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The lactose carrier of *Klebsiella pneumoniae* M5a1; the physiology of transport and the nucleotide sequence of the *lac*Y gene

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A comparison has been made between the physiology and amino acid sequence of the lactose carriers of *Klebsiella pneumoniae* M5a1 and *Escherichia coli* K-12. The membrane transport of lactose was much weaker in *Klebsiella* than in *E. coli*. On the other hand *o*-nitrophenylgalactoside uptake by *Klebsiella* was distinctly greater than with *E. coli*. In spite of the differences in sugar transport between the two organisms, the amino acid sequences of the respective lactose carriers were remarkably similar (60% of the amino acids are identical).

Introduction

A number of bacteria are capable of growing on lactose as a sole source of carbon. *Klebsiella pneumoniae* is one organism that possesses this property. A comparison with *Escherichia coli*, another lactose-positive cell in which the lactose operon has been extensively studied, shows that the arrangement of genes in the two cell types is similar [1]. As with *E. coli*, *Klebsiella* possesses a *lac*I gene producing a repressor, a *lac*Z gene coding for β-galactosidase and a *lac*Y gene which produces the lactose transport protein. Compared with the corresponding *E. coli* sequence [2,3] the nucleotide sequence of the *Klebsiella* T17R1 *lac*I and *lac*Z genes are 49% and 61% conserved [4]. The *Klebsiella lac*I gene is transcribed in the opposite direction to the *lac*I of *E. coli* [4].

A striking difference in the physiology of lactose metabolism in the two organisms is that *K. pneumoniae* is only weakly lactose positive in fermentation tests and grows on lactose at half the rate of *E. coli* [5,6]. Although the lactose carrier of *Klebsiella* is capable of accumulating the non-metabolizable thiomethyl-β-D-galactoside, it shows only half the activity of the carrier in *E. coli* [7].

Since there appeared to be significant differences between the lactose fermentation properties of the two organisms it was considered of interest to compare the transport properties in the two organisms and then to determine the nucleo-

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Abbreviations: TMG, thiomethyl-β-D-galactoside; ONPG, *o*-nitrophenyl-β-D-galactoside; IPTG, isopropylthio-β-D-galactoside.

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tide sequence of the *lac*Y gene in *K. pneumoniae* and compare the amino acid sequence with that of *E. coli* [8]. The conserved regions of the polypeptide might provide some insight as to the essential amino acid sequence for lactose recognition and transport. The results indicate that the pattern of sugar transport is different in the two organisms. In spite of this rather different sugar recognition there is considerable similarity to the *E. coli lac*Y gene (60% conservation of the amino acid sequence).

Materials and Methods

Bacterial strains and plasmids. The *Klebsiella pneumoniae* strain used in this work was M5a1. For transport comparisons *E. coli* strain 3000 (*lac*-I + Z + Y + A +) was used. DW2 was isolated from a TN10-containing strain of *E. coli* by the method used to obtain DW1 as described elsewhere [9]. Other strains used in this study are given in Table I.

The *lac*Y gene of *Klebsiella pneumoniae* M5a1 was cloned into pBR322 by Hitchin and Reeve [11]. This plasmid (pHE7) consists of a *Hin*dIII fragment of approximately 2500 base pairs which contains the *lac*Y gene. When an *E. coli* strain containing β-galactosidase (*lac*Z$^+$) but no membrane carrier (*lac*Y$^-$) was transformed by the plasmid pHE7 the transformants became lactose positive.

A promoter mutation that elevated the expression of the *Klebsiella lac*Y gene in plasmid pHE7 was isolated as follows: DW2/pHE7 was streaked on melibiose MacConkey plates. After 18 h incubation at 37°C all clones were white but red papilli appeared after another 24 h at room temperature. These melibiose-positive cells were restreaked to purify. Plasmid DNA from these melibiose-positive mutants and from the parental strain were isolated. The DNA from each strain was treated with *Hin*dIII and the pBR322 DNA and the 2500 bp insert DNA isolated. When normal *lac*Y insert was ligated to the mutant pBR322 the melibiose-positive phenotype was obtained. When *lac*Y from the mutant was ligated to the normal pBR322 a normal phenotype was obtained. It was concluded that the mutation was in pBR322, presumably the Tet promoter.

