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Phosphoenolpyruvate-Dependent Phosphotransferase System Enzyme III and Plasmid-Encoded Sucrose Transport in *Escherichia coli* K-12

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The phosphoenolpyruvate-dependent carbohydrate:phosphotransferase system enzyme II^{Scr}, specific for and regulated by sucrose, was analyzed in derivatives of *Escherichia coli* K-12 carrying the sucrose plasmid pUR404. Enzyme II^{Scr}, coded for by gene *scrA* of the plasmid, depended for its transport and phosphorylation activity directly on the phosphotransferase system enzyme $III^{Glc,Scr}$, coded for by the chromosomal gene *crr*.

Among the *Enterobacteriaceae*, only the tribe *Klebsielleae* is characterized by a stable sucrose-positive phenotype. For the other tribes, including the closely related *Vibrionaceae*, an unstable sucrose phenotype is typical. The genes for such variable metabolic properties (e.g., for lactose, raffinose, or sucrose fermentation), labeled "d" or "D" in *Bergey's Manual* (3), normally seem to be located on (transmissible) plasmids of hitherto unknown origin (4, 8, 14, 18). Thus, plasmid-free strains of *Escherichia coli* K-12 or *Salmonella typhimurium* LT2 are unable to take up and ferment sucrose or to mutate to a sucrose-positive phenotype (21). Among epidemiologically related strains of these bacteria, however, this phenotype is common and invariably coupled to the presence of plasmids (1, 4, 8, 15, 18, 22). A better understanding of the interrelationship between plasmid- and chromosome-encoded metabolic functions might provide clues as to the possible phylogenetic origin of metabolic plasmids.

A conjugative plasmid was isolated recently from a clinical sucrose-positive strain of *S. typhimurium* (22) and named pUR400 (15) to comply with genetic nomenclature rules (12). The plasmid was subsequently transferred by mating to a prototrophic strain of *E. coli* K-12, to which it confers jointly the sucrose-positive and tetracycline-resistant phenotypes. This sucrose utilization is mediated by two plasmid-encoded functions (15): a phosphoenolpyruvate (PEP)-dependent carbohydrate:phosphotransferase system (PTS) enzyme II^{Scr} (EII^{Scr}) is plasmid encoded. This enzyme is not normally found in *E. coli* K-12; it is specific for sucrose and is dependent on the host cell general PTS proteins enzyme I (EI) and HPr. Bacterial PTSs (13) consist of two cytoplasmic, nonsubstrate-specific proteins, EI and HPr, and of a series of substrate-specific and membrane-bound enzymes II (EII). Mutants defective in the *pts* operon and its proteins EI and HPr are pleiotropic, because these proteins are required for the uptake and phosphorylation of all PTS-dependent substrates. In addition, due to lowered levels of intracellular cAMP, such *pts* mutants are pleiotropically negative for most catabolic pathways. The latter effects can be suppressed either by the addition of cAMP to growing cultures or by constitutive expression of the generally inducible catabolic operons (13). However, mutants defective in a single EII are specifically deficient only in the transport of the substrates taken up through this enzyme (9, 10, 13). The genes encoding such enzymes are scattered over the genome of *E. coli* K-12 (for nomenclature, see Table 1). The second function involved in sucrose utilization is a β-D-fructofuranoside fructohydrolase (EC 3.2.1.26), hereafter called hydrolase, which has been shown to hydrolyze sucrose-6-phosphate, the immediate product of sucrose uptake through EII^{Scr}, into D-glucose-6-phosphate and D-fructose. The transport system and the hydrolase are coordinately inducible by sucrose or D-fructose in the medium, and both are expressed constitutively in the plasmid derivative pUR404 (15).

In *E. coli* K-12 and in *S. typhimurium*, most EIIs consist of a single membrane-bound protein (5, 9, 10). However, for the major D-glucose transport system of *E. coli* K-12 and its membrane-bound EIIB', coded for by the gene *glcA* (formerly *ptsG*), an additional cytoplasmic phos-

phocarrier protein, $EIII^{Glc}$, is required (10, 13, 16). This protein, coded for or regulated by the gene *crr* (13; J. Lengeler and P. W. Postma, unpublished data), has been isolated and characterized recently from *S. typhimurium* (16). Strains of *E. coli* K-12, defective in $EIII^{Glc}$ due to a mutation in the locus *crr*, are specifically defective only in the transport and phosphorylation of substrates of the major D-glucose transport system, whereas other PTS substrates are taken up normally (10; e.g., strains LM1, LM11, and derivatives in Table 1). Consequently, this enzyme has been considered up to now to be required only for the activity of the EIIB' transport system (13, 16).

