Glucose transport in *Escherichia coli*

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1. INTRODUCTION

1.1. Different mechanisms of glucose transport and their distribution

Glucose plays a special role among sugars because it can directly enter the glycolytic pathway. For many eucaryotic cells glucose is the only useful energy source. Bacteria can utilize a much greater variety of sugars, but given a choice prefer glucose. The presence in many bacteria of two different glucose permeases and the influence of glucose on metabolic regulation further corroborate its predominant role. In cells growing in the presence of glucose, the synthesis of enzymes that process nutrients less readily metabolizable than glucose is strongly reduced (glucose effect, catabolite repression, diauxic growth [1,2]).

*Escherichia coli* make use of a mechanism for glucose uptake which characteristically couples sugar transport with sugar phosphorylation. It depends on phosphoenolpyruvate as the phosphoryl donor, a requirement which establishes a direct link between the uptake and the glycolysis of the sugars. Such a mechanism which couples transport of a substrate with its chemical modification is termed group translocation. P-enolpyruvate dependent sugar transport is found in most facultative and obligatory anaerobic bacteria [3,4] but not in eucaryotes including yeast [5]. It is used by *E. coli* and *Salmonella typhimurium* for the transport of glucose, of other hexoses (e.g. mannose, N-acetyl glucosamine) and of the hexitols (e.g. mannitol, sorbitol).

1.2. The bacterial phosphotransferase system (PTS)

The transfer of the phosphoryl group from phosphoenolpyruvate to the sugar occurs in two steps. The first step, the transfer to the membrane-bound sugar permeases is catalyzed by two cytoplasmic proteins termed enzyme I and HPr. The second step, transfer from the permeases to the sugar substrates is catalyzed by the permeases themselves (Fig. 1). Enzyme I and HPr, together with the different permeases constitute a multienzyme system known as the bacterial phosphotransferase system (PTS). It was discovered by Kundig, Gosh, and Roseman in 1964 [6]. Subsequent work from Roseman's and other groups showed that the PTS is more than just a sugar transport system and is equally important for the regulation of the bacterial catabolism and for chemotaxis. For two comprehensive reviews see [9,10].

1.3. The glucose permeases of *E. coli*

*E. coli* and *S. typhimurium* both have two glucose-specific permeases. One, the glucose permease proper, is specific for glucose and the non-
Fig. 1. The bacterial phosphotransferase system. Enzyme I and HPr are two cytoplasmic proteins which sequentially transfer phosphoryl groups from P-enolpyruvate (PEP) to the sugar-specific permeases (enzyme II and enzyme II/III complexes). Enzymes I and HPr each contain one phosphorylation site (P), the permeases contain two phosphorylation sites located on two different subunits (III\textsuperscript{Glc}/II\textsuperscript{Glc}, III\textsuperscript{Glc}/II\textsuperscript{Ser}) or on a single polypeptide (II\textsuperscript{Man}, II\textsuperscript{Nag}, II\textsuperscript{BgI}). In the second case a Ala-Pro containing hinge peptide (symbolized by a caret) connects the two domains each containing one phosphorylation site (P). The amino acid sequence identity between corresponding domains is 35–40% (dotted) and ca. 50% (diagonal lines). The segment containing the catalytically essential Cys-421 of nGlo (solid) is conserved but transposed to the very N-terminus of nBgI and nNag. There is no obvious sequence similarity between the III\textsuperscript{Man}/II\textsuperscript{Man} complex and other PTS permeases. Complementation between nOlo and C-terminally truncated nNag [7], and between nNag and nOlo in the absence of nIGk [8] has been observed (broken arrows).

metabolizable analog methyl(α-D-glucopyranoside (aMG). It consists of two subunits, II\textsuperscript{Glc} and III\textsuperscript{Glc}, which are encoded by two separate genes, ptsG and err respectively. The second permease is specific for glucose, mannose and other sugars with different substituents at carbon 2 of the hexose ring. It is designated mannose permease because it is required for growth on mannose as the only carbon source. It consists of three subunits (III\textsuperscript{Man}, II-\textsuperscript{Man}, II-M\textsuperscript{Man}) which are encoded by the pslLPM transcriptional unit. Kundig and Roseman [11] started to biochemically characterize the two permeases, work which has been continued in this laboratory [12,13] and has more recently been taken up by myself [14–16].

