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A functional protein hybrid between the glucose transporter and the *N*-acetylglucosamine transporter of *Escherichia coli*

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Abstract

The glucose and *N*-acetylglucosamine-specific transporters (II^{Glc}/III^{Glc} and II^{GlcNAc}) of the bacterial phosphotransferase system mediate carbohydrate uptake across the cytoplasmic membrane concomitant with substrate phosphorylation. The two transporters have 40% amino acid sequence identity. Eight chimeric proteins between the two transporters were made by gene reconstruction. All hybrid proteins could be expressed, some inhibited cell growth, and one was active. The active hybrid transporter consists of the transmembrane domain (residues 1–386) of the II^{Glc} subunit and the two hydrophilic domains (residues 370–648) of II^{GlcNAc}. The N-terminal hydrophilic domain of II^{GlcNAc} contains the transiently phosphorylated cysteine-412. The hybrid protein is specific for glucose, which indicates that the sugar specificity determinant is in the transmembrane domain and that the cysteine from which the phosphoryl group is transferred to the substrate is not part of the binding site. The protein sequence (LKTPGRED) at which the successful fusion occurred has the characteristic properties of an interdomain oligopeptide linker (Argos, P., 1990, *J. Mol. Biol. 211*, 943–958).

Keywords: bacterial phosphotransferase system; chimeric protein; cysteine mutation; glucose transport; interdomain sequence; linker

The transporters for glucose and *N*-acetylglucosamine of *Escherichia coli* belong to a group of structurally and functionally related transporters known as enzymes II of the bacterial phosphotransferase system (for comprehensive reviews see Postma & Lengeler, 1985; Postma, 1987; Meadow et al., 1990). All enzymes II have the following properties in common: They couple translocation of the substrate to phosphorylation, they contain two sequentially acting phosphorylation sites, and they function as sugar-specific receptors in chemotaxis (reviewed in Lengeler & Vogler, 1989; Taylor & Lengeler, 1990). Phosphoenolpyruvate is the phosphoryl donor, and the phosphoryl groups are transferred via two cytoplasmic proteins to the transporters and hence to substrates as follows: P-enolpyruvate → enzyme I (site 1) → HPr (site 2) → transporter (sites 3 and 4) → substrate. All proteins involved become transiently phosphorylated. The phosphorylation sites 1, 2, and 3 are histidyl residues. Site 4 is either a cysteine or a histidine depending on the particular transporter. The transporters themselves consist of at least three functional units that are either domains of a single polypeptide chain or polypeptide subunits in a complex. The hydrophobic unit, which is predicted to span the membrane between six and eight times, contains the sugar-binding site. The two hydrophilic units that contain the phosphorylation sites are oriented toward the cytoplasm. The transporters differ in substrate selectivity, amino acid sequence, the chemical nature of phosphorylation site 4 (a cysteine or a histidine), and the sequential arrangement of the domains in the polypeptide subunit(s) (reviewed in Robillard & Lolkema, 1988; Erni, 1989, 1992).

The glucose and *N*-acetylglucosamine transporters have about 40% amino acid sequence identity, and their functional units are colinearly arranged (Peri & Waygood,

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Abbreviations: II^{Glc}, transmembrane subunit of the glucose transporter; III^{Glc}, hydrophilic subunit of the glucose transporter; II^{GlcNAc}, *N*-acetylglucosamine transporter; II^{Mtl}, mannitol transporter; αMG, methyl-α-D-glucopyranoside; octyl-POE, polydisperse octyl-oligooxyethylene; G, a DNA segment of *ptsG* or a protein segment of II^{Glc}; E, a DNA segment of *nagE* or a protein segment of II^{Nag}.

