The fructose permease of Escherichia coli, the fructose-specific Enzyme II of the phosphoenolpyruvate-dependent phosphotransferase system (PTS), contains a duplicated IIB domain. The protein therefore consists of three distinct domains, B, B', and C (N-terminal to C-terminal), joined by flexible linkers and is thus designated FruB'BC. The N-terminal B' domain was removed using molecular genetic techniques, and the truncated Enzyme II (FruBC) was characterized relative to the wild-type enzyme both in vivo and in vitro. Using molecular genetic techniques, and the truncated Enzyme II (FruBC) was characterized relative to the wild-type enzyme both in vivo and in vitro. In vivo, FruBC exhibited depressed fermentation characteristics at low fructose concentrations. [14C]Fructose uptake measurements revealed reduced rates only when the permease was rate-limiting for transport. In vitro, FruBC exhibited a 10-fold lower affinity for its phosphorylating protein, the IIA-FPr diphosphoryl transfer protein (DTP), than was observed with the wild-type enzyme, and the maximal velocity of fructose phosphorylation was 7-fold depressed. Because the fructose-1-phosphate-[14C]fructose transphosphorylation reaction appeared normal, we conclude that the loss of the B' domain primarily affected phosphoryl transfer between the IIA and IIB domains of the permease. A mutant FruB'BC derivative with cysteine 112 replaced by serine (C112SFruBC) was inactive as a phosphoryl carrier and a sugar transport protein. Expression of the plasmid-encoded mutant protein inhibited the in vivo activity of the chromosomally encoded wild-type fructose permease, but it did not observably affect the activities of the mannitol or glucitol PTS permeases or of non-PTS sugar permeases. Further, the presence of the detergent extracted mutant protein inhibited the activity of the detergent solubilized wild-type or FruBC enzyme. In contrast, the wild-type FruB'BC permease was apparently epistatic over the truncated FruB'BC permease in vivo. The experiments reported 1) show that the B' domain of the fructose permease functions to facilitate phosphoryl transfer between DTP and the permease, 2) establish the essentiality of cysteine 112 in the B domain of the permease, 3) provide evidence that a functional fructose permease consists of an oligomer in which both IIB domains must be active for the enzyme to catalyze normal rates of phosphoryl transfer and transport, 4) suggest that a single B' domain in the oligomeric Enzyme II is sufficient to allow high efficiency phosphoryl transfer between the IIA domain of DTP and the IIB domain of the permease, and 5) show that the B' domain is not important for oligomerization.

The permeases of the bacterial phosphotransferase system (PTS) are multidomain Enzyme II complexes that mediate the detection, transport, and phosphorylation of their sugar substrates (Robillard and Lolkema, 1988; Lolkema and Robillard, 1992; Postma et al., 1993). They consist minimally of three distinct domains termed IIA, IIB, and IIC, which may be found fused or dissociated in various combinations and various orders with each other and with other PTS protein domains (Saier and Reizer, 1992, 1994). They accept a phosphoryl group from HPr(his-P), which in turn is phosphorylated with phosphoenolpyruvate (PEP) as the ultimate phosphoryl donor in a reaction catalyzed by Enzyme I of the PTS. The phosphoryl transfer chain of the PTS can be generalized as shown in Scheme I, where S1 and S2 represent two different sugar substrates of the PTS. The upper scheme is representative of the major class of PTS permeases found in almost all eubacteria, which includes the glucose, fructose, mannitol, and glucitol permeases of Escherichia coli, whereas the lower scheme is representative of a minor class of PTS permeases, which includes the mannose permease of E. coli (Lolkema and Robillard, 1992; Meins et al., 1993; Buhr et al., 1994).

