Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 4052–4055, June 1989
Biochemistry

Site-directed mutagenesis of the phosphocarrier protein, III^{Glc}, a major signal-transducing protein in *Escherichia coli*

(phospho*enol*pyruvate:glycose phosphotransferase system/phosphohistidine)

KATHLEEN A. PRESPER, CING-YUEN WONG, LINDA LIU, NORMAN D. MEADOW, AND SAUL ROSEMAN

McCollum–Pratt Institute and the Department of Biology, The Johns Hopkins University, Baltimore, MD 21218

Contributed by Saul Roseman, March 10, 1989

ABSTRACT **The glucose-specific phosphocarrier protein (III^{Glc}) of the bacterial phospho*enol*pyruvate:glycose phosphotransferase system (PTS) is a major signal transducer that mediates the intricate interplay among extracellular signals (PTS and non-PTS sugars), cytoplasmic and membrane proteins (PTS and non-PTS transporters), and adenylate cyclase. To further define the central role of III^{Glc} in these multiplex signaling mechanisms, we have used site-directed mutagenesis to construct three mutant III^{Glc} proteins containing single amino acid changes; Phe-3 was replaced with tryptophan ($[Trp^3]III^{Glc}$), and His-75 and the active-site His-90 were replaced with glutamine ($[Gln^{75}]III^{Glc}$ and $[Gln^{90}]III^{Glc}$, respectively). $[Trp^3]III^{Glc}$ resembles the wild-type protein in most properties and should be valuable for spectrophotometric experiments. In contrast, clear differences between mutant and wild-type proteins were observed with both $[Gln^{75}]III^{Glc}$ and $[Gln^{90}]III^{Glc}$ in *in vitro* sugar phosphorylation assays. As predicted, $[Gln^{90}]III^{Glc}$ with a modified active site cannot be phosphorylated. Unexpectedly, $[Gln^{75}]III^{Glc}$ accepts but cannot transfer phosphoryl groups, suggesting His-75 may also be a critical amino acid for III^{Glc}-mediated signaling mechanisms. The physiological effects of these mutations are briefly described.**

The multifunctional phospho*enol*pyruvate:glycose phosphotransferase system (PTS) comprises a complex array of reversibly phosphorylated proteins that effect the concomitant uptake and phosphorylation of PTS sugar substrates. The PTS also acts as a signal transducer in the interplay of several other protein networks (e.g., non-PTS transport systems and adenylate cyclase) required for the maintenance of bacterial cellular metabolism (for review, see refs. 1 and 2). Our original hypothesis (3) proposed that the *crr* gene product, III^{Glc} (the phosphocarrier protein of the phosphotransferase system specific for glucose and methyl α-D-glucopyranoside), can deliver multiple regulatory signals in response to the extracellular concentrations of solute sugar nutrients, and, depending on the phosphorylation state of III^{Glc}, these signals are transduced by different pathways within the bacterial cell. The current working model for III^{Glc}-mediated regulatory functions is summarized in Fig. 1. Our ultimate goal is to dissect the signaling mechanisms in each of the cellular responses involving the pivotal PTS protein, III^{Glc}.

Structure–function studies of III^{Glc} are obviously germane to any mechanism(s) postulated to define the interactions outlined in Fig. 1. In the present study, we describe the construction of III^{Glc} mutant proteins with site-directed mutations of three critical amino acid residues: (*i*) We substituted glutamine for histidine at position 90 ($[Gln^{90}]III^{Glc}$) to investigate the role of His-90 in the phosphorylation–dephosphorylation functions of III^{Glc}; the presumptive phosphoryl-accepting active site in III^{Glc} has been identified as N-3 (4) of His-90 (6). (*ii*) The only other histidine residue in the protein, His-75, was also replaced by glutamine ($[Gln^{75}]III^{Glc}$); both histidyl residues are present within a proposed regulatory domain (7) conserved near the C terminus of five transport system proteins found in Gram negative and Gram positive organisms. (*iii*) In the third mutation tryptophan was substituted for Phe-3 in the N-terminal heptapeptide region ($[Trp^3]III^{Glc}$); the N terminus of III^{Glc} (4, 8) appears to be required for interaction with IIB^{Glc}, the membrane receptor protein. We also report preliminary characterization of the three mutant III^{Glc} molecules and their *in vitro* and *in vivo* physiological effects.

