Effects of Mutations and Truncations on the Kinetic Behavior of IIAGlc, a Phosphocarrier and Regulatory Protein of the Phosphoenolpyruvate Phosphotransferase System of Escherichia coli

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IIAGlc, a component of the glucose-specific phosphoenolpyruvate:phosphotransferase system (PTS) of Escherichia coli, is important in regulating carbohydrate metabolism. In Glc uptake, the phosphotransfer sequence is: phosphoenolpyruvate → Enzyme I → HPr → IIAGlc → IICBGlc → Glc. (HPr is the first phosphocarrier protein of the PTS.) We previously reported two classes of IIAGlc mutations that substantially decrease the P-transfer rate constants to/from IIAGlc. A mutant of His75 which adjoins the active site (His75Glu) (H75Q), was 0.5% as active as wild-type IIAGlc in the reversible P-transfer to HPr. Two possible explanations were offered for this result: (a) the imidazole ring of His75 is involved in a charge delocalization and (b) H75Q disrupts the hydrogen bond network: Thr73His75, phospho-His90. The present studies directly test the H-bond network hypothesis. Thr73 was replaced by Ser, Ala, or Val to eliminate the network. Because the rate constants for phosphotransfer to/from HPr were largely unaffected, we conclude that the reaction is not correct. In the second class of mutants, proteolytic truncation of seven residues of the IIAGlc N terminus caused a 20-fold reduction in phosphotransfer to membrane-bound IICBGlc from Salmonella typhimurium. Here, we report the phosphotransfer rates between two genetically constructed N-terminal truncations of IIAGlc (Δ7 and Δ16) and the proteins IICBGlc and IIBGlc (the soluble cytoplasmic domain of IICBGlc). The truncations did not significantly affect reversible P-transfer to IIBGlc, but substantially decreased the rate constants to IICBGlc in E. coli and S. typhimurium membranes. The results support the hypothesis (Wang, G., Peterkofsky, A., and Clore, G. M. (2000) J. Biol. Chem. 275, 39811–39814) that the N-terminal 18-residue domain “docks” IIAGlc to the lipid bilayer of membranes containing IICBGlc.

The phosphoenolpyruvate phosphotransferase system (PTS) is a major pathway for the uptake of carbohydrates in the eubacteria (1, 2).

Sugars that are PTS substrates are phosphorylated as they are translocated across the cell membrane, as illustrated schematically in Fig. 1 for the Glc-specific uptake systems of Escherichia coli and Salmonella typhimurium.

The PTS also serves several regulatory functions, including the inhibition of the uptake of several carbohydrates that are not PTS substrates and indirect regulation of carbon metabolism on a wide scale by control of the activity of adenylate cyclase (3). E. coli, the regulatory function is served largely (1, 2, 4) by the 18.1-kDa phosphocarrier protein of the glucose-specific PTS, IIAGlc (in the older literature, this protein was called IIIGlc). Unphosphorylated IIAGlc represses transcription of the uptake systems of three non-PTS carbon sources (lactose, maltose, and melibiose) by inhibiting the uptake of inducer (5), and, in the case of glycerol, which is taken up by facilitated diffusion, by inhibiting glycerol kinase (2). Extensive evidence suggests that [P]IIAGlc is a potent stimulator of adenylate cyclase (3). Therefore, both the presence or absence of IIAGlc, and its state of phosphorylation, are of importance for the regulation of cell growth. The state of phosphorylation of IIAGlc is determined by the relative flux of phospho-groups between it and HPr or IICBGlc, the membrane-associated glycoside transporter.

Structural studies of IIAGlc by NMR and x-ray crystallography (6–8) have shown that it comprises two domains: 1) an N-terminal domain that is unstructured in solution with the following amino acid sequence: (Met)-Gly-Leu-Phe-Asp-Lys-Leu-Lys-Ser-Leu-Val (6–8) have shown that it comprises two domains: 1) an N-terminal domain that is unstructured in solution with the following amino acid sequence: (Met)-Gly-Leu-Phe-Asp-Lys-Leu-Lys-Ser-Leu-Val-Asp-Lys-Thr-Gly; the N-terminal Met is quantitatively removed post-translationally, and is not numbered, and 2) a compact domain that consists of the remaining 150 residues, including the active site, His90 (9, 10). The present report concerns two aspects of the IIAGlc structure thought to play important roles in the phosphotransfer reactions as follows.

