Analysis of Sucrose Catabolism in *Klebsiella pneumoniae* and in Scr⁺ Derivatives of *Escherichia coli* K12

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In contrast to a previous report, strains of *Klebsiella pneumoniae* were found to take up and phosphorylate the disaccharide sucrose via the phosphoenolpyruvate-dependent carbohydrate phosphotransferase system (PTS). In addition to the two soluble and general components enzymeI and HPr of the PTS, a sucrose-specific enzymeII^{Scr} (gene *scrA*), together with the enzymeIII, coded for by the gene *crr*, were needed for the vectorial phosphorylation of sucrose to generate intracellular sucrose 6-phosphate. This sugar phosphate is hydrolysed by a hydrolase (invertase, gene *scrB*) to generate glucose 6-phosphate and free fructose. The latter is converted to fructose 6-phosphate by an ATP-dependent fructokinase (gene *scrK*), an enzyme which is part of the sucrose and not of the fructose catabolic pathway. Analysis of different mutants of *K. pneumoniae* strain 1033, and of *Escherichia coli* K12 derivatives carrying R'*scr* plasmids isolated from *K. pneumoniae*, showed that the genes *scrA*, *B*, and *K*, together with a gene *scrR* for a repressor, form a genetic unit located on the chromosome of *K. pneumoniae*. These genes and the corresponding sucrose metabolic pathway are very similar to a previously described *scr* system encoded on plasmid pUR400 and found in other enteric bacteria.

INTRODUCTION

Metabolism of the disaccharide sucrose (D-glucopyranosyl- β 1,2-D-fructofuranoside) by bacteria has been studied in detail in *Bacillus subtilis* (Lepesant *et al.*, 1976; Aymerich *et al.*, 1986; Amory *et al.*, 1987), in the cariogenic oral streptococci (St Martin & Wittenberger, 1979; Mimura *et al.*, 1984) and in a clinical isolate of a sucrose-positive strain of *Salmonella* carrying the sucrose plasmid pUR400 (alias pSCR53) (Wohlhieter *et al.*, 1975; Schmid *et al.*, 1982; Lengeler *et al.*, 1982). In these organisms, sucrose is taken up and phosphorylated by a specific enzymeII^{Scr} (EII^{Scr}) of the phosphoenolpyruvate (PEP) dependent carbohydrate phosphotransferase system (PTS) to yield, in combination with a soluble enzymeIII (EIII), sucrose 6-phosphate. This phosphate is hydrolysed by an intracellular sucrose-6-phosphate hydrolase (sucrase or invertase) into glucose 6-phosphate and fructose. In Gram-positive bacteria, extracellular glycosyltransferases (e.g. a levansucrase) have also been found which transfer the sucrose moieties to growing glucans or fructans, and a levanase which hydrolyses the high-polymer fructan called levan (references in Lepesant *et al.*, 1976; St Martin & Wittenberger, 1979; Aymerich *et al.*, 1986; Amory *et al.*, 1987). Molecular analysis of pUR400 in mutants of *Escherichia coli* K12, a strain naturally unable to metabolize sucrose, revealed that the genes *scrA* for EII^{Scr} and *scrB* for the invertase were clustered with two additional genes, *scrK* and *scrY*, coding for hitherto unknown functions in an *scr* operon regulated by a repressor (gene *scrR*) (Schmid *et al.*, 1988). No plasmid *scr* genes coding for glycosyltransferases or an EIII were detected, the latter being encoded by the chromosomal gene *crr*.

Abbreviations: EI, EII, EIII, enzymeI, II, III; PEP, phosphoenolpyruvate; PTS, phosphotransferase system.

In Gram-negative bacteria, a different sucrose-catabolic pathway involving uptake of free sucrose and its cleavage into glucose and fructose has been described for '*Aerobacter aerogenes*' PRL-R3 (Kelker *et al.*, 1970), an organism recently reclassified as *Klebsiella pneumoniae* var. *oxytoca* (Mortlock, 1982). Uptake systems for sucrose were not analysed in this strain, but the PTS was claimed not to be involved. Instead, phosphorylation of intracellular glucose and fructose by ATP-dependent kinases was proposed. These conclusions apparently were corroborated by the presence of a fructokinase in cells of '*A. aerogenes*' pregrown on sucrose and fructose, yielding fructose 6-phosphate in an ATP-dependent reaction.

In view of the differences found between the plasmid-encoded metabolic pathway and that described for *K. pneumoniae* var. *oxytoca*, we reinvestigated the metabolism of sucrose in a naturally Scr⁺ strain of *Klebsiella pneumoniae* 1033 and also in strain PRL-R3. Our data indicate that in both organisms a chromosomally encoded and PTS-dependent sucrose metabolic pathway similar to the plasmid-encoded one is present, which always includes a soluble fructokinase apparently encoded by the gene *scrK*.