Restriction mapping. In order to isolate the *lac*Y gene the plasmid DNA of pHE7 was prepared by the method of Birnboim and Doly [13]. Plasmid DNA was then digested with *Hin*dIII to remove the insert and the digest subjected to gel electrophoresis in 1% agarose plus ethidium bromide. The DNA bands were identified under long-wavelength ultraviolet light. Slots were cut in the gel immediately in front of and behind the 2.5 kb band and small pieces of ion-exchange paper (DE81 from Whatman) were inserted into the slots. The gel was returned to the electrophoresis bath and current applied until the DNA had moved forward into the paper. The paper was removed, washed twice with 100 mM NaCl, 10 mM Tris (pH 8), 0.1 mM EDTA and the DNA extracted from the paper with two aliquots of 1 M

TABLE I

STRAINS

Name	Genotype	Source
Bacteria		
Klebsiella pneumoniae M5a1	*lac*I+Z+Y+	F. Ausabel and E. Kashket
E. coli 3000	*lac*I+Z+Y+A+	J. Monod [10]
E. coli DW2	*lac*I+ ΔZY *mel*A$^+$ΔB	This paper
Plasmids [a]		
pHE7	*Klebsiella lac*Y	Hitchin and Reeve [11]
pHE7 (promoter)	*Klebsiella lac*Y with a promoter mutation	This paper
pHE7 (HIS 210)	*Klebsiella lac*Y with Arg-210 changed to histidine	This paper
pTE18	*E. coli lac*Y	Teather et al. [12]

[a] All plasmids are derivatives of pBR322.

NaCl, 1 mM EDTA. The DNA solution was then extracted with phenol and the DNA precipitated with ethanol and dried. The *Hin*dIII insert was digested with *Hin*cII and with *Pst*I and the fragments separated by agarose electrophoresis.

The DNA fragments were ligated to the replicative form of either M13 mp18 or M13 mp19 which had been linearized by digestion with *Hin*dIII, *Hin*cII, *Pst*I or a combination of two of these restriction enzymes. After transfection of strain JM101 single clear plaques were picked from soft agar plates containing 5-bromo-4-chloro-3-indolyl-β-D-galactoside. Single-stranded DNA from the recombinant M13 virus was purified and subjected to electrophoresis on agarose gel to determine the size of the insert.

Nucleotide sequencing. The dideoxynucleotide chain termination method of Sanger et al. [14] was used for sequencing. In many experiments a kit (New England Biolabs) consisting of enzymes and 15-bp primer was used. When GC compression was encountered the 'Sequenase' kit (U.S. Biochemical, Cleveland, OH) was used.

Oligonucleotide primers. A major fraction of the sequencing was carried out by oligonucleotide (17 bp) primers prepared with a DuPont Coder 300 Synthesizer (DuPont Biotechnology Systems, Wilmington, DE).

Site-specific mutagenesis. Oligonucleotide site specific mutagenesis was carried out by the method of Eckstein [15,16]. Reagents for this procedure were provided by Amersham (RPN 2322).

Sugar transport assays. Cells were grown at 37°C in Medium 63 [17] containing 1% Tryptone (DIFCO) plus 0.5 mM isopropyl-β-thiogalactoside to mid-logarithmic phase. They were then centrifuged, washed once with 100 mM potassium phosphate buffer (pH 7) and resuspended in a small volume of the same buffer at 0°C. Prior to the assay cells were equilibrated at 25°C in phosphate buffer. Radioactive sugar was then added and 0.2 ml samples (containing approx. $5 \cdot 10^8$ cells) were filtered through a 25 mm membrane filter (pore size = 0.65 μm). The cells on the filter were then washed with 5 ml phosphate buffer. Filters were placed in plastic vials and radioactivity was determined by liquid scintillation counting using Liquiscint (National Diagnostic, Somerville, NJ).

