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INTERRELATIONSHIP OF THE PHAGE λ RECEPTOR PROTEIN AND MALTOSE TRANSPORT IN MUTANTS OF *ESCHERICHIA COLI* K12

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Summary

(1) The λ receptor protein in maltose-induced cells of *Escherichia coli* K12 is a major protein of the outer membrane and therefore amounts to about 100 000 copies per cell.

(2) 17 λ-resistant derivatives of the λ-sensitive strain *E. coli* W3110 were isolated independently; all were impaired in maltose transport and showed a much lower growth rate on maltotriose except one mutant, *E. coli* W3110/21 which resembled the wild type parent in that it grew equally fast on maltotriose and maltose. All mutants lacked the λ receptor protein.

(3) The presence of the maltose-inducible λ receptor protein in the missense mutant *E. coli* CR63 was demonstrated by SDS-gel electrophoresis of isolated outer membranes. The molecular weight of 47 000 daltons corresponded to the size of the wild type receptor protein. Four derivative strains with additional *lam*B mutations were obtained by selecting for mutants resistant to a λ phage with an extended host range (λh). They all grew equally fast on maltose and maltotriose but only one contained as much λ receptor protein as the original strain. There was no correlation between the amount of residual receptor protein in these mutants and the initial rate of transport which ranged from 13 to 47% of the transport rate of the parent strain *E. coli* CR63.

(4) Maltose-1-phosphate, trehalose, melibiose, sucrose, raffinose, and cellobiose at 2 mM up to 11 mM concentrations did not compete with maltose uptake. Growth of cells on trehalose of melibiose induced the maltose transport system to 20 or 7%, respectively, of the level observed in maltose-grown cells, measured as initial rate of maltose uptake.

Introduction

It has recently been shown that phage and colicin receptor proteins in the outer membrane of *Escherichia coli* are involved in the uptake of substrates.

For example the lack of the λ receptor function increases the apparent Michaelis constant, K_m, for maltose transport by a factor of 100—500 without influencing the maximal rate of transport [1]. These data suggest that the rate of maltose diffusion across the outer membrane is facilitated by the λ receptor protein. The receptor protein becomes only important in maltose transport when maltose is present in the medium at low concentrations.

It is desirable to extend these observations to higher homologes of maltose to see whether the requirement for the receptor becomes stricter with increasing molecular weight of the substrate. Indeed it was claimed that maltotriose even at 10 mM concentration is not transported into *lam*B mutants [1,2]. In contrast, as will be shown in this paper *lam*B missense mutants grow on maltotriose as fast as the wild type and only nonsense mutants show a markedly reduced growth rate. We studied some aspects of the maltose-λ receptor interrelationship in order to compare it with the various receptor-facilitated transport systems known to date [3—15]. Our final goal is to test whether or not a uniform picture emerges of the function of receptor proteins in the translocation of substrates across the outer membrane.

Materials and Methods

Strains and culture conditions

The original strains *E. coli* K12 W3110 and *E. coli* K12 CR63 and their derivatives were grown either on M63 medium [16] consisting of 13.6 g KH_2PO_4/2 g $(NH_4)_2SO_4$/0.2 g $MgSO_4 \cdot 7\ H_2O$/0.5 mg $FeSO_4 \cdot 7\ H_2O$ in 1 l deionized water, adjusted to pH 7.0 with KOH, or on ML medium [17] consisting of 10 g Bacto Tryptone/5 g Yeast Extract (Difco)/5 g NaCl, in 1 l deionized water, pH 7.2. Growth plates were prepared using 8 g Tryptone/5 g Yeast Extract/5 g NaCl/15 g agar (solid agar) or 6 g agar (soft agar plate) per 1 l deionized water.

The virulent phage λ strain and the virulent λ derivative with an extended host range, λh, were obtained from H. Schuster, Berlin. Selection of λ- and λh-resistant *E. coli* mutants of the λ-sensitive *E. coli* K12 W3110 strain, abbreviated from now on as *E. coli* W3110, and the λ-resistant but λh-sensitive *E. coli* K12 CR63 strain, abbreviated from now on as *E. coli* CR63, was performed as described by Thirion and Hofnung [18].

