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L-Sorbose Metabolism in *Klebsiella pneumoniae* and Sor$^+$ Derivatives of *Escherichia coli* K-12 and Chemotaxis Toward Sorbose

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L-Sorbose degradation in *Klebsiella pneumoniae* was shown to follow the pathway L-sorbose → L-sorbose-1-phosphate → D-glucitol-6-phosphate → D-fructose-6-phosphate. Transport and phosphorylation of L-sorbose was catalyzed by membrane-bound enzyme II^{sor} of the phosphoenolpyruvate-dependent carbohydrate:phosphotransferase system, specific for and regulated by this ketose and different from all other enzymes II described thus far. Two soluble enzymes, an L-sorbose-1-phosphate reductase and a D-glucitol-6-phosphate dehydrogenase, were involved in the conversion of L-sorbose-1-phosphate to D-fructose-6-phosphate. This dehydrogenase was temperature sensitive, preventing growth of wild-type strains of *K. pneumoniae* at temperatures above 35°C in the presence of L-sorbose. The enzyme was distinct from a second D-glucitol-6-phosphate dehydrogenase involved in the metabolism of D-glucitol. The *sor* genes were transferred from the chromosome of nonmotile strains of *K. pneumoniae* by means of a new R'*sor*$^+$ plasmid to motile strains of *Escherichia coli* K-12. Such derivatives not only showed the temperature-sensitive Sor$^+$ phenotype characteristic for *K. pneumoniae* or Sor$^+$ wild-type strains of *E. coli*, but also reacted positively to sorbose in chemotaxis tests.

In many bacteria, transport and concomitant phosphorylation of a series of carbohydrates are catalyzed by a set of membrane-bound and substrate-specific enzymes II (EII) of the phosphoenolpyruvate (PEP)-dependent carbohydrate: phosphotransferase system (PTS). In the presence of PEP and two cytoplasmic proteins, enzyme I (EI) and HPr, EII's are phosphorylated either directly by these protein kinases or by the intermediate of a third phosphocarrier protein enzyme III (EIII). In the phosphorylated form, EII's catalyze with a high affinity the vectorial phosphorylation and the translocation of their substrates through the cytoplasmic membrane into the cells (12, 19, 22). EII's, furthermore, have a primary role as the chemoreceptors in bacterial chemotaxis for carbohydrates taken up through these transport systems (PTS-carbohydrate) (1, 5, 9, 12). None of the methyl-accepting chemotaxis proteins is involved in the transduction of a signal from EII's to the tumble regulator of flagella. Instead, an alteration in the level of phosphorylation of some component of the PTS or of a molecule phosphorylated by the PTS during the translocation of a substrate triggers the chemotactic signal (11, 12, 17, 18). The signal itself remains to be determined.

The nonmotile bacterium *Klebsiella pneumoniae* and its motile relative *Escherichia coli* diverged in their evolution an estimated 35 million years ago (6). It thus seemed interesting to transfer the structural gene for an EII normally not found in *E. coli* from the chromosome of *K. pneumoniae* into *E. coli* K-12 to test whether this extraneous EII could still be coupled to the signal-transducing machinery of *E. coli*. We chose to transfer the genes for catabolism of L-sorbose (Sor), a ketose never fermented by wild-type cells of *E. coli* K-12 but always by strains of *K. pneumoniae*. The data of Kelker et al. (7) indicated the existence of an EII of the PTS involved in the transport and phosphorylation of Sor in a strain of *Aerobacter aerogenes*, later renamed *K. pneumoniae*. Unfortunately, due to the lack of radioactively labeled Sor and appropriate mutants, no further characterization of the EII involved was given.

In the present communication, the transfer of the *sor* genes from *K. pneumoniae* to *E. coli* K-12, the Sor metabolic pathway, and the enzymes involved and their participation in transport and chemotaxis are described. The implications of the results for our model on signal transduction in EII-mediated chemoreception are discussed. Further data on the isolation and characterization of the mutants used and on the mapping of the *sor* genes on the chromosome of *K. pneumoniae* will be given in a separate communication (G. A. Sprenger and J. W. Lengeler, manuscript in preparation). In an independent study, D. K. Olukoya (University of Reading, England, personal communication) came to similar conclusions on Sor metabolism and the genes involved in Sor$^+$ strains of *E. coli*.

MATERIALS AND METHODS

Chemicals. L-[$U-^{14}C$]sorbose was from CEA, Gif-sur-Yvette, France, and D-[^3H]glucitol was from New England Nuclear Corp., Dreieich, Federal Republic of Germany. All other materials were of commercial origin.