METHODS

Bacteria and plasmids. These are described in Table 1. All derivatives of *K. pneumoniae* 1033, strain KAY2026 (Sprenger & Lengeler, 1984), are auxotrophic for L-arginine and guanine and sensitive to bacteriophage P1kc.

Culture media and growth conditions. Complex tryptone broth (LT), Lennox broth (LB), phosphate-buffered minimal medium (MM), and the MacConkey agar plates containing 1% (w/v) of the carbohydrate to be tested, have been described (Lengeler & Lin, 1972). In minimal media, amino acids and nucleosides were added to 20 µg ml⁻¹, carbohydrates to 2 g l⁻¹. In growth determinations, one OD₄₂₀ corresponded to 5 × 10⁸ bacteria ml⁻¹.

Isolation of mutants. Wild-type strain KAY2026 was mutagenized with ethyl methanesulphonate as described by Tanaka *et al.* (1967). Carbohydrate-negative mutants were enriched with streptozotocin (Lengeler, 1979) and auxotrophic mutants with nalidixic acid treatment (Sprenger *et al.*, 1986). Preparation of P1 transducing lysates and transductions with P1 were done as described (Arber, 1960; Sprenger & Lengeler, 1987). Isolation of R⁺scr⁺ plasmids and their transfer was done as described (van Gijsegem & Toussaint, 1982; Sprenger & Lengeler, 1984). For the construction of *K. pneumoniae* Hfr strains, we introduced into the Lac⁻ strain KAY2209 the temperature-sensitive F'ts114 *lac*⁺::Tn10 from *Salmonella typhimurium* TT628 (Chumley *et al.*, 1979). After selection for growth on MM plus lactose and tetracycline (10 µg ml⁻¹) plates at 42 °C, larger colonies were picked and tested for potential Hfr abilities by crossing with suitable recipient strains of *K. pneumoniae*.

Preparation of cell-free extracts and enzyme measurements. Cell extracts from late-exponential-phase cells were prepared (Tanaka *et al.*, 1967; Lengeler & Lin, 1972), and transport of ¹⁴C-labelled sucrose (Schmid *et al.*, 1982) and carbohydrate phosphate formation by the ion-exchange filter binding method (Lengeler *et al.*, 1971) were tested as described. Sucrose and sucrose-6-phosphate hydrolase (EC 3.2.1.26; invertase) activities were measured either with toluene-treated cells (Schmid *et al.*, 1982) or with freshly prepared sonicated extracts, since the activity was lost rapidly. In both cases, a coupled enzyme assay mixture (System Glucose; Merck) with end-point measurement of NADH formation was used. Fructokinase (EC 2.7.1.4) activities were measured in two different ways. Method (1) was a coupled assay with phosphoglucose-isomerase and glucose-6-phosphate dehydrogenase (Boehringer Mannheim) as auxiliary enzymes monitoring the formation of NADPH. The assay mixture contained, in 0.1 M-Tris/HCl pH 7.5: 5 mM-ATP, 10 mM-MgCl₂, 5 mM-NADP, 5 mM-fructose, phosphoglucose-isomerase (3 U ml⁻¹), glucose-6-phosphate dehydrogenase (3 U ml⁻¹) and cell extracts. The assay was run at 25 °C. The incubation mixture (without fructose) was preincubated for 15 min at 25 °C. Method (2) was a direct assay with ¹⁴C-labelled substrate. The assay mixture contained in 0.1 M-Tris/HCl pH 7.5: 5 mM-ATP, 10 mM-MgCl₂, 1 mM-[¹⁴C]carbohydrate (800 Bq µmol⁻¹) and extracts of sonicated cells. Incubation was at 25 °C. Formation of labelled sugar phosphates was measured on ion-exchange filters (Whatman DE81) (Lengeler *et al.*, 1971). Protein determinations were done by the Lowry method with bovine serum albumin as standard and with correction for the Tris buffer.

Chemicals. D-[¹⁴C]Glucose, D-[¹⁴C]fructose, D-[¹⁴C]mannose and [U-¹⁴C]sucrose were purchased from NEN Chemicals. [U-¹⁴C]Sucrose purified by column chromatography was a generous gift from Dr K. Schmid. PEP (Sigma), phosphoglucose-isomerase, glucose-6-phosphate dehydrogenase (Boehringer Mannheim), the System Glucose (Merck) and all other chemicals were of commercial origin.

