Nucleotide sequence of the *rhaR*–*sodA* interval specifying *rhaT* in *Escherichia coli*

C. GARCIA-MARTIN, L. BALDOMÀ, J. BADÍA and J. AGUILAR*

Department of Biochemistry, School of Pharmacy, University of Barcelona, Diagonal 643, 08028 Barcelona, Spain

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The precise location of the *rhaT* gene, encoding rhamnose permease, has been established between *sodA* and *rhaC* at 3605–3607 kb of Kohara's physical map, which corresponds to 88.4 min on the *Escherichia coli* chromosomal map. The dependence of the activity of the *rhaT* product on the function of *rhaC*, the rhamnose operon regulatory gene, was established by measuring rhamnose transport in wild-type and *rhaC*-deficient strains. The sequence of the *sodA*–*rhaC* interval displayed a single ORF corresponding to *rhaT*, which is transcribed counterclockwise on the *E. coli* chromosome. The ORF was shown to be preceded by a ribosome binding consensus sequence and a catabolite repression protein consensus sequence. The derived amino acid sequence displayed very low homology with any other permease and was clearly dissimilar to the homologous group formed by the xylose, arabinose, galactose and several glucose transporters. Analysis of the *rhaT* primary sequence identified potential membrane-spanning regions, possibly defining a protein structure model different from the one corresponding to the above-mentioned homologous group.

Introduction

Rhamnose utilization requires the function of a specific rhamnose transport system (Baldomà *et al.*, 1990) which, although it has never been demonstrated experimentally, has been assumed to be induced by the presence of the methylpentose. Thus, the structural *rhaT* gene, encoding rhamnose permease, would be under the control of the rhamnose regulatory gene *rhaC*, formed by two overlapping genes, *rhaR* and *rhaS* (Tobin & Schleif, 1989). These regulatory genes control the three other structural genes forming the rhamnose operon: *rhaA*, encoding rhamnose isomerase; *rhaB*, encoding rhamnulose kinase and *rhaD*, encoding rhamnulose-1-phosphate aldolase (Power, 1967).

The *rhaT* gene has been mapped in the *rha* locus at 88.4 min on the *Escherichia coli* chromosome, separated from *rhaA*, *rhaB* and *rhaD* by their regulatory gene *rhaC* (Power, 1967; Badía *et al.*, 1989; Baldomà *et al.*, 1990). The precise location of the three structural genes has been determined by complementation analysis of mutants with different alterations in rhamnose metabolism

and by enzymic assays of strains transformed with recombinant plasmids containing different parts of the cloned rhamnose operon (Badía *et al.*, 1989). In the case of *rhaT*, the gene has been located in a 4.6 kb *Bam*HI–*Eco*RI fragment encompassing *rhaC* by analysis of cotransduction with *glpK* and other *rha* markers, and also by complementation of rhamnose transport mutants characterized by their ability to grow on rhamnose at concentrations of 50 mM, but not at 10 mM (Baldomà *et al.*, 1990). The resulting gene order (counter-clockwise) is thus *glpK*...*rhaT*–*rhaR*–*rhaS*–*rhaB*–*rhaA*–*rhaD*. The *rhaT* gene has been cloned and its product identified by expression in a T7 RNA polymerase/promoter system yielding a 23 kDa protein that has been shown to be located in the membrane (Baldomà *et al.*, 1990). In this report we present the precise location of *rhaT*, which is shown to be under the control of *rhaC*, and we specify and analyse the nucleotide sequence of the *rhaR*–*sodA* interval which contains only the ORF encoding *rhaT*.

Methods

Bacterial strains and plasmids. The *E. coli* strains and plasmids used are listed in Table 1. The strain XL1Blue was used as a host for the Bluescript recombinant plasmids. Mutations in *rhaC* and *rhaA* were obtained by insertion of Tn5 by infecting strain ECL1 (10⁹ cells ml⁻¹) with phage λ 467 (*b221 cIts857 rex::Tn5 Oam29 Pam80*) at a m.o.i. of

* Author for correspondence. Tel. 3 330 79 63; fax 3 490 82 74.