1988; Rogers et al., 1988). The glucose transporter consists of two subunits, II^{Glc} and III^{Glc}. III^{Glc} (18 kDa) is a hydrophilic protein that contains phosphorylation site 3 (His-90; Dörschug et al., 1984). II^{Glc} (52 kDa) consists of a transmembrane domain (approximately 380 N-terminal amino acids) and a hydrophilic domain (approximately 100 C-terminal amino acids), which contains phosphorylation site 4 (Cys-421; Nuoffer et al., 1988; Pas & Robillard, 1988). III^{Glc} could be shown to allosterically enhance the catalytic activity of II^{Glc} in vitro (Erni, 1986). The *N*-acetylglucosamine transporter (II^{GlcNAc}) consists of a single polypeptide chain (71 kDa). The domains of II^{GlcNAc} equivalent to II^{Glc} and III^{Glc} are fused through a peptide segment containing two alanine-proline repeats. Similar alanine-proline repeats are present in other proteins and are the hallmark of a particular type of flexible hinge that connects structurally independent domains (Erni, 1989).

Here we describe experiments aimed at identifying the domain(s) of the II^{Glc} subunit and of II^{GlcNAc} involved in substrate binding. A question of particular interest was whether the peptide segments containing the cysteines from which the phosphoryl groups are transferred to the substrate also contribute to the substrate specificity of the binding site.

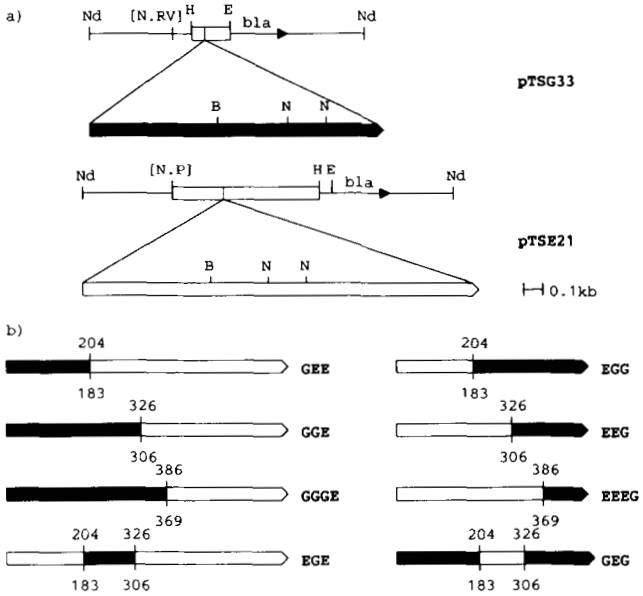

Fig. 1. Restriction maps of the plasmids used for the expression of hybrid proteins. **a**: Restriction maps of the parent plasmids pTSG33, encoding II^{Glc} (filled), and pTSE21 encoding II^{GlcNAc} (open). The structural genes are shown as inserts fivefold enlarged with respect to the chromosomal noncoding and pBR322 sequences. The positions of the extra restriction sites are indicated in the insert. Nd, *Nde*I; E, *Eco*RI; H, *Hin*dIII; B, *Bam*HI; N, *Nru*I; [N.P], blunt-ended and ligated *Nru*I and *Pst*I. **b**: Schematic drawing of the recombined structural genes encoding the hybrid proteins.

Results

Cloning and overexpression of II^{GlcNAc}

Escherichia coli LR2-168 (*nagE*) was infected with a mini-Mu lysate obtained from strain RP7029, and transductants fermenting GlcNAc were selected on McConkey agar plates. As GlcNAc is a good substrate for mannose transporter (II^{Man}, III^{Man}), but Man is not a substrate for II^{GlcNAc}, the GlcNAc-fermenting transductants were transferred to McConkey Man, and transductants that did not ferment Man were further analyzed. The mini-Mu plasmids from clones scoring positive were digested with different restriction endonucleases and the fragments ligated into the corresponding sites of pJF119EH. The insert was trimmed by sequential deletion of the flanking sequences. The progress of deletion from the 5′ end was monitored by assaying for isopropylthiogalactoside-dependent expression of II^{GlcNAc}. Once the endogenous promoter was deleted or overrun by transcription from the tacP promoter of pJF118EH, the cells stopped growing upon addition of IPTG. A comparison of the phosphotransferase activity of II^{GlcNAc} encoded by the different plasmids showed that plasmids with *nagE* under the control of the endogenous constitutive promoter afforded the highest activity. In contrast, strains transformed by plasmids with *nagE* under the control of tacP immediately stopped growing upon induction with IPTG and expressed only negligible II^{GlcNAc} activity. However, upon phosphorylation with ^{32}P-enolpyruvate, II^{GlcNAc} could always be detected as a strong protein band migrating slightly faster than phosphoenzyme I on sodium dodecyl sulfate (SDS) polyacrylamide gels. For the experiments described below, two inserts containing *nagE* under the endogenous promoter were recloned into pBR322 to afford plasmids pTSE1 and pTSE21. A schematic restriction map of pTSE21 is shown in Figure 1a. The inserts in pTSE1 and pTSE21 contained the same 0.8-kb upstream sequence but differed with respect to the length of the 3′ sequence. II^{GlcNAc} activity could be solubilized with the detergent octyl-POE as described for II^{Glc} (Erni et al., 1982). While this work was in progress, the DNA sequence of *nagE* was published (Peri & Waygood, 1988; Rogers et al., 1988). The published sequence and restriction map was used for the work described below.