RESULTS AND DISCUSSION

Uptake and phosphorylation of sucrose

Uptake and phosphorylation of labelled sucrose (K_m 10 µM) could be detected in cells of *K. pneumoniae* strains KAY2026 and PRL-R3 after growth on fructose and the fructose-

Table 1. *Bacteria and plasmids*

The genetic nomenclature is according to Bachmann (1983) and Postma & Lengeler (1985). The *pts* mutations of *K. pneumoniae* strains were classified according to *in vitro* complementation tests with known *ptsI* (JWL191 *ptsI*, Lengeler *et al.*, 1981) and *ptsH* mutants (LBG1605 *ptsH*, Gershanovitch *et al.*, 1977) of *E. coli* K12. All strains of *E. coli* K12 carry, in addition to the markers indicated in the table, the following markers: F⁻ *thi-1 argG6 hisG1 metB1 tonA2 supE44 rpsL104 galT6 xyl-7* Kba (Ts).

Strain	Relevant genetic markers	Origin or source
<i>K. pneumoniae</i>		
PRL-R3	Wild-type	R. P. Mortlock via E. C. C. Lin
1033	F ⁻ Arg ⁻ Gua ⁻	E. C. C. Lin
KAY2026	1033 P1 sensitive	Sprenger & Lengeler (1984)
KAY2027	2026 <i>ptsI101</i>	
KAY2030	2026 <i>manI, A101</i>	This work
KAY2033	2030 <i>ptsH102</i>	
KAY2035	2030 Scr ⁻ * <i>galK101 manA, I101</i>	Sprenger & Lengeler (1984)
KAY2038	2026 <i>scrB101</i>	
KAY2039	2026 <i>scrA101</i>	This work
KAY2062	2030 <i>galK101/pULB113 Km^R Tc^R Ap^R</i>	
KAY2209	2026 <i>dha-102 rha-103 galE Mel⁻ lacY</i>	Sprenger & Lengeler (1987)
KAY2400†	Hfr PO (<i>leu ara thr malB</i>)	
KAY2403†	Hfr PO (<i>metB rha mtl malA</i>)	This work
KAY2151	<i>scrB101 thr-103</i>	
KAY2156	<i>sor-101 rpoB101 scrB101</i>	This work
KAY2161	<i>scrA101 mel-101 leu-101</i>	
KAY2173	<i>ara-101 thr-102 scrA101 malA101 galK102</i>	Sprenger & Lengeler (1987)
KAY2213	<i>metB101</i>	
<i>E. coli</i> K12		
L191	F ⁻ <i>ptsI</i>	Lengeler <i>et al.</i> (1981)
LR2-167	F ⁻ <i>manA, I nagE</i>	
LR2-175	F ⁻ <i>manA, I nagE glcA fruA</i>	J. Lengeler Lengeler <i>et al.</i> (1982)
LM1	F ⁻ <i>manA, I nagE crr</i>	
JWL263	JWL191 <i>ptsI/F'198 pts⁺::Tn10</i>	This work
GSL28	LR2-175/pR'A01 Scr ⁻ ‡ Km ^R	
GSL29	LM1/pR'A01 Scr ⁻ ‡ Km ^R	
GSL43	LR2-175/pR'A03 <i>scr⁺</i> Km ^R	
GSL59	LR2-175/pR'A02 <i>scr⁺</i> Km ^R	
Plasmids		
pULB113	RP4::Mu3A, Ap ^R Km ^R Tc ^R Tra ⁺	van Gijsegem & Toussaint (1982)
pGSL1	Indigenous plasmid of KAY2026, 5.7 kb DNA, <i>mob⁺</i>	This work
pR'A01	pULB113 Scr ⁻ ‡	
pR'A02	pULB113 <i>scr⁺</i>	
pR'A03	pRLB113 <i>scr⁺</i>	
F'ts114	<i>lac⁺ zzzf-20::Tn10</i>	Chumley <i>et al.</i> (1979)
F'198	<i>pts⁺</i>	Curtis & Epstein (1975)

* Strain KAY2035 carries an *scr*-mutation preventing expression of all sucrose enzymes.

† KAY2400 is an Hfr-derivative of KAY2209 carrying F'ts *lac⁺::Tn10* and injecting genes counter-clockwise starting with *leu, ara, thr*. KAY2403 is a similar Hfr injecting the genes counter-clockwise starting with *metB, rha, mtl*.

‡ Constitutive expression of the sucrose genes.

containing saccharides sucrose, raffinose (Table 2) and lactulose (data not shown) but not on glucose or glycerol. Dialysed extracts and washed membrane fractions from preinduced cells showed a PEP-dependent sucrose phosphorylation not seen in the presence of ATP, provided that the soluble components enzymeI (EI) and HPr of the PTS were also supplied (Tables 2 and 3). Interestingly, the phosphorylation depended on the presence of the soluble EIII of the PTS, product of the gene *crr*. This dependence of a membrane-bound and sucrose-specific EII^{Scr} upon EIII has also been observed in the pUR400-encoded sucrose system (Lengeler *et al.*, 1982).