Bacteria. The properties of the strains are given in Table 1. All *K. pneumoniae* strains labeled KAY were sensitive to bacteriophage P1 and derived from strain 2002, itself an F$^-$ *arg gua* derivative of strain 1033-5P14 (22). The latter, originally described as *A. aerogenes*, had, according to the Enterotube Roche, the Fermotube Roche, and several additional tests (lactose, cellobiose, or Sor fermentation; methyl red test; phage typing test), all the properties of a typical *K. pneumoniae*, especially the lack of flagella and the inability to mutate to a Fla$^+$ phenotype (data not shown). A full description of strain 2002 and the isolation and characteriza-

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TABLE 1. Origin, phenotype, and genotype of the bacteria used

Strain	Origin	Reference	Relevant markers[a]
K. pneumoniae			
2002		22	F^- *arg gua fla* Sor(Ts)
KAY2026[b]	2002		P1 sensitive
KAY2027	KAY2026		*ptsH*
KAY2028	KAY2026		*ptsI*
KAY2029	KAY2026		Gut^{pc} Sor(Ts)
KAY2036	KAY2026		Sor^{pc} gut^+
KAY2040	KAY2026		Sor(Tr)
KAY2135	KAY2026		Gut^{pc} Sor(Ts) gua^+
E. coli K-12			
L163		9	F^- $gutR^+$ $gutAp^+$ $gutAo^+$ $gutA^+$ *gutD50*
LM1	LR2-167	12	F^- *crr manAI nagE*
LR2-175	LR2-167		F^- *glcA fruA manAI nagE*
GSL21	L163		pR'sor^+ Sor(Ts) Gut^- Ap^r Km^r Tc^r
GSL24	LM1		pR'sor^+ Sor(Ts) Glc^- Ap^r Km^r Tc^r
GSL25	LR2-175		pR'sor^+ Sor(Ts) Glc^- Fru^- Man^- Nag^- Ap^r Km^r Tc^r
MXR		23	pULB113 Ap^r Km^r Tc^r Tra^+ (Mu3A)

[a] The genetic nomenclature is that of Bachmann (4) and Lengeler et al. (12), using *glcA* for *ptsG*, *fruA* for *ptsF*, *manA* for *ptsM*, *manI* for *man*, *nagE* for *ptsN*, *gut* for *srl* according to Lin (15), and *sor* for Sor genes. Mu3A designates the presence of a mini-Mu derivative of mutator phage Mu in the RP4 derivative pULB113 (23). For mutants listed previously, only the altered markers are given.

[b] The isolation and characterization of these strains and plasmids will be given in a separate communication (Sprenger and Lengeler, in preparation). All of the KAY strains were derived from strain 2002.

[c] The superscript p indicates the presence of an unknown pleiotropic mutation in the *gut* or *sor* operon, respectively, which eliminates the expression of all enzyme activities.

tion of its derivatives will be given in a separate communication (Sprenger and Lengeler, in preparation). The *E. coli* K-12 strains L163 (lacking the *gutD*-coded D-glucitol-6-phosphate [Gut6p] dehydrogenase), LM1 (lacking the *crr* gene-coded enzyme III^{glc}), and MXR with its plasmid pULB113 have been described previously (9, 12, 23). After its transfer into strain KAY2026, an R'sor^+ plasmid carrying the *sor* genes of its host strain was isolated from pULB113. The isolation and characterization of this plasmid are described elsewhere (Sprenger and Lengeler, in preparation). Strain LR2-175, finally, was a derivative of the *E. coli* K-12 mutant LR2-167 (12) and lacks all known EII of the PTS specific for hexoses and hexosamines.

Culture media and growth conditions. The media and the plates used have been described previously (9). In minimal media, amino acids were added to 20 μg/ml, vitamins to 5 μg/ml, and carbohydrates to 10 mM. In growth determinations, one absorbance unit at 420 nm corresponds to 4.75×10^8 bacteria per ml for *E. coli* and 5×10^8 bacteria per ml for *K. pneumoniae* strains. To obtain cultures of *E. coli* with highly motile cells, growth was at 30°C in minimal glycerol medium (with 10 mM Sor if needed for induction) at a slow rotation rate.

Chemotaxis assays. The capillary tube assay for chemotaxis described by Adler was performed in a slightly modified form, as described previously (12). The number of bacteria accumulating in 1 h at 30°C inside a 1-μl micropipette (Drummond Scientific, Colo.) was determined by plating out its content on tryptone plates.