The Catalytic Role of Amino Acids Close to His90—Transient-state (rapid quench) kinetic methods were adapted to study the phosphotransfer reactions of the PTS (11). Application of these methods to the kinetics of a site-directed mutation of His90 (H75Q) (9), which lies very close to His90 in the tertiary structure, showed that the mutation reduced the rate constants for phosphotransfer to and from HPr by a factor of 200.

Gln is isosteric with His, and structural studies of the (H75Q) IIAGlc mutant (12) indicated a virtually undetectable change around the active site. Two hypotheses were offered to explain the 200-fold decrease in the rate constants. (a) His5 stabilizes the negative charge on P-His90 by virtue of a hydrogen bond and, consequently, delocalizes the negative charge on the intermediate (Fig. 2) (12). This delocalization of the charge would be substantially decreased in the Gln mutant. (b) The active site contains a possible “proton relay” network consisting of...
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FIGURE 1. A diagram of the Glc-specific PTS from E. coli or S. typhimurium. The phosphorylated amino acid in each of the four proteins is indicated. There are five phosphotransfer reactions, each designated by the Roman numeral used throughout the text. The glucose permease, IIC\textsuperscript{Glc}, is shown separated into its two domains, the phosphorylation domain IIB\textsuperscript{Glc}, which extends into the cytoplasm, and the sugar recognition and binding domain IIC\textsuperscript{Glc}, which is an integral membrane domain. Enzyme I is active only as a homodimer of 64-kDa subunit monomers; HPr is 9.1 kDa; IIA\textsuperscript{Glc} is 18.1 kDa; and IIC\textsuperscript{Glc} is a homodimer of 50.4-kDa subunit monomers (1, 2). Enzyme I and HPr are called “general proteins,” because they are common to all of the sugar-specific proteins in a bacterial species. HPr is a branch point in the flow of phospho-groups to the sugar-specific proteins. The ratio of IIA\textsuperscript{Glp} to IIA\textsuperscript{Glc} is important for the regulation of carbohydrate metabolism, and this ratio is determined by the relative flux of phospho-groups between IIA\textsuperscript{Glc} and HPr or IIC\textsuperscript{Glc}.

FIGURE 2. Structures of the active sites of wild-type and H75Q IIA\textsuperscript{Glc}. A, wild-type IIA\textsuperscript{Glc}; B, wild-type [PIIIA\textsuperscript{Glc}P]; C, H75QIIA\textsuperscript{Glc}; D, H75Q[PIIIA\textsuperscript{Glc}]P. Taken from Pelton et al. (12) with permission.
Thr$^{73}$-His$^{75}$-P-His$^{90}$. Whether proton transfer might take place between Thr$^{73}$ and His$^{75}$ during the phosphotransfer reaction had not been investigated.

In the present studies, the validity of the second hypothesis was tested by eliminating the H-bond network by substituting amino acids (Ser, Ala, or Val) for Thr$^{73}$ but maintaining His$^{75}$. The rate constants for phosphotransfer between these mutants and HPr were measured.

The Function of the Unstructured N-terminal Domain—Early kinetic studies of IIA$^{Glc}$ by steady-state methods showed that $[P]IIA^{Glc}$ is a Michaelis-Menten substrate of IICB$^{Glc}$ with a definable $V_{\text{max}}$ and $K_m$ (13, 14). A modified form of IIA$^{Glc}$ was isolated that was much less active kinetically (a few percent of wild type) (13). This protein was a truncated form of IIA$^{Glc}$, lacking 7 residues at the N terminus. The truncation is catalyzed by what appears to be a specific membrane protease (15). The proteolysis occurs between Lys$^7$ and Ser$^8$ (13, 15).