MATERIALS AND METHODS

Plasmids and *Escherichia coli* Bacterial Host Strains. Strain XL1-Blue {*recA1 lac*$^-$ *endA1 gyrA96 thi hsdR17 supE44 relA1*λ^- [F′ *proAB lacI*^{q} *lacZ*ΔM15 Tn*10* (tet^r)]} (9), helper phage R408, and phagemid pBluescript SK$^-$ were obtained from Stratagene, and CJ236 [*dut1 ung1 thi1 relA1*/pCJ105 (*Cm*^{r})] was from Bio-Rad. The host strain (DS166) used for expression of mutant and wild-type *crr* genes is a recA derivative (10) of *E. coli* strain DG40, shown by Wolfgang Epstein (personal communication) to carry a deletion that begins within the *ptsI* gene and extends into or beyond the *cysA* gene. The following DS166 transformants harbor the indicated phagemids: SR1600, pBluescript SK$^-$; SR1601, wild-type *crr*; SR1602, His-90 → Gln; SR1603, His-75 → Gln; SR1604, Phe-3 → Trp. Cells were grown aerobically at 37°C in LB medium (11), supplemented with appropriate antibiotics.

Mutagenesis. Kits for mutagenesis and dideoxy-nucleotide sequencing were from Bio-Rad and Bethesda Research Laboratories, respectively. The Muta-Gene *in vitro* mutagenesis kit (Bio-Rad) was used with the modifications of the manufacturer's procedure indicated below. Mutagenic oligonucleotides were assembled on a Biosearch 8750 automated DNA synthesis apparatus using β-cyanoethylphosphoramidite methodology (12). The template for mutagenesis was pBluescript SK$^-$ containing the *Eco*RI–*Pst* I restriction fragment of pDS45 (10); this 1.5-kilobase fragment contains the structural gene for III^{Glc}, but lacks the genes for HPr and enzyme I. Recombinant phagemids were introduced into the *dut ung* strain CJ236 by transformation (13). Packaged single-stranded DNA was generated by superinfecting transfor-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: III^{Glc}, the phosphocarrier protein of the phosphotransferase system specific for glucose and methyl α-D-glucopyranoside; PTS, phospho*enol*pyruvate:glycose phosphotransferase system; $[Trp^3]III^{Glc}$, $[Gln^{75}]III^{Glc}$, and $[Gln^{90}]III^{Glc}$, the Phe-3 → Trp, His-75 → Gln, and His-90 → Gln III^{Glc} mutant proteins, respectively; IIB^{Glc}, glucose-specific integral membrane protein of the PTS; HPr, histidine-containing phosphocarrier protein of the PTS; αMeGlc, methyl α-D-glucopyranoside.