Transport and enzyme tests. Standard uptake tests for 25 μM D-glucitol or 5 μM Sor, the preparation of cell extracts by ultrasonic treatment and membrane fractions by centrifugation (2 h at 100,000 × *g*), as well as the EI + HPr and EII tests, have been described in detail previously (9, 13). For reconstruction experiments (see Fig. 3), membranes of strains KAY2026 and GSL25 were isolated from ultrasonic and French pressure cell extracts as the 100,000 × *g* pellet after two washings. To reduce the amount of EI + HPr apparently entrapped inside the membrane vesicles, an additional ultrasonic treatment of the membranes followed by a last washing was necessary. Determinations of the enzyme L-sorbose-1-phosphate (Sor1P) reductase were done by the method of Anderson and Simkins (3), using D-fructose-1-phosphate as a substrate, MOPS (morpholinepropanesulfonic acid) (0.25 M, pH 6.2) instead of MES (morpholineethanesulfonic acid) buffer, and ultrasonic cell extracts or toluenized cells for enzymes. Toluenization was done by adding 10 μl of toluene per ml to cells resuspended in minimal medium at 0°C and mixing for 60 s on a Vortex-type mixer before incubating for an additional 10 min on a roller drum. This toluene treatment at 0°C in tubes covered with Parafilm prevented early evaporation of toluene, allowed the test of sensitive enzymes, and gave specific activities comparable to activities measured in ultrasonic or French pressure cell extracts (data not shown). Tests of the Gut6P dehydrogenase activities were done as described previously (13) in Tris buffer (0.1 M, pH 7.5) using 3(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazoliumbromide plus phenazine methosulfate as electron acceptors and cell extracts or toluene-treated cells from cultures pregrown at 30°C. To test the thermal inactivation of enzyme activities, 250 μl of toluene-treated cells was shifted from 0°C to either a 30°C or a 37°C water bath. After 10 min, the activity in an unheated control sample and the remaining activity in the 30°C sample and a portion of the 37°C sample were determined. Further portions of the 37°C sample were thereafter shifted to one of the temperatures indicated in Fig. 4, heated for an additional 10 min, and tested.

RESULTS

Growth on Sor. Although most strains of *E. coli*, e.g., strains K-12, B, or C, were unable to ferment Sor, *Klebsiella* strains normally were able to grow on this ketose, e.g., *K. pneumoniae* 1033-5P14 and its derivatives used in the present study. For strain KAY2026, however, growth on Sor could only be detected at temperatures below 33°C and after a prolonged lag. Furthermore, a culture growing at 30°C on Sor almost immediately stopped further growth after a shift of the temperature to 42°C (Fig. 1A). Also, during growth on glycerol at 42°C, the addition of Sor caused a growth inhibition in the Sor^+ derivatives of *E. coli* K-12 (Fig. 1B) and in all strains of *K. pneumoniae* tested thus far (data not shown). This could be taken as an indication of the presence of a temperature-sensitive enzyme involved in Sor metabolism, since growth on D-glucitol (Fig. 1A) and on other PTS- or non-PTS-carbohydrates was not temperature sensitive (data not shown).

The Sor(Ts) phenotype was not restricted to strain KAY2026 and its parent strain 2002 but was also observed in strain M5A1 and in five other wild-type strains of *K. pneumoniae* and even in a Sor^+ wild-type strain of *E. coli*,

all freshly isolated from decaying organic material (data not shown). After transfer of the chromosomal sor^+ gene from strain KAY2026 by means of the plasmid R'sor^+ (derived from pULB113) into strains of *E. coli* K-12, several Sor^+ derivatives were isolated (Table 1). These too showed the typical Sor(Ts) phenotype (Fig. 1B).

Growth inhibition at elevated temperatures is not to be confused with the growth inhibition caused by Sor in all strains of *E. coli* K-12 and at all temperatures (8, 20, 21, 24). The latter is due to the accumulation of Sor1P or L-sorbose-6-phosphate in the cells and catalyzed by EII^{glc} and EII^{fru} coded for by the genes *glcA* (*ptsG*) and *fruA* (*ptsF*) (21; Lengeler, unpublished results). Thus, strain LR2-175, which lacks EII^{glc} and EII^{fru}, was resistant to Sor, whereas its derivative GSL25 carrying the plasmid R'sor^+ had regained the Sor(Ts) phenotype.

Sor transport and catabolic pathway. Based on data from a strain of *A. aerogenes*, Kelker et al. (7) proposed the following metabolic pathway for Sor degradation in enterobacteria: Sor → Sor1P → Gut6P → Fru6P. Furthermore, they proposed the participation of (i) an EII of the PTS, not coordinately regulated with the remaining of the enzymes; (ii) an NAD(P)H_2-dependent Sor1P reductase; and (iii) an NAD-dependent Gut6P dehydrogenase, apparently common to the Sor and D-glucitol metabolic pathways. Since, however, neither an uncoupling of the transport system and the remaining enzymes during induction nor the participation of one common enzyme regulated by two different inducing substrates had been observed in any of the EII-initiated pathways analyzed thus far (10), we retested the crucial steps in our strains.