To isolate mutants with defects in sucrose transport and metabolism, strain KAY2026 was mutagenized with ethyl methanesulphonate and treated with the selective agent streptozotocine

Table 2. *Sucrose enzyme activities in strains of K. pneumoniae*

All values given are the mean of three independent measurements.

Strain	Carbon source*	Sucrose transport†	EII ^{Scr} ‡	Invertase§	Fructokinase
KAY2026	Glc	0.01	ND	0.01	≤ 2
	Glyc	0.01	12	0.02	≤ 2
	Fru	0.48	82	1.25	19
	Scr	1.20	142	2.29	51
	Raf	1.10	158	5.75	46
KAY2033 (<i>ptsH</i>)	Glyc	0.01	10	0.02	≤ 2
	Fru	0.01	42	2.47	12
PRL-R3	Glyc	0.08	23	0.10	≤ 2
	Fru	0.72	73	0.70	28
	Scr	1.92	137	1.46	134

ND, Not determined; ≤, activities at the limit of detection.

* The cells were grown to late-exponential phase on minimal medium containing 0.1% Casamino acids and 0.4% of the carbon source indicated (for abbreviations see text).

† Uptake of [¹⁴C]sucrose (initial concentration 0.56 µM) is given in nmol min⁻¹ (mg protein)⁻¹.‡ EII^{Scr} activity was tested using 0.56 µM-[¹⁴C]sucrose, purified membrane vesicles of the strains and cultures indicated and membrane-free cytoplasmic extracts of KAY2026 or PRL-R3 from cells pregrown on D-glucitol. Specific activities are given in pmol min⁻¹ (mg protein)⁻¹.§ Invertase activity was tested with 53 mM-sucrose using the System Glucose enzyme assay mixture (Merck). Activities are in µmol min⁻¹ (mg protein)⁻¹.|| Fructokinase activity was determined with the phosphoglucose-isomerase/glucose-6-phosphate dehydrogenase coupled assay and is given in nmol min⁻¹ (mg protein)⁻¹.

(Lengeler, 1979). A series of pale colonies on MacConkey sucrose plates was isolated and characterized, among them mutants with pleiotropic growth defects on typical PTS carbohydrates, e.g. D-mannitol (Mtl), D-glucitol (Gut), N-acetylglucosamine (Nag) and L-sorbose (Sor), but still positive on non-PTS carbohydrates such as D-galactose (Gal), raffinose (Raf), melibiose (Mel) and glycerol (Glyc) (Table 4). The addition of 5 mM-cyclic-AMP to such growing cells did not relieve the Scr⁻ phenotype. *In vitro* complementation tests using cell extracts from known *ptsI* and *ptsH* strains of *E. coli* (Table 1) showed that strain KAY2027 lacked EI activity while strain KAY2033 was defective in HPr (Table 2, and F. Titgemeyer, personal communication). These results were corroborated by *in vivo* complementation tests showing that both strains were complemented by F₁₉₈*pts*⁺ from *E. coli* K12 and by an R⁺*pts*⁺ isolated from KAY2026. Neither the *ptsI* nor the *ptsH* mutant of *K. pneumoniae* was negative for the fermentation of D-glucose (Glc), D-mannose (Man) and D-fructose (Fru) (Table 4). For these carbohydrates one or several non-PTS transport systems and metabolic pathways, e.g. a quinoprotein-dependent glucose dehydrogenase system (Neijssel *et al.*, 1983), are still active in Pts⁻ mutants. Scr⁺ mutants from both strains, however, invariably were Pts⁺ revertants, further supporting the conclusion that sucrose is a typical PTS carbohydrate in *K. pneumoniae*.

Besides such pleiotropic mutants, single Scr⁻ mutants were also found, e.g. KAY2039 (Table 5). This strain lacked sucrose transport and EII^{Scr} phosphorylating activity, but retained the hydrolase and fructokinase activity. It thus appears to be a *scrA* mutant in the structural gene for the EII^{Scr} transport system. The lowered inducibility of the hydrolase and kinase by external sucrose, which cannot be taken up at a normal rate, compared to a normal induction by fructose taken up through the fructose-PTS is characteristic for transport-negative mutants (Schmid *et al.*, 1982). It is due to a residual transport activity in the mutant strain sufficient for a low induction at the higher inducer concentration used (10 mM) and/or to contaminating fructose. No evidence for the existence of an efficient non-PTS transport system for free sucrose as proposed by Kelker *et al.* (1970) could be found in the different *K. pneumoniae* strains tested thus far (data not shown).