2. GLUCOSE PERMEASE (II\textsuperscript{Glc}/III\textsuperscript{Glc} COMPLEX)

2.1. Quaternary structure

III\textsuperscript{Glc} is a hydrophilic protein of $M_r$ 18,556 [17,18]. It is only loosely associated with the II\textsuperscript{Glc} subunit and after breaking the cells is found in the cytoplasmic fraction [19,20]. II\textsuperscript{Glc} is an integral membrane protein of $M_r$ 50,645. A hypothetical model of its membrane topology, which is based on hydropathy analysis [15], is shown in Fig. 2. According to this model two hydrophobic regions comprising 50% of the II\textsuperscript{Glc} sequence could span the membrane six times. The two regions are separated by a sequence of about 120 hydrophilic residues. The C-terminal end of II\textsuperscript{Glc} comprising about 90 amino acids is also polar. Biochemical experiments (see 2.2.1.) suggest that both hydrophilic regions are exposed at the cytoplasmic face of the membrane. The N-terminus of II\textsuperscript{Glc} has a strong helical hydrophobic moment. If it is arranged as an α-helix, the non-polar amino acids protrude from one face, the polar side chains including three lysins from the opposite face of

Fig. 2. Speculative model of the II\textsuperscript{Glc}/III\textsuperscript{Glc} complex. The amphiphilic N-terminus of II\textsuperscript{Glc} is shown as a ‘surface-seeking’ helix on the cytoplasmic face of the membrane bilayer. Two non-contiguous hydrophobic segments of II\textsuperscript{Glc} spanning the membrane four and two times respectively constitute the transmembrane domain of the glucose permease. An internal hydrophilic segment and the C-terminus of II\textsuperscript{Glc} together with III\textsuperscript{Glc} are shown to constitute the cytoplasmic domain. The N-terminus of III\textsuperscript{Glc} is essential for binding to II\textsuperscript{Glc} [29]. Three residues are suggested to be part of the glucose phosphorylation site: His-91 of III\textsuperscript{Glc} (solid square [27]), Cys-421 of II\textsuperscript{Glc} (solid circle) and a not yet identified His of II\textsuperscript{Glc} (open square). Shown as an insert is the helical wheel projection of the amphiphilic N-terminus of II\textsuperscript{Glc}. The hydrophilic amino acids are underlined.
the helix (Fig. 2, insert). This amphipilic helix is strikingly similar to mitochondrial targeting/prefacequences [21,22], but its function in the permease is not yet known. One possibility is that it aligns parallel to the cytoplasmic face of the membrane [23], and by locally disturbing the integrity of the lipid bilayer [24] facilitates insertion of the protein into the membrane. Similar amphipilic helices have subsequently been found at the N-termini of other PTS permeases and appear to be a structure characteristic of these proteins [25].

2.2. Functions

2.2.1. Transport and phosphorylation: \( \text{II}^{\text{Glc}} \) and \( \text{III}^{\text{Glc}} \) each contain an active center, which is transiently phosphorylated [14,26]. Phosphorylation of \( \text{III}^{\text{Glc}} \) occurs at His-91 [27]. The phosphorylated residue of \( \text{II}^{\text{Glc}} \) is believed to be a histidine of the 120 residue hydrophilic segment, but it has not yet been chemically identified.

Purified \( \text{II}^{\text{Glc}} \) is sufficient to catalyze the phosphorylation exchange between glucose-6-phosphate and glucose at equilibrium [28], indicating that this protein contains both glucose recognition (binding) and glucose phosphorylation sites. Phosphorylated \( \text{II}^{\text{Glc}} \) (P-II\(^{\text{Glc}}\)) is also sufficient to phosphorylate glucose [14]. If assayed in vitro, this process is, however, slow and accompanied by hydrolysis of the phosphoprotein. Only if \( \text{III}^{\text{Glc}} \) is also present, phosphorylation transfer from P-II\(^{\text{Glc}}\) to glucose is rapid and almost quantitative. The two subunits together rather than \( \text{II}^{\text{Glc}} \) alone must therefore be the functional form of the glucose permease. Association between \( \text{III}^{\text{Glc}} \) and \( \text{II}^{\text{Glc}} \) was also demonstrated by immunoprecipitation of the two subunits with antisera directed against either of the two proteins [14]. The N-terminal seven amino acids of \( \text{III}^{\text{Glc}} \) appear particularly important for binding to \( \text{II}^{\text{Glc}} \) because their proteolytic removal results in a \( \text{III}^{\text{Glc}} \) species which can no longer efficiently transfer the phosphoryl group to \( \text{II}^{\text{Glc}} \) [29]. In Fig. 2, it is therefore proposed that \( \text{II}^{\text{Glc}} \) and \( \text{III}^{\text{Glc}} \) form a complex and that a phosphorylated histidine (open square) of \( \text{II}^{\text{Glc}} \), Cys-421 (solid dot) of \( \text{II}^{\text{Glc}} \) and His-91 (solid square) of \( \text{III}^{\text{Glc}} \) together constitute the sugar phosphorylation site.