Of the PTS permeases, the fructose-1-phosphate-forming fructose permeases are both the most widespread in nature and the most complex. Many Gram-negative and Gram-positive bacteria utilize only fructose via the PTS (Saier, 1977; Romano and Saier, 1992; Mitchell et al., 1993; Titzemeyer et al., 1994, 1995), and it has consequently been postulated that a fructose-specific permease may have been the primordial system that gave rise to the major class of PTS permeases (Saier et al., 1985; Wu and Saier, 1990a, 1990b).

Sequence analyses of fructose permeases in Gram-negative bacteria have revealed that the proteins that comprise these permeases have unique combinations of PTS protein domains linked together. For example, the fru operon in E. coli encodes a tridomain, diphosphoryl transfer protein (DTP) in which IIA'^ is linked to a fructose-inducible HPr-like domain called FPr via a central domain of unknown function (Geese et al., 1989; Wu et al., 1990, 1990; Reizer et al., 1994b). The membranal Enzyme II possesses three domains, two B domains and a C domain, with the domain order: B'BC (Prior and Kornberg, 1988; Wu and Saier, 1990b). E. coli also possesses cryptic operons encoding fructose-like PTS permeases (Reizer et al., 1995).
Overproduction and Purification of Enteric Bacterial PTS Proteins—The overproduction and purification of E. coli Enzyme I and HPr have been described (Reizer et al., 1992). The DTP encoded within the fructose (fru) operon of Salmonella typhimurium (Geese et al., 1989) was overproduced as follows: an NdeI restriction site was introduced in the initiation codon of the fruB gene encoding DTP by site-specific mutagenesis (Reizer et al., 1989). The fruBC operon was digested with the NdeI and SacI restriction enzymes and was ligated into the NdeI-XhoI-digested pET19B plasmid (Novagen, Madison, WI). This procedure results in attachment to DTP of an N-terminal polyhistidyl sequence.

Overproduction of his-tagged DTP was accomplished following transfer of the resultant plasmid (pRFuB) to E. coli strain BL21(DE3) and growth of this plasmid-bearing strain in LB medium (10 liters) in the presence of 0.15 mM isopropyl β-thiogalactoside (4 h at 37°C). Cells were harvested, washed three times by centrifugation, and resuspended in 20 mM Tris-HCl, pH 7.5, containing 0.1 mM phenylmethylsulfonyl fluoride. They were then ruptured by three passages through a French pressure cell at 10,000 psi. Cell debris was removed by centrifugation (10,000 × g for 10 min at 4°C), and the clarified extract was loaded onto an immobilized Ni²⁺ column (Novagen, Madison, WI; column height, 5 cm; column diameter, 2 cm). The column was washed with 20 mM Tris-HCl buffer, pH 7.9, containing 0.5 mM NaCl and 60 mM imidazole. DTP was eluted with the same buffer with the imidazole concentration increased to 1 M. DTP was obtained with an estimated purity of 80–90% as judged by SDS-polyacrylamide gel electrophoresis. This protein was found to complement a fruB-negative mutant of E. coli in vivo (Chin et al., 1989) and proved to catalyze [¹⁴C]fructose phosphorylation in the presence of Enzyme I and membranes isolated from fructose grown S. typhimurium strain LT-2 cells in in vitro assays. It was phosphorylated with [¹⁴C]PEP and purified Enzyme I and was capable of transferring this phosphoryl moiety to homologous IIA, IIC, and NPr (Powell et al., 1995).

Polymerase Chain Reaction Amplification of Wild-type, Truncated, and Mutant fru Genes from Chromosomal DNA of E. coli Strain TG1—The two primers used to amplify the wild-type fruA gene (encoding the wild-type fructose permease, designated FruBC) were: left primer (35-mer, coding strand), 5'-GGCGAATTCAT(ATG)AAAA-GTCCAGGCTGCGGATCCGC-3'; right primer (39-mer, complementary strand), 5'-CGAAGACCTGTGCATTACGCTGGCGCTGTCGATTATCGAC-3' (The ATG start codon is underlined and in parentheses.) The two primers used to amplify the gene encoding the truncated FruA permease comprising only the B and C domains (designated FruBC) were: left primer (33-mer, coding strand), 5'-GGCGAATTCATGCTTGCCGAAACGCGTAGTTGCGGTG-3' (The ATG start codon is underlined and in parentheses). The two primers used to amplify the gene encoding the truncated DTP, a permute with a cysteine to serine substitution at site 112 (designated C112S FruBC) were: left primer (50-mer, coding strand), 5'-GGCGAATTCGCGGTAGAAATGCTGCTGCGGATCCGC-3' and right primer same as the right primer used for the full-length gene.