Table 3. *EII^{Ser}* activities in *K. pneumoniae* strains KAY2026 and PRL-R3

Washed and purified membranes from sucrose-grown cells of strains KAY2026 and PRL-R3 were tested for phosphorylation of [¹⁴C]sucrose (0.56 µM), using membrane-free cytoplasmic extracts of the strains and mutants indicated as the source of EI, HPr and EIII. PEP or ATP (5 mM) was added as indicated. The relatively high values for tests without PEP or with ATP are due to incomplete dialysis of such extracts. *EII^{Ser}* activity is expressed in pmol min⁻¹ (mg protein)⁻¹. The mean values of two measurements are given.

Source of:			<i>EII^{Ser}</i> activity
Membranes	Soluble extracts	Additions	
—	KAY2026	+ PEP	≤ 1
KAY2026	—	—	≤ 1
KAY2026	KAY2026	— PEP	50
KAY2026	KAY2026	+ PEP	159
KAY2026	KAY2026	+ ATP	53
KAY2026	LR2-167	+ PEP	140
KAY2026	L191 <i>ptsI</i>	+ PEP	≤ 1
KAY2026	LM1 <i>crr</i>	+ PEP	≤ 1
KAY2026	L191 + LM1	+ PEP	25
PRL-R3	PRL-R3	+ PEP	132
PRL-R3	LR2-167	+ PEP	88
PRL-R3	L191 <i>ptsI</i>	+ PEP	≤ 1
PRL-R3	L191 + LM1	+ PEP	50

Table 4. *Phenotypes of different mutant strains of K. pneumoniae and E. coli* K12

The different strains were tested on McConkey indicator plates containing 1% of the carbohydrates indicated (for abbreviations see text). Increasing reactions from negative (—) to strong (3+) are indicated.

Strain	Origin	Genotype* or phenotype	Man	Glc	Fru	Scr	PTS†	Non-PTS‡
<i>K. pneumoniae</i>								
PRL-R3		Wild-type	3+	3+	3+	3+	3+	3+
KAY2026		Wild-type	3+	3+	3+	3+	3+	3+
KAY2027	KAY2026	<i>ptsI</i>	2+	2+	2+	—	—	—
KAY2030	KAY2026	<i>manA,I</i>	—	3+	3+	3+	3+	3+
KAY2033	KAY2030	<i>ptsH</i>	—	2+	2+	—	—	2+
KAY2035	KAY2030	<i>Scr</i> [—]	—	3+	3+	—	3+	3+
KAY2038	KAY2026	<i>scrB</i>	3+	3+	3+	(—)‡	2+	3+
KAY2039	KAY2026	<i>scrA</i>	3+	3+	3+	—	3+	3+
<i>E. coli</i> K12								
LM1		<i>crr nagE manA,I</i>	—	—	2+	—	3+	2+
GSL29	LM1/R'A01	<i>Scr</i> [—]	—	—	2+	—	3+	2+
LR2-175		<i>fruA glcA nagE manA,I</i>	—	—	—	—	3+	2+
GSL28	LR2-175/R'A01	<i>Scr</i> ⁺	—	—	—	3+	3+	2+
GLS43	LR2-175/R'A03	<i>Scr</i> ⁺	—	—	—	3+	3+	2+
GSL59	LR2-175/R'A02	<i>Scr</i> ⁺	—	—	—	3+	3+	2+

* Only relevant mutations are indicated. Mutations of the parent strain are not repeated in derivatives.

† The PTS carbohydrates tested were D-mannitol, D-glucitol, *N*-acetylglucosamine and L-sorbose; the non-PTS carbohydrates were D-melibiose and raffinose. Wild-type strains of *E. coli* K12 are *Ser*[—] and *Raf*[—], but positive for the other carbohydrates unless they carry the mutations indicated.

‡ The *scrB* mutant KAY2038 grows slowly on sucrose due to the presence of a second hydrolase normally involved in raffinose metabolism.

A final proof for the conclusion that sucrose is taken up and phosphorylated in *K. pneumoniae* through an *EII^{Ser}* of the PTS which also required EIII was that GSL29, a *crr* derivative of *E. coli* K12 lacking this EIII, remained *Scr*[—] after transfer of an *R'scr*⁺ plasmid (Table 4), that *Scr*⁺ revertants invariably had regained EIII activity, and furthermore that strain LR2-175, lacking all hexose-specific EIIs of the PTS, became *Scr*⁺ after obtaining an *R'scr*⁺ (strains GSL28, 43 and 59 in Table 4).

Table 5. *Sucrose enzyme activities in different mutant strains*

Details are as described in the legend to Table 2. In EII^{Scr} tests involving strains of *E. coli* K12, the cell-free extracts were from the wild-type strain LR2-167. The mean values of three independent measurements are given for activity tests.