Binding between \( \text{II}^{\text{Glc}} \) and \( \text{III}^{\text{Glc}} \) on the other hand is not strong enough to allow copurification of the two proteins. And, indeed, the function of \( \text{III}^{\text{Glc}} \) in regulation of a number of enzymes, which are most likely spatially separated from the glucose permease, almost necessitates that \( \text{III}^{\text{Glc}} \) be loosely bound to \( \text{II}^{\text{Glc}} \). It is therefore conceivable that the association is only transient and might be coupled with dephosphorylation/rephosphorylation of \( \text{III}^{\text{Glc}} \).

Both monomeric and dimeric forms of purified \( \text{II}^{\text{Glc}} \) can be observed in vitro [28]. Purified, non-phosphorylated dimeric \( \text{II}^{\text{Glc}} \) can be isolated by velocity sedimentation in a glycerol gradient and covalently cross-linked by either the oxidative formation of an intersubunit disulfide involving Cys-421 or by cross-linking with glutaraldehyde. However, all attempts at identifying the dimeric form of phosphorylated \( \text{II}^{\text{Glc}} \) have failed, suggesting that \( \text{II}^{\text{Glc}} \) is monomeric during catalytic turnover. In agreement with this interpretation, \( \text{II}^{\text{Glc}} \) sedimenting as a monomer through a glycerol gradient catalyzes the phosphoryl exchange between glucose-6-phosphate and glucose [28].

\( \text{II}^{\text{Glc}} \) contains three cysteines. If Cys-421 is exchanged for a serine, \( \text{II}^{\text{Glc}} \) can neither be phosphorylated by phosphorylated \( \text{III}^{\text{Glc}} \) nor catalyze phosphoryl exchange between glucose-6-phosphate and glucose at equilibrium. If Cys-204 and Cys-326 are replaced by serine, \( \text{II}^{\text{Glc}} \) activity is unaffected but the stability of the protein is reduced in vivo (C204S) and in vitro (C204S and C326S) [30]. Cys-421 is therefore envisaged to be a catalytically important part of the phosphorylation site (Fig. 2, solid circle) but most likely not the phosphorylated residue. Interaction of this site with \( \text{II}^{\text{Glc}} \) requires that it be accessible from the cytoplasmic side. Since the same site also participates in sugar phosphorylation, glucose should be phosphorylated only after exit from the transmembrane part of the permease. As a consequence, transport and phosphorylation must be consecutive rather than concomitant events. Two observations support the mechanistic model, Wild-type \( \text{II}^{\text{Glc}} \) can phosphorylate intracellular glucose without concomitant transport (see 2.2.3.), and mutant forms of \( \text{II}^{\text{Glc}} \) ('uncoupled' \( \text{II}^{\text{Glc}} \), [31]) facilitate glucose transport along a concentration
gradient although they are unable to phosphorylate glucose. It is not yet known which parts of the \( \text{II}^{\text{Glc}} \) molecule constitute the transport pathway for glucose.

2.2. Regulation: \( \text{II}^{\text{Glc}} \) is an inducible permease and its synthesis is regulated at the level of transcription. However, there is also evidence for post-translational regulation of \( \text{II}^{\text{Glc}} \) activity. Several investigators found that the rate of \( \alpha \text{MG} \) accumulation by intact cells increased when the membrane potential was decreased, e.g. during growth under anaerobic conditions or after addition of uncouplers and respiratory chain inhibitors (reviewed in [9]). There exists as yet no uncontested explanation of this effect. Regulation by a reversible dithiol : disulfide interconversion as proposed by Robillard and Konings [32] appears unlikely because bacteria expressing mutant \( \text{II}^{\text{Glc}} \) with serine in place of Cys-204 and Cys-326 react like wild-type cells to respiratory chain inhibitors [30]. If Cys-421, which is required for transport and phosphorylation, were the target for regulation, a change of \( V_{\text{max}} \) and not of \( K_M \) (as observed) would be expected. Conceivably, a conformational change affecting the \( K_M \) of \( \text{II}^{\text{Glc}} \) could be induced by interaction of a protein dipole with the transmembrane electrical field [33], or alternatively, the increased accumulation of \( \alpha \text{MG} \) is only apparent, due to a decreased, possibly energy requiring rate of \( \alpha \text{MG} \) expulsion and not to increased \( \text{II}^{\text{Glc}} \) activity.