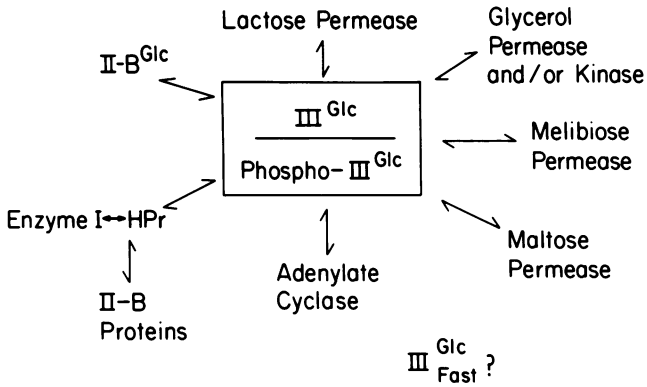

FIG. 1. Regulatory interactions of III^{Glc} (1, 2). As a component of the glucose-specific PTS (III^{Glc}/IIB^{Glc}), the III^{Glc} and/or phospho-III^{Glc} proteins are signal transducers between the phosphorylated histidine-containing phosphocarrier protein of the PTS (HPr), the glucose-specific integral membrane protein of the PTS (IIB^{Glc}), and glucose or methyl α-D-glucopyranoside (αMeGlc). III^{Glc} is also a negative effector of the transport systems of at least four non-PTS carbon sources (lactose, maltose, melibiose, and glycerol), whereas phospho-III^{Glc} is either inactive or a positive effector. In addition, phospho-III^{Glc} is thought to be an activator of adenylate cyclase, thereby regulating the expression of a number of catabolic and transport system operons. Finally, the III^{Glc} protein itself may be metabolically regulated; two electrophoretically distinguishable forms of III^{Glc}, III$^{Glc}_{Slow}$ and III$^{Glc}_{Fast}$, have been isolated (4). III$^{Glc}_{Slow}$ is the active form of III^{Glc} in the glucose phosphorylation reaction and III$^{Glc}_{Fast}$ is generated by a specific membrane protease (5) that cleaves the N-terminal heptapeptide from III$^{Glc}_{Slow}$. III$^{Glc}_{Fast}$ is fully capable of accepting phosphate from phospho-HPr, but shows low relative phosphotransfer activity (4). The physiological importance of the two III^{Glc} species and/or the heptapeptide is unknown.

mants (two cycles) with the helper phage R408. Purified uracil-containing DNA was the template in the *in vitro* mutagenesis reactions; the following synthetic primers were used to direct the mutations: 5′-CAGTTTATCCCACAAACCCAT-3′, for Phe-3 → Trp; 5′-AGAGAATGCCTGGTTGGTT-3′, for His-75 → Gln; 5′-TACCGAACTGGACGAACA-3′, for His-90 → Gln (mismatched nucleotides are underlined). After transformation into *E. coli* XL1-Blue, cells carrying mutant phagemids were identified by colony screening with ^{32}P-labeled mutagenic oligonucleotide probes. Superinfection of transformants with R408-generated single-stranded DNA for sequence analysis (14). Mutation-bearing phagemids were subsequently transformed into DS166 cells and double-stranded DNA isolated from these cells was resequenced using the K/RT sequencing system as described by the manufacturer (Promega Biotec).

Secondary-Structure Prediction Analyses. The hydropathicity profile and secondary-structure prediction programs of the Bionet National Computer Resource for Molecular Biology (IntelliGenetics, Mountain View, CA) were used for computer analysis of protein secondary structure. The Kyte and Doolittle (15) and Hopp and Woods (16) methods were used for computing average hydropathicity and hydrophilicity profiles, respectively. Protein secondary structures were predicted using the Chou and Fasman algorithm (17).

Isolation of Mutant III^{Glc} Proteins. Partially purified mutant proteins were isolated from transformed DS166 cells by a modification of procedure A described in ref. 4; initially, cell homogenates were treated with 0.5% Celite 503 (J. T. Baker) instead of protamine sulfate, and the preparative isoelectric focusing and gel electrophoresis steps were omitted. The quantities of mutant and wild-type III^{Glc} proteins were determined by rocket immunoelectrophoresis (4).

Assays for III^{Glc}. III^{Glc} proteins were assayed *in vitro* (4) using homogeneous preparations of *E. coli* enzyme I and HPr and partially purified IIB^{Glc} (18). Protein was measured by the method of Bradford (19).

Phosphoacceptor and phosphotransfer activities of III^{Glc} proteins were monitored using ^{32}P-labeled phospho*enol*pyruvate (20) in the presence and absence of αMeGlc as indicated. Protein products were separated by NaDodSO$_4$/polyacrylamide gel electrophoresis (21) and labeled proteins were detected by autoradiography.

RESULTS AND DISCUSSION

Construction and Structural Properties of the III^{Glc} Mutant Proteins. The method of Kunkel *et al.* (22) was used to construct mutant plasmids. Codon changes were confirmed by DNA sequence analysis and the results are shown in Fig. 2. The mutation TTC → TGG changes Phe-3 to Trp (Fig. 2*A*), CAC → CAG changes His-75 to Gln (Fig. 2*B*), and CAC → CAG changes His-90 to Gln (Fig. 2*C*).