To test which of the host cell's cytoplasmic or membrane-bound proteins and enzymes of the PTS are directly involved in sucrose transport and phosphorylation, we crossed pUR404 with constitutive expression of the sucrose metabolic enzymes into a series of *E. coli* K-12 mutants carrying known mutations in the *pts* or in the EII genes, which have been described before (10). Glycerol-grown cells of the wild-type strain DS409(pUR404) exhibited a high uptake rate of ^{14}C-labeled sucrose, in contrast to plasmid-free strains, in which no sucrose uptake was detectable (Table 1). The plasmid pUR404 was also transferred to strains lacking all known PTSs for hexoses and hexosamines, except for the inducible EII^{Fru} coded for by the gene *fruA* (formerly *ptsF*). Strain L332(pUR404), which carries the mutations in genes *mglA-C*, *galP*, *nagE* (formerly *ptsN*), *manA* (formerly *ptsM*), and *glcA* (formerly *ptsG*) of strain LR2-168 and thus lacks the transport systems for D-galactose, β-galactosides, *N*-acetylglucosamine, D-mannose, and D-glucose (10), had a sucrose-positive phenotype and a high uptake of this disaccharide (Fig. 1 and Table 1). The three EIIs specific for and regulated by D-mannitol, D-glucitol, and galactitol were not induced under the test conditions, and elimination of EII^{Fru} by mutation of the *fruA* gene in strain L332 did not affect its sucrose-positive phenotype (data not shown). When pUR404 was transferred to the *crr* mutant LM1 or LM11 (except for the $glcA^+$ *crr* genotype isogenic to the *glcA* crr^+ strain LR2-168), such derivatives (e.g., L330 or L331 in Table 1) retained their sucrose-negative phenotype and remained unable to take up sucrose (Fig. 1). Two hundred sucrose-positive revertants of L330 were isolated subsequently and tested. All had simultaneously regained a glucose-positive phenotype and sucrose as well as α-methylglucoside transport activity. A few representative colonies tested (e.g., L33OR1 in Table 1) had also regained $EIII^{Glc}$ activity. Furthermore, it was shown by P1 transduction that for L33OR1 the genotype became crr^+ again (data not shown). Among 200 D-glucose-positive revertants of L330, about half became sucrose positive also and had regained $EIII^{Glc}$ activity. The other half (e.g., L33OR2 in Table 1) remained sucrose negative and still lacked $EIII^{Glc}$ activity. This was confirmed by in vitro phosphorylation tests (10)

TABLE 1. Properties of the strains used

Strain	Plasmid	Origin	Genotype[a]	Phenotype[b]				Transport activity[c]		$EIII^{Glc,Scr}$[d]
				Scr	Glc	Nag	Suc	Scr	αMeGlc	
DS409	pUR404		Wild type	3+	3+	3+	+	1.87	NT[e]	NT
L314			Wild type	−	3+	3+	+	<0.001	32.70	1.00
LR2-167		L314	*glcA*$^+$ *crr*$^+$ *manA nagE*	−	3+	−	+	<0.001	26.20	1.00
LR2-168		LR2-167	*glcA crr*$^+$ *manA nagE*	−	−	−	+	<0.001	0.10	1.00
L332	pUR404	LR2-168	*glcA crr*$^+$ *manA nagE*	3+	+	−	+	0.86	0.10	1.00
LM1		LR2-167	*glcA*$^+$ *crr manA nagE*	−	(+)	−	−	<0.001	0.10	<0.01
L330	pUR404	LM1	*glcA*$^+$ *crr manA nagE*	−	(+)	−	−	0.008	0.30	<0.01
L330R1	pUR404	L330	*glcA*$^+$ *crr*$^+$ *manA nagE*	3+	3+	−	+	1.37	30.00	1.00
L330R2	pUR404	L330	*glcA*$^+$ *crr manA*$^+$ *nagE*	−	2+	2+	−	0.01	0.10	<0.01
LM11		LR2-167	*glcA*$^+$ *crr manA nagE*	−	−	−	+	<0.001	0.10	<0.01
L331	pUR404	LM11	*glcA*$^+$ *crr manA nagE*	−	−	−	+	<0.001	0.40	<0.01

[a] The genetic symbols are according to references 2 and 10 with the following modifications: *glcA* (formerly *ptsG*) designates the gene coding for the D-glucose enzyme $EIIB'^{Glc}$, *manA* (formerly *ptsM*) codes for the D-mannose enzyme EII^{Man}, and *nagE* (formerly *ptsN*) codes for the *N*-acetyl-D-glucosamine enzyme EII^{Nag}.