2.2.3. Other functions of the glucose permease: (i) The glucose permease is part of a chemotactic system regulating the swimming behavior of the cell in response to changing concentrations of glucose [34,35]. Both, \( \text{II}^{\text{Glc}} \) and \( \text{II}^{\text{I}} \text{Glc} \) appear to contain transmitter-receiver modules, i.e. amino acid sequence motives characteristic of two-component regulatory systems found in procaryotes [36]. Chemotactic function, like transport and phosphorylation, depends on enzyme I and HPr [34]. It is, however, not clear whether the cytoplasmic components are part of the signal relay between the receptor and the flagellar motor or are required only to poised \( \text{II}^{\text{Glc}} \) in a 'receptive state'. Because both, PTS activity and sensory signalling in chemotaxis involve protein phosphorylation relays [37,38] with phosphorylation of histidyl side chains [39], 'cross-talk' between the two systems can be envisaged. A drain of phosphoryl groups from the chemotactic system into the PTS system could occur whenever the steady state concentration of phosphorylated PTS components decreases during transport of PTS substrates. This drain could eventually lead to dephosphorylation of CheY and in consequence a decreased frequency of tumbles [38].

(ii) The \( \text{III}^{\text{Glc}} \) subunit of the glucose permease plays an important role in metabolic regulation by modulating the activities of the adenylcyclase, of membrane permeases, which are not components of the PTS, and of certain metabolic enzymes. The affinity of \( \text{III}^{\text{Glc}} \) for these target proteins depends on the phosphorylation state of \( \text{III}^{\text{Glc}} \). During active glucose transport, \( \text{III}^{\text{Glc}} \) turns over and therefore is dephosphorylated most of the time. In the absence of glucose, phosphorylated \( \text{III}^{\text{Glc}} \) accumulates ([40] reviewed in [9]).

(iii) \( \text{II}^{\text{Glc}} \) phosphorlylates not only glucose in transit across the cytoplasmic membrane but also intracellular glucose. It can thus complement glucokinase activity. \( E. \text{coli} \) strain ZSC112L which is unable to metabolize glucose because it lacks \( \text{II}^{\text{Glc}} \), \( \text{II}^{\text{Man}} \) and glucokinase is also unable to grow on maltose as the only carbon source. These cells accumulate maltose, but for unknown reasons cannot utilize it. Instead, they secrete large amounts of glucose derived from maltose. After transformation with a plasmid encoding \( \text{II}^{\text{Glc}} \), the same cells stop secreting glucose and grow normally [30]. Similarly, phosphorylation of intracellular glucose is catalyzed by the mannose permeases of \( \text{Streptococcus lactis} \) [41] and \( E. \text{coli} \) [Erni, unpublished].