The nucleotide sequence data reported in this paper have been submitted to EMBL and have been assigned the accession number X60699.

Table 1. *E. coli* strains and plasmids used

Strain or plasmid	Relevant genotype	Source or reference
<i>E. coli</i>		
strains		
ECL1	HfrC <i>phoA8 relA1 tonA22 T2^r</i> (λ)	E. C. C. Lin*
JA117	ECL1 <i>rhaC::Tn5</i>	This study
JA118	ECL1 <i>rhaA::Tn5</i>	This study
JA107	ECL1 <i>rhaT::Tn5</i>	Baldomà <i>et al.</i> (1990)
JA109	MC4100 <i>rbs7 pcnB80 rhaT::Tn5 recA::cat</i>	Baldomà <i>et al.</i> (1990)
XL1-Blue	<i>recA1 lac⁻ endA1 gyrA96 thi hsdR17 supE44 relA1 {F' proAB lacZ M15 Tn10}</i>	Stratagene
Plasmids		
Bluescript	Ap ^r <i>lacZ</i>	Stratagene
pLB2	<i>rhaTC</i> (4.6 kb <i>EcoRI</i> - <i>Bam</i> HI fragment) in pBR322, Ap ^r	Baldomà <i>et al.</i> (1990)
pCG1	<i>rhaT</i> (1.6 kb <i>Pst</i> I- <i>Sma</i> I fragment) in Bluescript, Ap ^r	This study

* E.C.C. Lin, Harvard Medical School, Boston, MA, USA.

0.5 (Bruijn & Lupsky, 1984) and selected by their inability to grow on 10 mM-L-rhamnose. The *rhaC* mutant (strain JA117) was identified by the absence of all the enzyme activities of L-rhamnose metabolism (for assays, see below) when cells were grown on 0.5% casein acid hydrolysate in the presence of 10 mM-rhamnose. The *rhaA* mutant (strain JA118) was identified by the absence of rhamnose isomerase activity in its extract, while the rhamnulose kinase and rhamnulose-1-phosphate aldolase levels were the same as those found in wild-type strain ECL1 when grown under the same conditions.

Media and growth conditions. For enzyme and transport assays, cells were grown aerobically in minimal medium (Boronat & Aguilar, 1979) containing 0.5% casein acid hydrolysate and 10 mM-L-rhamnose as inducer.

Selection of Bluescript recombinant plasmids was performed on LB-agar plates containing X-Gal (30 μ g ml⁻¹), IPTG (10 μ g ml⁻¹) and ampicillin (100 μ g ml⁻¹).

DNA manipulation. Plasmid DNA was isolated from *E. coli* by the boiling method described by Sambrook *et al.* (1989). For large-scale preparation, DNA in a crude sample was purified by chromatography on a Qiagen column (Diagen). DNA manipulations such as restriction endonuclease cleavage, ligation, transformation, Southern transfers and hybridization were performed as described by Sambrook *et al.* (1989). DNA fragments were purified using a kit (Geneclean, Bio101).

Nucleotide sequencing. For DNA sequencing, the DNA fragments were subcloned into appropriate Bluescript plasmids (Stratagene) and exonuclease III was used to generate two sets of overlapping deletions of opposite polarity (Henikoff, 1984). The DNA sequence was determined by using the dideoxy-chain termination procedure of Sanger *et al.* (1977) and a sequencing kit (T7 Sequencing Mixes; Pharmacia LKB). Double-stranded plasmid DNA was prepared for use as a template. In order to resolve the numerous sequencing gel compressions, 7-deaza-dGTP and 7-deaza-dITP were used instead of dGTP.