Reagents. Lactose, melibiose and TMG were purchased from Sigma. [^{35}S]Deoxyadenosine 5'-triphosphate and [^{14}C]lactose were from Amersham. [^{3}H]Melibiose was from the French Atomic Energy Commission and was a gift from Dr. Gérard LeBlanc. [^{3}H]ONPG was obtained from New England Nuclear. The phage M13 mp18 and M13 mp19, restriction enzymes, DNA polymerase I (Klenow Fragment), T4 DNA ligase and other reagents for sequencing were obtained from New England Biolabs. Isopropyl-β-D-thiogalactoside (IPTG) and 5-bromo-4-chloro-3-indolyl-β-D-galactoside were obtained from Boehringer Mannheim.

Proton uptake. The method of West [18] was used for the measurement of proton uptake. Cells were washed twice in 120 mM choline chloride plus 1 mM 2-mercaptoethanol and resuspended in the same medium to a density approximately equivalent to 70 mg dry weight/ml. The suspension was diluted 1/5 in choline chloride containing 10 mM KSCN. A sample of cells (2.5 ml) was placed in a 3 ml plastic vial with the lid cut to fit over an inserted pH electrode. A small vent in the lid was used for the introduction of nitrogen and a second vent for substrate addition. N_2 was passed through one vent and passed over the surface of the suspension, which was mixed with a magnetic stirrer. After 30–40 minutes the assay was started by the introduction of a small volume of anaerobic TMG.

Results

Sugar transport by Klebsiella M5a1

A comparison was made of sugar transport in *Klebsiella* M5a1 with that in *E. coli*. *Klebsiella pneumoniae* M5a1 grows on lactose as a sole source of carbon and energy, although its growth is about half the rate of *E. coli* 3000 (at a lactose concentration of 6 mM). On lactose MacConkey indicator plates (30 mM sugar) the M5a1 clones show red centers while *E. coli* are completely red in color. This indicates that lactose fermentation is somewhat less pronounced in *Klebsiella*.

When washed cells (induced with 0.5 mM IPTG) were exposed to radioactive lactose at a concentration of 0.1 mM, *E. coli* took up the sugar much more effectively than *Klebsiella* cells (Fig. 1). Since the discrepancy between the trans-

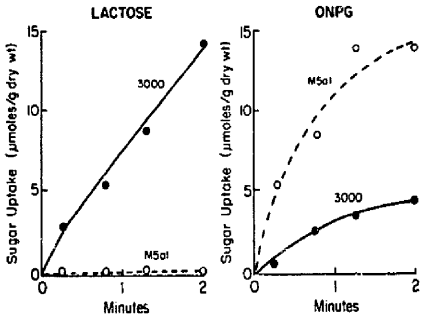

Fig. 1. Lactose and β-ONPG transport by *K. pneumoniae* M5a1 and *E. coli* 3000. The left hand panel shows an experiment in which transport was initiated by the addition of [^{14}C]lactose to give a final concentration of 100 μM (0.1 μCi/ml). The right-hand panel shows an experiment in which [^{3}H]β-ONPG was added to give a final concentration of 50 μM (0.2 μCi/ml).

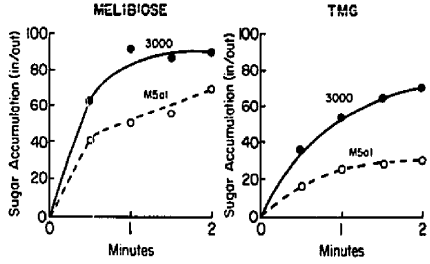

Fig. 2. Uptake of melibiose and TMG by *K. pneumoniae* M5a1 and *E. coli* 3000. The left-hand panel shows an experiment in which transport was initiated by the addition of [^{3}H]melibiose to give a final concentration of 100 μM (0.5 μCi/ml). In the experiment shown in the right hand panel TMG was added to give 100 μM (0.1 μCi/ml).

port rates in the two strains is much greater at low than at high substrate concentrations it is probable that the affinity for lactose by the M5a1 carrier is less than that in *E. coli*.