3. THE MANNOSE PERMEASE COMPLEX (\( \text{III}^{\text{Man}} / \text{II}^{\text{P-Man}} / \text{II}^{\text{M-Man}} \))

3.1. Quarternary structure

\( \text{II}^{\text{P-Man}} \) and \( \text{II}^{\text{M-Man}} \) are membrane bound and probably membrane spanning proteins of \( M_r \) 27636 and 31016 respectively (Fig. 3b). \( \text{III}^{\text{Man}} \) is a hydrophilic protein of \( M_r \) 35016 and is present in both, the cytoplasmic and the membrane fraction. A complex containing all three subunits can
be purified to homogeneity. Alternatively, III\textsuperscript{Man} and a subcomplex consisting of II-P\textsuperscript{Man} and II-M\textsuperscript{Man} can be purified separately and sugar phosphorylation activity can be reconstituted by combining the two preparations [16]. As in the glucose permease (see 2.2.1.), the association between membrane subunit (II\textsuperscript{Man}, II\textsuperscript{Glc}) and hydrophilic subunit (III\textsuperscript{Man}, III\textsuperscript{Glc}) is weak and probably reversible (Fig. 3c). If purified mannose permease is assayed in vitro, it can be stimulated two-fold with extra III\textsuperscript{Man} suggesting that either some of the mannose permease complexes dissociated and lost their III\textsuperscript{Man} subunit during purification, or that dissociation of III\textsuperscript{Man} and II\textsuperscript{Man} occurs during catalytic turnover leading to their dilution in the incubation medium. Attempts to dissociate II-P\textsuperscript{Man} and II-M\textsuperscript{Man} resulted in complete loss of activity. Preliminary attempts at the reconstitution of the II\textsuperscript{Man} subcomplex from two crude membrane extracts each containing only one of the two subunits also failed. The subunit stoichiometry of the mannose permease is not yet known. III\textsuperscript{Man} alone forms a homodimer in vitro, which above pH 7.0, but not below pH 6.5, is partly resistant against dissociation by sodium dodecylsulfate during gel electrophoresis. This pH dependence indicates that a histidine residue, possibly His-86 is important for dimerization (see 3.2.1.).

3.2. Functions

3.2.1. Transport and phosphorylation: Of the three subunits only III\textsuperscript{Man} has so far been analyzed in detail. Inspection of its amino acid sequence revealed a 20 to 28 residue long segment consisting of Ala-Pro repeats and a few Lys (Fig. 4). Limited proteolysis of III\textsuperscript{Man} with trypsin affords two fragments, suggesting III\textsuperscript{Man} to consist of two domains. The 13 kDa fragment (P13 domain) extends from the N-terminus of III\textsuperscript{Man} to Lys-127 in the hinge region. The 20 kDa fragment (P20 domain) starts at Ala-148 in the hinge and probably extends to the C-terminus of III\textsuperscript{Man}. In the presence of the II\textsuperscript{Man} subcomplex and the cytoplasmic phosphoryl carrier proteins enzyme I and HPr, the two separate domains together catalyze the phosphorylation of mannose. Both domains become transiently phosphorylated in this process. P13 at the imidazole N-3 of His-10 and P20 at N-1 of His-175 (Erni and Kocher, unpublished results). P13, the N-terminal domain, is phosphorylated by the cytoplasmic phosphoryl carrier protein HPr. P20 is phosphorylated by P13. Phosphoryl exchange between the domains is reversible. Like intact III\textsuperscript{Man}, P13 has a tendency to dimerize, particularly in the phosphorylated state. P20 and the II\textsuperscript{Man} subunits together catalyze the phosphoryl exchange between mannose-6-phosphate and mannose at equilibrium. P20 must therefore contain both a binding site for the II\textsuperscript{Man} subcomplex and (part of) the active site for sugar phosphorylation. II-P\textsuperscript{Man} and II-M\textsuperscript{Man}, in contrast, are not phosphorylated. By reconstructing the ptsL gene (Fig. 4), (i) single amino acids (Phe, Cys, His) were introduced into the hinge, (ii) the hinge was shortened, (iii) increased up to sevenfold in size, and (iv) the two domains were expressed as individual polypeptides. Some of the
Fig. 4. Modifications of the Ala-Pro rich hinge peptide linking the P13 and the P20 domains of the III\textsuperscript{Man} subunit. Changes at the hinge, the end of the P13 domain and the start of the P20 domain are shown in lower-case letters. Cells expressing plasmid-encoded modified forms of III\textsuperscript{Man} were tested for fermentation of mannose on McConkey indicator plates and their growth rates determined in a medium containing mannose as the only carbon source. The different III\textsuperscript{Man}, P13 and the P20 were purified and their sugar phosphorylation activity assayed in vitro. A: wild-type sequence. B: modifications due to the introduction of unique restriction sites at the beginning, in the middle, and at the end of the hinge sequence of \textit{ptsL}. C: in-frame fusion of the two domains after removal of the hinge sequence. D, E: duplication of the hinge region and further elongation by random, non-functional polypeptide segments. F: P13 and P20 are expressed as individual polypeptides. The hinge sequence of \textit{ptsL} is replaced by a heteroduplex encoding a translational stop and a ribosome binding site appropriately positioned upstream of ATG encoding the first Met of P20.