Transport and phosphorylation of Sor by EII^{sor}. After growth at 30°C on Sor or a mixture of Sor and glycerol, strain KAY2026 showed a high accumulation of ^{14}C-labeled Sor (Fig. 2). The K_m value for Sor was 30 μM (data not shown). This transport activity was low in cells pregrown on glycerol and, in contrast to the EII^{gut} activity coded for by *gutA* of the *gut* operon (9), not induced by the presence of D-glucitol in the medium, whereas the latter EII activity was not induced by Sor in the medium (Table 2).

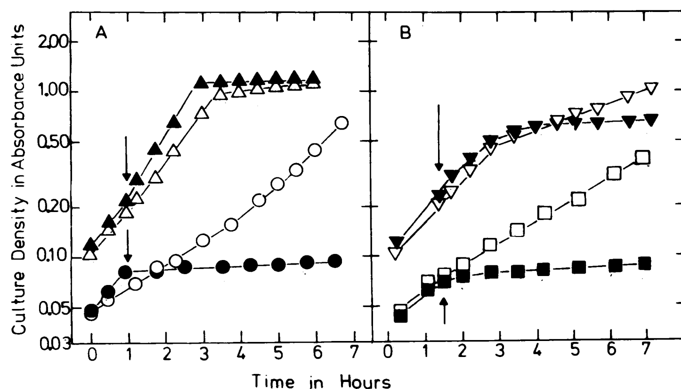


FIG. 1. Growth of *K. pneumoniae* and Sor^+ strains of *E. coli* K-12 on Sor. (A) Cells of strain KAY2026 pregrown at 30°C in minimal medium containing Sor (○ and ●) or D-glucitol (△ and ▲) were reinoculated into fresh medium and grown. After about one generation, one batch was shifted to 42°C (indicated by the arrow) (●, ▲), and further growth was followed (B) Cells of strain GSL21 *gutD* Sor^+ were tested for growth on Sor at 30°C (□) and 42°C (■) as described in (A). Cells of strain GSL25 *glcA fruA* Sor^+ were pregrown on minimal medium containing glycerol at 30°C. After about one generation, 10 mM Sor was added, and one batch was kept at 30°C (▽) while the other was shifted to 42°C (▼) at the times indicated by arrows.

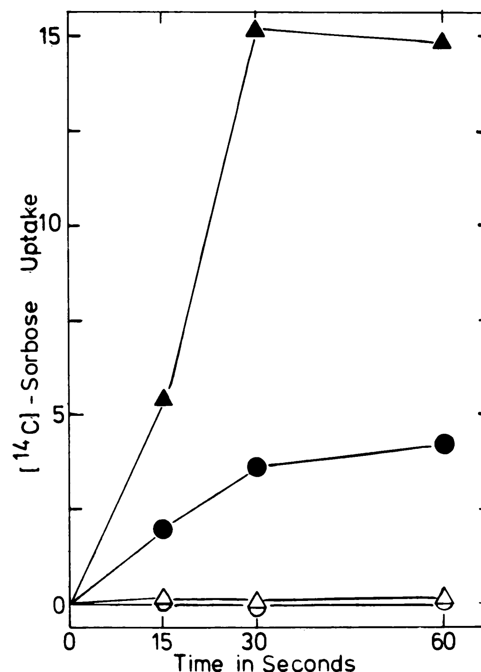


FIG. 2. Uptake of Sor. Cells pregrown at 30°C in minimal medium with glycerol plus Sor were tested for uptake of L-[^{14}C]sorbose (5 μM). The activity is expressed in nanomoles per minute per milligram of protein. *K. pneumoniae* KAY2026 gut^+ sor^+ (▲), KAY2027 *ptsH* and KAY2028 *ptsI* (△), KAY2036 gut^+ Sor^- (○), *E. coli* K-12 strain LR2-175 *glcA fruA manA nagE* (△), and GSL25 *glcA fruA manA nagE* sor^+ (●) were used.

Derivatives of strain KAY2026 lacking either the general PTS proteins EI (strain KAY2028) and HPr (KAY2027) or EII^{sor} coded for by *sorA* in the *sor* operon (KAY2036), as well as the *E. coli* K-12 mutant LR2-175 lacking all known EII's of the PTS specific for hexoses and hexosamines, had no detectable Sor transport activity (Fig. 2). Sor^+ derivative GSL25 of the latter, in contrast, did show Sor transport activity, although at a lower level than in the corresponding strains of *K. pneumoniae* (Fig. 2). This activity was only inducible by Sor. Strain GSL24, lacking the EIII of the PTS coded for by *crr*, had a Sor^+ phenotype and Sor transport activity (data not shown).