The transport rate of *o*-nitrophenyl-β-galactoside (ONPG), on the other hand, is considerably better in *Klebsiella* than in *E. coli*. The 2 min uptake of ONPG (0.1 mM) by M5a1 was about 3-times greater than by 3000. With both ONPG and lactose there is hydrolysis of the sugar by the intracellular β-galactosidase. In the case of *E. coli* it is known that the activity of the enzyme exceeds the activity of the transport system. Since these two sugars are rapidly hydrolyzed within the cell the sugar entry is thermodynamically 'down hill' (i.e., no sugar accumulation occurs). In the case of *Klebsiella* it is probable that the transport rate is also rate limiting although this has not been studied in detail.

Two sugars were tested which are not metabolized in these cells and marked intracellular accumulation was observed. When *E. coli* cells were exposed to 0.1 mM TMG they accumulated the sugar in 2 min to an intracellular concentration 70-times that in the external medium. Cells of M5a1 accumulated the sugar 30-fold. Melibiose accumulation shows a similar pattern (Fig. 2). There is a separate α-galactoside transport system in both *Klebsiella* and in *E. coli*, but it is not induced under the conditions of these experiments.

The relative affinities of several sugars for the lactose carrier were tested by studying their inhibition of TMG transport. In *E. coli* 3000 lactose and thiodigalactoside produce a strong inhibition of TMG transport while ONPG is somewhat less effective (Table II). The carrier of *Klebsiella pneumoniae* M5a1, on the other hand, is strongly inhibited by ONPG while lactose (1 mM) has no effect. Table II also shows the percentage inhibition of these systems by two different concentrations of *p*-hydroxymercuribenzoate. M5a1 is more

TABLE II

INHIBITION OF TMG TRANSPORT

Inhibitor	% inhibition	
	E. coli 3000	*K. pneumoniae* M5a1
Lactose (1 mM)	86	0
Thiodigalactoside (1 mM)	100 [a]	67
o-Nitrophenylgalactoside (4 mM)	93	99
o-Nitrophenylgalactoside (1 mM)	68	99
p-Hydroxymercuribenzoate (0.1 mM)	89	100 [b]
p-Hydroxymercuribenzoate (0.01 mM)	40	92

[a] Uptake in the presence of 1 mM thiodigalactoside was taken as the 'blank' (100% inhibition) and subtracted for the other values for *E. coli* 3000.

[b] Uptake in the presence of 0.1 mM *p*-hydroxymercuribenzoate was taken as the 'blank' and subtracted from the other values for *K. pneumoniae* M5a1.

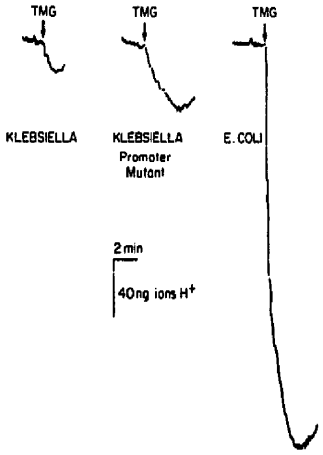

Fig. 3. Proton uptake induced by TMG in DW2/pHE7 and DW2/pTE18. The plasmid pHE7 contains the *lac*Y gene of *Klebsiella* while pTE18 contains the *lac*Y gene of *E. coli*. The method for H^+ uptake is described in Materials and Methods. Thiomethylgalactoside (25 µl of 500 mM) was added at the arrow. Down represents alkalinization of the medium.

sensitive to this sulfhydryl-modifying compound than is *E. coli* 3000.