Adsorption rate of phage λ to whole cells

Cells were grown in 1 ml tryptone/yeast medium in the presence of 0.4% maltose to stationary phase. They were spun down, taken up in 0.5 ml 2 mM $MgSO_4$, shaken for 20 min at 37° C and then 0.5 ml of a suspension of phage λ or λh ($1—2 \cdot 10^9$ plague forming units) was added. Samples of 0.05 ml were withdrawn at 0, 2, 3, 4, 5, 8, 10 and 15 min after phage addition and diluted into 5 ml of an ice cold solution of 2 mM $MgSO_4$. The cells were spun down (15 min, 2000 × *g*) and 10^{-6} and 10^{-7} dilutions of the supernatants were plated on the indicator strains.

Extraction and purification of λ receptor

We followed the methods of Randall-Hazelbauer and Schwarz [19]. The

receptor activity during the preparation was followed in the Tris/EDTA/cholate extract of the cells, after treatment with chloroform/methanol, and in the fractions of the two QAE-Sephadex chromatographies as described.

Gel electrophoresis of receptor preparations in the presence of sodium dodecyl sulfate (SDS)

To identify the λ receptor protein, purified cell extracts and outer membranes of cells grown on maltose to induce the maltose operon were subjected to SDS polyacrylamide gel electrophoresis. We largely followed the procedure of Lugtenberg et al. [20], but instead of staining the proteins with Fast Green FCF, we visualized the protein bands with a solution of 1.25 g Coomassie Brilliant Blue in 450 ml methanol/acetic acid 1 : 1 (v : v) for 2 h. The gels were destained with an aqueous solution containing 7% acetic acid and 5% methanol.

For estimation of the molecular weight of the λ receptor protein by SDS polyacrylamide gel electrophoresis the standard proteins phosphorylase *a* (M_r 94 000), bovine serum albumin (M_r 67 000), ovalbumin (M_r 43 000) and chymotrypsinogen (M_r 25 000) were used [29].

Results

Phage λ-resistant E. coli mutants

We used two strains (*E. coli* W3110 and *E. coli* CR63) to isolate λ-resistant *E. coli* mutants. *E. coli* W3110 is originally λ-sensitive; out of 20 resistant mutant colonies picked from λ-containing agar plates, 17 mutants grew on maltose as sole carbon source. The other three mutants did not grow at all on maltose; presumably these are regulatory mutants in which not only the λ receptor protein gene (*lam*B) in the *mal*B operon but also other functions required to take up and catabolize maltose are not expressed. The other strain, *E. coli* CR63, carries a point mutation in the *lam*B gene which confers resistance against normal λ phage but not against λh strains, which have an extended host range [19]. Of 20 λh-resistant mutants isolated, only 4 grew on maltose as sole carbon source and they were studied further. All of the λ-resistant *E. coli* W3110 derivatives were simultaneously resistant against λh.

Growth of λ resistant strains on maltose and maltotriose

All but one of the λ-resistant mutants of *E. coli* W3110 grew faster on maltose than on maltotriose (Fig. 1). The exceptional strain, *E. coli* W3110/21 grew as fast as the wild type strain on maltose or maltotriose (Fig. 1). The growth rates of the point mutant, CR63, and of the 4 λh-resistant derivatives (CR63/1, 4, 5, 9) were equal on maltose or maltotriose and were comparable with λ-sensitive strains (Table I). The lack of the λ receptor function apparently becomes effective in some mutants and not in others. As will be shown later, we verified in all cases that the inability of phage λ to grow on these strains was due to failure to adsorb to the cells, and not to interference in later stages of phage development.