The two primers used to amplify the gene encoding the truncated DTP, with a cysteine to serine substitution at site 112 (designated C112S FruBC) were: left primer (50-mer, coding strand), 5'-GGCGAATTCGCGGTAGAAATGCTGCTGCGGATCCGC-3' and right primer same as the right primer used for the full-length gene. In these two constructions an additional ATG codon encoding cysteine 112 has been changed to a TCC codon encoding a serine residue (Cys→Ser).

Two-step Subcloning of the Amplified DNA Fragments into the proK1 Expression Vector—The amplified double-stranded DNA fragments were first subcloned into the pCR™ cloning vector using the Invitrogen TA cloning™ kit (Invitrogen Corporation, San Diego, CA). The double-stranded DNA fragments encompassing the respective fruB and fruBC genes were obtained by cutting the pCR™ recombinant plasmids simultaneously with EcoRI and HindIII. DNA Amplification fragment was then cloned into the proK1 expression vector (proK1). The proK1 recombinant plasmid carrying the wild-type fruA gene (encoding the FruBC wild-type permease) was designated pB7C. proK1 recombinant plasmids carrying...
truncated fruA genes (encoding FruBC and C112S FruBC mutant permeases) were designated pBC and pC112SBC, respectively. Expression of the Recombinant Plasmids in HK1376—The recombinant pROK1 plasmids were transferred by electroporation into the recipient fruA strain HK1376 for expression. These plasmids were again subjected to restriction analysis to verify their correct identity. The NcoI restriction fragments of FruBC and FruBC were also checked by DNA sequence analysis of plasmids pBC and pBC using the M13 reverse primer (−48) (Biolabs NEN), and the USB Sequenase version 2.0 DNA sequencing kit (Amersham Corp.).

In Vivo Fructose Uptake Measurements—Bacteria were grown overnight at 37 °C with agitation in minimal Medium 63 supplemented with 1 µg/ml thiamine, 0.2% casamino acids, 0.2% fructose, and 100 µg/ml ampicillin. The same medium (5 ml) was then inoculated with an aliquot of the overnight culture (initial A600 = 0.05). Cultures were grown at 37 °C with agitation to an A600 of 0.6. Bacteria were then washed twice with Medium 63 containing 40 µg/ml chloramphenicol, resuspended in the same medium in aliquots of 0.5 ml at A600 = 0.2, and placed on ice. Cell suspensions were pre-equilibrated at room temperature for 15 min prior to the addition of [14C]fructose (10 µM; 20 µ Ci/µmol). At t = 30, 60, and 90 s, aliquots (150 µl) of each bacterial suspension were applied to Millipore filters (pore size, 0.45 µm), washed twice with minimal medium, dried, and submerged in Biosafe NACSintillation fluid (3 ml) before counting in a Beckman LS230 liquid scintillation counter.

In Vitro Fructose Phosphorylation Assays—Bacteria were grown overnight at 37 °C with agitation in LB medium supplemented with various carbon sources and inducers as indicated. Cells were pelleted by centrifugation and washed three times. For 1-liter cultures, the washed pellets were resuspended in 20 ml of 20 mM Tris-HCl, pH 7.5, containing 1 mM dithiothreitol and 0.1 mM phenylmethylsulfonyl fluoride. The resuspended bacteria were disrupted by passage through a French pressure cell at 10,000 psi. Urea-butanol extracted membranes were prepared and dialyzed as described by Saier et al. (1977).