alterations decrease but they never completely abolish III\textsuperscript{Man} activity. Therefore neither the exact amino acid composition nor the exact length of the hinge appear critical for III\textsuperscript{Man} function. These results and the additional observation of bidirectional phosphoryl exchange between III\textsuperscript{Man} and

Fig. 5. Ala-Pro rich segments are functional modules in proteins. Shown are the relevant amino acid sequences. The position of these sequences in the proteins is schematically indicated: Solid dots indicate flexible hinges between functional domains leading to the coupling of active sites (III\textsuperscript{Man}, II\textsuperscript{Nag}, II\textsuperscript{Bgl}, E2). Notched dots indicate that the Ala-Pro rich segment contains a site for posttranslational, proteolytic processing of the protein. See text for references.
isolated P13 domains suggest that the phosphoryl groups are transferred from P13 to P20 domains located on different subunits in the dimeric III\textsuperscript{Man} complex rather than between domains on a single subunit. This hypothesis is now being tested with heterodimeric III\textsuperscript{Man} consisting of subunits each containing only one intact phosphorylation site.

By computer assisted sequence comparison Ala-Pro containing peptide modules were found in other procaryotic and eucaryotic proteins including two PTS permeases (Fig. 5, see 5.2.). The following functions can be assigned to them: In the poly-Ig receptor the Ala-Pro rich segment might include the proteolytic cleavage site between the secretory component and the transmembrane part of the protein [42]. In the endo-\beta-N-acetylglucosaminidase H precursor [43] and the cytochrome C3 precursor [44] Ala-Pro rich segments separate the signal sequence from the mature part of the protein and contain the posttranslational cleavage site. In the pyruvate dehydrogenase complex three Ala-Pro rich segments form flexible hinges between the three lipoyl domains and the catalytic domain [45–47]. In the outer membrane protein OmpA [48] and in IgG light chains [49,50] they separate distinct protein domains.

3.2.2. Other functions of the mannose permease: Like glucose permease, the mannose permease is a chemotactic receptor [34,35] and can phosphorylate intracellular glucose (see 2.3.3). Unlike III\textsuperscript{Gc}, III\textsuperscript{Man} so far does not appear to have a regulatory function.

An intriguing additional function of the II\textsuperscript{Man} subcomplex is its role during infection of E. coli by bacteriophage λ. Elliott and Arber [51] characterized E. coli mutants which were resistant against infection by phage λ and at the same time unable to metabolize mannose. They also found that some but not all mannose transport mutants were λ resistant. When the λ resistant mutant E. coli WA2127 was transformed with plasmids encoding subunits of the mannose permease, II\textsuperscript{P-Man} and II-M\textsuperscript{Man} together turned out to be sufficient for infection by phage λ while III\textsuperscript{Man} was not required [16]. It is still a mystery how λ DNA and/or the phage tail proteins (probably gpV or gpH [52]) interact with the II\textsuperscript{Man} subcomplex, and how the II\textsuperscript{Man} subunits could facilitate the entry of phage λ DNA across the cytoplasmic membrane. Compared with mannose transport, λ penetration appears less sensitive to structural changes of the II\textsuperscript{Man} subunits. II-P\textsuperscript{Man} and II-Man with C-terminal deletions no longer transport mannose but continue to mediate λ penetration, although in one case at a reduced rate [16]. This difference could be due to distinct transport mechanisms for the different substrates but also reflect that penetration of a single DNA molecule is sufficient to cause cell lysis while multiple transport of mannose is required to support cell growth.

4. PURIFICATION OF GLUCOSE PERMEASES

The purification procedures for glucose- and mannose permease from wild-type and overproducing bacteria starts with detergent extraction of membranes at alkaline pH. The detergents of choice for solubilization turned out to be octylPOE (octyl-polyoxyethylene) for the glucose permease and MEGA-9 (nonanoyl N-methylglucamide) for the mannose permease [28,53]. For all subsequent purification steps octyl-POE was found optimal. The next step, isoelectric focusing of the membrane extract in a sucrose gradient is particularly efficient, because most of the membrane proteins precipitate while the permeases remain in solution. The reason for this selectivity is not understood. After isoelectric focusing the proteins are already up to 90% pure and 60%-90% of the sugar phosphorylation activity is recovered. The glucose permease can then be purified to homogeneity by chromatofocusing. The mannose permease, in contrast, is inactivated by chromatofocusing but can be further purified by gel filtration. Inclusion of phospholipids in the gel filtration buffer increases the recovery of activity. The II\textsuperscript{Man} subcomplex can be purified exactly as the complete mannose permease complex, although preparations tend to be less pure. Only a limited overexpression of II\textsuperscript{Man} is tolerated by the cell, while III\textsuperscript{Man} alone can be expressed to a level where it is the most prominent protein in the cell, apparently without affecting cell viability. III\textsuperscript{Man}
is present in the cytoplasmic fraction and in the low salt wash of the membrane fraction. It can be precipitated from both pools with ammonium sulfate and then purified to almost homogeneity by stepwise elution from a phosphocellulose column. Because of the unusually high lysine content of the P20 domain III\textsuperscript{Man} binds strongly to this resin and can thus be separated from practically all other cytoplasmic proteins.