Choice of sites for the joining of gene segments

Unique restriction sites were introduced by site-directed mutagenesis into three homologous segments in the *ptsG* and *nagE* genes (Fig. 2a). The first site was introduced in the hydrophobic domain between the N-terminal long stretch of hydrophobic character and a characteristic hydrophilic segment, clearly discernible in the hydropathy plots of II^{GlcNAc} and II^{Glc}. The second site was set within the hydrophobic domain in what could be a hydrophilic loop between two membrane-spanning segments of II^{Glc}

a)

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II^Glc      NPVVAFGIYGFIERCLVPFGLHHIWNV (216)
              **  ||*|||*|x|+| ||| | |
II^GlcNAc   G-ALGSGIFGFINRLLIPTGLHQVLNT (195)

II^Glc      YIIHAILAGLAFFICILLGMRDGTSFS (338)
            |**||*| |*****x |||** | |||
II^GlcNAc   YLLHALLTGISLFVATLLGIHAGFSFS (318)

II^Glc      FRVLIKALDLKTPGRED-----ATEDA (393)
            | **|****|||||x||     *||*|
II^GlcNAc   FSLVIRMFNLKTPGREDKEDEIVTEEA (381)
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b)

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          L   I   F   A   I   G   V   A       (68/61)
        TTG ATT TTT GCG ATC GGT GTC GCA
          A   C   C   C           G

              G   K   R   F   V   P   I       (155/135)
            GCT AAA CGC TTT GTG CCG ATC
              C                       T

Q   I   G   E   X   T   N   A   A   G   X   V   F   H   G   D   (236/215)
CAG ATT GGT GAA   ACC AAC GCA GCA GGT   GTT TTC CAC GGC GAC
                            G   G                       T

              L   K   T   P   G   R   E       (387/370)
            CTG AAA ACG CCG GGT CGT GAA

          D   A   C   I   T   R   L   R       (426/417)
        GAC GCA TGT ATT ACC CGT CTG CGC
              C       C
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Fig. 2. Sequence comparisons. **a**: Sequences of II^{Glc} and II^{GlcNAc} at the crossover points (x). **b**: Comparison of codon usage in segments of the *ptsG* and *nagE* structural genes encoding identical amino acid sequences. Shown are the amino acid sequence, the DNA sequence of *ptsG*, and the sequence differences in *nagE*. The numbers refer to the amino acid residues in II^{Glc}/II^{GlcNAc}.

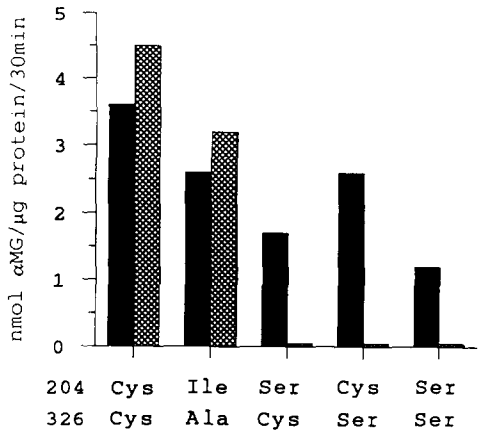

Fig. 3. Phosphotransferase activity of membrane-bound and detergent-solubilized II^{Glc} mutants. The activities of the wild-type protein (Cys-204, Cys-326) and of mutants with different amino acids in positions 204 and 326 are given as nmol αMG 6-phosphate per μg of total membrane protein. Washed membranes are designated by closed bars; detergent-solubilized proteins by cross-hatched bars. The plasmids encoding these mutants are precursors of the plasmids shown in Figure 1. The cysteine to serine mutants are of Nuoffer et al. (1988).