Strain	Carbon source	Sucrose transport	EII ^{Scr}	Invertase	Fructokinase
<i>K. pneumoniae</i> KAY2026	Glyc	0.01	12	0.02	≤2
	Fru	0.48	82	1.25	19
	Scr	1.20	142	2.29	51
	Raf	1.10	158	5.75	46
KAY2035 (Scr ⁻)	Glyc	0.03	9	≤0.01	≤2
	Fru	0.04	≤5	≤0.01	≤2
	Scr*	0.03	13	0.07	2
	Raf	0.04	≤5	1.21	5
KAY2038 (<i>scrB</i>)	Glyc	0.02	10	≤0.01	3
	Fru	0.69	154	≤0.01	23
	Scr*	0.35	64	0.08	26
	Raf	0.59	76	1.83	28
KAY2039 (<i>scrA</i>)	Glyc	0.01	≤5	0.04	8
	Fru	0.06	≤5	0.84	21
	Scr*	0.01	≤5	0.15	12
	Raf	0.09	8	6.36	41
<i>E. coli</i> K12 LR2-175	Glyc	≤0.01	≤2	0.01	≤2
	Fru*	≤0.01	≤2	0.01	≤2
GSL28 (pR'A01)	Glyc	0.33	39	0.51	30
	Fru*	2.15	262	3.68	71
GSL59 (pR'A02)	Scr	0.94	84	0.84	78
	Glyc	0.01	5	0.02	≤2
	Fru*	0.85	101	1.01	8
	Scr	1.03	153	3.43	35

* Cultures grown on glycerol and the carbon source indicated.

Hydrolysis of sucrose 6-phosphate

The existence of an extracellular sucrose hydrolase (invertase) in sucrose-grown cells of '*Aerobacter aerogenes*' PRL-R3 (Kelker *et al.*, 1970) has been reported as well as an enzyme which hydrolyses both sucrose 6-phosphate with a high affinity and sucrose with a lower affinity in sucrose-grown cells of *E. coli* K12 containing pUR400 (Schmid *et al.*, 1982). A similar activity was found in cell extracts of *K. pneumoniae* KAY2026 and PRL-R3 as well as of R'^{scr}⁺-containing cells of *E. coli* K12 (GSL28, GSL59), after pregrowth on sucrose, fructose and raffinose (Tables 2 and 5). The activity was also present in the Pts⁻ mutants and in the *scrA* mutant KAY 2039, but not in the Scr⁻ mutants KAY2035 and KAY2038 after pregrowth on sucrose and fructose. All strains of *K. pneumoniae*, including the latter ones, showed hydrolase activity after growth on raffinose. This trisaccharide induces a second hydrolase, different from ScrB, involved in raffinose metabolism, and similar to an invertase encoded on *raf* plasmids of certain pathogenic enterobacteria (Schmid *et al.*, 1979). KAY2038 thus appears to be a *scrB* mutant retaining sucrose transport/phosphorylation and fructokinase activity.

An ATP-dependent fructokinase for intracellular fructose

Pregrowth of PRL-R3 on sucrose and fructose induced an ATP-dependent fructokinase activity converting the ketose to fructose 6-phosphate (Kelker *et al.*, 1970). Kinase-negative mutants had no detectable impairment of sucrose or fructose metabolism provided that the fructose-PTS pathway was still present. In contrast to the fructokinase, transport and concomitant phosphorylation of external fructose by the fructose-PTS yielded fructose 1-phosphate in PRL-R3 (Kelker *et al.*, 1970). Since the kinase was coregulated with the sucrose-invertase and not with the other fructose-degrading enzymes, the authors concluded that it

probably had a physiological function in sucrose metabolism, namely the phosphorylation of intracellular fructose derived from the hydrolysis of intracellular sucrose. Inducibility by external sucrose and fructose, however, could not be explained.

After pregrowth on sucrose and fructose, we also found fructokinase activity in sonicated cell extracts of PRL-R3. This activity was not found in extracts from cells grown on glucose or glycerol. Furthermore, a similar activity was found in cell extracts of strain KAY2026 after pregrowth on sucrose, fructose or raffinose (Table 2). It was located in the soluble cytoplasmic fraction and showed a strict dependence on ATP for which PEP could not substitute. The enzyme did not appreciably phosphorylate D-mannose or L-sorbose, nor was the phosphorylation of 1 mM-D-fructose inhibited by 10 mM of these sugars (data not shown). It thus resembled the inducible fructokinase described by Kelker *et al.* (1970), and not the mannofructokinase (gene locus *mak*) which is cryptic in wild-type strains of *E. coli* K12 and *S. typhimurium* (Sebastian & Asensio, 1972; Saier *et al.*, 1971; J. W. Lengeler, unpublished results), or the constitutive glucokinase (gene locus *glk*) (Curtis & Epstein, 1975).

