JOURNAL OF BACTERIOLOGY, Sept. 1997, p. 5783–5788 Vol. 179, No. 18
0021-9193/97/$04.00+0

Identification of a New Porin, RafY, Encoded by Raffinose Plasmid pRSD2 of *Escherichia coli*

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Received 24 March 1997/Accepted 16 July 1997

The conjugative plasmid pRSD2 carries a *raf* operon that encodes a peripheral raffinose metabolic pathway in enterobacteria. In addition to the previously known *raf* genes, we identified another gene, *rafY*, which in *Escherichia coli* codes for an outer membrane protein (molecular mass, 53 kDa) similar in function to the known glycoporins LamB (maltoporin) and ScrY (sucrose porin). Sequence comparisons with LamB and ScrY revealed no significant similarities; however, both *lamB* and *scrY* mutants are functionally complemented by RafY. Expressed from the *tac* promoter, RafY significantly increases the uptake rates for maltose, sucrose, and raffinose at low substrate concentrations; in particular it shifts the apparent K_m for raffinose transport from 2 mM to 130 μM. Moreover, RafY permits diffusion of the tetrasaccharide stachyose and of maltodextrins up to maltoheptaose through the outer membrane of *E. coli*. A comparison of all three glycoporins in regard to their substrate selectivity revealed that both ScrY and RafY have a broad substrate range which includes α-galactosides while LamB seems to be restricted to malto-oligosaccharides. It supports growth only on maltodextrins but not, like the others, on raffinose and stachyose.

The first step in carbohydrate metabolism of gram-negative bacteria is the permeation of the substrate through the outer membrane. Molecules up to a mass of about 600 Da can enter the periplasm through the general porins OmpF and OmpC in *Escherichia coli*; however, tetrasaccharides and larger molecules need specific glycoporins, such as LamB or ScrY (for recent reviews, see references 16 and 31).

The maltoporin LamB of *E. coli*, which also functions as a phage λ receptor (19), is required for maltodextrin catabolism. In contrast to the general porins, it contains specific sugar-binding sites that facilitate diffusion of oligosaccharides through the pore (4, 14, 15). Moreover, the maltoporin also enhances the uptake of mono- and disaccharides (e.g., glucose and trehalose) at low substrate concentrations. The gene *lamB*, which is part of the chromosomal *mal* regulon, is normally induced by maltose, maltodextrins, and trehalose. It seems, however, that the endogenously induced level of the *mal* regulon is high enough for LamB to play an important general role in the carbohydrate transport of *E. coli* (8, 12).

The second glycoporin, ScrY (11, 22, 23), was found to be involved in the sucrose catabolic pathway encoded by the enterobacterial conjugative plasmid pUR400 (25). Although the sequence similarity between LamB and ScrY is very low, the latter can substitute for the maltoporin in *lamB* mutants, except for the function as a λ receptor (23). ScrY also contains sugar-binding sites (26).

Here we describe a third glycoporin in enterobacteria, which is also encoded by a conjugative plasmid first isolated from an *E. coli* strain found in a chicken egg (7). This plasmid, pRSD2, is known to enable *E. coli* K-12 cells to grow on raffinose by providing a raffinose permease (encoded by *rafB*), an invertase (encoded by *rafD*), and an α-galactosidase (encoded by *rafA*). These known *raf* genes were shown to constitute a *raf* operon (*rafABD*), which is negatively regulated by the RafR repressor (*rafR*) (1). However, a gene coding for a glycoporin was not found earlier.

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture conditions. *E. coli* strains and plasmids used in this study are listed in Table 1. Cells were aerobically grown either in minimal phosphate medium (29) supplemented with 0.2% carbohydrates or in Lennox broth (13) without glucose (LBo). Antibiotics were used at concentrations of 10 mg/liter for tetracycline, 25 mg/liter for chloramphenicol, and 100 mg/liter for ampicillin, kanamycin, and spectinomycin.

DNA techniques and analysis. Isolation of plasmids, restriction analysis, and cloning procedures were carried out as described elsewhere (3). The nucleotide sequence of *rafY* was determined by the method of Sanger with the T7 polymerase sequencing kit from Boehringer Mannheim according to the supplier's protocol. Both strands of overlapping restriction fragments were sequenced. Computer analysis was done by using the BLAST programs and database services provided by the National Center for Biotechnology Information, Bethesda, Md.