and II^{GlcNAc}. The third site was positioned at the point of maximum DNA similarity. This position corresponds to the region of transition between the N-terminal hydrophobic domain and the C-terminal hydrophilic domain in both II^{Glc} and II^{GlcNAc}. Two restriction sites in II^{Glc} were chosen to substitute Cys-204 and Cys-326 by isoleucine and alanine, respectively, because these cysteines are neither essential for II^{Glc} function nor conserved in II^{GlcNAc} (Nuoffer et al., 1988). In contrast to the previously described substitutions of Cys-204 and Cys-326 by serine, which destabilize II^{Glc} to the extent that it does not insert quantitatively into the membrane and can no longer be solubilized with detergents (Nuoffer et al., 1988), the substitutions of the cysteines by hydrophobic residues do not interfere with protein stability and function (Fig. 3). The conservative L183I substitution in II^{GlcNAc} had no effect on phosphotransferase activity (results not shown). The other DNA changes did not alter the amino acid sequence.

Expression of eight different hybrid proteins between II^{Glc} and II^{GlcNAc}

Six dipartite and two tripartite hybrid genes were constructed by reciprocal exchange of corresponding restriction fragments between *ptsG* and *nagE* as schematically shown in Figure 1b. A *recA* derivative of strain LR2-168 was transformed, and transformants were scored on McConkey agar plates containing GlcNAc and Glc, respectively. Only one hybrid gene encoded a fully functional protein (see below). Four transformants formed small colonies on McConkey agar plates, indicating that the hybrid proteins had a deleterious effect on cell growth. To confirm that the hybrid proteins were expressed, a minicell-producing strain was transformed with the recombinant plasmids. In all cases plasmid-encoded ^{35}S-labeled proteins could be detected by autoradiography (Fig. 4a). The amount of some of the hybrid proteins was reduced with respect to wild-type II^{Glc} and II^{GlcNAc}, indicative of reduced stability. In two cases, smaller fragments could be detected with an anti-II^{Glc} antiserum (Fig. 4b). The antiserum, which is a pool of four monoclonal antibodies (Meins et al., 1988), reacts only with hybrid proteins containing the C-terminal hydrophilic domain of II^{Glc} and does not cross-react with the homologous domain of II^{GlcNAc}. A polyclonal rat anti-II^{Glc} serum also reacted with the same hybrid proteins containing the C-terminal domain of II^{Glc} (results not shown), indicating that the hydrophilic C-terminal domain only was sufficiently immunogenic to elicit antibodies under the standard conditions used for immunization (Erni et al., 1982).

Characterization of a functional fusion protein between II^{Glc} and II^{GlcNAc}

Although all hybrid proteins with fusion points within the hydrophobic domain were inactive and some were unstable, one hybrid protein with the crossover point in the C-terminal hydrophilic domain turned out to be active. This hybrid consists of amino acid residues 1-386 of the II^{Glc} subunit and of residues 369-640 of II^{GlcNAc}. Residues