PTS-dependent sugar phosphorylation assays were performed essentially as described previously (Reizer et al., 1989, 1992). The final volume was 50 µl, the PEP concentration was 5 mM, and the concentration of [14C]fructose (5 µCi/µmol) was 10 µM unless otherwise specified. When urea-butanol extracted membranes were used, purified components of the PTS (E. coli Enzyme I and DTP) were added as detailed in the figure legend. Transphosphorylation assays were performed essentially as described previously with rate-limiting amounts of urea-butanol washed membranes (Saier et al., 1977) and assay mixtures (final volume, 200 µl) containing 10 mM fructose-1-phosphate, 10 µM [14C]fructose (5 µCi/µmol), and other constituents as described previously (Saier et al., 1977).

Preparation of Lubrol Solubilized Proteins—E. coli strains HK1376 (pB BC), HK1376 (pB BC), and HK1376 (pC112SBC) were grown at 37 °C in Medium 63 supplemented with thiamine (1 µg/ml), casamino acids (0.2%), ampicillin (100 µg/ml), and glucose (0.2%). At A600 = 0.6–0.7, IPTG (10−3 M) was added, and growth was followed to A600 = 1.0–1.5. Bacteria were collected by centrifugation and washed, and the pellets from 1-liter volumes of cell cultures were resuspended in 20 ml of 20 mM Tris-HCl, pH 7.5, containing 1 mM dithiothreitol. Cells were broken by passage through a French Press (10,000 psi) at 4 °C. After removal of cell debris, membranes were collected by centrifugation at 100,000 x g for 90 min at 4 °C.

Membrane proteins were solubilized with 0.5% deoxycholate by shaking at 20 °C for 30 min in a buffer solution consisting of 20 mM Tris-HCl, pH 8.4, 0.2 M NaCl, and 1 mM dithiothreitol in a total volume of 25 ml. The suspension was then centrifuged at 100,000 × g for 90 min at 4 °C. The supernatant was finally dialyzed against 20 volumes of 20 mM Tris-HCl, pH 8.4, 1 mM dithiothreitol, and 0.5% Lubrol PX (TDL buffer). Dialysis was continued for 40 h with two changes of buffer.

RESULTS

Topological Model of the E. coli Fructose Permease—Fig. 1A presents a topological model of the E. coli fructose permease protein (FruB’BC). This model is based on TOP-PRED (Sipos and von Heijne, 1993) analyses for the protein members of the fructose IIC family (Reizer et al., 1995). The TOP-PRED program predicted nine transmembrane helical segments for the E. coli Enzyme IICfru, for the demonstrably homologous IIC domains of the fructose PTS permeases of R. capsulatus and X. campestris, and for the IIC protein domains encoded within the frw and frv operons of E. coli (Reizer et al., 1994a, 1995). The linear domain structure of wild-type FruB’ BC is presented in Fig. 1B where the relative domain sizes are portrayed to scale. The two mutant forms of the fructose permease (FruB’C and C112S FruB’C) that were constructed for this study are diagrammed in Fig. 1C and D, respectively.

In Vivo Properties of FruB’BC, FruB’C, and C112S FruB’C—As summarized in Table I, cells expressing FruB’BC or FruB’C were capable of efficient fructose fermentation if the fructose concentration was ≈ 0.5%. At a fructose concentration of 0.2%, however, FruB’BC bearing cells fermented fructose weakly, whereas cells possessing FruB’ BC gave a strong response. The C112S FruB’ protein appeared nonfunctional at all fructose concentrations tested based on these fermentation criteria (Table I). The same strains were tested for growth in liquid minimal salts medium containing 0.2% casamino acids as well as 0.2% fructose. The growth rates were the same for all three strains, showing that fructose was not growth inhibitory (data not shown).