Plasmid construction. To construct pUSL01, a 3.5-kb *Eco*RI/*Hin*dIII fragment carrying *rafR* and *rafA* from pRSD2 was cloned into pSU19. This plasmid was then used to clone *rafY* as a 4.4-kb *Sma*I fragment from pRSD2 into the *Eco*RV site in *rafA* to yield pUSL7 (Fig. 1). pUSL714 is a pBR322 derivative with *rafY* inserted as a 2.5-kb *Eco*RV fragment into the *Sca*I site within the *bla* gene. Using this plasmid, we inactivated *rafY* by an insertion of the *kan* gene from Tn*1737Km* (30) into the *Hpa*I site of *rafY*, yielding pUSL715, which was then used for the allelic exchange of *rafY* in pRSD2 and pRSD2-1.

Gel electrophoresis and transport assays. Outer membrane fractions were prepared from exponentially growing cells by a two-step sucrose gradient centrifugation, and membrane proteins were separated by gel electrophoresis as described earlier (23). Initial uptake rates for [^{3}H]raffinose, [^{14}C]sucrose, [^{14}C]lactose, and [^{14}C]maltose were determined by filtration of the cells 30, 60, and 90 s after the addition of the substrates (25).

Nucleotide sequence accession number. The nucleotide sequence presented in this paper was entered into GenBank under accession no. U82290.

RESULTS

Raffinose plasmid pRSD2 enables a *lamB* mutant to grow on maltotetraose. The *E. coli* K-12 mutant PS9, which lacks the *lamB*-encoded maltoporin, is unable to grow on maltotetraose as a sole carbon source. When the raffinose plasmid pRSD2-1, expressing the *raf* genes constitutively, was transferred to PS9 growth on maltotetraose was restored. The inducible plasmid pRSD2, in contrast, required low concentrations of melibiose (0.3 mM) for the induction of the *raf* operon to promote growth. PS9, harboring the previously known *raf* genes *rafA*, *-B*, *-D*, and

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant characteristics	Reference or source
E. coli K-12		
PS9	F^- λ^+ $LamB^-$	23
TKW22851	*polA*	18
Plasmid		
F′ scr35-6	Tra^+ Tc^r *lacIZ*$^+$*Y*$^+$ *scrK*$^+$*YA*$^+$*B*$^+$*R*	23
pRSD2	Tra^+ Raf^+	7
pRSD2-1	Tra^+ *rafRA*$^+$*B*$^+$*D*$^+$*Y*$^+$	24
pUR400	Tra^+ Tc^r Scr^+	25
pUR404	Tra^+ Tc^r *scrK*$^+$*Y*$^+$*A*$^+$*B*$^+$*R*	25
pRU600	Cm^r *rafA*$^+$*B*$^+$*D*$^+$ in pACYC184	1
pPSO101	Cm^r *scrA*$^+$ in pACYC184	23
pPSO112	Ap^r Sp^r *scrY*$^+$ in pBR322	23
pTROY9	Tc^r *lamB*$^+$	10
pUSL01	Cm^r *rafR*$^+$*A*$^+$ in pSU19	This work
pUSL7	Cm^r *rafY*$^+$ inserted in *rafA* of pUSL01	This work
pUSL20	Tra^+ Km^r *kan*$^+$ inserted in *rafY* of pRSD2	This work
pUSL21	Tra^+ Km^r *kan*$^+$ inserted in *rafY* in pRSD2-1	This work
pUSL70	Cm^r *rafY*$^+$ in pSU19	This work
pUSL77	Cm^r *lacI*^{q} with P_{tac} in front of *rafY*$^+$ of pUSL70	This work
pUSL714	Tc^r *rafY*$^+$ in pBR322	This work
pUSL715	Tc^r Km^r *kan*$^+$ inserted in *rafY* of pUSL714	This work

a deleted allele of *rafR* (repressor) cloned in pRU600, was not able to metabolize maltotetraose. These findings prompted us to search for another *raf* gene on pRSD2 encoding a porin that allows diffusion of oligosaccharides through the outer membrane of *E. coli.*

