9.1 \cdot 10^{-6}$
{18} 4-β-D-Maltopentaosyl-D-glucopyranose	±	−	21	79	$5.4 \cdot 10^{-4}$	$9.1 \cdot 10^{-7}$

[a] The growth of *E. coli* on the given substrate as sole carbon source (+, good growth; ±, slow growth; −, no growth).

[b] The effect of analogues at 0.5–1 mM concentration, when bacteria were growing on glycerol as sole carbon source (±, slight inhibition; −, no inhibition).

[c] The influence of 10 μM and 100 μM analogues was tested on the rate of transport of 1 μM [^{14}C]maltose.

similar affinities. The most striking difference of binding affinities was found upon introduction of a negative charge into the derivatives; analogues {11} and {12} were particularly poorly bound in comparison to other analogues of similar size. However, the presence of additional α,1 → 4 linkages as in {13} permitted good binding by maltose-binding protein. These results suggest that the binding protein recognizes α,1 → 4 linkages between glucose residues rather than the sugar at the reducing end of the molecule.

Discussion

The interaction of 15 maltooligosaccharide analogues with the maltose/maltodextrin transport system of *E. coli* has been investigated. A comparison of the binding affinities of these and comparably sized maltooligosaccharides towards maltoporin and maltose-binding protein has also been undertaken.

The presence of either an α- [13] or β-linked methyl substitution at the 1-position of maltose or maltodextrins has only a marginal effect on recognition by the maltose transport system or binding to maltoporin or maltose-binding protein. Analogues {4} and {5} were effective inhibitors of maltose transport. The absence of a 5-methoxy group from the reducing end glucose, as in analogues {6} and {7}, caused a marked reduction in transport inhibition as well as binding affinities. This would suggest that the 6-position is more

important than the 1-position in maltose for interacting with transport components.

The absence of a ring structure at the reducing end, or even a reducing group as in maltitol{8} did not abolish binding by either maltoporin or maltose-binding protein, although there was a 60-fold reduction in affinity of binding protein. This reduction in affinity was less noticeable when additional α,1 → 4-linked glucoses were present as in {9} and {10}; with maltohexaitol, the binding affinities were comparable to that of maltohexaose. It may be concluded that the structure of the reducing sugar in maltodextrins is not significant in binding to either maltoporin or maltose-binding protein, except perhaps at the 6-position. Even introduction of a negative charge (as in {13}) or a nitrophenyl substitution (as in {16}) did not significantly reduce binding by the two proteins provided that at least four or five α,1 → 4-linked glucose residues were present in the ligand.

It was therefore surprising and interesting that maltohexaitol, which was an excellent ligand for both binding protein and maltoporin, was a relatively poor inhibitor in maltose transport assays. Clearly, binding by these proteins was not in itself sufficient for recognition by the maltose/maltodextrin transport system.

There also appeared to be a lack of correlation with other analogues of the ability of maltoporin or maltose-binding protein to bind analogues effectively and the inhibitory effect on transport. 4-Nitrophenyl α-D-maltotetraoside {16} was bound with higher affinity by both maltoporin or maltose-binding protein than maltotetraose, yet was much less inhibitory for maltose transport than maltodextrins. A similar lack of correlation between binding and transport inhibition was found with {18} in which the only difference from maltohexaose was in the presence of a β- rather than an α-linkage between the reducing glucose and the rest of the molecule.

Several explanations for the lack of correlation between the binding and transport-inhibitory effects of the analogues are possible. The first possibility is that the binding affinities measured were not the affinities in the intact bacterium when transporting sugars. This possibility is somewhat unlikely but difficult to exclude. A second possibility is that the binding affinities towards maltoporin did not reflect the ability of analogues to penetrate the pore. This possibility is also difficult to exclude totally, but recent data on the good correlation between pore properties and binding affinities have been obtained [23]. A third and most intriguing possibility is that another component in addition to maltoporin or maltose-binding protein is influential in determining the transport specificity of the system. There is some additional evidence to support this suggestion; we have recently obtained mutants able to grow on {18} and the mutation permitting growth maps in *malG*, another component of the system (D. Maris, K.-S. Lee and T. Ferenci, unpublished results). This mutation causes a drastic change in the specificity of transport, suggesting that MalG protein is also significant in determining the overall specificity of the maltose/maltodextrin transport system. The influence of other components with yet unidentified binding properties may also need to be considered to get a full understanding of the specificity of the transport system.

Acknowledgements

This research was supported by the C. & V. Ramaciotti Foundations. The assistance of Susan Weston with the maltose-binding protein purification is gratefully acknowledged.

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