Preparation of cell extracts and enzyme assays. Cell extracts were prepared as described by Boronat & Aguilar (1979). L-Rhamnose isomerase activity was determined from the initial rate of ketose formation measured by the cysteine-carbazole method (Takagi &

Sawada, 1964). L-Rhamnulose kinase and L-rhamnulose-1-phosphate aldolase activities were determined as described by Chiu & Feingold (1964, 1969).

Transport assays. L-Rhamnose permease activity was determined by the rate of L-[¹⁴C]rhamnose [210 mCi mmol⁻¹ (7.77 GBq mmol⁻¹); CEA, Gif-sur-Yvette, France] uptake by whole cells. Cells were harvested and the assay was performed as described for fucose transport by Hacking & Lin (1976) using a rhamnose concentration of 0.2 mM [0.8 mCi mmol⁻¹ (29.6 MBq mmol⁻¹)].

Results and Discussion

Induction of rhamnose permease

The dependence of the structural genes on *rhaC* function has been experimentally demonstrated for *rhaA*, *rhaB* and *rhaD* but not for *rhaT*, a gene generally assumed to belong to the rhamnose operon. The control of *rhaT* expression by *rhaC* was shown by measuring rhamnose transport in cells, derived from wild-type strain ECL1, which are deficient for rhamnose metabolism. As shown in Fig. 1, strain JA118, a rhamnose isomerase mutant, incorporated rhamnose to saturation in 2–3 min, whilst the wild-type strain, ECL1, incorporated the labelled sugar at the same rate and displayed no saturation. Strain JA117 with a mutation in *rhaC*, and hence unable to induce the rhamnose metabolic enzymes, was completely deficient in rhamnose transport. As predicted, an absence of rhamnose transport was also observed in the *rhaT* mutant strain JA107 (Fig. 1). Thus it may be concluded that *rhaC* is required for *rhaT* function. The *rhaT* gene, although inevitably transcribed separately from the other three structural genes as a consequence of the gene order in the rhamnose operon (Baldomà *et al.*, 1990), must be coordinately expressed with these genes under the control of this regulatory gene.

Identification of the *rhaT* gene in plasmid pLB2

Additional evidence for the presence of the *rhaT* gene in the cloning plasmid pLB2 (Baldomà *et al.*, 1990) came from the location of the Tn5 insertion that blocked rhamnose transport in strain JA107 used in isolation of the clone. For this mapping, chromosomal DNA of strains JA107 and ECL1 was digested with *Bam*HI and, after Southern transfer, genomic fragments were analysed using the 2.2 kb *Bgl*II fragment as a probe (Fig. 2). From the number and size of the labelled fragments, the Tn5 insertion was located in the 1.6 kb *Pst*I-*Sma*I fragment at 0.6 kb from the *Pst*I restriction site (not shown), thus localizing *rhaT* in this fragment. Furthermore, sequence data presented in Fig. 4 showed a single ORF between the identified sequences of *rhaR* and *sodA*, leaving no more space for any other ORF that could lead to erroneous identification of *rhaT*.

Fig. 1. Dependence of rhamnose transport on the function of *rhaC* and *rhaT*. Time course of L-[¹⁴C]rhamnose uptake by whole cells is plotted for wild-type ECL1 (○), rhamnose-isomerase-deficient JA118 (●), rhamnose-permease-deficient JA107 (□) and rhamnose-regulator-minus JA117 (■).

Fig. 2. (a) Fragment of the restriction map of Kohara *et al.* (1987) encompassing the *rhaT* gene. The restriction sites for several enzymes are indicated in the horizontal open bars, which are labelled as follows: B, *Bam*HI; H, *Hind*III; E, *Eco*RI; EV, *Eco*RV; Bg, *Bgl*I; K, *Kpn*I; P, *Pst*I; Pv, *Pvu*II. (b) Restriction map of the insert of plasmid pLB2 and its correspondence to the map of Kohara *et al.* (1987). (c) Deletion analysis of the 1.6 kb *Pst*I–*Sma*I insert of plasmid pCG1. The thick line represents the DNA fragment of pCG1. Thinner lines below pCG1 represent the fragments of several subclones constructed by deletion of pCG1, which were used for the complementation analysis of *rhaT* mutations (Table 2). The solid bar represents the *rhaT* coding region. The arrow indicates the direction of transcription.