Cloning of *rafY*. Speculating that the putative porin gene (*rafY*) could map within or near the *raf* operon, we first cloned both adjacent regions from pRSD2 into the low-copy vector pUSL01, a pSU19 derivative containing the genes *rafR* and *rafA*. We used this vector to avoid a possible unregulated overexpression of *rafY*, which could be deleterious to the cells, as is the sucrose porin, ScrY (23). The first evidence for a porin was obtained with plasmid pUSL7 (Fig. 1), in which a 4.4-kb *Sma*I fragment from the downstream region of gene *rafD* was cloned into pUSL01. PS9 cells harboring pUSL7 could grow slowly on maltotetraose. This is probably due to a very low expression of *rafY*, because normal growth was achieved with plasmid pUSL70 (Fig. 1), in which *rafY* had been cloned as a 2.5-kb *Eco*RV fragment downstream of the *lacZ* promoter in pSU19.

Nucleotide and deduced amino acid sequences of *rafY*. A stretch of 2 kb (map positions 6.4 to 8.4 in Fig. 1) has been sequenced, thus overlapping the previously known sequence of the *raf* operon by 100 bp at the *Sal*I site at map position 6.5. An open reading frame with a length of 1,392 bp was found, starting 599 nucleotides downstream from the 3′ end of *rafD* and showing the same transcriptional polarity as the known *raf* genes (Fig. 1 and 2). The deduced amino acid sequence predicts a protein of 464 mostly hydrophilic residues (calculated molecular mass, 52.7 kDa). A more detailed discussion of this sequence and its comparison with *lamB* and *scrY* is given below.

RafY is an outer membrane protein. To determine the cellular location of the *rafY* gene product, we first cloned the strong P_{tac} promoter together with the *lacI*^{q} allele in front of *rafY* as shown in Fig. 1. The resulting plasmid, pUSL77, allowed a controlled overexpression of *rafY* by the addition of IPTG (isopropyl-β-D-thiogalactopyranoside) to the growth medium. PS9 cells harboring this plasmid were grown in LBo medium supplemented with chloramphenicol. Usually after about 2 h of induction cells were harvested and fractionated. Outer membranes were prepared from a two-step sucrose gradient, and membrane proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described before (23). In Fig. 3 the patterns of outer membrane protein bands of IPTG-induced samples (lanes 3 and 4) are compared with those of the plasmid-free control strain, PS9, and uninduced cells of PS9/pUSL77 (lanes 1 and 2, respectively). In samples from induced cultures two additional bands appeared, corresponding to proteins with relative molecular masses of about 52 kDa. Supposing that one of the two bands represents the mature form of the porin, this result would be in good

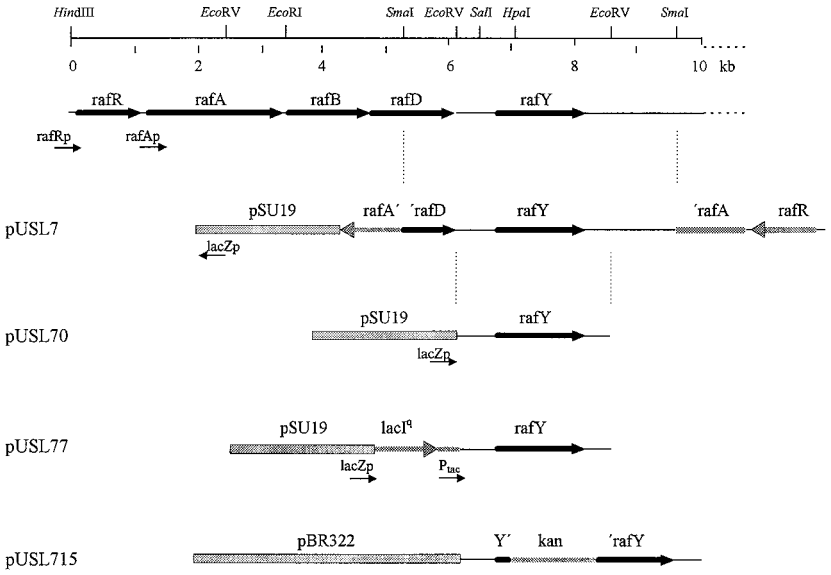

FIG. 1. Map of the *raf* operon (top) and subclones of *rafY*. A physical and genetic map of the *raf* operon from pRSD2 is given along with relevant restriction sites. The region from the *Hin*dIII site (0 kb) to the *Sal*I site at 6.5 kb was taken from Aslanidis et al. (1); mapping of the extended region is a result of this study. The construction of plasmids is described in Results or Materials and Methods. P_{tac}, *trpEp-lacZp* hybrid promoter (9). Vertical dotted lines indicate insertions.