A function for the N-terminal domain of IIA$^{Glc}$ is suggested by recent studies of the physical properties of the domain. These suggest that it can form an amphipathic helix that serves as a membrane anchor (16, 17). In the present work, we investigate the effects of two different length truncations (6 or 17 residues) of the N-terminal domain of IIAGlc on phosphotransfer rates with the soluble proteins HPr and IIB$^{Glc}$, and the membrane proteins IICB$^{Glc}$ from S. typhimurium and E. coli.

EXPERIMENTAL PROCEDURES

Materials—All buffer salts and other reagents were of the highest purity commercially available. The pH of all buffers is reported at the temperature and concentration at which they were used. Methyl-$\alpha$-[U-$^{14}$C]glucoside was synthesized from [U-$^{14}$C]glucose (NEC 042, PerkinElmer Life Sciences) by the method of Bollenback (18), and the products were purified by the method of Austin et al. (19). The enzy- matic synthesis of $[^{32}P]$PEP (20) was performed with modifications as described previously (11).

Bacterial Strains, Plasmids, and Growth Media—All strains were grown on Luria-Bertani broth containing the required antibiotics and inducers. E. coli strain BL21(DE3) (F$^{ompT}$ hsdSB (rB$^-$ mB$^-$) gal dcm (DE3)) (Novagen) was used as the host for the pET21a plasmids containing the mutated genes for IIA$^{Glc}$.

Construction of Site-directed Mutants of IIA$^{Glc}$—A mutagenesis method based on a one-step PCR was used to construct plasmids containing deletions of either the first 8 or the first 17 codons of the gene. These codons include the N-terminal Met, which is not assigned an amino acid sequence number because it is removed post-translationally in vivo. Thus, the gene contains 169 codons, but the mature protein contains 168 amino acids, so that the number designating an amino acid residue is smaller than its codon number by one. In the case of the 8 codon deletion, Ser$^3$ was replaced by Met, so that the encoded protein is identical to the proteolytically truncated protein previously isolated, except that Met now replaced Ser as the first residue. This protein is referred to as (Δ5)IIA$^{Glc}$. To test the effect of a more extensive truncation of the N-terminal domain, Thr$^{17}$ was replaced by Met, creating a protein that is 16 amino acids residues shorter than the mature, native protein and lacks virtually the entire N-terminal domain of IIA$^{Glc}$ (18 amino acids). This protein is referred to as (Δ16)IIA$^{Glc}$.

The plasmid pDS35 (21) containing the $\text{cm}$ gene was used as the template. PCR was performed by using two sets of primers. For S8M, the primers were: SS112 (5′-GGCGCCATTTTTCACTGCCAGAATTCTTACTTCTTGATGC-3′) containing the EcoRI site of restriction and S8M (5′-GGCGCCATTTTTCACTGCCAGAATTCTTACTTCTTGATGC-3′) containing the EcoRI site of restriction and S8M (5′-GGCGCCATTTTTCACTGCCAGAATTCTTACTTCTTGATGC-3′) containing the Ndel restriction site. For T717M the primers were: SS112 and T17M (5′-GACGACAAAGAAGCATATGGGAAACTTTGAG-3′) containing the Ndel site. After the PCR reaction, PCR products were restricted with Ndel and EcoRI and then cloned into plasmid pET21a (Novagen).

Construction of T73S, T73A, and T73V mutations was performed by using the method based on a two-step PCR as described (22). For T73A the primers were: T73A1 (5′-ATCTTTGAACCAACCAGCA-3′) and T73A2 (5′-TGCGTGTTGATGCTTGGGAAACTTTGAG-3′) containing the T73A mutation. For T73S the primers were: T73S1 (5′-ATCTTTGAAGCTCAACCAGCA-3′) and T73S2 (5′-TGCGTGTTGATGCTTGGGAAACTTTGAG-3′) containing the T73S mutation. For T73V the primers were: T73V1 (5′-ATCTTTGAGCTCAACCAGCA-3′) and T73V2 (5′-TGCGTGTTGATGCTTGGGAAACTTTGAG-3′) containing the T73V mutation.