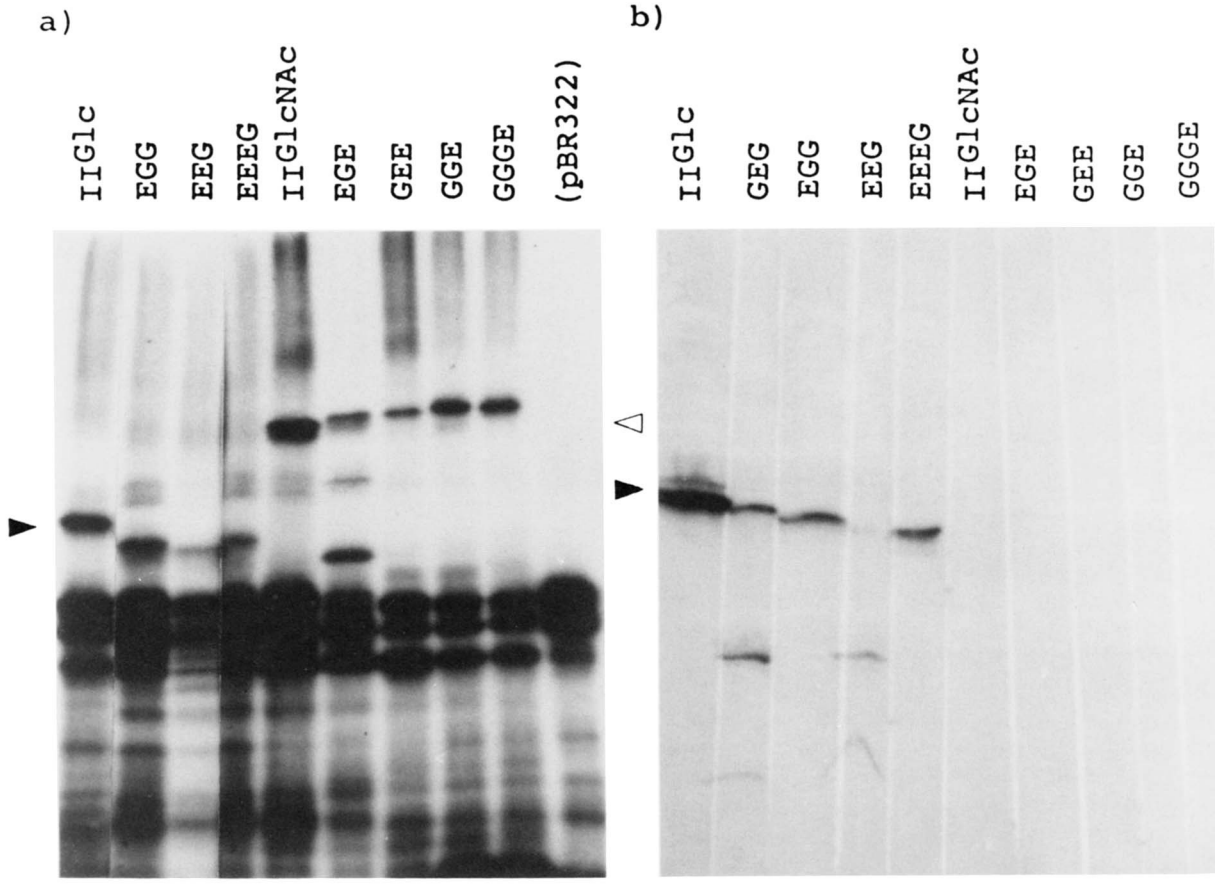

Fig. 4. Expression of hybrid proteins between II^{Glc} and II^{GlcNAc}. **a**: Expression in minicells. The hybrid protein GEG could not be expressed in minicells (not shown). **b**: Immunoblots of membrane proteins. The blot was incubated with a pool of four monoclonal antibodies against II^{Glc} and stained with lactoperoxidase coupled to anti-mouse IgG. Only hybrids containing the C-terminal hydrophilic domain of II^{Glc} are recognized by these four antibodies. II^{Glc} is designated by the closed arrowhead; II^{GlcNAc} by the open arrowhead.

386 and 369 are two homologous arginines at the crossover point. Membranes prepared from cells expressing this hybrid protein had the same specific phosphotransferase activity as membranes from cells expressing II^{Glc}. However, although II^{Glc} activity completely depends on added III^{Glc}, the hybrid protein is independent of or at best very little stimulated by III^{Glc} (Table 1). The hybrid protein is specific for Glc but does not phosphorylate GlcNAc, indicating that the N-terminal hydrophobic domain contains the sugar-binding site. The catalytically essential cysteine (phosphorylation site 4 of the transporter) from which the phosphoryl group is transferred to the sugar is derived from II^{GlcNAc} (Cys-412) in the hybrid protein. It appears that this cysteine and the highly conserved sequence surrounding it are not part of the sugar specificity determinant but, e.g., might be in a flexible loop that closes down when the substrates appear on the cytoplasmic surface of the transmembrane domain. The reciprocal fusion between the N-terminal 369 residues of II^{GlcNAc} and the C-terminal 91 residues of II^{Glc} is inactive (results not shown).