Evidence for proton-β-galactoside co-transport was provided by measurement of proton uptake on the addition of sugar by the method of West [18]. When TMG was added to give a final concentration of 5 mM the external medium showed alkalinization. West [18] has shown that such behavior is associated with proton entry into the cells. With M5a1 definite proton uptake with TMG addition was observed but the rate was only about 20% of that of *E. coli* 3000. Additional experiments were carried out in *E. coli* cells containing the *lac*Y gene of the two cell types cloned separately onto the plasmid pBR322. Proton uptake data with such cells are shown in Fig. 3. Addition of TMG to cells containing the plasmid with *Klebsiella lac*Y was followed by alkalinization. A cell containing a similar plasmid but with a promoter mutation showed a greater rate of proton uptake. These cells with the promoter mutation were found to transport all substrates at rates about twice that of the parent. Cells containing the cloned *lac*Y gene of *E. coli* showed a much more pronounced alkalinization on the addition of TMG.

Sequencing of the lacY gene of Klebsiella M5a1

Hitchins and Reeve [9] have recently cloned the chromosomal *lac*Y gene of *Klebsiella pneumoniae* M5a1 into the plasmid vector pBR322. The insert consisted of a 2500 bp *Hin*dIII fragment containing the *lac*Y gene plus a portion of the adjacent *lac*Z gene. This *Hin*dIII fragment was digested with several restriction enzymes and the restriction sites for the two most useful enzymes (*Hin*cII and *Pst*I) are shown in Fig. 4.

The DNA fragments resulting from the digestion with these three restriction enzymes were cloned into M13 and several were sequenced. The smaller fragments from *Hin*cII digestion were subcloned into both M13 mp18 and M13 mp19. A small (200 bp) *Hin*cII fragment (cloned in both orientations) revealed a possible Shine-Delgarno sequence plus an open reading frame. A 17-bp primer with a sequence identical to a region within this *Hin*cII fragment was synthesized. This primer provided an additional length of sequence information that overlapped the *Hin*cII fragment. Next, a region was picked from the newly sequenced region and another primer was synthesized. By this stepwise procedure the complete gene was sequenced (Fig. 4). In addition, the

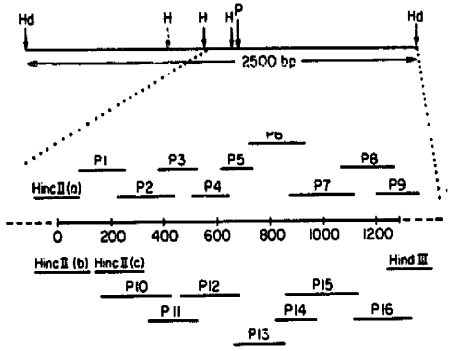

Fig. 4. Restriction map and sequencing strategy. At the top is shown the restriction map of the *Hin*dIII fragment which includes the *Klebsiella lay*Y gene. Below is given the pattern of sequencing of the two strands of the *lac*Y gene from *K. pneumoniae* M5a1. P1–P9 represent sequences of the coding strand obtained from synthetic primers. *Hin*cII (a) represents a sequence obtained with the universal primer. Sequences indicated below the line (including P10–P16) are sequences of the anticoding strand. Data from *Hin*cII (b), *Hin*cII (c) and *Hin*dIII were obtained with the universal primer.

entire gene was sequenced in the opposite strand. The position of each sequenced region is given in Fig. 4.

The inferred amino acid sequence is 416 amino acids in length (Fig. 5). This corresponds to a molecular weight of 46049. The *Klebsiella* sequence shows alternating regions of hydrophobic and hydrophilic residues in a pattern very similar to that seen for the lactose carrier of *E. coli*. The *Klebsiella* protein is one amino acid shorter than the *E. coli* protein, since *Klebsiella* has five additional amino acids on the N-terminus but six amino acids less at the C-terminus. There was 60% conservation of the amino acid sequence. A somewhat greater conservation was found in the hydrophobic segments (presumed membrane spanning regions) than the hydrophilic regions.