Fructose uptake rates were measured with 10 µM [14C]fructose after growth under inducing (Fig. 2A) or noninducing (Fig. 2B) conditions. After growth under fructose-induced conditions, where the fructose permease proteins were present in excess, there was no significant difference in the rate or extent of fructose uptake by cells bearing FruB’BC or FruB’C. By contrast, C112S FruB’C was essentially inactive. Glycerol grown cells (uninduced for fru operon expression) took up [14C]fructose much more slowly than did the fructose-induced cells, and the truncated FruB’C permease was detectably less
active than the wild-type permease. These results show that loss of the B’ domain has a perceptible effect on fructose transport and metabolism under the conditions employed only when the fructose permease is rate-limiting for uptake.

In Vitro Properties of Fru BC and Fru B—Fig. 3 shows the results of an in vitro experiment in which crude extracts from cells possessing the wild-type (Fru BC) and truncated (Fru B) proteins were examined for [14C]fructose phosphorylation after growth of the cells in the presence of fructose. The wild-type Fru BC enzyme phosphorylated [14C]fructose efficiently with endogenous DTP, and its activity was stimulated slightly by the addition of excess purified DTP. In contrast, the truncated Fru BC enzyme exhibited low endogenous activity, but the activity of this preparation was greatly stimulated, essentially to the wild-type level, by the addition of excess DTP. The control strain (HK1376 (pROK1)) was completely inactive either with or without added DTP. These results show that the Fru BC protein exhibits appreciable levels of activity only in the presence of excess DTP.

The kinetic properties of Fru BC were compared with those of Fru BC when studied with excess purified enzyme and variable concentrations of purified DTP in the presence of a constant fructose concentration (Fig. 4A). The truncated protein lacking the B’ domain exhibited a 7-fold depressed maximal phosphoryl transfer velocity, and it exhibited a 10-fold higher Km value for DTP (300 μM versus 30 μM). When the same two enzymes were examined as a function of fructose concentration (1–10 mM) in the presence of constant amounts of DTP, straight lines were obtained on the double reciprocal plot (Fig. 4B). The calculated Km values for fructose (assuming a ping-pong bi bi mechanism) (Segal et al., 1975) were 5.4 μM and 1.6 μM for Fru BC and Fru B, respectively.

The fructose-1-phosphate[14C]fructose transphosphorylation reaction (Saier et al., 1977) was also studied. For this experiment, urea-butanol-extracted membrane preparations were used (see “Experimental Procedures”). The two enzymes (present in rate-limiting amounts) gave the same rate of transphosphorylation (42 pmol of fructose phosphorylated per min per milligram protein ± 10%). Because transphosphorylation does not involve DTP and is a reflection of the affinities of the enzyme for fructose and fructose-1-phosphate (Saier et al., 1977), the results suggest that the primary defect resulting from the loss of the B’ domain involves phosphorl transfer from DTP to the B domain of the fructose permease.

Negative Dominant Phenotype of the C112S Fru BC Permease over the Wild-type Permease and Positive Dominant Phenotype of the Wild-type Permease over the Fru BC Permease in Vivo—Plasmids pBC and pC112SBC encoding the truncated permease lacking the B’ domain and the truncated C112S mutant permease, respectively, were individually transferred to E. coli strain TG1, which encodes the wild-type fructose permease on the chromosome. Fermentation responses were recorded on MacConkey agar plates in the presence of varying concentrations of fructose either with or without 1 mM IPTG to induce synthesis of the plasmid-encoded permeases. The results are summarized in Table I. Strain TG1 exhibited a strong fermentation response regardless of the fructose concentration used, and the same was observed when pBC was expressed. However, when pC112SBC was present, the fermentation response was somewhat diminished at fructose concentrations of 0.2 and 0.5%. Inclusion of 1 mM IPTG in the agar medium, which enhanced expression of the plasmid-encoded mutant permease, strongly inhibited the fermentation response at all sugar concentrations (see Table I).
of two other PTS sugars (mannitol and glucitol) as well as three non-PTS sugars (l-arabinose, maltose, and melibiose) (see description in Table II). In no case did high level expression (IPTG-induced) of pC112SBC give rise to fermentation inhibition. Thus, the inhibitory effect of pC112SBC was specific to the fructose permease.