Purification and Characterization of HPr, $[^{32}P]$HPr, IIA$^{Glc}$, $[^{32}P]$IIA$^{Glc}$, IIB$^{Glc}$-6His, and $[^{32}P]$IIB$^{Glc}$-6His—Homogeneous IIA$^{Glc}$ (23) and HPr (24) were isolated as described. The various site-directed mutants of IIA$^{Glc}$ were purified by the same methods used for the wild-type protein. The T73V mutant of IIA$^{Glc}$ behaved somewhat differently during the purification and required a second passage on the ion exchange column to obtain homogeneous protein. IIA$^{Glc}$ was tested for proteolysis by the use of polycaylamide gel chromatography (13). IIB$^{Glc}$-6His was purified by the method of Buhr et al. (25), except that a Superose 12 HR 10/30 column (Amersham Biosciences) was substituted for Sephadex G75. The final preparations were apparently homogeneous as judged by SDS-PAGE. The methods previously described (11) were used to determine the concentrations of HPr, IIA$^{Glc}$, and IIB$^{Glc}$-6His. $[^{32}P]$HPr, $[^{32}P]$IIA$^{Glc}$, and $[^{32}P]$IIB$^{Glc}$-6His were prepared as described previously, with the same attention to the accuracy of the specific activity of the $[^{32}P]$PEP (11).

Sugar Phosphorylation Assay for PTS Activity and the Determination of Specificity Constants—The PEP-driven sugar phosphorylation assay was performed as reported (26, 27). Membrane suspensions (28) for these assays were prepared from E. coli strain ZSC112A G harboring plasmid pCB30 (encoding wild-type IICB$^{Glc}$) and S. typhimurium, strain PP1133 (29). To determine $V_{\text{max}}$ and $K_m$ (IIA$^{Glc}$), the assay mixture contained fixed concentrations of the following substances in a volume of 0.1 ml: 50 mM Tris/Cl$^-$ buffer, pH 8.0; 10 mM KF; 5 mM MgCl$_2$; 10 mM PEP; 5 mM methyl-$\alpha$-[U-$^{14}$C]glucoside (5 Bq/nmol); 8 nM Enzyme I (this is 5 units, where a unit is defined as the quantity of enzyme that produces 1 μmol of sugar phosphate in 30 min at 37 °C); and 6 μM HPr. The assays contained <0.2 unit of IICB$^{Glc}$; the concentration of E. coli IICB$^{Glc}$ was ~16 nm and S. typhimurium IICB$^{Glc}$ ~20 nm as determined (28). The concentrations of IIA$^{Glc}$, either wild type or truncated, were 3, 5, 20, and 60 μM, $V_{\text{max}}$ and $K_m$ (IIA$^{Glc}$) were calculated by the methods of Ednie-Hofstee and Hanes (30), and the values were averaged.

The specificity constant of IICB$^{Glc}$ for IIA$^{Glc}$ is defined as $k_{\text{cat}}/K_m$ (IIA$^{Glc}$) (30), where $k_{\text{cat}}$ is defined as $V_{\text{max}}/[\text{IICB}^{Glc}]_{\text{total}}$. We emphasize that the specificity constant is mathematically equal to the rate constant in a reaction catalyzed by an enzyme with a ping-pong mechanism.

Rapid Quench Assays—The present study employed the same rapid quench apparatus described previously, including all the details for its set-up and use, treatment with bovine serum albumin to eliminate adsorption of protein, etc. (11). The quench solution for experiments with HPr and IIA$^{Glc}$ was 3 mM KOH with 9 mM urea, used as one volume of quench to two volumes of reaction mixture (11). For experiments with IIA$^{Glc}$ and IIB$^{Glc}$ the quench solution was 0.3 mM KOH, 9 mM urea, also used as one volume of quench to two volumes of reaction mixture (28). Analysis of the quenched reactions by gel-filtration chromatography using high-performance liquid chromatography grade columns at pH 12.3 was also as described (11). Preparation of the solutions for rapid quench experiments required large dilutions from stock solutions of Enzyme I and HPr, and a change from the
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\[ [P]HPr + IIAGlc \rightleftharpoons HPr + [P]IIAGlc : \quad k_{III} \quad k_{IV} \quad III  \\
 [P]IIAGlc + IIBGlc \rightleftharpoons IIAGlc + [P]IIBGlc : \quad k_{IVA} \quad k_{IVa} \quad IVA  \\
 [P]IIAGlc + IICBGlc \rightleftharpoons IIAGlc + [P]IICBGlc : \quad k_{IV} \quad k_{IVa} \quad IV  \\