On the basis of several considerations, it seems unlikely that the mutated *crr* genes encode truncated or drastically altered III^{Glc} molecules: (*i*) The side chain of glutamine is smaller and has greater flexibility than histidine, but Lowe *et al.* (24) have reported that the amide nitrogen of the side chain of glutamine may be superimposed to occupy the same position as N-3 of histidine; the substitution of an indolyl group (tryptophan) for a phenyl group (phenylalanine) is very conservative with respect to size and aromaticity. (*ii*) The structural prediction methods of Kyte and Doolittle (15), Hopp and Woods (16), and Chou and Fasman (17) were used to analyze the derived primary amino acid sequences of the mutant proteins. When compared to the wild-type sequence, the results suggest that the mutations do not result in major perturbations of the wild-type III^{Glc} structure (data not shown). (*iii*) Each of the III^{Glc} proteins encoded by the mutant *crr* genes is indistinguishable from wild-type III^{Glc} when analyzed by native and NaDodSO$_4$/polyacrylamide gel electrophoresis (unpublished results and Fig. 3). (*iv*) Both the III$^{Glc}_{Slow}$ and III$^{Glc}_{Fast}$ species (4) were isolated from all strains transformed with the mutant genes and are electrophoretically identical to the respective wild-type III^{Glc} species. (*v*) The mutant forms of III$^{Glc}_{Slow}$ are processed *in vitro* by the membrane protease (5) that removes the N-terminal heptapeptide from wild-type III$^{Glc}_{Slow}$ (data not shown). (*vi*) The mutant proteins are reactive with antibodies raised against wild-type III^{Glc}.

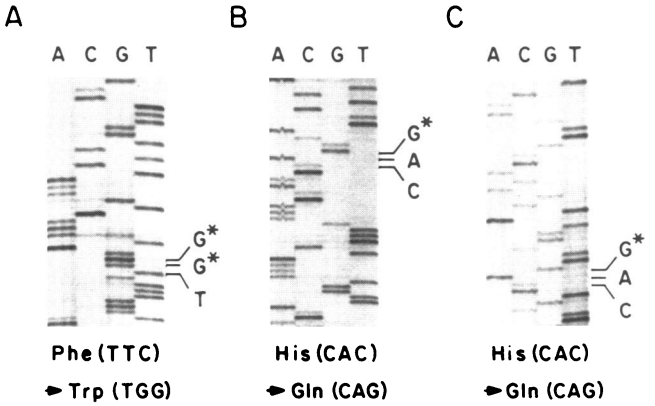

FIG. 2. DNA sequence analysis of mutant *crr* genes. Double-stranded DNA was isolated from DS166 transformants and sequenced using reverse transcriptase. Sequences shown are the antisense strands numbered as in ref. 23; asterisks denote nucleotide substitutions. (*A*) Nucleotides 2281–2320; TGG codons for tryptophan at positions 2293–2295. (*B*) Nucleotides 2481–2521; CAG codons for glutamine at positions 2509–2511. (*C*) Nucleotides 2548–2579; CAG codons for glutamine at positions 2554–2556.

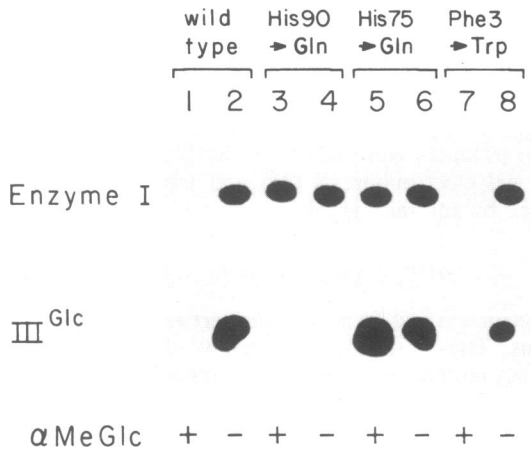

FIG. 3. Phosphoacceptor and phosphotransfer activities of III^{Glc} proteins. The assay mixtures included 2 mM [^{32}P]phospho*enol*pyruvate, 0.1 M αMeGlc when present, and the following PTS proteins: enzyme I at 1 μM, HPr at 6.6 μM, III^{Glc} proteins at 1 μM, and IIB^{Glc} at 0.06 μM. Proteins were separated by $NaDodSO_4$/PAGE and ^{32}P-labeled proteins were detected by autoradiography. The portion of the gel containing proteins migrating to the positions of homogeneous enzyme I and III^{Glc} is shown; except for phospho-HPr (not shown), no other labeled protein bands were observed. Lanes: 1 and 2, wild-type III^{Glc}; 3 and 4, $[Gln^{90}]III^{Glc}$; 5 and 6, $[Gln^{75}]III^{Glc}$; 7 and 8, $[Trp^{3}]III^{Glc}$. +, αMeGlc added; −, no αMeGlc added.