[b] The phenotypes for sucrose (Scr), D-glucose (Glc), and *N*-acetylglucosamine (Nag) are expressed as generation times in minutes [3+, 50 to 55 min; 2+, 150 min; +, ca. 600 min on 10 mM substrates; (+), slow growth on plates with 50 mM D-glucose; −, no growth]; Suc^+ or Suc^- indicates a succinate-positive or -negative phenotype.

[c] Transport activities for sucrose (0.05 μM) and α-methylglucoside (αMeGlc; 25 μM) are expressed as nanomoles per minute per milligram of protein.

[d] The amount of $EIII^{glc,scr}$ was determined in toluenized cell extracts followed by rocket immunoelectrophoresis, using antiserum against purified factor III (16), and is given in relative units.

[e] NT, Not tested.

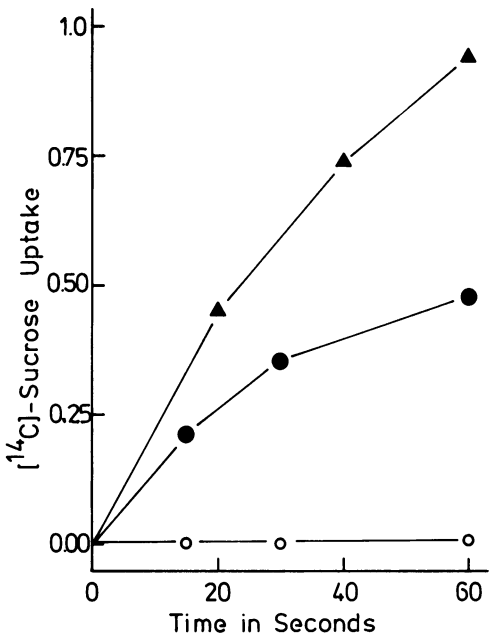

FIG. 1. [^{14}C]sucrose uptake in different mutants. Washed cells, after exponential growth in minimal glycerol medium, were exposed to [^{14}C]sucrose (0.05 μM; purified by Sephadex G-15 chromatography) as described before (9). Strains L33OR1 (▲) and L332 (●) and strains L330, L331, L33OR2, and LR2-167 (○). Uptake activity is expressed in nanomoles per milligram of protein.

and, in collaboration with P. W. Postma, by rocket immunoelectrophoresis of toluenized cell extracts of a few typical mutants, using antiserum against purified EIII^{Glc} (16). The latter class of revertants invariably had regained one of the other D-glucose transport systems not requiring EIII^{Glc}, usually the *manA* (formerly *ptsM*)-coded EII complex EII^{Man} (data not shown).

Final proof for a direct role of EIII^{Glc} in the uptake and phosphorylation of sucrose by plasmid-encoded EII^{Scr} was obtained from in vitro phosphorylation and complementation tests. When purified membranes of strain L330 (*crr* pUR404) were mixed with cell extracts of strain LR2-168 (*crr*$^+$), we found a strong PEP-dependent phosphorylation of sucrose (Fig. 2). Cell extracts of the *crr* mutant LM1, however, did not stimulate this reaction, nor could ATP substitute for PEP. The sucrose-negative phenotype of strain L330 or L331 (data not shown) thus is not due to a deficiency of EII^{Scr} in the membranes, but to the lack of the cytoplasmic EIII^{Glc} of the PTS.