SCHEME 1. The balanced equations for the phosphotransfer reactions between [P]IIAGlc and HPr or IIBGlc. The signs of the rate constants are positive for reactions proceeding from left to right. The balanced equations are for the scheme of reactions used in the kinetic simulator, Kinsim. The convention for numbering the reactions is adapted from Rowher et al. (38). By this convention, the data reported previously (11) pertained to Reaction III. Reaction IVA applies to IIBGlc, the molecularly cloned, cytoplasmic domain of Enzyme IICBGlc. "IIAGlc" pertains to the wild-type protein as well as to the mutant forms used in this report.

| TABLE 1 Rate constants for phosphotransfer between HPr and IIAGlc, wild type, and mutants |
|---------------------------------|---------------------------------|
| IIAGlc                          | Reaction                      |
|                                 | \( k_{III} \) | \( k_{IV} \) | \( n \) | \( K_{eq} \) |
| wt                             | 60 \pm 14 | 42 \pm 8 | 3 | 1.4 |
| T73A                            | 38 \pm 3  | 20 \pm 3  | 2 | 2.8 |
| T73S                            | 44 \pm 5  | 16 \pm 9  | 2 | 2.8 |
| \( \Delta \eta \)              | 35 \pm 6  | 27 \pm 11 | 3 | 1.3 |
| \( \Delta 16 \)                | 34 \pm 7  | 30 \pm 3  | 3 | 1.1 |

The values for \( k_{III} \) and \( k_{IV} \), obtained at pH 7.5, are similar to those obtained previously at pH 6.5 (11), which were \( 61 \times 10^6 \text{ mol}^{-1} \text{ s}^{-1} \) and \( 47 \times 10^6 \text{ mol}^{-1} \text{ s}^{-1} \), respectively.

One of the experiments with this protein was started in the reverse direction, i.e., with HPr as the acceptor and \([\text{32P}]\text{phospho-IIAGlc} \) as the donor. The rate constants derived from experiments started from opposite directions were in good agreement, indicating that there are no significant concentrations of intermediate complexes between the two reacting proteins prior to the last step, transfer of the phosphoryl group to the acceptor, and separation of the proteins to yield the products.

Function of the N-terminal Domain

In the PTS reaction sequence (Fig. 1), IIAGlc accepts a phosphoryl group from \([P]HPr\) and is a donor to the IIB (cytoplasmic) domain of the membrane protein, IICBGlc. The reactions studied here with the cloned, soluble IICBGlc domain are referred to as Reaction IVA in Scheme 1.

We previously reported that proteolysis of the seven N-terminal residues of IIAGlc from S. typhimurium reduced its activity in sugar phosphorylation assays to a few percent of the activity of the full-length protein (13). In the present studies, we test the role of the N-terminal domain of IIAGlc on its kinetic properties by constructing and assaying two truncations, \((\Delta \eta)\text{IIAGlc} \) and \((\Delta 16)\text{IIAGlc} \), as described under "Experimental Procedures."

The phosphotransfer properties of the truncations were tested with the soluble proteins, HPr and IIBGlc, and with the membrane protein IICBGlc. A differential effect on the rate constants might indicate whether the N-terminal domain requires membrane lipid to exert its effect.

Effects of Truncations on Phosphotransfer Reactions with HPr—Experimental and theoretical progress curves for phosphotransfer Reaction III between \((\Delta \eta)\text{IIAGlc} \) and HPr are shown in Fig. 3, and a summary of six experiments is given in Table 1. Truncation has only a small effect on Reaction III, reducing both \( k_{III} \) and \( k_{IV} \) by a factor of about two, and the length of the truncation did not matter.