***In Vitro* Sugar Phosphorylation Assays.** As a first step in analyzing the phospho*enol*pyruvate-dependent phosphotransferase signaling mechanism, the functional integrity of the engineered polypeptides was evaluated in complete *in vitro* phosphorylation assays (4). Comparable yields of sugar phosphate were obtained from incubations containing equivalent concentrations of either wild-type III^{Glc} or $[Trp^{3}]III^{Glc}$. Predictably, no detectable sugar phosphate could be isolated from assays that included the active-site modified $[Gln^{90}]III^{Glc}$. Unexpectedly, $[Gln^{75}]III^{Glc}$ yielded similar results. The proteins containing the His → Gln substitutions do not complement one another in mixing experiments. Furthermore, neither His → Gln mutant inhibits the activity of wild-type III^{Glc}. Thus, the lack of phosphocarrier activity of these protein preparations is not caused by an inhibition of sugar phosphorylation.

The overall phosphocarrier activity of the substrate, III^{Glc}, is dependent upon two specific reactions: (*i*) acceptance of a phosphoryl group from phospho-HPr and (*ii*) IIB^{Glc}-catalyzed transfer of the phosphoryl group from phospho-III^{Glc} to sugar. Hence, both the phosphoacceptor and phosphotransfer ability of the mutant proteins were assessed and the results are shown in Fig. 3. The wild-type protein is phosphorylated in the absence (lane 2) of αMeGlc and the [^{32}P]phosphoryl group is discharged in the presence (lane 1) of an excess of sugar; HPr and IIB^{Glc} were present at low levels in all incubation mixtures. Identical results were obtained with $[Trp^{3}]III^{Glc}$ (lanes 8 and 7, respectively). As expected, when His-90 is replaced with glutamine, the active-site mutant protein cannot accept the phosphoryl group (lane 4) and consequently is not a donor (lane 3). The most interesting result was obtained with $[His^{75}]III^{Glc}$. We expected this mutant protein to behave similarly to wild-type III^{Glc}_{Slow} (see Fig. 1) and indeed $[Gln^{75}]III^{Glc}_{Slow}$ does act as a phosphoryl acceptor (lane 6). However, phosphorylated $[Gln^{75}]III^{Glc}_{Slow}$ is unable to donate the phosphoryl group to the αMeGlc substrate of IIB^{Glc} (lane 5). In this sense, $[Gln^{75}]III^{Glc}_{Slow}$ resembles wild-type III^{Glc}_{Fast}.

With the assumption that none of the amino acid substitutions led to gross conformational changes, the results summarized above verify the critical role of His-90 in the molecular mechanism of glucose translocation. On the other hand, the effects of the Gln-75 mutation are not readily explained. One interpretation of the data is that the His-75 → Gln substitution results in a local modification of protein structure and/or chemistry (due to the slightly smaller size of glutamine as compared to histidine or to the loss of proton-donating capability) that locks phospho-III^{Glc} in a conformation where the bound phosphoryl group is stabilized and cannot be transferred. In view of the suggestion by Poolman *et al.* (7) that the III^{Glc}-like domains present in several widely divergent transport system proteins may serve a regulatory function, we cannot exclude the possibility that both the His-75 and His-90 residues play an important role in the group translocation process by some unknown mechanism, perhaps by phosphate migration from one histidine residue to the other.

Phenotypic Properties of III^{Glc} Mutants. Coupling of the PTS signal transduction pathway to alternate protein signaling pathways is one mechanism by which sugar nutrients can elicit a variety of intracellular responses. Preliminary studies on the *in vivo* behavior of the mutant *crr* gene products are summarized in Table 1. *E. coli* cells were transformed with plasmid DNA encoding wild-type and mutant III^{Glc} proteins. We emphasize that a functional enzyme I is not present in the *ptsI crr* host strain and the plasmids do not carry the gene (*ptsI*) for enzyme I. Thus, in the *E. coli* genetic background examined, it should be possible to evaluate the cellular signaling effects of dephosphorylated III^{Glc} on non-PTS target proteins.