Among a series of additional *pts* mutants derived from KAY2026 and its parent strain 2002, none was able to grow on Sor (with or without the addition of 5 mM cAMP) or to take up and phosphorylate this ketose. Furthermore, all Sor^+ revertants of such *pts* mutants had regained the general PTS protein activities (data not shown), further supporting the existence of a Sor-specific EII^{sor}. The phosphorylation of Sor by purified membranes from induced cells of strain KAY2026 or GSL25 was tested directly (Fig. 3). This

TABLE 2. Induction of Sor and D-glucitol transport activities in strain KAY2026[a]

Inducer	Uptake (nmol/min/mg of protein)	
	Sor	D-Glucitol
None	0.05	0.20
Sor	13.40	0.20
D-Glucitol	0.05	7.20

[a] Cells of strain KAY2026 pregrown at 30°C on glycerol, Sor, or D-glucitol were tested for 5 μM Sor and 25 μM D-glucitol uptake.

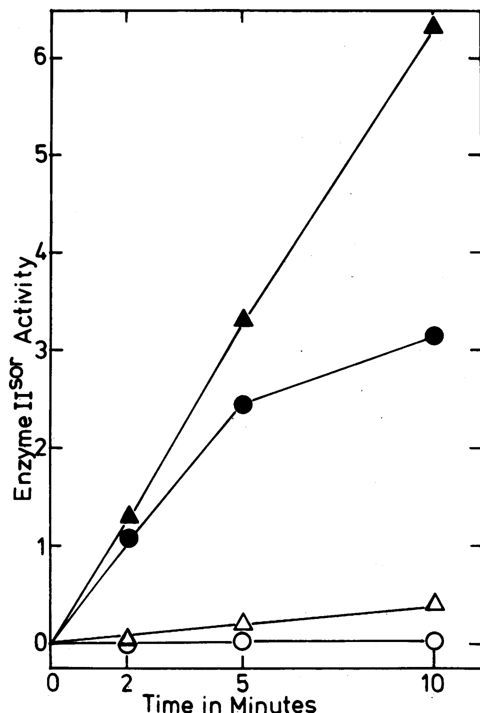


FIG. 3. In vitro test of EII^{sor} activity. A purified and washed membrane fraction was used to test the phosphorylation of L-[^{14}C]sorbose in combination with the membrane-free supernatant from cells of strain KAY2026 pregrown on sucrose or *N*-acetylglucosamine which contained EI + HPr activity. Shown here are the supernatant of strain KAY2026 with membranes from Sor-grown cells of strain KAY2026 (▲) or strain GSL25 (●); KAY2026 without PEP (△) and with ATP instead of PEP (○); membranes from induced cells without supernatant of KAY2026 (EI + HPr) (○); membranes from KAY2026 pregrown on D-glucitol (uninduced) and from strain LR2-175 pregrown on glycerol plus Sor (○). All activities are expressed in nanomoles per minute per milligram of protein.

phosphorylation depends on the simultaneous presence of EI, HPr, PEP (for which ATP was not a substitute), and purified membranes. Furthermore, membranes from induced Sor$^+$ strains but not from cells induced by D-glucitol or *E. coli* LR2-175 gave a positive reaction. Again, membranes from strain GSL25 showed a lower activity than membranes from strain KAY2026, perhaps indicating an incomplete complementation and expression of the *K. pneumoniae*-derived proteins and genes in the *E. coli* host cell.

The final conclusion from the transport and in vitro phosphorylation tests was, consequently, that a Sor-specific and regulated EII^{sor} of the PTS was involved in Sor metabolism and that this EII^{sor} was none of the EII's described thus far, did not depend on EIII^{glc}, and was not normally found in *E. coli* K-12. Strain LR2-175, indeed, lacks EII^{glc} for D-glucose, EII^{fru} for D-fructose, EII^{man} for D-mannose, EII^{nag} for N-acetylglucosamine, and EII^{bgl} for β-glucosides coded for by *glcA* (*ptsG*), *fruA* (*ptsF*), *manA* (*ptsM*), *nagE* (*ptsN*) and *bglC* (*ptsB*), respectively, whereas the hexitol-specific systems were not expressed. Yet its derivative, GSL25, had a Sor$^+$ phenotype.

Sor1P reductase. In accordance with the data of Kelker et al. (3, 7), an NAD(P)H_2-dependent reductase was detectable in Sor$^+$ cells of *K. pneumoniae* and in Sor$^+$ derivatives of *E. coli* K-12 (80 nmol/min per mg of protein), but not in wild-type cells of this organism (≤2.5 nmol/mg of protein). This activity was inducible by Sor but not by D-glucitol and appeared coordinately induced with EII^{sor} and one of two (see below) distinct Gut6P dehydrogenase activities. The reductase activity was stable at high temperatures (data not shown).