Transport of maltose into λ-sensitive and λ-resistant strains

Growth of the various strains was measured at millimolar maltose concen-

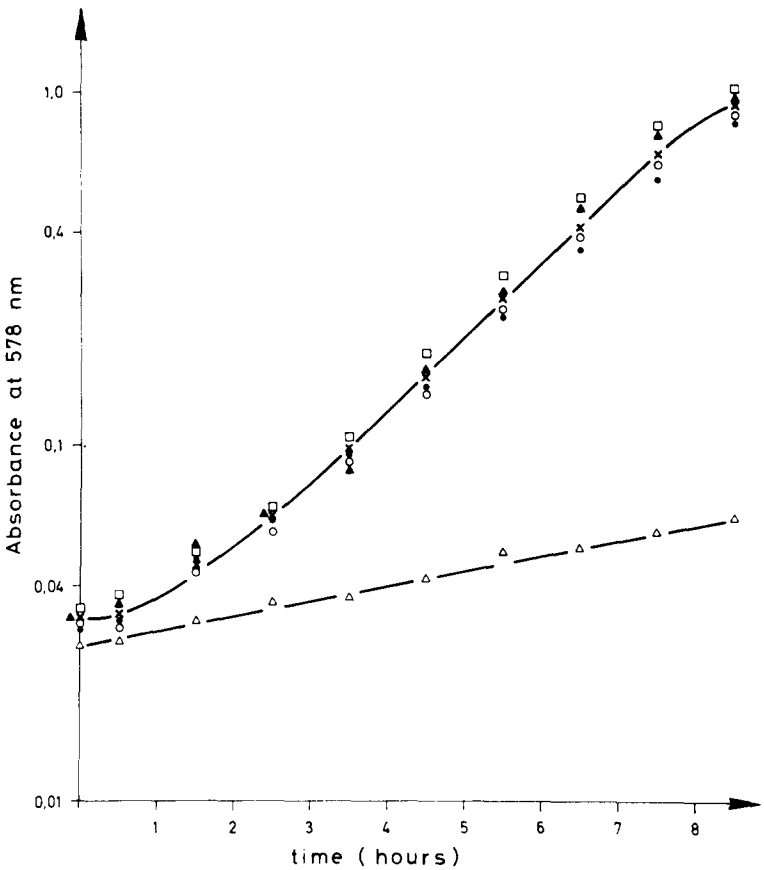

Fig. 1. Growth of the *E. coli* K12 strains W3110 (X———X), W3110/10 (○———○), CR63/9 (▲———▲) all on 0.1% maltose and W3110/10 (△———△) and W3110/21 (●———●) and CR63/9 (□———□) all grown on maltotriose. Stationary phase cells grown overnight in M63 medium containing either 0.1% maltose or maltotriose were diluted into the same medium to an adsorbance of 0.05 at 578 nm. The cultures were shaken at 37° C and growth was followed by measuring the absorbance at 578 nm.

trations (Fig. 1). Since transport can be measured accurately at micromolar concentrations, differences in transport rates among wild type, missense, and nonsense mutants in the *lam*B gene can be studied. The λ-sensitive wild type strains took up maltose much faster than the mutants; the transport capacity differed, among the mutants, too. Table I summarizes the initial rates of transport for all mutants studied and for the starting strains, *E. coli* W3110 (λ-sensitive) and *E. coli* CR63 (λh sensitive). The transport rate into the missense mutant, *E. coli* CR63, was lower than into the wild type, *E. coli* W3110. However, it was significantly faster than into all our simultaneously λ and λh resistant mutants of *E. coli* W3110 and *E. coli* CR63. When the initial transport rate exceeded 0.04 nmol/min per sample, the strains grew on maltose as fast as on maltotriose; the derivative *E. coli* W3110/21 is an exception.

The λ receptor protein might be in the membrane but inaccessible to the phage [17] but still function as "pore" for the passage of maltose. It was therefore necessary to demonstrate the presence or absence of the λ receptor protein

TABLE I

PROPERTIES OF THE *E. COLI* STRAINS

=, growth rate on maltose equals that on maltotriose and corresponds with the growth rate of a λ sensitive wild type strain; ≠, growth rate on maltotriose lower than on maltose; 0, no growth on maltose and maltotriose as sole carbon source; +, maltose inducible λ receptor protein identified on SDS polyacrylamide gels after electrophoresis of outer membranes; (+), reduced amounts of receptor protein (see Fig. 4); —, no λ receptor protein detectable. The values for the initial rates of transport are based on at least two independent experiments which deviated from each other not more than 20%.