5. COMPARISONS OF GLUCOSE AND MANNOSE PERMEASE WITH OTHER TRANSPORT PROTEINS

5.1. Glucose permease of \textit{E. coli} and \textit{S. typhimurium}

\textit{E. coli} and \textit{S. typhimurium} are closely related enteric bacteria. Comparison of the II\textsuperscript{Glc} subunits of both origin showed the following: Both proteins have the same electrophoretic mobility and within experimental errors the same amino acid composition. They, however behave differently during isoelectric focusing and chromatofocusing. II\textsuperscript{Glc} of \textit{E. coli} focuses around pH 9, II\textsuperscript{Glc} of \textit{S. typhimurium} at pH 6.5. Two monoclonal antibodies raised against II\textsuperscript{Glc} of \textit{E. coli} on an immuneblot nor neutralize \textit{E. coli} activity. Four other monoclonal antibodies and polyclonal sera cross-react with proteins of both origins. II\textsuperscript{Glc} and III\textsuperscript{Glc} subunits from both species function equally in all combinations [28].

5.2. Hexose permeases of the phosphotransferase system

Although the glucose- and the mannose permease appear to function similarly and have overlapping substrate specificity, their amino acid sequences are completely different. In particular the mannose permease is different from all PTS permeases which are known so far. In contrast, sequence similarities exist between the glucose permease and the permeases specific for N-acetyl glucosamine (II\textsuperscript{Nag} [54]), sucrose (II\textsuperscript{Scr} of \textit{Klebsiella pneumoniae} and \textit{Bacillus subtilis} [55,56]), and \(\beta\)-glucosides (II\textsuperscript{Bgl}) [57,58]). They are schematically summarized in Fig. 1. Most obvious is the strong homology between the III\textsuperscript{Glc} subunit of the glucose permease and the C-terminal 200 amino acids of II\textsuperscript{Bgl} and II\textsuperscript{Nag}. Also strongly similar are the amino acid sequences of the membrane domains of II\textsuperscript{Glc} and II\textsuperscript{Nag} on one hand, and of II\textsuperscript{Scr} and II\textsuperscript{Bgl} on the other hand. Present in all four proteins, either close to the C-terminus (II\textsuperscript{Glc} and II\textsuperscript{Nag}) or close to the N-terminus (II\textsuperscript{Scr} and II\textsuperscript{Bgl}) is the segment containing the functionally important Cys-421 of II\textsuperscript{Glc} [30].

A different kind of similarity can be detected in the segments linking the 200 C-terminal amino acids of II\textsuperscript{Bgl} and II\textsuperscript{Nag} corresponding to III\textsuperscript{Glc}, to their membrane domains that correspond to II\textsuperscript{Glc} of the glucose permease. They contain two Ala-Pro repeats and a Lys as the only conserved residues (Fig. 5). In view of the observations made with III\textsuperscript{Man} and other multidomain proteins (see 3.2.1.), it is likely that these Ala-Pro repeats, too, confer hinge properties. By combining the results of sequence comparison and biochemical analysis it can probably be generalized that all permeases of the PTS, whether they consist of one, two or three different protein subunits, always contain two phosphorylation sites, which can be localized either on different subunits or on two domains of a single polypeptide.

5.3. Comparison with eukaryotic transport proteins

Amino acid sequence similarities exist between the mammalian glucose carriers and the arabinose as well as the xylose transporters of \textit{E. coli} [59], and between the major drug resistance gene product from myeloma cells and the subunits from bacterial maltose as well as histidine transport systems [60–62]. No similarities between the permeases of the phosphotransferase system and eukaryotic proteins have been discovered so far.

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