Table 2. Complementation of the *rhaT* mutation of strain JA109 by different plasmids obtained after deletion of pCG1

Cells of strain JA109 transformed with different plasmids were grown on casein acid hydrolysate in the presence of 10 mM-L-rhamnose as inducer. L-Rhamnose uptake was determined as described in Methods.

Plasmid	L-Rhamnose uptake (nmol min ⁻¹ per 10 ⁹ cells)
pCG1	7.2
pCG1.2	0.0
pCG1.3	0.0
pCG1.9	7.5
pCG1.13	0.0
pCG1.14	0.0

Fig. 3. Sequencing strategy for the 1.6 kb *Pst*I–*Sma*I fragment in plasmid pCG1. The arrows indicate the start-point, direction and extent of sequence determined from each subclone. The open rectangle at the bottom of the figure shows the extent of the *rhaT* gene.

Determination of the *rhaT* coding region

To locate *rhaT* with precision in the 4.6 kb *Bam*HI–*Eco*RI fragment of plasmid pLB2 encompassing *rhaC* (Fig. 2), this construction was further digested with several restriction enzymes. By comparing the restriction map of the *rhaT* region with the corresponding region of the physical map of Kohara *et al.* (1987), we found that the *rhaT* gene is located between 3605–3607 kb on this physical map. However, due to the inversion of this region present in the strain used by Kohara *et al.* (1987) this location does not correlate with the genetic map (Bachmann, 1990) in which *rhaT* is mapped at around 88.4 min on the chromosome. Sequence analysis of the *rhaT* region has shown that the start codon of the ORF of the *rhaT* gene is proximal to the *Bam*HI site and distal to the *Eco*RI site on plasmid pLB2 (Fig. 2). Accordingly, the *rhaT* gene is transcribed counter-clockwise on the *E. coli* chromosome.

10 20 30 40 50 60
 CTGCAGGGTGGTGCCTTTTTTCAGACCTTTCCAGAACAGGCTGTGGTTAGCGTGACCGCC
 70 80 90 100 110 120
 AGCGTTGTTGCGCAGTACGGTTTTCTTGCTGCTGGCAGCTGGTCCAGTTTGGTGATCAG
 130 140 150 160 170 180
 CTCTTCAACCGGCAGGTTGGCAAATTCTGGCAGGCTTTCCAGCGCCGCGTTGGCGTTGTT
 190 200 210 220 230 240
 TACGTAGGTCTGATGGTGTGGTGGTGGATTTCATGGTCTGCTTATCGAAGTGCGG
 250 260 270 280 290 300
 TTCCAGGGCATCGTAAGCATACGGCAGGGATGGCAGGGTATAGCTCATATTCATCTCCAG
 310 320 330 340 350 360
 TATTGTCGGGCGGCCGATTGTTAATGCCGCGTAAGCAGTTGGTTCATTATAGTTAATTAA
 370 380 390 400 410 420
 ATGATATTGAAAATGATTATCAATGCCGTACTTTTCGTAAGGGTATGGTTTTGCAGGAAA
 430 440 450 460 470 480
 ATGCCCGAGATGTGAAGCAAATCACCCACTTAATGCCGTGATTGCCAGTAAATCGACAAC
 490 500 510 520 530 540
 GGCGGCAACAGGCGAAAGGTTAATCGACAGCACGATTTTACACTCATCTCGTCGGAGAT
 550 560 570 580 590 600
 GTGACGCGACGAAAAATGATGAGGATAAGAAGATGAGTAACGCGATTACGATGGGGATAT
 M S N A I T M G I
 610 620 630 640 650 660
 TTTGGCATTGATCGGCGCGGCCAGTGCAGCCTGTTTTACGCTCCGTTCAAAAAAGTAA
 F W H L I G A A S A A C F Y A P F K K V
 670 680 690 700 710 720
 AAAAATGGTCATGGGAAACCATGTGGTCAGTCGGTGGGATTGTTTCGTGGATTATTCTGC
 K K W S W E T M W S V G G I V S W I I L
 730 740 750 760 770 780
 CGTGGGCCATCAGCGCCCTGTTACTACCGAATTTCTGGGCGTATTACAGCTCGTTTAGTC
 P W A I S A L L L P N F W A Y Y S S F S
 790 800 810 820 830 840
 TCTCTACGCGACTGCCTGTTTTCTGTTCCGCGCTATGTGGGGGATCGGTAATATCAACT
 L S T R L P V F L F G A M W G I G N I N