Discussion

Why is the II^{Glc}-II^{GlcNAc} hybrid with the crossover at Arg 386/369 functional when the other constructs are not? This site was chosen on the basis of maximum sequence similarity at the level of DNA (23 identical nucle-

Table 1. *Sugar phosphorylation activity of II^{Glc}/III^{Glc} and the functional II^{Glc}-II^{GlcNAc} hybrid protein*[a]

	Plus III^{Glc}	Minus III^{Glc}
pTSG31 (II^{Glc})	10.8 ± 0.7	<0.05
pGGGE (II^{Glc}-II^{GlcNAc})	11.8 ± 0.3	8.8 ± 0.6

[a] Washed membranes from *E. coli* LR2-168 transformed with plasmids pTSG31 and pGGGE, respectively, were assayed in the presence of phosphoenolpyruvate, partially purified enzyme I, HPr, and in either the presence or absence of partially purified III^{Glc}. The specific activity is given as nmol αMG 6-phosphate/μg membrane protein/30 min incubation.

otides) rather than polypeptide sequence. It is located approximately 40 amino acid residues upstream of the CITRLR sequence containing the phosphorylatable Cys-421 and Cys-412 of II^{Glc} and II^{GlcNAc}, respectively. Similar sequences (LKTPGRED and LKTPGRD) are present in the same distance upstream from the CITRLR sequence in the glucose transporter of *Bacillus subtilis* (Gonzy-Tréboul et al., 1989; Sutrina et al., 1990) and the MalX protein of *E. coli* (Reidl & Boos, 1991). Van Weeghel et al. (1991) have identified a flexible region in the mannitol transporter (II^{Mtl}) at which they could genetically dissect the polypeptide into two complementing subunits. This region of II^{Mtl} is also 40 residues upstream from the catalytic cysteine-384 (CDAGMG). It appears that guided by a comparison of the DNA sequence, an interdomain region has been identified in II^{Glc} and II^{GlcNAc}. Indeed, this sequence (LKTPGRED), like the corresponding but different sequence in II^{Mtl}, consists of amino acid residues that are frequent in protein segments of high flexibility (Karplus & Schulz, 1985) and in oligopeptides that link the domains of multidomain proteins (Argos, 1990). According to Argos (1990), these oligopeptides preferentially contain Thr, Ser, Gly, Ala, Pro, Asp, Lys, Gln, and Asn; have an average length of 6.5 residues; are about average in flexibility compared with other protein regions; and the majority (60%) are in coil or bend structures. The *N*-acetylglucosamine transporter and the mannitol transporter of *E. coli* and the glucose transporter of *B. subtilis* (Sutrina et al., 1990) have a very similar overall domain organization, with a hydrophobic N-terminal domain that binds the substrate, a hydrophilic domain containing the catalytically essential cysteine (site 4) and a C-terminal domain with the phosphorylation site 3 (which transfers the phosphoryl group from phospho-HPr to site 4).

Although II^{GlcNAc} and II^{Glc}/III^{Glc} display over 40% amino acid sequence identity, little similarity can be detected at the level of DNA, and not a single six-nucleotide restriction site is conserved. If all DNA segments in II^{Glc} and II^{GlcNAc} encoding identical stretches of seven and eight adjacent amino acid residues are compared, only the DNA segment encoding the LKTPGRED sequence is identical in both genes, whereas synonymous codons occur in the other sequences (Fig. 2b). Wu et al. (1990) proposed that similar DNA sequences encoding the linkers between protein domains may also provide hot spots for genetic recombination and therefore facilitate the rearrangement of genes encoding functional protein units. In order to test this hypothesis, and in search of a hybrid protein with relaxed substrate specificity, more hybrid proteins between II^{Glc} and II^{GlcNAc} are now being made in vivo by using the RecE recombination pathway that mediates recombination between partially homologous DNA sequences (Keim & Lark, 1990).