Two recent studies of the interacting complexes of HPr and IIAGlc (34) and IIBGlc and IICBGlc (35) have shown that the N-terminal domain of IIAGlc remains unstructured in both interactions. The weak effects of truncation on the kinetics of phosphotransfer are consistent with these observations.

Effects of Truncations on Reactions with IIBGlc6His—A progress curve of P-transfer between the truncated IIAGlc proteins and IIBGlc is given in Fig. 4, and the rate constants for this reaction are summarized in Table 2. Both truncations resulted in an increased rate constant for P-transfer,
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**TABLE 2**

| IIAGlc | \( k_{\text{cat}} \) (S-1 M-1) | \( k_{\text{cat}}/K_{\text{m}} \) (S-1 M-1) | \( n \) | \( K_{\text{eq}} \) (M) |
|--------|-----------------|-----------------|-----|--------|
| wt + IICBGlc | 3.7 ± 0.3 | 8.3 ± 2.3 | 4 | 0.2 |
| \( \Delta7 \) + IICBGlc | 10 ± 0.7 | 14 ± 2 | 2 | 0.7 |
| \( \Delta16 + \text{IICBGlc} \) | 12 | 15 | 1 | 0.8 |

\( ^a \) The values in parentheses are for \( k_{\text{cat}} \) and \( k_{\text{cat}}/K_{\text{m}} \).

\( ^b \) These data are from Ref. 28 and are shown here so that they can be compared to the data with the cloned domain.

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**FIGURE 3. Phosphotransfer reactions of HPr with \( \Delta16 \) IIAGlc.** Phosphotransfer reaction between \( ^{32} \)P HPr and \( \Delta16 \) IIAGlc. Experimental and theoretical progress curves (solid and dashed lines) fitted manually using Kinsim are shown for the transfer reactions conducted as described under “Experimental Procedures.” Time points at 30 s were obtained by hand mixing. Initial concentrations (after mixing): \( ^{32} \)P HPr = 20 nM; HPr = 7.5 nM; \( \Delta16 \) IIAGlc = 26 nM; the HPr is produced by hydrolysis of \( ^{32} \)P HPr during storage.

A, progress curves shown on a logarithmic time scale. B, the first 0.6 s of the progress curve shown on a linear time scale. The rate constants from the model are: \( k_{\text{cat}} = 42 \times 10^6 \text{ M}^{-1} \text{s}^{-1} \) and \( k_{\text{cat}}/K_{\text{m}} = 32 \times 10^6 \text{ M}^{-1} \text{s}^{-1} \), and the calculated equilibrium constant for Reaction III is: \( K_{\text{eq}} = 1.2 \). These data are included in Table 1. Progress curves for the phosphotransfer reaction between HPr and the IIA\(^{\text{Glc}}\) Thr\(^{73}\) mutants are very similar to the one shown here.

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**FIGURE 4. Phosphotransfer reactions of \( \Delta7 \) IIAGlc with IIBGlc-6H.** Phosphotransfer reaction between \( ^{32} \)P \( \Delta7 \) IIAGlc and IIBGlc-6H. Experimental and theoretical progress curves (solid and dashed lines) fitted manually using Kinsim are shown for the transfer reactions conducted as described under “Experimental Procedures.” Time points at 30 s were obtained by hand mixing. Initial concentrations (after mixing): \( ^{32} \)P \( \Delta7 \) IIAGlc = 33 nM; \( \Delta7 \) IIAGlc = 5 nM; IIBGlc-6H = 38 nM; the \( \Delta7 \) IIAGlc is produced by hydrolysis of \( ^{32} \)P \( \Delta7 \) IIAGlc during storage. A, progress curves shown on a logarithmic time scale. B, the first 0.6 s of the progress curve shown on a linear time scale. The rate constants from the model are: \( k_{\text{cat}} = 13 \times 10^6 \text{ M}^{-1} \text{s}^{-1} \) and \( k_{\text{cat}}/K_{\text{m}} = 11 \times 10^6 \text{ M}^{-1} \text{s}^{-1} \), and the calculated apparent equilibrium constant for Reaction Ia is therefore \( K_{\text{eq}} = 1.2 \). These data are included in Table 2.