Strain *E. coli* K12	Growth rate on maltose and maltotriose	Initial rate of maltose transport (nmol/min per sample)	Receptor protein
W3110 λ^a	=	1.46	+
W3110 λ^r, (16 strains)	≠	0.016—0.040	—
W3110/21 λ^r	=	0.026	—
CR63 $\lambda^r\lambda h^s$	=	0.38	+
CR63/1 $\lambda^r\lambda h^r$	=	0.18	(+)
CR63/4 $\lambda^r\lambda h^r$	=	0.062	(+)
CR63/5 $\lambda^r\lambda h^r$	=	0.052	(+)
Cr63/9 $\lambda^r\lambda h^r$	=	0.12	+
CR63 $\lambda^r\lambda h^r$ (5 strains)	0	0.04	—

in the outer membrane to interpret the range of mutants obtained.

We studied in particular the exceptional mutants *E. coli* W3110 λ^r/21. This mutant is unable to adsorb λ (Fig. 2). The time course of phage adsorption to the wild type and to another derivative is shown for comparison. When the same strains were extracted with a mixture containing deoxycholate/Tris/

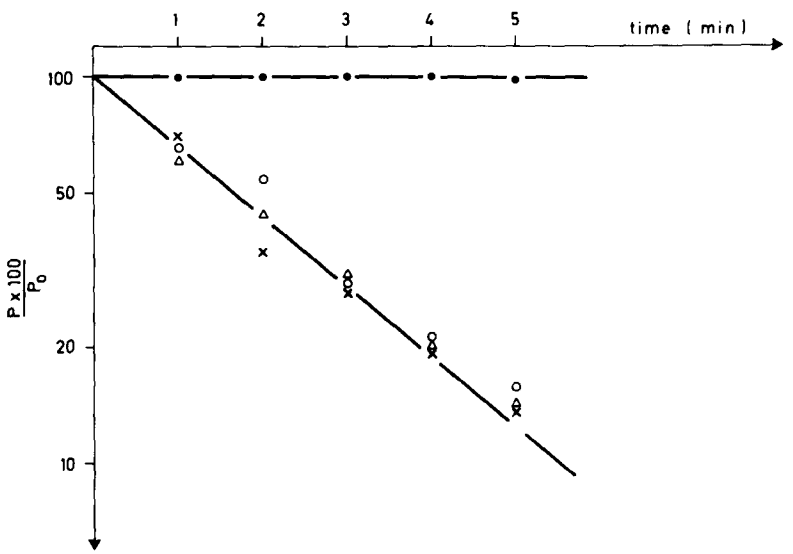

Fig. 2. Inactivation of phage λ by adsorption to the *E. coli* K12 strains W3110 (X———X), W3110 in the presence of 2 mM maltose (○———○) or maltotriose (△———△) and the λ resistant derivatives W3110/4 or W3110/21 (●———●). P_0 denotes the input phage numbers, P the number of phages remaining in the supernatant after adsorption has been allowed to the cells. The procedure used is described in Materials and Methods.

EDTA, the extract of the λ-sensitive strain *E. coli* W3110 reduced the initial phage titer from 600 to 4 plaque-forming units in the case of λ and from 600 to 10 in the case of λh, whereas no reduction occurred with extracts of strains *E. coli* W3110 λ^r/4 and *E. coli* W3110 λ^r/21. The λh resistant mutants of *E. coli* CR63 also failed to adsorb λ and λh (data not shown).

To examine whether the receptor protein in *E. coli* W3110 λ^r/21 is present but inactive, we purified the extract by two chromatographies on QAE-Sephadex. Since no activity could be measured, the purification was performed identically to the successful procedure employed for the receptor protein of the λ-sensitive *E. coli* W3110 strain [19]. The corresponding fractions were electrophoresed in SDS polyacrylamide gels. As shown in Fig. 3, no protein band with the electrophoretic mobility of the λ receptor protein appeared. The identical growth rates of *E. coli* W3110 λ^r/21 on maltose and maltotriose, therefore, can not be explained by an existing λ receptor protein deficient in λ adsorption but functional in maltose and maltotriose uptake. The transport measurement with [^{14}C] maltose (Table I) support this conclusion.