FIG. 2. Nucleotide sequence of *rafY* with the translated amino acid sequence in one-letter code shown beneath. The indicated *Sal*I restriction site in the first line corresponds to map position 6.5 in Fig. 1. Amino acids in the N- and C-terminal parts of RafY considered as characteristic for porins are underlined. The putative ribosome binding site preceding *rafY* is double underlined and italicized.

agreement with the molecular weight calculated from the nucleotide sequence of *rafY*. The second band could be either pre-RafY molecules or a breakdown product of RafY. Similar results were also obtained by using the T7 expression system with different host strains (data not shown). These results indicate that the two bands are not a host- or vector-specific artifact.

RafY decreases the apparent K_m value of raffinose transport through LacY. Since diffusion through the outer membrane is the first step in the uptake of a substrate in gram-negative bacteria, the presence of a porin for this substrate should increase the permeability of the membrane and consequently the overall transport rate. Moreover, because transport through the inner membrane usually follows a saturation kinetic, porins should lower the apparent K_m (K_m^{app}) value for the overall uptake of a substrate by increasing the substrate concentration in the periplasm but should not influence V_{max}, as was shown for the LamB-dependent maltose transport in *E. coli* (28). To test RafY in regard to its porin-specific properties, we measured the raffinose uptake by whole cells at various substrate concentrations in the presence and absence of RafY. Since the substrate specificity of the chromosomal lactose permease (LacY) in *E. coli* is very similar to that of the plasmid-encoded raffinose permease (RafB) (1), we could use IPTG-induced PS9 cells which had been transformed with pUSL70 carrying *rafY* under the control of *lacZp*. The Michaelis-Menten plot in Fig. 4 shows the expected K_m^{app} shift from 2 mM to 130 μM when RafY was present, while V_{max} did not change. Within the concentration range we used, the plot is linear, as was also found for maltose transport by Szmelcman et al. (28) when tested under similar conditions. Obviously, diffusion of raffinose through the general porins is high enough for the low-affinity lactose permease. Otherwise a biphasic plot would have been obtained, as described for porin mutants in which diffusion through the outer membrane becomes limiting (6, 15).

Construction and phenotype of a *rafY* mutant. To test further the physiological relevance of RafY in the pRSD2-mediated raffinose metabolism, we constructed a pRSD2 derivative in which the *rafY* gene had been inactivated by an insertion. A *kan* gene was cloned into the *Hpa*I site of *rafY* in pUSL714, thereby inactivating *rafY*. The resulting plasmid, pUSL715 (Fig. 1), was then transformed into the *polA* mutant TKW22851, harboring pRSD2 and transformants selected on kanamycin plates. Since pUSL715 cannot be replicated in the *polA* strain, mutants were selected in which the *kan* gene was inserted into the Raf plasmid by homologous recombination via the *rafY* regions at both sides of the *kan* gene. Following the same procedure, *rafY* was also inactivated in the constitutive *raf* operon of

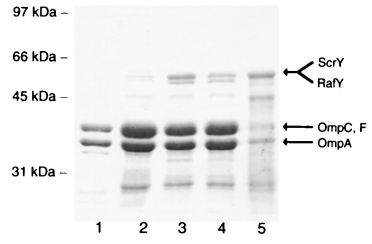

FIG. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of outer membrane proteins from cells grown in LBo medium. PS9 (lane 1) and PS9/pUSL77 (lane 2) were grown without inducer. Lanes 3 and 4 contain membranes from PS9/pUSL77 induced by 10 mM IPTG for 2 and 4 h, respectively. For comparison, a membrane preparation from PS9/pPSO112 expressing the sucrose porin (ScrY) after induction by fructose (0.2%) and IPTG (10 mM) is shown in lane 5. Molecular mass markers are at the left.