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      850      860      870      880      890      900
ACGGCCTGACCATGCGTTATCTCGGCATGTCGATGGGAATTGGCATCGCCATTGGCATT
Y  G  L  T  M  R  Y  L  G  M  S  M  G  I  G  I  A  I  G  I

      910      920      930      940      950      960
CGTTGATTGTCGGTACGCTGATGACGCCAATTATCAACGGCAATTTGATGTGTTGATTA
T  L  I  V  G  T  L  M  T  P  I  I  N  G  N  F  D  V  L  I

      970      980      990      1000      1010      1020
GCACCGAAGGCGGACGCATGACGTTGCTCGGCGTTCTGGTGGCGCTGATTGGCGTAGGGA
S  T  E  G  G  R  M  T  L  L  G  V  L  V  A  L  I  G  V  G

      1030      1040      1050      1060      1070      1080
TTGTAAGTTCGCGCCGGGCGAGTTGAAAGAGCGCAAGATGGGCATTAAAGCCGAAGAGTTCA
I  V  T  R  A  G  Q  L  K  E  R  K  M  G  I  K  A  E  E  F

      1090      1100      1110      1120      1130      1140
ATCTGAAAAAGGGCTGGTGGTGGCGGTGATGTGCGGCATTTTCTCTGCCGGGATGTCCT
N  L  K  K  G  L  V  L  A  V  M  C  G  I  F  S  A  G  M  S

      1150      1160      1170      1180      1190      1200
TTGCGATGAACGCCGCAAAACCGATGCATGAAGCCGCTGCCGCACTTGGCGTCGATCCAC
F  A  M  N  A  A  K  P  M  H  E  A  A  A  A  L  G  V  D  P

      1210      1220      1230      1240      1250      1260
TGATGTCGCTCTGCCAAGCTATGTTGTATCATGCGGCGGCGGCGGCGATCATTAACTCG
L  Y  V  A  L  P  S  Y  V  V  I  M  G  G  G  A  I  I  N  L

      1270      1280      1290      1300      1310      1320
GTTTCTGTTTTATTCTGCTCTGGCAAAGTGAAGGATTTGTGCTAAAAGCCGACTTCTCGC
G  F  C  F  I  R  L  A  K  V  K  D  L  S  L  K  A  D  F  S

      1330      1340      1350      1360      1370      1380
TGGCAAAATCGCTGATCATTACAAATGTGTTACTCTCGACACTGGGCGGGTTGATGTGGT
L  A  K  S  L  I  I  H  N  V  L  L  S  T  L  G  G  L  M  W

      1390      1400      1410      1420      1430      1440
ATCTGCAATTCTTTTTCTATGCCTGGGGCCACGCCCGCATTCCGGCGCAGTATGACTACA
Y  L  Q  F  F  F  Y  A  W  G  H  A  R  I  P  A  Q  Y  D  Y