As shown in Fig. 5, inhibition of [14C]fructose uptake was also observed following growth of the pC112SBC bearing strain in the presence of IPTG, although no inhibition was observed when the pBC bearing strain was grown under the same conditions. The transport results agree with the fermentation responses recorded in Table II, showing that C112S FruBC exhibits a negative dominant phenotype over the wild-type allele.

Negative Dominance of C112S FruBC over FruB and FruBC in Vivo—The wild-type fructose permease (FruB BC) from strain HK1376 (pBC), the truncated permease (FruBC) from strain HK1376 (pBC), and the mutant fructose permease from strain HK1376 (pC112SBC) were extracted with deoxycholate from isolated membranes and transferred to 0.5% Lubrol as outlined under “Experimental Procedures” (Jacobson et al., 1983). The diazylated extracts were then mixed as specified in the legend to Fig. 6. As can be seen from the data reproduced in Fig. 6, the presence of the C112S mutant fructose permease inhibited the activity of FruBC. The same was observed for the wild-type permease (data not shown). These results are in agreement with the negative dominant phenotype exhibited by C112S FruBC in vivo.

DISCUSSION

The fructose permease of E. coli is unique among PTS permeases in several respects: 1) It is the only known permease in E. coli to possess its own HPr-like protein, FPr (Saier et al., 1970); 2) it is the only one that has its IIA domain covalently linked to one of the general energy coupling proteins of the PTS (Geerse et al., 1989; Wu et al., 1990; Reizer et al., 1994a, 1994b); and 3) it is the only one that has an Enzyme II complex with an internally repeated IIB domain (Wu and Saier, 1990a). These unique features of the fructose PTS and other features discussed previously (Saier, 1977; Saier et al., 1985) have led to the hypothesis that an ancient fructose permease evolved into the first PTS and that this primordial PTS functioned to initiate the glycolytic cycle.
Negative dominance of plasmid-encoded C112S FruBC permease over chromosomally encoded wild-type FruB' BC and positive dominance of chromosomally encoded wild-type FruB' BC over plasmid-encoded FruBC

Fermentation responses of strain TG1 were recorded on fructose-MacConkey agar plates with fructose present at 0.2, 0.5, or 1% as indicated with or without 1 mM IPTG. Fermentation responses were recorded as follows: ++, wild-type response; +, a less strong response; ±, a weak response; −, no response. In addition to the responses recorded, all three strains were examined for their fermentation responses with and without IPTG on MacConkey fermentation plates containing 0.5% mannitol, arabinose, or maltose or 1% glucose or melibiose. Wild-type fermentation responses were recorded for all three strains on all five carbon sources regardless of the presence of IPTG. Overexpression of the truncated permease lacking the B' domain was also examined at lower concentrations of fructose (0.15 and 0.10%) with and without IPTG. In no case did expression of the truncated permease noticeably inhibit the fructose fermentation response of strain TG1. Strains used were: none, TG1; pC112SBC, TG1 (pC112SBC); pBC, TG1 (pBC).