Gut6P dehydrogenase. According to Kelker et al. (7), only one Gut6P dehydrogenase is responsible for growth on both Sor and D-glucitol in *A. aerogenes*. This activity, furthermore, was claimed to be induced by both carbohydrates. Since for all other PTS-carbohydrates tested thus far (10), the major EII substrate was the only inducer, we considered the possibility that here too two distinct Gut6P dehydrogenases were present, one inducible by Sor and the other by D-glucitol. One could, perhaps, have been missed previously due to its temperature sensitivity and general lability in cell extracts. In the *sor*$^+$ *gut*$^+$ strain KAY2026, inducible Gut6P activity was observed after growth on Sor and on D-glucitol. These, however, were two distinct activities: one, inducible by Sor, was present in the mutant KAY2029 *sor*$^+$ Gut$^-$, whereas the other, remaining in strain KAY2036 Sor$^-$ *gut*$^+$, was inducible by D-glucitol (Table 3). As predicted, the latter was found to map in the *gut* operon, coded for by *gutD*, whereas the former maps in the *sor* operon, coded for by *sorD* (Sprenger and Lengeler, in preparation). Both isoenzymes had the same pH optimum (pH 7.6) and were sensitive to Na_2CO_3. Most interestingly, both were temperature sensitive (Fig. 4), although to a different degree. The *gutD*-coded enzyme had its $T_{1/2}$ at 28°C; the *sorD*-coded enzyme had its $T_{1/2}$ at 50°C. When a derivative of strain KAY2026 able to grow on Sor at 42°C was selected, this derivative KAY2040 had a *sorD*-coded activity with a $T_{1/2}$ of 60°C (Fig. 4), whereas its *gutD*-coded activity remained unaltered.

Although the *gutD*-coded dehydrogenase was more temperature sensitive than the *sorD*-coded activity, strains of *K. pneumoniae* were able to grow at 42°C on D-glucitol (Fig. 1) but not on Sor. Strains of *E. coli* K-12, in contrast, have a Gut(Ts) phenotype (9). After the introduction of the *sor* genes into *E. coli*, such strains became able to grow on D-glucitol at 42°C after pregrowth on Sor, but remained Gut(Ts) after pregrowth on D-glucitol at 30°C. Conversely, Sor$^-$ mutants of *K. pneumoniae* which lacked the *sorD*-coded dehydrogenase activity transiently stopped growth at 42°C on D-glucitol. It remains to be shown which type of complicated regulation of the two dehydrogenases involved was responsible for this phenotype (see below).

Chemotaxis of Sor$^+$ strains of *E. coli* K-12 toward Sor. Wild-type strains of *E. coli* K-12, although unable to grow on Sor, showed high attraction when tested for chemotaxis toward this ketose, characteristic for many non-metabolizable analogs (2). The EII's involved in this reaction are EII^{glc}, EII^{fru}, and EII^{man}, which are also involved in transport and phosphorylation of Sor in strains of *E. coli* K-12 (12, 21).

TABLE 3. Induction of Gut6P dehydrogenase activities in *K. pneumoniae*[a]

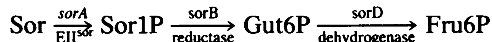
Inducer	Gut6P dehydrogenase (μmol/min/mg of protein)		
	KAY2026 *sor*$^+$ *gut*$^+$	KAY2029 *sor*$^+$ Gut$^-$	KAY2036 Sor$^-$ *gut*$^+$
None	≤0.01	≤0.01	≤0.01
Sor	0.55	0.82	0.02
D-Glucitol	0.36	≤0.01	0.40

[a] Cells pregrown at 30°C on glycerol eventually supplemented with Sor or D-glucitol and treated with toluene were tested for Gut6P dehydrogenase activity.

Strain LR2-175 (Fig. 5), which lacks these EII's showed only a low reaction to Sor. Whether the remaining stimulation was caused by a contaminating carbohydrate or a residual activity of the EII is unknown at present. GSL25, a Sor$^+$ derivative of strain LR2-175, in contrast, showed a high reaction in the capillary tube chemotaxis test. Its low threshold value (ca. 5 μM) and the characteristic decrease of the reaction at concentrations above 1 mM are typical for attractants with a high affinity for their chemoreceptor. At higher concentrations of the attractant in the tube, indeed, enough substrate diffuses into the pond to prevent effectively further entry of the cells into the tube. The maximum of the reaction of strain GSL25 to Sor is comparable to the reaction of cells of *E. coli* K-12 to the better PTS-carbohydrate attractants (12) and was observed only with cells pregrown on Sor.