Why should the reciprocal fusion between the N-terminal 369 residues of II^{GlcNAc} and the C-terminal 91 residues of II^{Glc} be inactive? Linking II^{GlcNAc} with the hydrophilic domain of II^{Glc} might cause a slight conformational strain in the latter and thus compromise binding of III^{Glc}. The noncovalent interaction of the III^{Glc} subunit with the II^{Glc} portion of the fusion protein is likely to be more sensitive to conformational changes than the interaction between the respective functional units in the II^{Glc}-II^{GlcNAc} fusion where the association is covalent and therefore concentration independent. This hypothesis is now being tested by fusing the III^{Glc} subunit to the C-terminus of the II^{GlcNAc}-II^{Glc} fusion protein.

Materials and methods

Bacterial strains, phages, plasmids, and media

The following bacterial strains, phages, and plasmids were used: *Escherichia coli* K12 with relevant genotypes: LR2-168, *manI nagE ptsG lacY1 galT6 xyl-7* (Lengeler et al., 1981). A *recA*$^-$ derivative of LR2-168 prepared by conjugative transfer of *recA56* from strain JC10240, HfrKL16 *recA56 srl-300*::Tn10 (gift of W. Epstein, University of Chicago); UT580, Δ(*lac-proAB*) *supD/F'lacI*^{q} *ZΔM15 proA*$^+$*B*$^+$ (gift of C. Lark, University of Utah), WK6mutS, Δ(*lac-proAB*) *galE strA mutS215*::Tn10/*F'lacI*^{q} *ZΔM15 proA*$^+$*B*$^+$ (Stanssens et al., 1989); P678-54, minicell-producing strain (Reeve, 1979); RP7028, Mucts (gift of J.S. Parkinson, University of Utah); RP7029, MudII4042 (gift of J.S. Parkinson, University of Utah). Bacteriophages: M13K07, M13 helper phage (Stanssens et al., 1989); Mucts, Mu with temperature-sensitive repressor *c*ts62 (Groisman et al., 1984). Plasmids: MudII4042, Mucts62 *A*$^+$*B*$^+$ Cm^r *rep*P15A *lac*(*'ZYA*)931 (Groisman et al., 1984). pJF118EH, *tacP lacI*^{q}, Ap^r (Fürste et al., 1986). pMa/c5-8, phagemids for mutagenesis (Stanssens et al., 1989). pTSG33: a derivative of pTSG3 (Erni & Zanolari, 1986) with one *Eco*RI site next to the *Hin*dIII site, and the *Nru*I/*Eco*RV fragment of the Tet^r gene deleted.

Unless stated otherwise, cells were grown to stationary phase in LB broth (1% Difco Bacto tryptone, 0.5% Oxoid Bacto yeast extract and 1% NaCl) at 37 °C on a rotary shaker. McConkey plates containing 0.4% Glc or GlcNAc were prepared as indicated by Difco. For growth of plasmid-containing cells, media were supplemented with 100 μg/mL ampicillin or 20 μg/mL of chloramphenicol.

Plasmid DNA techniques

Plasmid DNA was prepared by standard techniques (Maniatis et al., 1982). Restriction endonucleases were used as recommended by the suppliers.

Cloning of nagE

In vivo cloning of *nagE* with a mini-Mu bacteriophage containing a high copy number plasmid replicon was performed as described (Groisman et al., 1984). A mini-Mu lysate obtained from *E. coli* RP7029 was used to infect the recipient strain LR2-168 lysogenic for Mucts. Chloramphenicol-resistant and GlcNAc-fermenting transductants were selected on McConkey agar plates. Two mini-Mu plasmids from II^{GlcNAc} overexpressing transductants were digested with different restriction nucleases and the fragment mixtures ligated into the corresponding sites of pJF118EH. The recombinant plasmid with the shortest insert was selected for further trimming of the insert from both ends with exonuclease III. One insert with the flanking polylinkers of pJF118EH was transferred into the *Eco*RI site of pBR322 to afford plasmid pTSE1 with a 2.8-kb insert containing in addition to *nagE* approximately 0.8 kb of upstream sequence. Another trimmed insert containing the same 0.8-kb upstream sequence and in addition a 1.2-kb downstream sequence was recombined with pBR322 opened at *Nru*I and *Hin*dIII to afford plasmid pTSE21 (Fig. 1a). The restriction maps of the two inserts were obtained by comparison with the restriction map that was published while this work was in progress (Rogers et al., 1988).