Site-specific mutagenesis of Klebsiella arginine-210 to histidine

One of the nto the mutations in *E. coli* that is known to reduce transport activity is a change from histidine-205 to arginine. Since the *Klebsiella* sequence contains arginine at position 210 (corresponding to position 205 of the *E. coli* numbering) it was considered of interest to substitute a histidine for arginine-210 by site-specific mutagenesis and determine whether the transport activity in *Klebsiella* would be increased. The Eckstein method [15,16] was used to carry out this substitution. The product was inserted into the *Hin*dIII site of pBR322 (tetracycline gene).

E. coli cells (DW2) containing a plasmid with either the normal *Klebsiella Y* gene (Arg-210) or the mutant Y gene (His-210) were tested for transport of two sugars. When cells were exposed to 100 μM radioactive lactose the cell with the normal Y gene (Arg-210) accumulated the sugar 12-fold in 7 min while the mutant gave 13-fold. With ONPG (100 μM) as substrate both cells accumulated the sugar 18-fold in 7 min. Thus, no alteration in transport activity was found to be associated with a change of Arg-210 to histidine.

Discussion

The physiological properties of the *Klebsiella* lactose carrier are rather different from those found in *E. coli*. While lactose transport in *Klebsiella* is quite poor at low sugar concentrations, at high sugar concentrations it is adequate for modest fermentation rates and for growth on lactose as a sole source of carbon (albeit at a slower rate than *E. coli*). Melibiose and TMG transport are each about half that of *E. coli*. Proton transport associated with TMG uptake in *Klebsiella* was quite weak compared with *E. coli*. On the other hand transport of ONPG by M5a1 was distinctly better than by *E. coli*.

The nucleotide sequencing data indicate a considerable degree of similarity of amino acid sequence of the lactose carrier between the *Klebsiella* M5a1 and another *Klebsiella* strain (T17R1). Buvinger and Riley [4] have reported data on the first third of the *lac*Y gene of a *Klebsiella pneumoniae* strain T17R1. Of the N-terminal 133 amino acids reported for T17R1 113 are identical to those in the present study with M5a1.

In spite of the differences in sugar transport between *Klebsiella pneumoniae* M5a1 and *E. coli* 3000 the amino acid sequences of the respective lactose carriers were remarkably similar. There are 411 residues which can be directly compared (omitting the six C-terminal residues of the *E. coli* sequence for which there is no *Klebsiella* counterpart). Of these amino acid residue 60% are identical. The conservation at the N-terminal 18 amino acid residues is poor. This portion of M5a1 lactose carrier protein possesses an extra five amino acid residues compared with *E. coli* and the subsequent 13 amino acid residues are poorly conserved, suggesting that this region is not essential for lactose transport. These data are consistent with the findings of Bocklage and Müller-Hill [19] that the first eight amino acid residues of the carrier in *E. coli* may be deleted and the carrier maintains full biological activity.

The C-terminal sequences also differ in the two organisms. Only two of the terminal 11 amino acid residues of M5a1 are the same as those found in *E. coli*. It is known that the terminal few amino acid residues of the *E. coli* protein may be removed by carboxypeptidase without losing transport activity [20]. Likewise carboxypeptidase treatment sufficient to remove epitopes required for antibody binding did not reduce transport activity [21]. In addition Hobson, Gho and Müller-Hill [22] failed to find transport-negative mutants in

TABLE III

*lac*Y SEQUENCE COMPARISONS BETWEEN *E. COLI* AND TWO *KLEBSIELLA* STRAINS

Analysis of amino acid differences	*Klebsiella* M5a1 vs. *E. coli*	*Klebsiella* M5a1 vs. *Klebsiella* T17R1
Number of residues compared	411	428
Percent different	40%	11%
Residues the same: percent with silent base changes [a] analysis of silent codon differences [b]	55%	37%
Divergence: uncorrected [c]	42%	40%
Divergence: corrected [d]	61%	57%

[a] This excludes the amino acids methionine and tryptophan (4 and 9, respectively, in the two comparisons) which have no silent base substitutions.

[b] Ref. 23.

[c] Calculated as the number of silent base changes as percent of 0.273 multiplied by the total number of bases.