In accordance with the accompanying paper (15), it can be concluded that pUR404-encoded transport and phosphorylation involves a sucrose-specific EII^{Scr}, cytoplasmic proteins EI and HPr of the PTS, and cytoplasmic EIII^{Glc}, coded for by the chromosomal genes *ptsI*, *ptsH*, and *crr*, respectively. At the moment, we can only speculate as to why EIIB'^{Glc} and EII^{Scr} require an additional cytoplasmic EIII^{Glc,Scr}, whereas six other EIIs tested thus far in *E. coli* K-12 do not. These include a newly discovered EII^{Tre}, specific for and regulated by trehalose (D-glucose-1,2-α-D-glucose) (Lengeler, unpublished data), which in *Vibrio parahaemolyticus* seems to depend on an EIII^{Glc} (7). Moreover, in EII phosphorylation, EIII^{Glc} has been implicated with catabolite repression as a regulator of internal cAMP levels (references in 13 and 16) and with transient repression as a regulator of intracellular hexose phosphate levels (references in 10). Such an implication seems to be supported by the observation (Lengeler, unpublished data) that sucrose in *E. coli* K-12(pUR404) strains equals D-glucose in the gen-

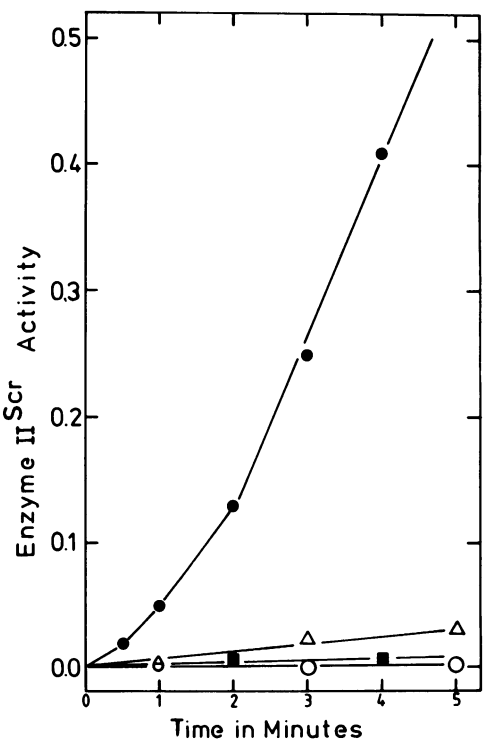

FIG. 2. In vitro reconstitution of EII^{Scr}/EIII^{Glc,Scr}-dependent phosphorylation of [^{14}C]sucrose. Membranes of strain L330 (○), isolated from ultrasonic cell extracts after two washings as the 200,000 × *g* pellet (10), and ultrasonic extracts of strain LR2-168 (△) or LM1 (○) were tested alone or in the combinations L330 × LR2-168 (●) and L330 × LM1 (■) for the PEP-dependent phosphorylation of 0.1 μM [^{14}C]sucrose as described (9). Activities are expressed in nanomoles per milligram of protein.

eration of catabolite or transient repression and inducer exclusion, whereas trehalose does not.

The tight coupling of plasmid- and chromosome-encoded enzymes in sucrose metabolism, together with the high transmissibility of pUR404 among the *Enterobacteriaceae*, is compatible with a hypothesis, suggested before (8), that sucrose plasmids may have originated in the *Klebsielleae*. In apparent contradiction to this hypothesis, it has been claimed that sucrose metabolism in *Klebsiella pneumoniae* (formerly *Aerobacter aerogenes*) does not involve a PTS EII, but rather uptake of free sucrose and an ATP-dependent phosphorylation of the hydrolysis products (6). By contrast, the pathway proposed now (15) resembles the sucrose metabolism in most of the gram-positive bacteria analyzed thus far, regardless of whether the corresponding genes are located on plasmids or on the chromosome (1, 11, 17, 19, 20). The published data on *Aerobacter* (6), however, do not rule out a PTS-dependent pathway, since neither all intermediates and enzymes involved nor mutants for each step have been determined. Consequently, we analyzed again the sucrose-metabolism in two different strains of *K. pneumoniae* and found it to be very similar to the pUR 404 pathway, except for the *scr* genes being located on the chromosome (J. Lengeler and G. Sprenger, manuscript in preparation). This similarity included the same intermediates, similar enzymes and their regulation, and a cytoplasmic $EIII^{Glc,Scr}$ having antigenic properties similar to those of the analogous enzymes from *E. coli* K-12, *S. typhimurium* (16), and probably *V. parahaemolyticus* (7). The ability of plasmid-encoded sucrose genes to recombine with the *E. coli* K-12 chromosome is another indication of a close relationship of the two DNAs involved (1). In conclusion, the *Klebsielleae* indeed appear to be potential candidates for the origin of the sucrose plasmid pUR404 in its present form, a hypothesis which could be further tested by DNA-sequencing experiments now underway.

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