Inducible fructokinase activity could also be found in the *Scr*⁻ mutants KAY2038 and KAY2039 (Table 5), in agreement with the assumption that they carry *scrA* and *scrB* point-mutations respectively. A lowered activity was finally found in the *ptsH* mutant KAY2033, but none in the *ptsI* mutant KAY2027. This was probably due to the inability of this strain to take up the free fructose needed for induction. When the *scr* genes from KAY2026 were transferred to strains of *E. coli* K12 lacking all hexose-specific PTS, mannofructokinase and glucokinase (e.g. LR2-175), the inducible fructokinase activity was still expressed after pregrowth on sucrose, further corroborating the conclusion that there exist three distinct kinases (Table 5 and data not shown).

From the transport and enzyme tests and the results on mutants presented thus far, we can conclude that sucrose metabolism in *K. pneumoniae* involves a sucrose-specific EII^{Scr} which, upon transport, yields sucrose 6-phosphate. The sucrose 6-phosphate is hydrolysed to glucose 6-phosphate and fructose, and the latter phosphorylated by a fructokinase to fructose 6-phosphate. This sucrose-PTS-dependent catabolic pathway is found not only in strain KAY2026, but also in PRL-R3, an observation which contradicts previous conclusions by Kelker *et al.* (1970). Furthermore, no extracellular glycosyltransferases liberating glucose or fructose as in the Gram-positive bacteria have yet been detected. This is in agreement with the observation that *scrA* mutants lacking only the EII^{Scr} transport had a *Scr*⁻ phenotype while host cells lacking all transport systems for free glucose and fructose remained *Scr*⁺.

Regulation of the sucrose enzyme activities

As shown above, sucrose transport, EII^{Scr} phosphorylation, sucrose-6-phosphate hydrolase and fructokinase activities were coordinately induced in cells of strains KAY2026, PRL-R3 and their derivatives (Tables 2 and 5). All activities were high after growth on sucrose, raffinose and fructose, intermediate on lactulose (D-galactopyranosyl-1,4-D-fructose) and low on glycerol or glucose. Fructose, the only common moiety to all inducing substrates, or a derivative thereof, seems to be the inducer for the *scr* system, as has been described for the pUR400 system (Schmid *et al.*, 1988). In contrast to sucrose uptake, raffinose transport was induced only by raffinose, and fructose transport only by fructose. Furthermore, the *scrA* mutant KAY2038 retained uptake activity for fructose and raffinose, and the *ptsH* mutant KAY2033 retained that for fructose. Consequently, such cells retain inducibility by fructose and raffinose while sucrose barely induces the sucrose enzymes (Tables 2 and 5).

The sucrose plasmids R'A02 and R'A03 conferred an inducible phenotype to their *E. coli* K12 hosts GSL59 or GSL43. This seems to indicate that, analogous to the pUR400 system (Schmid *et al.*, 1988), a repressor gene *scrR* maps close to the other *scr* genes and has been cloned. Among several R'*scr*⁺ plasmids, one (R'A01, Table 5) expressed the four sucrose activities in a semi-constitutive way in its *E. coli* K12 host GSL28. Since the constitutive phenotype conferred by R'A01 remained unaltered after transfer of the plasmid into KAY2026, the plasmid most probably carries a promoter/operator mutation (data not shown).

The Scr⁻ strain KAY2035, finally, lacked all four activities inducible by sucrose or fructose, perhaps indicative of the presence of a promoter-negative or a polar mutation in an otherwise coordinately expressed *scr* operon. KAY2035/R'A01-diplogenetic derivatives became Scr⁺ and expressed all activities constitutively, further supporting this assumption (data not shown).

Cloning and mapping of the chromosomal scr genes from K. pneumoniae

E. coli K12 neither grows on sucrose, nor do the cells contain any detectable sucrose-metabolic enzymes, due to the lack of the corresponding genes (Le Minor *et al.*, 1973; Schmid *et al.*, 1982; Table 5). The general PTS proteins EI, HPr and EII (genes *ptsI*, *ptsH* and *crr*) of *E. coli* K12 are able to complement *K. pneumoniae* specific EIIs (Sprenger & Lengeler, 1984; F. Titgemeyer, personal communication). We therefore transferred the genes for sucrose utilization from strain KAY2026 by means of pULB113 derivatives (R'A01 to R'A03, Table 4) into different strains of *E. coli* K12. All sucrose-positive derivatives grew with a mean generation time of 60 min on this disaccharide and expressed the four sucrose-metabolic activities in an inducible way, except for those containing R'A01 which, as stated above, expressed the *scr* genes semi-constitutively (Table 5). Sucrose, fructose and, in *lacI* mutants, also raffinose and lactulose acted as inducers. Neither *ptsI*, *ptsH*, *cya*, *crp* nor *crr* mutants containing sucrose plasmids grew on sucrose, while derivatives lacking all hexose-specific PTS showed the Scr⁺ phenotype (Table 4 and data not shown).