In searching for a faster method to check the other mutants, we found that after induction by maltose the λ receptor protein became such a prominent band in gel electropherograms of outer membrane preparations that no further purification was required. A set of outer membrane preparations of *E. coli* CR63, grown on maltose (gels 1, 7) or glycerol (gels 2, 8), and the λh-resistant mutants CR63/1 (gels 3, 9), CR63/4 (gels 4, 10), CR63/5 (gels 5, 11), CR63/9 (gels 6, 12), all grown on maltose as sole carbon source (Fig. 4), demonstrate the large amount of λ receptor protein upon maltose induction in those mutants which still synthesize the protein in normal amounts (*E. coli* CR63 and CR63/9).

In the λh-sensitive starting strain, *E. coli* CR63, grown on glycerol, no receptor protein could be detected. The λh-resistant derivatives, grown on maltose, expressed the receptor protein to very different extents. Resistance to λh cannot be explained by this reduction in the amount of the receptor protein because few receptors, which could not be seen on the gels, suffice to infect cells. They may be missense mutants which in addition express the *lam*B gene to various extents or the modified protein cannot properly be integrated in the membrane so that reduced amounts end up in the outer membrane. We favour the latter interpretation for the reason that the *lam*B gene is coordinately regulated with the other 3 genes in the *mal*B region whose products are necessary for maltose transport [18,21,22] and our data exhibit no correlation between the amount of receptor protein in the outer membrane and the ability to transport maltose. The λh-resistant strain *E. coli* CR63/9 contained as much receptor protein but had an initial rate of maltose transport only 31% as great as the λ-resistant starting strain *E. coli* CR63 (Table I). In the λh-resistant derivative, *E. coli* CR63/1, the receptor protein could barely be detected on the gel, despite an initial transport rate 47% the size of that of *E. coli* CR63. The other two λh-resistant strains, CR63/4 and CR63/5, contained more receptor protein than CR63/1 but maltose transport was low, 16 and 13% of the rate of *E. coli* CR63.

All the λ-resistant derivatives of *E. coli* W3110 studied lack the λ receptor protein (data not shown).