For each corresponding strain and non-PTS sugar tested, quantitative growth patterns (Table 1) in minimal medium generally confirmed qualitative fermentation responses observed on rich indicator medium: (*i*) Strains SR1601, SR1603, and SR1604 grew slowly on maltose, glycerol, and melibiose,

Table 1. Phenotypes of III^{Glc} mutants

		Maltose		Glycerol		Melibiose*		Lactose*		Galactose	
Strain	Protein	Gen., hr	Ferm.	Gen., hr	Ferm.	Gen., hr	Ferm.	Gen., hr	Ferm.	Gen., hr	Ferm.
SR1600	None†	3.9	++	2.8	+	3.0	+	2.8	+++	1.8	++
SR1601	III^{Glc}	∞‡	−	9.3	−	7.6	∓	2.7	++	2.8	+++
SR1602	$[Gln^{90}]III^{Glc}$	3.2	+	5.7	∓	3.6	∓	1.7	++	1.9	++
SR1603	$[Gln^{75}]III^{Glc}$	∞	−	∞	−	4.9	∓	2.5	++	3.3	++
SR1604	$[Trp^{3}]III^{Glc}$	∞	−	4.6	−	4.1	+	2.1	++	2.2	++

Generation times (Gen.) were determined at 37°C in medium 63M (25) containing ampicillin (15 μg/ml), thiamine hydrochloride (5 μg/ml), thymidine (40 μg/ml), isoleucine (25 μg/ml), valine (25 μg/ml), and leucine (25 μg/ml), 120 μM cysteine, 120 μM methionine, and 0.5% sugar. Inocula were grown in LB medium; 1-ml aliquots were transferred to 1-liter flasks containing 200 ml of medium 63M and rotated at 215 rpm. Generation times are calculated from exponential phases of growth. Fermentation properties (Ferm.) of plasmid-bearing strains were determined on MacConkey agar indicator plates (without lactose) containing ampicillin (30 μg/ml) and 1% sugar. Fermentation responses were recorded as follows: from + to +++, positive to strongly positive fermentation; ∓, weak fermentation; −, no fermentation.

*Lactose operon is constitutively expressed in all strains; melibiose is a substrate of both the melibiose and the lactose transporters.

†Strain harbors pBluescript SK^{-}.

‡Generation time in excess of 10 hr.

which were also fermented poorly; [Trp^3]III^{Glc} and [Gln^{75}]III^{Glc} behave like the wild-type protein and regulate the utilization of these three non-PTS sugars, albeit to various degrees. (*ii*) In contrast, growth rates of strain SR1602 were similar to the parent strain, SR1600; [Gln^{90}]III^{Glc} behaves like a *crr* mutant and appears to have lost the regulatory functions of wild-type III^{Glc}. (*iii*) β-Galactosidase measurements show that the lactose operon is constitutively expressed in all the strains (unpublished results), which may account for the observed insensitivity of the lactose uptake system. (*iv*) The constitutive lactose operon also affected melibiose utilization; melibiose is a substrate of both the melibiose and lactose transporters. (*v*) Consistent with previous results (26, 27), galactose utilization is relatively insensitive to PTS-mediated regulation.

Since the interactions between III^{Glc} and the non-PTS proteins are stoichiometric, not catalytic (25, 27–29), then one potential explanation for the results shown in Table 1 is that the strains harboring the plasmids may produce different quantities of the corresponding mutant and wild-type III^{Glc} proteins. Each of the strains were, therefore, grown under the same conditions and extracts were assayed for the III^{Glc} proteins by rocket immunoelectrophoresis; all strains contained comparable quantities of immunoreactive III^{Glc} (data not shown). Thus, the differences in physiological responses must be attributed directly to the amino acid substitutions.

All of the results thus lead to the conclusions that both histidine residues are essential for phosphotransfer reactions but that only the active site His-90 is required for regulation of non-PTS systems. Why His-75 should be required for phospho-III^{Glc} to act as a phosphoryl-group donor remains to be elucidated. The answer to this intriguing question may shed significant light on the mechanism of action of this important PTS protein.