      1450      1460      1470      1480      1490      1500
TCAGTTGGATGCTGCATATGAGTTTCTATGTATTGTGCGGCGGTATCGTCGGGCTGGTGC
I  S  W  M  L  H  M  S  F  Y  V  L  C  G  G  I  V  G  L  V

      1510      1520      1530      1540      1550      1560
TGAAAGAGTGGAAACAATGCAGGACGCCGTCCGGTAACGTGTTGAGCCTCGGTTGTGTGGT
L  K  E  W  N  N  A  G  R  R  P  V  T  C  •

      1570      1580      1590      1600      1610      1620
GATTATTGTCGCCGCTAACATCGTCGGCATCGGCATGGCGAATTAATCTTTCTGCGAATT

      1630      1640      1650      1660
GAGATGACGCCACTGGCTGGGCGTCATCCCGGTTTCCCGGG

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rhaR

Fig. 4. Nucleotide sequence of the *rhaR-sodA* interval specifying *rhaT*. The coding region of *rhaT* has been translated and is indicated using the single letter amino acid code. The putative Shine-Dalgarno sequence is boxed. The inverted repeat constituting a good catabolite repression protein consensus binding site is underlined twice. The position and direction of genes *rhaR* and *sodA* encoded in the complementary strand are also indicated. Stop codons are indicated by a dot.

Restriction sites for *Pst*I and *Sma*I in plasmid pLB2 specified a 1.6 kb fragment that was subcloned into a Bluescript vector yielding plasmid pCG1 (Fig. 2), which also complemented *rhaT*. The assay required the *pcnB* mutation, present in strain JA109, to avoid toxicity effects (Baldomà *et al.*, 1990).

Since the *rhaT* gene product has been described by Baldomà *et al.* (1990) as a protein with an estimated molecular mass of 23 kDa, the ORF encoding *rhaT* would probably not exceed the limits of the *Pst*I–*Sma*I fragment. This would also be true if, as indicated by Maiden *et al.* (1988) for other hydrophobic proteins, the 23 kDa estimated by electrophoresis for rhamnose permease corresponded to more than 30 kDa or a protein of over 300 amino acids. These data imply that the *Pst*I–*Sma*I fragment includes the entire *rhaT* and that no part of the gene was beyond its limits.

Several nuclease deletions were obtained from plasmid pCG1. The deletions were checked for their capacity to complement *rhaT* mutant strain JA109 by determining the rhamnose uptake of this strain when transformed with the plasmids containing the different deletions (Table 2). Since only pCG1 and pCG1.9 restored L-rhamnose uptake, the limits of *rhaT* were thus determined more precisely.

Nucleotide sequence of the *rhaT* coding region

Plasmid pCG1 was used for sequencing the genomic insert and a diagram of the sequencing procedure is shown in Fig. 3. The sequence of the 1661 bp *Pst*I–*Sma*I fragment, derived from both DNA strands and fully overlapped, displayed an ORF from positions 573 to 1544 encoding a single 323 amino acid polypeptide of 35320 Da (Fig. 4) which was identified as rhamnose permease on the basis of the complementation data indicated above. In the 3'-flanking region of the *rhaT* ORF, the complementary sequence at the 3' end of *rhaR* (Tobin & Schleif, 1989) was identified. In addition, 5' to *rhaT*, another ORF started for a divergently transcribed gene which corresponded to *sodA* as described by Takeda & Avila (1986). Normal codon usage was found for the *rhaT* ORF according to the method of Ikemura (1981).

Features of the *rhaT* primary sequence

A good ribosome binding site is found at position 561–565, 8 nucleotides upstream of the ATG initiation codon of *rhaT* (Fig. 4). A TGTGA motif appears at position 431–435 with a highly conserved inverted repeat 6 nucleotides downstream, constituting a good catabolite repression protein consensus binding site (Fig. 4). However, no clear –10 or –35 consensus sequences for

Fig. 5. Hydropathic profiles of (a) L-rhamnose permease and (b) xylose-H⁺ transporter. Hydropathy values (Kyte & Doolittle, 1982) for a window of nine amino-acid residues were averaged and plotted according to the position of the middle residue along the length of each sequence. Bars denote predicted membrane-spanning β -strands for L-rhamnose permease.