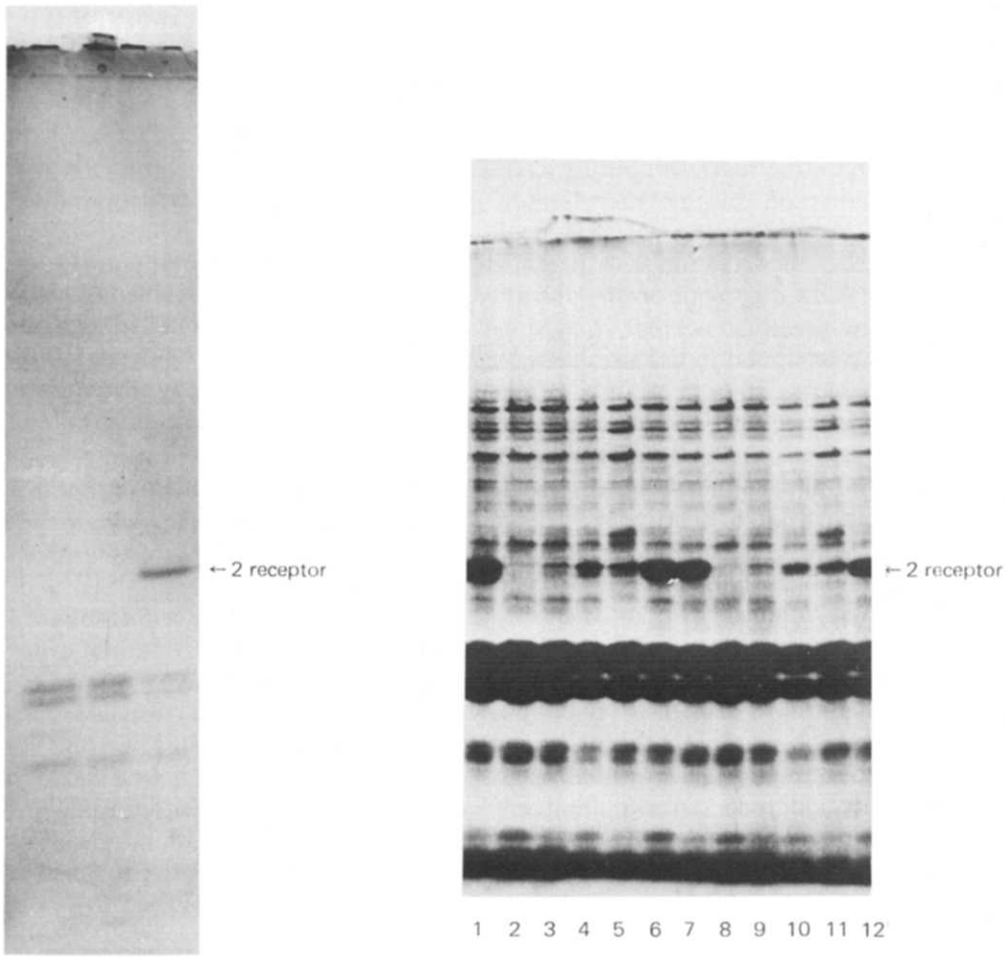

Fig. 3. Polyacrylamide gel electrophoresis in the presence of SDS of the λ receptor preparations of the λ resistant strain *E. coli* W3110/21 (two left gels) and of the λ sensitive parent strain *E. coli* W3110 (right gel) after two subsequent purification steps on columns of QAE-Sephadex. Note that the λ resistant strain lacks the upper protein band seen in the λ sensitive strain. The electrophoretic procedure is described in Materials and Methods.

Fig. 4. Polyacrylamide gel electrophoresis in the presence of SDS of outer membranes of the *E. coli* K12 strains CR63 grown on maltose (gels, 1, 7), on glycerol (gels 2, 8) and of the λh resistant derivatives CR63/1 (gels 3, 9), CR63/4 (gels 4, 10), CR63/5 (gels 5, 11), CR63/9 (gels 69/12) all grown on maltose. Cultures of 400 ml were grown to cell densities of $3 \cdot 10^8$/ml. The outer membranes were prepared by the method of Osborn et al. [28]. The outer membranes were taken up in 0.2 ml water, of which 90 μl were mixed with 110 μg of the sample buffer of the electrophoresis. Samples of 10 μl (right set of gels) and 15 μl (left set of gels) were applied to the individual gels.

Amount of λ receptor protein

The thickness and staining intensity of the λ receptor protein band in the gels correspond to those of the so called major proteins in the outer membrane, for which the number of copies per cell has been estimated to be in the range of 100 000 (23—26). The previously given figure of 6000 copies of λ receptor protein in maltose-induced cells, based on inactivation of λ by receptor extracts

[27], probably underestimated the real number of molecules by a factor of ten. This may be due to aggregation of the receptor molecules under the conditions of assaying inactivation of λ.

Size of λ receptor protein

The results demonstrated in Figs. 3 and 4 indicate which protein among several proteins on the polyacrylamide gels is the λ receptor. We therefore determined the molecular weight of the receptor protein by comparing its electrophoretic mobility in the presence of SDS with standard proteins of known size. The electrophoretic mohility of the λ receptor protein obtained corresponded to a molecular weight of 47 000 daltons. A size of 55 000 daltons was mentioned in the literature [27].

Studies on the specificity of maltose uptake

Since mutations in the *lam*B gene affect maltose, maltotriose transport and λ adsorption one could assume that maltose or maltotriose prevents binding of λ. No reduction of λ adsorption to whole cells of *E. coli* W3110 could be observed at 2 mM concentrations of both sugars (Fig. 2). We then measured whether other sugars inhibit maltose transport. Under the assay conditions used 2 mM maltose reduced uptake of [^{14}C]maltose by 98%. Maltose-1-phosphate, melibiose, trehalose, sucrose, raffinose or cellobiose at 2 mM up to 11 mM concentrations had no effect. Glucose inhibited maltose transport by 10%. When cells of *E. coli* W3110 have been grown in the presence of trehalose or melibiose transport of [^{14}C]maltose was 20% respectively, 7% of the initial transport rate of cells grown on maltose.