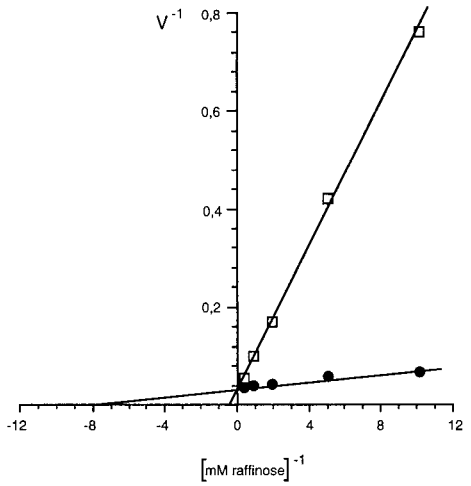

FIG. 4. Effect of RafY on the K_m^{app} value for raffinose uptake via the lactose permease (LacY). The initial uptake rates for [^{3}H]raffinose at various substrate concentrations were determined in PS9 without RafY (open squares) and in PS9/pUSL70 (filled circles) expressing *rafY* after induction by IPTG. Both strains were grown in LBo medium supplemented with chloramphenicol to select for plasmid maintenance. For induction of *lacY* and *rafY*, 2 mM IPTG was added to the cultures 1 h before the cells were assayed. Uptake rates are expressed in nanomoles per minute per milligram of protein and are plotted in a Lineweaver-Burk diagram. V, initial uptake rate.

pRSD2-1. The resulting plasmids, pUSL20 (inducible) and pUSL21 (constitutive), were checked for the *kan* insertion by PCR and finally transferred by conjugation into PS9. All exconjugants had the expected phenotype (Kan^r Tet^s), indicating that an exchange recombination rather than an insertion of pUSL715 had taken place.

Growth experiments in medium containing 3.4 mM raffinose as the sole carbon source showed that even at high raffinose concentrations cells lacking RafY have a markedly reduced growth rate (0.3) compared to that of the wild type (1.0) (Table 2). Since PS9/pUSL20 had no lag phase and because the constitutive mutant, PS9/pUSL21, showed a growth kinetic similar to that of the inducible strain, the slow growth is not caused by a delay of induction. Obviously, diffusion of raffinose through the outer membrane is the limiting step during growth on this substrate. When we used the tetrasaccharide stachyose as the substrate the presence of RafY became even more relevant. While the wild type could grow in minimal stachyose medium at a normal rate, the *rafY* mutant did not grow at all (Table 2).

Effect of RafY on the uptake of various oligosaccharides in *E. coli* K-12. In addition to the tetrasaccharides maltotetraose and stachyose, maltohexaose and maltoheptaose were also found to be substrates for RafY. Growth of PS9 on these oligosaccharides was strictly dependent on the presence of RafY (Table 2). In contrast, the generation time of PS9 with and without the raffinose porin was invariably 1 h in medium containing the disaccharide lactose, maltose, or sucrose (5 mM) as the sole carbon source. For growth experiments on sucrose PS9 cells harboring F′ scr35-6 were used. This plasmid contains the *scr* genes from pUR400 constitutively expressed, except for *scrY*, the gene for the sucrose porin, which is inactivated by a 35-bp insertion (23).

TABLE 2. Effects of glycoporins on the growth rate of *E. coli* K-12 on various oligosaccharides

Strain	Glycoporin	Growth rate on[a]:			
		Raf	Sta	Mal-6-ose	Mal-7-ose
PS9/pUSL21	None	0.3	0	0	0
PS9/pRSD2-1	RafY	1.0	1.0	0.8	0.6
PS9/pUSL21/pUR404	ScrY	1.0	1.0	0.9	0.7
PS9/pUSL21/pTROY9	LamB	0.3	0	0.7	0.7

[a] Cells were grown aerobically at 37°C in minimal medium containing 0.2% carbohydrates and appropriate antibiotics to select for plasmid maintenance. Growth rates are given as generations per hour. Raf, raffinose; Sta, stachyose; Mal-6-ose, maltohexaose; Mal-7-ose, maltoheptaose.