Site-directed mutagenesis

The gapped duplex method of Stanssens et al. (1989) was used. The *ptsG* gene was excised with *Eco*RI and *Hin*dIII from pTSG31 and ligated into the polylinker region of pMa/c5-8 to afford plasmids pMa/cG. The *nagE* gene was excised with *Bam*HI and *Sac*I from plasmid pTSE1 and ligated into pMa/c5-8 opened with *Bam*HI and *Hin*dIII to afford pMa/cE (to match *Sac*I and *Hin*dIII the recessed ends were filled in with Klenow fragment). The mutagenic oligonucleotides were synthesized by the phosphoramidite method (Applied Biosystems 380B) and purified by high performance liquid chromatography (Nuoffer et al., 1988). The oligonucleotide sequences were deduced from the published sequences of *nagE* (Rogers et al., 1988) and *ptsG* (Erni & Zanolari, 1986). The following **mismatch** oligonucleotides were used to introduce the indicated restriction sites; the *amino acid substitutions* resulting from these changes are also shown: in *ptsG*: CATCGAACG**GATC**CTGGTACCG, *Bam*HI, C204I; CATTCCCAA**TCGCGA**TTCTTCTG, *Nru*I, C326A; ACGCCGGG**TCGCGA**AGACGCG, *Nru*I, R386R; in *nagE*: GGGATCAG**GATCC**GGTTGATG, *Bam*HI, L183I; CAGCAGCG**TCGCGA**CAAACAGGC, *Nru*I, A306A; TTTATCT**TCGCGA**CCCGGCGT, *Nru*I, R369R. Plasmids were screened for the presence of the desired restriction site by restriction analysis. Between 20% and 50% of the plasmids scored positive. Restriction fragments carrying the additional restriction sites were transferred back from the mutagenesis vector into the plasmids pTSG31 and pTSE21.

Minicell assay

Minicells were prepared from the transformed strain P678-54 (Reeve, 1979) by the method described by Christen et al. (1983).

Small scale preparation of membranes and membrane extracts

Membranes were prepared and solubilized in 5% octyl-POE as described by Erni et al. (1982).

Assay for phosphoenolpyruvate:sugar phosphotransferase activity

Sugar phosphorylation activity was assayed by the ion-exchange method of Kundig and Roseman (1971) as detailed by Erni et al. (1982). The reaction mixture contained, per 0.1 mL, 100 μg phosphatidylglycerol from egg yolk (Sigma), 50 mM KP_i, pH 7.5, 2.5 mM dithiothreitol, 2.5 mM NaF, 5 mM $MgCl_2$, 1 mM P-enolpyruvate (Sigma), 0.5 mM [U-^{14}C]αMG (New England Nuclear; 250 mCi/mmol, diluted to 900 cpm/nmol) or 0.5 mM [U-^{14}C]GlcNAc (New England Nuclear; 250 mCi/mmol, diluted to 200 cpm/nmol), and either 10 μL (150 μg) of a cytoplasmic extract (Erni et al., 1982) as a source of soluble phosphoryl carrier proteins or partially purified enzyme I (5 μg; Weigel et al., 1982), pure HPr (1 μg; Beneski et al., 1982), and partially purified III^{Glc} (0.1 μg of protein purified through the gel filtration step; Meadow & Roseman, 1982). Incubation was for 30 min at 37 °C. Activity is expressed as nanomoles of sugar-phosphate formed after 30 min.

Other techniques

Standard polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate was performed as described (Erni et al., 1982). Protein samples were not boiled prior to electrophoresis. Standard methods for electroblotting (Towbin et al., 1979) and a pool of four monoclonal anti-II^{Glc} antibodies (Meins et al., 1988) together with lactoperoxidase coupled to anti-mouse IgG were used for immunodetection of II^{Glc} on nitrocellulose blots. Protein was determined by a Lowry assay as modified by Markwell et al. (1978).

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