Glucose and lactose induced maltose transport to less than 2% of maltose grown cells. It is unknown whether trehalose and melibiose can induce the maltose operon directly or whether they are converted into inducing products.

Discussion

The lack of a functional λ receptor protein reduced strongly the uptake of maltose at micromolar concentrations but did not effect significantly growth on maltose at millimolar concentrations. The λ receptor protein apparently facilitates the diffusion through the outer membrane. At high maltose concentrations, enough molecules pass across the outer membrane to satisfy the growth requirement without λ receptor present. The permeability barrier became more effective for maltotriose, in accordance with data of Nikaido and coworkers [30,31]. They showed that the diffusion rate of neutral saccharides decreases with increasing size of the molecules and that an exclusion limit exists near 550—650 daltons. The findings previously published on the function of the λ receptor protein in the uptake of maltodextrins [1,2,18] are largely supported by our results. However, maltotriose in our experiments supported growth of most of the λ-resistant mutants. Only the nonsense mutants of *E. coli* W3110 were impaired in the growth rate (Table I). The λ-resistant missense mutant, *E. coli* CR63, and their λh-resistant derivatives grew on maltotriose or maltose equally well. Thin layer chromatography showed that the maltotriose contained no maltose (detection limit 5%). The amount of receptor protein

found in the outer membrane of those maltose-induced derivative strains did not correlate with their rates of maltose transport (compare Table I and Fig. 4). The possibility that they are partial regulatory mutants, not only impaired in the expression of the *lam*B gene, but also in the genes for maltose permease and the maltose catabolyzing enzymes, is unlikely since they grow as fast on maltose and maltotriose as did λ-sensitive wild type strains.

The mutant *E. coli* W3110/21 also grew on both sugars equally well despite the complete lack of the λ receptor protein (Figs. 1 and 2). The comparison of the amylomaltase activity in this strain to the λ-sensitive wild type and another λ-resistant mutant which showed reduced growth on maltotriose revealed no difference. After growth on maltose we obtained the enzyme activities: *E. coli* W3110 (160 units/mg protein), W3110/21 (157 units), W3110/4 (174 units). The activities after growth on glycerol amounted to 32, 24, 38 units/mg protein. The values determined for the wild type corresponded with those in the literature [21]. The complete absence of the λ receptor protein in this mutant reduced the rate of maltose uptake as in other nonsense mutants (Table I), but the growth rate on maltotriose remained unaffected.

The main purpose of collecting more information about the interrelationship between maltodextrin uptake and the λ receptor was to compare it with the other receptor-facilitated uptake systems which we and others study. In contrast to the concentration dependent uptake of maltose into missense and nonsense mutants, *ton*A mutants lacking the T5 receptor proteins do not take up iron supplied as ferrichrome complex at various concentrations [4]. Revertants or partial revertants have fully or partially regained all the *ton*A dependent functions such as infection by the phages T5, T1, ϕ80, colicin M, sensitivity against the antibiotic albomycin and transport of ferrichrome [4]. The main difference in the maltose system and the systems which transport iron as ferrichrome or enterochelin complexes, or the vitamin B12 uptake [32], is that even high concentrations of maltose do not prevent binding of phage λ, whereas ferrichrome inhibits binding of phages T5, ϕ80 [5], killing of colicin M [3], ferric enterochelin prevents killing by colicin B [9], and vitamin B12 inhibits adsorption of phage BF23 [32]. This is in line with the observations that missense *lam*B mutants transport maltose to various extents, but some with rates very close to the wild type, even at low maltose concentrations.

The results suggest that binding of the λ phage and the specificity conferring interaction of maltose and the λ receptor are not identical reactions. The same situation seems to hold for the T6/colicin K receptor protein involved in the translocation of nucleosides across the outer membrane [15] since nucleosides do not inhibit T6 or colicin K binding. It is open at present whether in these cases different sites at the receptor proteins are involved or whether the number of sites differ which are important for binding of the macromolecules and the translocation of the small substrates. It looks as if the various systems only differ in the extent of the common binding sites of the phages, the colicins and the small molecules. It will be important to study mutants which have only amino acid exchanges in the receptor proteins to unravel the details of their multifunctional properties.

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