Table II

| Plasmid        | 0.2% Fructose | 0.5% Fructose | 1% Fructose |
|----------------|---------------|---------------|-------------|
|                | −IPTG         | +IPTG         | −IPTG        | +IPTG        | −IPTG         | +IPTG         |
| None           | ++            | ++            | ++          | ++          | ++            | ++            |
| pC112SBC       | ±             | −             | +           | ±           | +             | ±             |
| pBC            | ++            | ++            | ++          | ++          | ++            | ++            |

Fig. 5. Competition between FruB' BC and C112S FruBC for in vivo fructose transport. Plasmid pBC or pC112SBC was transferred into E. coli strain TG1 containing the wild-type chromosomal fruA gene, and cells were prepared for [14C]fructose uptake measurements as described under “Experimental Procedures” after growth of strain TG1(pBC) without (∅) or with (ї) IPTG or of strain TG1(pC112SBC) without (∅) or with (ї) IPTG. [14C]fructose uptake was measured as a function of time as described under “Experimental Procedures.”

Condition (b) is probably rate-limiting. Evidence for tight binding PTS enzyme complexes in vivo has been published previously (Gachelin, 1969; Saier and Staley, 1977; Saier et al., 1982). Because duplicated B' domain(s) are found in the R. capsulatus and X. campestris fructose permeases (Wu et al., 1990; de Crécy-Lagard et al., 1991) and a detached B' domain is encoded within the frw gene cluster of E. coli (Reizer et al., 1995), the findings reported here may prove applicable to several fructose permease systems.

Construction of the mutant C112S FrubC permease, lacking the putative phosphorylation site residue (Cys112) in the IIB domain, allowed us to show that this protein is inactive as expected (Lokkema and Robillard, 1992; Lengeler et al., 1994; Meins et al., 1993). They also allowed us to provide evidence that like the mannitol and glucose PTS permeases (see Introduction), the fructose permease is probably an oligomer. Because fructolization and the activity of the wild-type fructose permease were specifically inhibited by the presence of the C112S FrubC mutant permease, both in vivo and in vitro, whereas mannitol, glucitol, maltose, arabinose, and melibiose utilization was not affected, it must be concluded that high level expression of C112S FrubC did not interfere with membrane protein insertion generally but instead inhibited the permease directly. The fact that the C112S FrubC encoding gene was found to be epistatic over the wild-type gene (i.e. exhibited a negative-dominant phenotype both in vivo and in vitro) leads to the tentative conclusion that the individual subunits within the oligomeric Enzyme II are poorly active. These findings are in agreement with a previously reported observation with the X. campestris fructose permease (de Crécy-Lagard et al., 1991). In this case, a C-terminal truncation, eliminating the second half of the IIC domain, exhibited a negative dominant phenotype over the wild-type enzyme. However, they contrast with results reported in analogous experiments conducted with the mannitol permease of E. coli (van Weeghel et al., 1991; Boer et al., 1994).

Although the FrubC enzyme did not allow efficient fermentation of fructose at 0.2% sugar, the heterooligomeric protein (FrubC' BC/FruB' BC) in the merodiploid (TG1 (pBC)) apparently was capable of fermentation at low fructose concentrations as efficiently as the wild-type strain (TG1) (compare Tables I and II and see description of Table II). It therefore appears that although the heterooligomer, C112S FrubC/FruB' BC, exhibits depressed activity, the heterooligomer, FrubC' BC/FruB' BC, is as active as the wild-type homooligomer and substantially more active than the truncated homooligomer. All subunits must therefore possess active phosphorylation sites in their B domains in order for the permease to exhibit normal activity, but
the B’ domain may be required in only one of the subunits. This conclusion suggests that transport requires fully active B domains in the identical subunits of the Enzyme II complex but that association of DTP with the permease does not. The molecular basis for these interesting observations remains to be elucidated.

Acknowledgments—We thank Dr. Hans Kornberg for strain HK1376 and Mary Beth Hiller for expert assistance in the preparation of this manuscript.

REFERENCES

Boer, H., ten Hoeve-Duurkens, R. H., Schuurman-Wolters, G. K., Dijkstra, A., and the B9 manuscript. and Mary Beth Hiller for expert assistance in the preparation of this manuscript.