EIIs and the PTS are the chemoreceptors for PTS carbohydrates (Adler & Epstein, 1974; Lengeler *et al.*, 1981). Scr⁺ derivatives of *E. coli* K12 (which normally are unable to react to sucrose) carrying the *scr* genes from *K. pneumoniae* reacted positively in chemotaxis tests towards this disaccharide (data not shown). Since even derivatives like GSL28 and GSL59 (Table 4), lacking all glucose and fructose chemoreceptors and unable to react to these hexoses, reacted towards sucrose, the reaction must be directly towards sucrose. Here, as for the sorbose-PTS (Sprenger & Lengeler, 1984), an EII from the naturally non-motile bacterium *K. pneumoniae* still acted as a functional chemoreceptor in *E. coli* K12.

To find out whether the *scr* genes of *K. pneumoniae* were located on the chromosome or on a pUR400-like plasmid, we looked for plasmids in strain KAY2026. A small plasmid, pGSL1, of about 5.7 kbp was detected. It could be mobilized by the conjugative plasmid pULB113 (van Gijsegem & Toussaint, 1982) and transferred to *E. coli* K12, but never conferred a Scr⁺ phenotype on these host cells. Next, we used a series of F⁺ and F'⁺ derivatives of KAY2026 containing standard F' plasmids from *E. coli* K12 to test transfer of known markers from the *K. pneumoniae* chromosome not present in *E. coli* K12 (e.g. for L-sorbose, D-arabinitol, D-ribitol, citrate metabolism) or of standard auxotrophy markers into *E. coli* recipient strains, but never succeeded. Finally, the F'ts114::Tn10 plasmid was used according to Chumley *et al.* (1979) to select Hfr KAY2400 and Hfr KAY2403 (Table 1). No marker transfer to strains of *E. coli* K12 was ever observed. Hfr KAY2400 did transfer, however, the gene loci *leu*, *ara* and *thr* with a high efficiency to Str^R recipient *K. pneumoniae* strains carrying appropriate mutations, e.g. KAY2151 *thr-103*, KAY2161 *leu-101* and KAY2173 *ara-101 thr-102*. It transferred the genes *dha*, *sor*, *ilv*, *metB*, *rpoB*, *rha*, *mtl* and *malA* with decreasing efficiency, *scr* as well as *rpsL* with a low efficiency, and rarely (in conjugations of more than 45 to 60 min duration) *lysA*, *pts* and *his*. The second Hfr strain KAY2403 did not transfer the marker *leu*, *ara* and *thr*, but it did transfer *metB* into the Str^R recipient KAY2213 *metB101*. In an interrupted conjugation with KAY2156 involving the markers *dha*, *sor*, *rha*, *scr* and *rpsL*, KAY2403 did transfer the *scr* genes after about 30 min, locating them close to 65 min. Neither donor transferred the markers *pro*, *lac* or *gal*, also indicating counterclockwise gene transfer in conjugations. Unfortunately, the inherent instability of both Hfr strains did not allow a more precise mapping of the *scr* genes in the chromosome. Further attempts by P1 transduction, using the markers *pts*, *gut* (= *srl*), *fuc*, *thyA*, *rpsL*, *malA* and *mtl*, in a series of newly isolated mutants, of *K. pneumoniae* also failed. Finally, none of 27 different R' plasmids isolated from *K. pneumoniae* contained the *scr* genes, and none of the isolated R'scr⁺ plasmids were able to complement a large series of additional mutations in *E. coli* K12 recipient strains. The precise location of the *scr* genes in the chromosome of *K. pneumoniae* thus remains to be established.

A sucrose-PTS involving a sucrose-specific EII^{Scr} (gene *scrA*) which requires EIII (gene *scrB*) for full activity, a soluble invertase (gene *scrB*) and a soluble fructokinase (gene *scrK*) whose structural genes are apparently clustered in an *scr* regulon and inducible by fructose (or a fructose derivative) are features common to the sucrose systems from the chromosome of *K. pneumoniae* and from pUR400. This similarity also includes gene products of identical size and properties, repressor/operator pairs with a high cross-specificity, and a high similarity in DNA:DNA hybridization experiments, both DNAs showing a high (58 mol %) G + C content (R. Ebner, F. Titgemeyer, G. Sprenger & J. Lengeler, unpublished results). These results suggest a recent common origin of both systems. Further investigations at the molecular level are under way to clarify this origin.

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