[d] Corrected for multiple hits by the formula $-3\ln[1-(4/3)f]/4$, where f is the uncorrected divergence expressed as a fraction.

the extreme C-terminal region although such mutants were found throughout most of the remaining *lac*Y gene.

In Table III is shown a sequence comparison between the two *Klebsiella* strains and between M5a1 and *E. coli*. A calculation is made of divergence (uncorrected and corrected) by the method described by Ochman and Wilson [23]. The data indicates that the two *Klebsiella* strains differ from each other by almost the same extent as *Klebsiella* M5a1 and *E. coli* K-12.

In attempts to determine the amino acid residues essential for normal function of the lactose carrier of *E. coli* several laboratories isolated mutants and analyzed them for altered function and aminoacid substitutions. Site-specific mutagenesis has been used to generate several mutants [24–32]. In addition mutants have been obtained by selection for growth under specific conditions [33–35]. The mutations obtained with these two methods that lead to defective phenotype are given in Table IV. The *Klebsiella* sequence shows amino acid residues identical to normal *E. coli* in eleven out of thirteen positions shown in Table IV.

One example in Table IV in which the *Klebsiella* sequence differs from that of *E. coli* is in position 210, corresponding to *E. coli* histidine-205. Padan et al. [27] showed that when arginine was substituted for histidine at position 205 in the lactose carrier of *E. coli*, sugar transport was completely abolished. On the other hand, when histidine-205 was substituted by the neutral amino acids asparagine or glutamine, normal activity was obtained [28]. Thus, in the *E. coli* protein histidine is not essential in this position but arginine substitution prevents function. It was therefore surprising that the normal *Klebsiella* carrier possessed arginine in this position, and substitution by histidine by site specific mutagenesis did not increase the activity of the carrier. This presumably indicates that some internal compensation in the molecule occurs in the case of *Klebsiella* carrier which permits normal transport with either of the two amino acids at this position.

The second example in which the *Klebsiella* amino acid sequence differs from that of *E. coli* in

TABLE IV

MUTATIONS OF *E. COLI lac*Y THAT REDUCE OR ABOLISH TRANSPORT

Normal	Substitution	Reference
Gly 24	Glu, Arg	34
Tyr 26	Asp	35
Pro 28	Ser	34
Cys 148	Ser, Gly	24, 25, 30
Cys 154	Ser	26
Ala 177	Val, Thr	33
His 205	Arg	27
Tyr 236	Phe, Asn, Ser, His	33
Gly 262	Asp	35
Thr 266	Ile	35
Arg 302	Leu	31
His 322	Arg, Asn, Gln	27, 28
Glu 325	Ala	29