Buhr, A., Flükiger, K., and Erni, B. (1994) Mol. Microbiol. 17, 2111–2120

Reizer, J., Sutrina, S. L., Saier, M. H., Jr., Stewart, G. C., Peterkofsky, A., and Reddy, P. (1989) EMBO J. 8, 2111–2120

Reizer, J., Sutrina, S. L., Wu, L.-F., Deutscher, J., Reddy, P., and Saier, M. H., Jr. (1992) J. Biol. Chem. 267, 9158–9169

Reizer, J., Michotey, V., Reizer, A., and Saier, M. H., Jr. (1994a) Protein Sci. 3, 440–450

Reizer, J., Reizer, A., Kornberg, H. L., and Saier, M. H., Jr. (1994b) FEMS Microbiol. Lett. 118, 159–162

Reizer, J., Reizer, A., and Saier, M. H., Jr. (1995) Microbiology 141, 961–971

Robillard, G. T., and Lukkema, J. S. (1988) Biochim. Biophys. Acta 947, 493–519

Romano, A. H., and Saier, M. H., Jr. (1992) In The Evolution of Metabolic Function (Mortlock, R. P., ed) pp. 143–170, CRC Press, Inc., Boca Raton, FL

Saier, M. H., Jr. (1977) Bacteriol. Rev. 41, 856–871

Saier, M. H., Jr., and Reizer, J. (1992) J. Bacteriol. 174, 1433–1438

Saier, M. H., Jr., and Reizer, J. (1994) Mol. Microbiol. 13, 755–764

Saier, M. H., Jr., and Staley, J. T. (1977) J. Bacteriol. 131, 716–718

Saier, M. H., Jr., Simani, R. D., and Roseman, S. (1970) J. Bacteriol. 156, 5870–5873

Saier, M. H., Jr., Feucht, B. U., and Mora, W. K. (1977) J. Biol. Chem. 252, 8899–8907

Saier, M. H., Jr., Cox, D. F., Feucht, B. U., and Novotny, M. J. (1982) J. Cell. Biochem. 18, 231–238

Saier, M. H., Jr., Grenier, F. C., Lee, C. A., and Waygood, E. B. (1985) J. Cell. Biochem. 27, 43–56

Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Segal, I. H. (1975) Enzyme Kinetics: Behavior and Analysis of Rapid Equilibrium and Steady State Enzyme Systems, pp. 684–727, John Wiley & Sons, Inc., New York

Sipos, L., and von Heijne, G. (1993) Eur. J. Biochem. 213, 1333–1340

Titgemeyer, F., Walkenhorst, J., Cui, X., Reizer, J., and Saier, M. H., Jr. (1994) Res. Microbiol. 145, 89–92

Titgemeyer, F., Walkenhorst, J., Reizer, J., Stuiver, M. H., Cui, X., and Saier, M. H., Jr. (1995) Microbiology 141, 51–58

van Weeghel, R. P., van der Hoek, T. Y., Pas, H. H., Elferink, M., Keck, W., and Robillard, G. T. (1995) Biochemistry 34, 7168–7173

Wu, L.-F., and Saier, M. H., Jr. (1990a) Mol. Microbiol. 4, 1219–1222

Wu, L.-F., and Saier, M. H., Jr. (1990b) J. Bacteriol. 172, 7167–7178

Wu, L.-F., Tomich, J. M., and Saier, M. H., Jr. (1990) J. Mol. Biol. 213, 687–703
Function of the Duplicated IIB Domain and Oligomeric Structure of the Fructose Permease of *Escherichia coli*
Alain Charbit, Jonathan Reizer and Milton H. Saier, Jr.

*J. Biol. Chem.* 1996, 271:9997-10003.
doi: 10.1074/jbc.271.17.9997

Access the most updated version of this article at [http://www.jbc.org/content/271/17/9997](http://www.jbc.org/content/271/17/9997)

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at [http://www.jbc.org/content/271/17/9997.full.html#ref-list-1](http://www.jbc.org/content/271/17/9997.full.html#ref-list-1)