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**TABLE 3**

| IIAGlc | \( k_{\text{cat}}/K_{\text{m}} \) (S-1 M-1) | \( n \) |
|--------|-----------------|-----|
| wt + IICBGlc | \( 3.8 \pm 1.4 \) | 2 |
| \( \Delta7 + \text{IICBGlc} \) | \( 3.1 \pm 1.8 \) | 2 |
| \( \Delta7 + \text{IICBGlc} \) | \( 0.97 \pm 0.23 \) | 2 |

**E. coli and S. typhimurium.** The effects of truncation of E. coli IIAGlc on the specificity constants of IICBGlc from E. coli and S. typhimurium are shown in Table 3.

Truncation of IIAGlc reduces the specificity constant of E. coli IICBGlc by a factor of about 4 relative to intact IIA\(^{\text{Glc}}\), whereas the specificity constant of S. typhimurium IICBGlc is reduced by a factor of \(-12\), in reasonable agreement with the previously published results.

The 4-fold change with E. coli membranes may seem small, but may be very physiologically significant. In a previous paper (38) we report the results of transport experiments with whole cell and found that the pivotal protein in Glc transport was IICBGlc at its usual cellular levels.

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The differences in the effect of truncation of IIAGlc on its activity with E. coli and S. typhimurium IICBGlac membrane preparations...
that the N terminus has no large effect on phosphotransfer to and from protein IICBGlc. But for the N-terminal domain to specifically direct this activity when IIAGlc was truncated (N-terminal 7 amino acids) by a specific membrane protease (15). The results reported here with genetically engineered Δ7 and Δ16 amino acid truncations explicitly show that the N terminus has no large effect on phosphotransfer to and from the soluble proteins, HPi and IIIBGlc, but there is suggestive evidence for a weak interaction with IIIBGlc. This observation is important because IIIBGlc is a domain of the membrane protein, IICBGlc, that is affected more strongly by the truncations.

Our results can be explained and provide support for the contention by Wang et al. (16, 17) that the N-terminal domain forms an amphipathic helix in the presence of anionic phospholipids and their proposal that this helix enhances the interaction of [P]IIAGlc and IIICBGlc during phosphotransfer. In other words, that the N-terminal domain acts in “docking” of phospho-IIAGlc and IIICBGlc to the lipid bilayer and to interact with the IIBGlc domain of the membrane protein IICBGlc. But for the N-terminal domain to specifically direct this attachment in the correct position on a membrane containing innumerable proteins requires somewhat more than a nonspecific amphipathic helix. The kinetic results, although relatively small, suggest in fact that the N-terminal domain does play a role in the interaction between P-IIAGlc and IIICBGlc.

Perhaps the large effect observed with the truncated IIAGlc in the PTS sugar phosphorylation assay, especially with the S. typhimurium membranes, reflects loss of a synergistic effect in the IIAGlc truncations. That is, the role of the N-terminal domain is to form an amphipathic helix that binds to the lipid bilayer and to interact with the IIBGlc domain in IICBGlc.

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3. The observed amino acid sequence of IIAGlc from the two species are virtually identical, differing in only three residues (two are Ile for Val replacements, but one is a Pro in S. typhimurium) for the E. coli replacement. The primary sequences of the IICBGlc proteins are also very similar; a Blast comparison (36) showed 97% identity; only one of the differences produces a change in a charged residue, the replacement of Met249 in S. typhimurium with Lys in E. coli. There are, however, marked differences between the proteins from the two species. The reported isoelectric points for the “homogeneous” IICBGlc proteins are pH 6.5 for the S. typhimurium protein (29) and pH 9.0 for the E. coli IICBGlc (37), and the proteins react differently to antisera raised against the S. typhimurium protein (37). These results suggest a significant difference in folding of the two polypeptide chains, despite the great similarity in the amino acid sequences.