TABLE 3. Effects of glycoporins RafY, ScrY, and LamB on uptake rates for various oligosaccharides in a LamB$^-$ mutant of *E. coli* K-12

Strain	Relevant transport system(s)[a]	Glycoporin	Uptake rate[b]			
			Scr	Mal	Lac	Raf
PS9	LacY, MalEFGK	None	0	22	46	2
PS9/pPSO101	ScrA	None	34	–[c]	–	–
PS9/F′ scr35-6	ScrA	None	21	–	–	–
PS9/F′ scr35-6/pUSL70	ScrA	RafY	616	–	–	–
PS9/pUSL77	MalEFGK	RafY	–	553	–	–
PS9/pUSL70	LacY	RafY	–	–	42	52
PS9/pPSO101/pPSO112	ScrA	ScrY	1,732	–	–	–
PS9/pPSO112	MalEFGK	ScrY	–	866	–	–
PS9/pUR404	LacY	ScrY	–	–	60	34
PS9/pPSO101/pTROY9	ScrA	LamB	34	–	–	–
PS9/pTROY9	LacY, MalEFGK	LamB	–	1,592	26	3

[a] The lactose permease (LacY) and the maltose transport system (MalEFGK) were induced by 10 mM IPTG and 0.2% maltose, respectively. ScrA (EII^{Scr}) is constitutively expressed from both pPSO101 and F′ scr35-6.

[b] Uptake rates are given in picomoles per minute per milligram of protein at initial substrate concentrations of 670 nM.

[c] –, not determined.

At low substrate concentrations (e.g., 670 nM), however, a significant increase in the uptake rate for maltose (25-fold) and sucrose (29-fold) was found when RafY was provided by pUSL70 or pUSL77, while no significant change in lactose transport was observed under these conditions (Table 3). RafY apparently did not increase lactose uptake mediated either by the chromosomal lactose permease (LacY) or by the pRSD2-encoded raffinose permease (RafB).

Substrate range of RafY is similar to that of ScrY but differs remarkably from that of LamB. To allow a comparison of RafY, ScrY, and LamB in regard to their substrate specificities, the influence of the sucrose porin and the maltoporin on oligosaccharide transport in *E. coli* was analyzed in a way similar to that for RafY, described above. The results are summarized in Tables 2 and 3.

Growth experiments were carried out with PS9/pUSL21 expressing the *raf* genes (except *rafY*) constitutively. The sucrose porin was provided by the single-copy sucrose plasmid, pUR404, instead of by the multicopy plasmid, pPSO112, because of the deleterious effect of overexpressed *scrY* (23). The maltoporin was supplied by pTROY9, carrying *lamB* constitutively transcribed from an IS3 promoter. Both PS9/pUSL21/pUR404 and PS9/pUSL21/pTROY9 grew on maltohexaose and maltoheptaose at slightly reduced rates. However, while PS9/pUSL21/pUR404 could also grow on raffinose and stachyose at normal rates, PS9/pUSL21/pTROY9 could not. The slow growth on raffinose (0.3) cannot be attributed to LamB because the control strain, PS9/pUSL21, without any glycoporin showed the same growth rate (Table 2).

To determine the influence of LamB and ScrY on the transport kinetics of different oligosaccharides, we also measured the overall uptake of maltose via the *malEFGK* transport system and that of both lactose and raffinose through the lactose permease LacY. For sucrose uptake EII^{scr} was provided by pPSO101 carrying *scrA* transcribed from its own constitutive promoter (23). LamB was again supplied by pTROY9, and ScrY was supplied either by pPSO112 or by pUR404. (Multicopy plasmids carrying $lacI^q$, like pPSO112 or pUSL77, cannot be used when LacY has to be tested, because of the insufficient induction of the chromosomal *lac* operon even at high IPTG concentrations.) As estimated from the data shown in Table 3, the sucrose porin, ScrY, enhanced the uptake rates for sucrose (51-fold), maltose (39-fold), and raffinose (17-fold) but not significantly for lactose. LamB increased the maltose uptake 72-fold, but no effect was observed on the transport of sucrose, lactose, and raffinose under these conditions.