RNA polymerase σ^{70} binding are apparent, which is not unusual in a gene belonging to an operon under positive control (deCrombrughe *et al.*, 1984; Collado-Vides *et al.*, 1991).

The *rhaT* ORF encodes a highly hydrophobic protein (47% hydrophobic residues), as would be expected for a cytoplasmic membrane protein. Comparison of the deduced amino acid sequence of *rhaT* revealed no significant similarity to sequences in the GenBank protein data bank or to any of the transporters of the homologous group integrated by xylose, arabinose, galactose or different glucose transporters (Henderson, 1990). The highest score (18% homology) was for a 156 amino acid overlap with the *xylE* product, xylose permease (Davis & Henderson, 1987). This absence of homology seems to indicate that rhamnose permease belongs to a different family of transporters, perhaps integrated by as yet undescribed transport proteins other than those noted above. The rhamnose permease reported here does not have the two fundamental features indicated by Henderson & Maiden (1990) as initial clues to classify a permease in a homologous group, namely: 12 membrane-spanning helices and a repeated motif RXGRR, proposed to form a β -turn linking adjacent helices. Furthermore, none of the amino acid residues, consensus motifs or central hydrophilic regions conserved in the xylose, arabinose, galactose and different glucose transporters group are apparent in the *E. coli* rhamnose permease. Other transport proteins with dissimilar primary sequences have been reported, such as lactose, melibiose and fucose permeases (Henderson, 1990). None of them, however, presented homology with the rhamnose permease. The molecular

mass of the rhamnose permease of *Salmonella typhimurium* (37390 Da) is similar to that of *E. coli* (35320 Da) and lower than the molecular mass reported for other permeases (Henderson, 1990), but the *S. typhimurium* *rhaT* sequence has not been published and comparison is not possible.

In spite of the low homology between *E. coli* rhamnose and xylose permeases, the hydrophobicity profiles (Kyte & Doolittle, 1987) of the deduced amino acid sequences of the two proteins, are similar, but with two important differences (Fig. 5). First, the highly hydrophilic stretch of 50 amino acids of xylose permease (position 220–270) seems to be absent in rhamnose permease. Second, the hydrophobic domains determined by the sequences from amino acid 340 to the C-terminal end of xylose permease are absent in rhamnose permease.

Based on this hydropathy plot and on structure predictions of proteins, according to the Garnier algorithm (Garnier *et al.*, 1978), eight potential β -strand membrane-spanning regions of at least 10 amino acids were estimated and these are indicated in Fig. 5. This could lead to a closed β -barrel structure as described by Jähnig (1990), in a model for the folding of this transport protein, alternative to the twelve α -helices of the homologous group of transporters described by Henderson & Maiden (1990). Absence of homology to other transporters of which the structure has been established does not allow alignment and comparison to ascertain the features of the transmembrane regions.

The N-terminal part of the *rhaT*-derived amino acid sequence showed some similar features to those of signal peptides. The sequence displayed (i) presence of charged residues close to the N-terminal, (ii) a hydrophobic midsection with an Ile residue at position 9, usually conserved as a Leu or Ile at position 8 (Scripture & Hogg, 1983) or position 9 (Bedouelle *et al.*, 1980), and (iii) an Ala-X-Ala described as immediately preceding the cleavage site between the signal peptide and the mature protein (Scripture & Hogg, 1983). Determination of the N-terminal structure of the mature protein is required to confirm processing of this 19 amino acid putative signal peptide. It is worth noting that not all these features of the signal peptides are conserved in the homologous group of transporters analysed by Henderson, (1990).

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