DISCUSSION

The present data on Sor degradation in seven different strains of *K. pneumoniae* and in Sor$^+$ derivatives of *E. coli* K-12 basically confirmed the pathway proposed by Kelker et al. (7) for a strain of *A. aerogenes*:



$$\text{Sor} \xrightarrow[\text{EII}^{\text{sor}}]{sorA} \text{Sor1P} \xrightarrow[\text{reductase}]{\text{sorB}} \text{Gut6P} \xrightarrow[\text{dehydrogenase}]{\text{sorD}} \text{Fru6P}$$

The EII$^{\text{sor}}$ was induced coordinately with the other enzymes of the pathway by its major substrate, Sor. In a separate communication it will be shown that the structural genes *sorA* (for EII$^{\text{sor}}$), *sorB* (for Sor1P reductase), and *sorD* are clustered together with regulatory genes in the *sor* operon. The latter gene codes for a Gut6P dehydrogenase which is temperature sensitive and different from a second Gut6P dehydrogenase which is also temperature sensitive but coded for by *gutD* and involved in D-glucitol metabolism. Clustering of the structural gene for an EII together with the structural genes for the other enzymes of a catabolic pathway in an operon and consequently their coordinate induction by the major substrate of the EII had been observed previously for all EII-initiated pathways analyzed thus far. Only the D-glucose catabolic enzymes (also involved in glycolysis) and the D-mannose catabolic enzymes (also involved in cell wall biosynthesis) were not coordinately regulated with EII$^{\text{glc}}$ and EII$^{\text{man}}$, respectively (10).

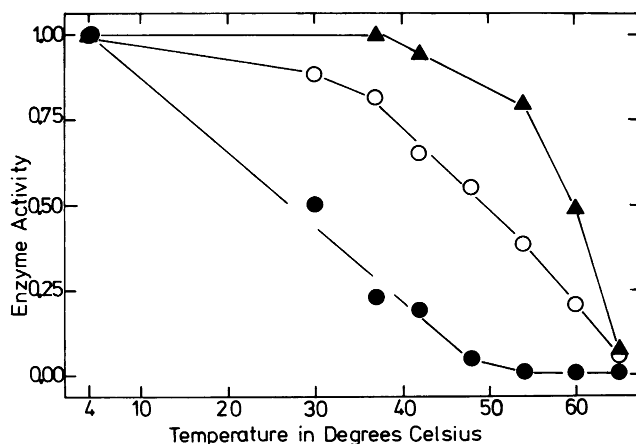


FIG. 4. Heat inactivation of Gut6P dehydrogenase activities. Freshly toluenized cells (1.5 × 10^9 bacteria per ml) pregrown exponentially on glycerol with Sor or with D-glucitol at 30°C were stored at 0°C in TRIS-hydrochloride buffer (0.1 M, pH 7.5). Samples were tested directly or after incubation for 10 min at the temperatures indicated. Activities are expressed as percent remaining activity of a control value. Activity from strains KAY2026 *gut*$^+$ *sor*$^+$, KAY2036 *gut*$^+$, *sor*$^-$, KAY2040 *gut*$^+$ Sor(Tr), and GSL25 *glcA fruA manA nagE sor*$^+$ pregrown on D-glucitol (●); strains KAY2026, KAY2135 *gut*$^-$ *sor*$^+$ and GSL25 pregrown on Sor (○); and strain KAY2040 Sor(Tr) grown on Sor (▲) is shown.

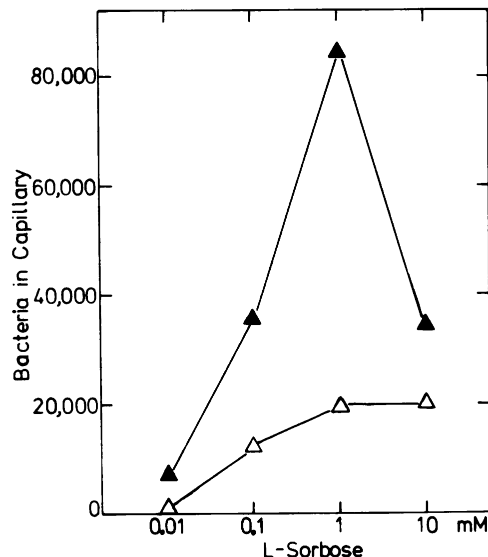


FIG. 5. Chemotaxis toward Sor. Fully motile cells of the *E. coli* K-12 strain LR2-175 *glcA fruA manA nagE* (△) and its isogenic derivative GSL25 *sor*$^+$ (▲) were grown at 30°C on minimal medium containing glycerol and Sor and tested for chemotaxis in the capillary tube test. After substraction of the value of a control tube containing wash medium, the number of bacteria per capillary was normalized to the value of a second control tube containing 1 mM L-aspartate.