DISCUSSION

Our results clearly identify the *rafY* gene product as a porin facilitating the diffusion of oligosaccharides up to maltoheptaose across the outer membrane of *E. coli* K-12. The gene was located 599 bp downstream from *rafD* of the *rafABD* operon of pRSD2. Although a transcription terminator structure was postulated behind the 3′ end of *rafD* (1), we consider *rafY* as part of the *raf* operon for the following reasons. (i) When the transcription-terminating omega fragment of pHP45ω (17) was inserted between *rafAp* and *rafY* in pRSD2-1, the resulting plasmid failed to complement the *lamB* mutant PS9, indicating that *rafY* is normally transcribed from the *rafAp* promoter (data not shown). (ii) *rafY* is inducible by melibiose and is constitutively expressed in a $RafR^-$ mutant (pRSD2-1). This phenotype would also correspond to a regulon model; however, we found neither the known *raf* operator sequence (2) in front of *rafY* nor elevated expression of *rafY* when it was cloned in the multicopy plasmid pUSL7. The function of the postulated transcription terminator could be to reduce transcription of *rafY* to a level that is nondeleterious yet is high enough to allow normal growth on oligosaccharides.

The deduced amino acid sequence of RafY corresponds to that of a protein of 464 amino acids. Similarly to other porins, RafY shows a rather hydrophilic amino acid composition (31), but it lacks significant sequence similarity to LamB and ScrY (9 and 14% identical amino acids, respectively). The N terminus of RafY has a positively charged amino acid (K-3) followed by a hydrophobic region and a glycine residue (G-7) as a helix breaker, which are characteristic for signal peptides of exported proteins. In addition, the structure of the C terminus fits the rule of Struyve et al. (27), according to which hydrophobic residues in outer membrane proteins are often found at positions 1, 3, 5, 7, and 9 from the carboxy end (V-456, V-458, F-460, Y-462, and F-464 in RafY) and that most porins end with a phenylalanyl residue.

Due to the lack of significant sequence similarities to both the sucrose and maltose porins, it is not possible to identify from sequence comparisons amino acid residues possibly involved in substrate binding, the "greasy slide" (21), or in the structural features of RafY, e.g., the N-terminal extension of ScrY compared to LamB (23).

For crystallographic analyses and electrophysiological in vitro studies of RafY, highly purified preparations of the porin are a prerequisite. We therefore cloned *rafY* behind the regulated *tac* promoter for controlled overexpression to facilitate purification of the raffinose porin. Indeed, RafY in the outer membrane increased after induction by IPTG, although to a lesser extent than expected (Fig. 3).

Our studies on the physiological relevance of RafY, ScrY, and LamB in regard to the uptake and metabolism of oligosaccharides allow a qualitative comparison of the substrate specificities of these three glycoporins (Tables 2 and 3). Both RafY and ScrY seem to be rather nonspecific, facilitating diffusion of all oligosaccharides tested. While in vitro studies on ScrY revealed a specific substrate binding (26), such experiments remain to be done for RafY. Thus, the designation "raffinose porin" reflects the genetic location of *rafY* as part of the *raf* operon rather than its molecular characteristics. LamB clearly differs from the others in its failure to support growth on stachyose and to increase sucrose transport under the conditions we used.

In *E. coli* the uptake of tetrasaccharides, such as stachyose, and longer oligosaccharides, like maltodextrins, requires glycoporins. The trisaccharide raffinose can penetrate the outer membrane; however, the growth rate is markedly reduced in the absence of RafY and ScrY. Growth on the disaccharides lactose, maltose, and sucrose at the usual substrate concentrations of about 5 mM is not affected by glycoporins, while at low concentrations the uptake of maltose and sucrose can be significantly increased by specific porins. Lactose is unique in that its uptake in *E. coli* K-12 is not enhanced by either RafY, ScrY, or LamB. This means that the diffusion of lactose through the general porin OmpC or OmpF is not the rate-limiting step during uptake, due to the low affinity of the lactose permease to lactose (K_m^{app}, about 1 mM). Glycoporins, such as those for sucrose and maltose, are apparently required only in disaccharide catabolic pathways with high-affinity transport systems (K_m^{app}, about 10 and 1 μM, respectively) (12) but not in low-affinity systems like the sucrose permease CscB (K_m^{app}, about 1 mM) (5, 20). For trisaccharides and larger carbohydrates, however, glycoporins became essential even in combination with low-affinity transport systems, as shown here for the plasmid-encoded raffinose system (K_m^{app}, about 1 mM).

ACKNOWLEDGMENTS

We thank Ulrike Siebers for preparing the photographs.

We also thank the Deutsche Forschungsgemeinschaft (SFB171 TP C4) and the Verband der Chemischen Industrie for financial support.

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