We thank Dr. Tomas Kempe, Director, Protein Nucleic Acid Laboratory, The University of Maryland, College Park, for synthesis of oligodeoxyribonucleotides and Eddie Stover for his expert technical assistance with the sugar phosphorylation assays. This work was supported by Grant MOU 88/02 from the Center of Marine Biotechnology and Grant 5 R37 GM38759 from the National Institutes of Health. C.-Y.W. was supported by Predoctoral Training Grant GM07231 from the National Institutes of Health. This is contribution 1427 from the McCollum–Pratt Institute.

1. Meadow, N. D., Kukuruzinska, M. A. & Roseman, S. (1984) in *The Enzymes of Biological Membranes*, ed. Martonosi, A. (Plenum, New York), Vol. 3, pp. 523–559.
2. Postma, P. W. & Lengeler, J. W. (1985) *Microbiol. Rev.* **49**, 232–269.
3. Postma, P. W. & Roseman, S. (1976) *Biochim. Biophys. Acta* **457**, 213–257.
4. Meadow, N. D. & Roseman, S. (1982) *J. Biol. Chem.* **257**, 14526–14537.
5. Meadow, N. D., Coyle, P., Komoryia, A., Anfinsen, C. B. & Roseman, S. (1986) *J. Biol. Chem.* **261**, 13504–13509.
6. Dörschug, M., Frank, R., Kalbitzer, H. R., Hengstenberg, W. & Deutscher, J. (1984) *Eur. J. Biochem.* **144**, 113–119.
7. Poolman, B., Royer, T. J., Mainzer, S. E. & Schmidt, B. F. (1989) *J. Bacteriol.* **171**, 244–253.
8. Jablonski, E. G., Brand, L. & Roseman, S. (1983) *J. Biol. Chem.* **258**, 9690–9699.
9. Bullock, W. O., Fernandez, J. M. & Short, J. M. (1987) *Biotechniques* **5**, 376–379.
10. Meadow, N. D., Saffen, D. W., Dottin, R. P. & Roseman, S. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 2528–2532.
11. Miller, J. H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
12. Sinha, N. D., Biernat, J. & Köster, H. (1983) *Tetrahedron Lett.* **24**, 5843–5846.
13. Davis, L. G., Dibner, M. D. & Battey, J. F. (1986) *Basic Methods in Molecular Biology* (Elsevier, New York), pp. 90–92.
14. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
15. Kyte, J. & Doolittle, R. F. (1982) *J. Mol. Biol.* **157**, 105–132.
16. Hopp, T. P. & Woods, K. R. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 3824–3828.
17. Chou, P. & Fasman, G. D. (1978) *Adv. Enzymol.* **47**, 45–148.
18. Bouma, C. L., Meadow, N. D., Stover, E. W. & Roseman, S. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 930–934.
19. Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254.
20. Roossien, F. F., Brink, J. & Robillard, G. T. (1983) *Biochim. Biophys. Acta* **760**, 185–187.
21. Laemmli, U. K. & Favre, M. (1973) *J. Mol. Biol.* **80**, 575–599.
22. Kunkel, T. A., Roberts, J. D. & Zakour, R. A. (1987) *Methods Enzymol.* **154**, 367–382.
23. Saffen, D. W., Presper, K. A., Doering, T. L. & Roseman, S. (1987) *J. Biol. Chem.* **262**, 16241–16253.
24. Lowe, D. M., Fersht, A. R., Wilkinson, A. J., Carter, P. & Winter, G. (1985) *Biochemistry* **24**, 5106–5109.
25. Mitchell, W. J., Misko, T. P. & Roseman, S. (1982) *J. Biol. Chem.* **257**, 14553–14564.
26. Saier, M. H., Jr., & Roseman, S. (1976) *J. Biol. Chem.* **251**, 6606–6615.
27. Mitchell, W. J., Saffen, D. W. & Roseman, S. (1987) *J. Biol. Chem.* **262**, 16254–16260.
28. Nelson, S. O., Scholte, B. J. & Postma, P. W. (1982) *J. Bacteriol.* **150**, 604–615.
29. Misko, T. P., Mitchell, W. J., Meadow, N. D. & Roseman, S. (1987) *J. Biol. Chem.* **262**, 16261–16266.