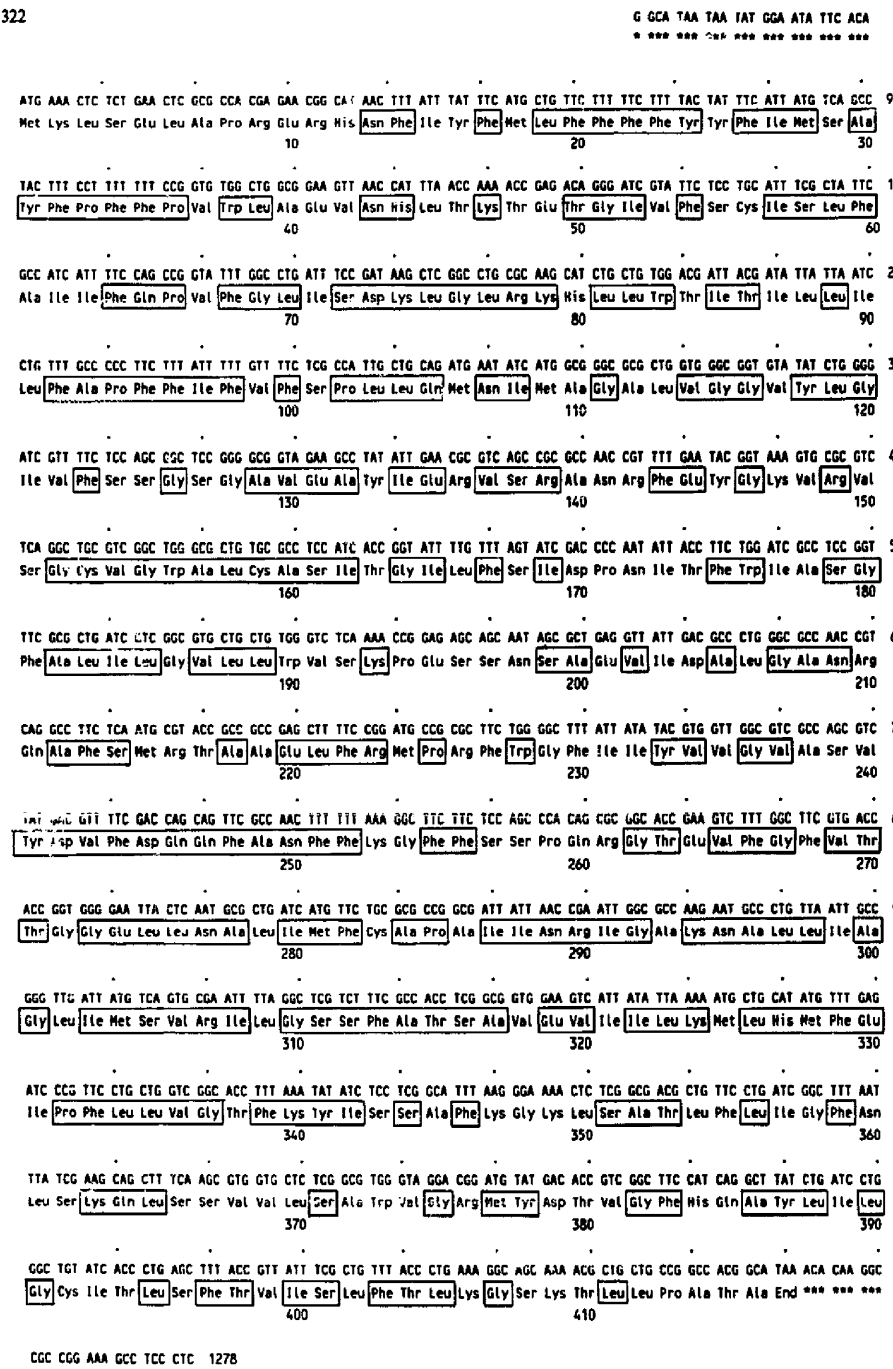

Fig. 5. Nucleotide sequence of the coding strand of the *K*lebsiella lacY gene. Inferred amino acid sequence is given below the nucleotide sequence. Nucleotide numbering is given at the end of each line; amino acid numbering is below the line. The boxed amino acids are those identical to the *E. coli* sequence. The *Klebsiella* protein contains five more amino acids at the N-terminal end and thus the numbering of the corresponding *E. coli* sequence is five less than the *Klebsiella* numbering.

the 13 mutations listed in Table IV is glycine-24 (corresponding to position 29 of the *Klebsiella* sequence). Overath et al. [34] found that when glycine-24 was replaced by glutamic acid or by arginine, transport was abolished, although sugar binding remained intact. The *Klebsiella* carrier shows a serine at this position. It is possible that it is the charge of the amino acid substitution at position 24 of *E. coli* that disrupts function in the two mutants mentioned above and that a variety of neutral amino acids could be tolerated.

Recently an interesting hypothesis concerning the biology of the lactose operon has been published by Boos [36]. He suggests that lactose is a relatively unimportant substrate in the life of *E. coli* (and perhaps other organisms). Other galactosides, however, are liberated in large quantities by the digestion of plant material. Plant membranes possess galactolipids which yield β-galactosylglycerol. This and other galactosides may contribute the major substrates in nature for the gene products of the lactose operon. If the 'natural' substrate for this carrier is not lactose but other galactosides, then *Klebsiella* may not be at such a disadvantage in nature as one might suppose from its poor affinity for lactose.

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