The biochemical similarity between the EII-initiated pathways and their enzymes and the arrangement of the corresponding structural and regulatory genes inside their operon and on the chromosome of *E. coli* K-12 prompted us to the speculation that they all were derived from a common operon (9, 10). The temperature-sensitivity of the two Gut6P dehydrogenases involved in D-glucitol and Sor metabolism, their regulatory interrelationship, and the position of the *sor* operon in *K. pneumoniae* between loci *rpo* and *malB* (Sprenger and Lengeler, in preparation) are further support for this hypothesis. Independently from us, D. K. Olukoya, University of Reading, England (personal communication) came to similar conclusions regarding the genes and enzymes involved in Sor metabolism for Sor$^+$ strains of *E. coli*.

Besides functioning in transport and in the regulation of peripheral catabolic pathways, EII's have a central role as the chemoreceptors in chemotaxis (1, 10, 12). The EII-mediated chemoreception does not involve methyl-accepting chemotaxis proteins, but instead requires phosphorylation-dephosphorylation (11, 12). It has been suggested that an alteration in the level of phosphorylation of some component of the PTS or a protein linked to the PTS during the translocation of a substrate triggers the chemotactical signal (12, 18). This signal, whose exact nature is still unknown, could be an alteration in the configuration of the phosphorylated and dephosphorylated EII itself, "sensed" directly by another chemotaxis protein. This hypothetical protein of the signal-transducing machinery then would have to be in direct physical contact with the EII's.

The integration of EII^{sor} from nonmotile cells of *K. pneumoniae* into the chemotaxis machinery of motile cells of *E. coli* K-12 seems to argue against this hypothesis. This EII, indeed, was distinct from all EII's of *E. coli* K-12 analyzed thus far and was not found in this organism due to the lack of the *sor* genes (24; unpublished results). These bacteria, which diverged in their evolution an estimated 35 million years ago, have accumulated 12% amino acid and 25% base pair exchanges, even in genes and in gene products subject to a strong selection (6, 16). It seems unlikely that none of these many amino acid exchanges should interfere with such a signal transduction by physical contact between an EII and another protein of the signal-transducing machinery. An $EIII^{glc}$-dependent EII^{scr}, specific for and regulated by sucrose, has been transferred from a *Salmonella typhimurium* plasmid to strains of *E. coli* K-12. This extrageneous EII^{scr} was integrated into the chemotaxis machinery (14, 18).

An alternative to signaling by direct physical contact of two proteins would be signaling by a biochemical coupling, e.g., of the EII-initiated signal through the general PTS proteins EI and/or HPr to a molecule of the remaining signal transducing machinery. The present data, as well as a series of previous data (1, 12, 18), are in agreement with this second model: (i) all EII's tested thus far were the chemoreceptors for their substrates; (ii) the stimulus in EII-mediated chemoreception was not the binding or dissociation of the substrate, but the reversible phosphorylation and dephosphorylation of the EII; (iii) mutants with a normal methyl-accepting chemotaxis protein-mediated chemoreception which had lost the capability to react to PTS-carbohydrates lacked one or both of the general PTS proteins EI and HPr; (iv) signal integration for all stimuli originating from EII's and the adaptation of the cells to these signals was caused primarily by EI and HPr and their regulation of the binding and phosphorylation capacity of all EII's; and (v) in vivo and in vitro complementation tests between EII's, EI, and HPr from *E. coli* K-12 and *K. pneumoniae* showed that the heterologous reconstitution reached half the value of a homologous one (Fig. 2 and 3; data not shown).

The hypothetical "phosphoryl-chemotaxis-protein" postulated as the next intermediate between the proteins EI and/or HPr and the tumble regulator could be (according to recent data of Black et al. [5] and unpublished data from our group) the enzyme adenylate cyclase. Its activity, indeed, seems regulated by the PTS (19), and one of its products, cGMP, seems to play a crucial role in tumble regulation (5).

Taken together, these data suggest the following model for chemotaxis to PTS-carbohydrates. During the translocation of a substrate through an EII, the substrate is phosphorylated, whereas the EII, thereafter HPr, and finally EI are dephosphorylated. This in turn triggers an alteration in the adenylate cyclase activity, the cGMP pool and/or in an as yet unidentified intermediate, leading eventually to a change in swimming behavior. At the same time, the signals from all EII's are integrated by the general PTS proteins through regulation of the binding and phosphorylation capacities of the EII's. If, however, substrates enter the cell at a high rate or over a longer period of time, a new equilibrium between EII activity, the level of phosphorylation of EI or HPr, and the adenylate cyclase activity is reached: the cell is adapted.

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