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A Novel Membrane Anchor Function for the N-terminal Amphipathic Sequence of the Signal-transducing Protein $IIA^{Glucose}$ of the *Escherichia coli* Phosphotransferase System*

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Enzyme $IIA^{Glucose}$ (IIA^{Glc}) is a signal-transducing protein in the phosphotransferase system of *Escherichia coli*. Structural studies of free IIA^{Glc} and the HPr-IIA^{Glc} complex have shown that IIA^{Glc} comprises a globular β-sheet sandwich core (residues 19–168) and a disordered N-terminal tail (residues 1–18). Although the presence of the N-terminal tail is not required for IIA^{Glc} to accept a phosphorus from the histidine phosphocarrier protein HPr, its presence is essential for effective phosphotransfer from IIA^{Glc} to the membrane-bound $IIBC^{Glc}$. The sequence of the N-terminal tail suggests that it has the potential to form an amphipathic helix. Using CD, we demonstrate that a peptide, corresponding to the N-terminal 18 residues of IIA^{Glc}, adopts a helical conformation in the presence of either the anionic lipid phosphatidylglycerol or a mixture of anionic *E. coli* lipids phosphatidylglycerol (25%) and phosphatidylethanolamine (75%). The peptide, however, is in a random coil state in the presence of the zwitterionic lipid phosphatidylcholine, indicating that electrostatic interactions play a role in the binding of the lipid to the peptide. In addition, we show that intact IIA^{Glc} also interacts with anionic lipids, resulting in an increase in helicity, which can be directly attributed to the N-terminal segment. From these data we propose that IIA^{Glc} comprises two functional domains: a folded domain containing the active site and capable of weakly interacting with the peripheral IIB domain of the membrane protein $IIBC^{Glc}$; and the N-terminal tail, which interacts with the negatively charged *E. coli* membrane, thereby stabilizing the complex of IIA^{Glc} with $IIBC^{Glc}$. This stabilization is essential for the final step of the phosphoryl transfer cascade in the glucose transport pathway.

The signal-transducing protein $IIA^{Glucose}$ (IIA^{Glc})[1] is an integral component of the phosphoenolpyruvate:sugar phosphotransferase system (PTS) in *Escherichia coli.* Glucose transport via the PTS initiates from phosphoenolpyruvate, which autophosphorylates enzyme I. Enzyme I transfers the phosphoryl group to the histidine-containing phosphocarrier protein, HPr, which in turn donates a phosphoryl group to IIA^{Glc}. Subsequently, IIA^{Glc} transfers the phosphoryl group to the solvent-exposed IIB domain of the membrane protein (1). In addition to its role in the PTS, IIA^{Glc} also modulates the activity of a number of other proteins, depending on its phosphorylation state. Although dephosphorylated IIA^{Glc} is a negative regulator of glycerol kinase (2) and various non-PTS permeases (1), phosphorylated IIA^{Glc} is a positive regulator of adenylyl cyclase activity (3). Structural studies on phospho- and dephospho-IIA^{Glc} by both x-ray crystallography (4, 5) and NMR spectroscopy (6, 7) have shown that the protein is composed of a globular core (residues 19–168) comprising a predominantly β-sheet sandwich and an unstructured N terminus (residues 1–18), which is invisible in electron density maps and highly mobile in solution. Recently, we determined the solution structure of the intact IIA^{Glc}-HPr complex by multidimensional NMR (8), and the N-terminal 18 residues remain disordered. Further, chemical shift mapping has shown that the N-terminal 18 residues are unperturbed upon binding of IIA^{Glc} to the isolated IIB domain of $IIBC^{Glc}$ (9), and our current structural studies on the IIA^{Glc}-IIB complex indicate that the N-terminal segment of IIA^{Glc} remains unstructured and is not involved in this protein complex.[2] Biochemical studies, on the other hand, have shown that whereas the presence of an intact N-terminal segment of IIA^{Glc} is not necessary for the transfer of phosphorus from HPr to IIA^{Glc} it is absolutely required for effective phosphoryl donation from IIA^{Glc} to $IIBC^{Glc}$ (10). The structural and functional role of the N-terminal portion of IIA^{Glc}, however, has not been elucidated. In this communication, we show that intact IIA^{Glc} and a synthetic peptide corresponding to the first 18 N-terminal residues of IIA^{Glc} interact with anionic phospholipids found in the membrane of *E. coli*, promoting the formation of an amphipathic helix. Based on these data, we propose a two-state model for IIA^{Glc} and discuss the significance of our observations in the context of sugar transport and the PTS in *E. coli.*

MATERIALS AND METHODS

Enzyme IIA^{Glc} from *E. coli* was expressed and purified as described (11) and quantified by UV spectroscopy at 257 nm. The N-terminal 18-residue peptide of IIA^{Glc} was synthesized by solid phase methods with the C-terminal end amidated and purified by reverse phase high pressure liquid chromatography (Commonwealth Biotechnologies, Inc.). The peptide was greater than 99% pure as judged by mass spectrometry and amino acid compositional analysis. Dioleoylphosphatidylglycerol (PG), phosphatidylethanolamine (PE) purified from *E. coli* membranes, and dioleolylphosphatidylcholine (dioleolyl-PC) were purchased from Sigma and were ≥ 98% pure. Vesicles were made by sonication of lipids in 10 mM phosphate buffer, pH 7, as reported elsewhere (12).

CD spectra were collected at 22 °C on a Jasco J-720 spectropolarim-

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[1] The abbreviations used are: IIX^{Glc}, enzyme $IIX^{Glucose}$; PTS, phosphoenolpyruvate:sugar phosphotransferase system; PG, dioleoylphosphatidylglycerol; PE, phosphatidylethanolamine; dioleolyl-PC, dioleolylphosphatidylcholine; Pep18, 18-residue synthetic peptide comprising the N-terminal 18 residues of IIA^{Glc}.

[2] Unpublished data.

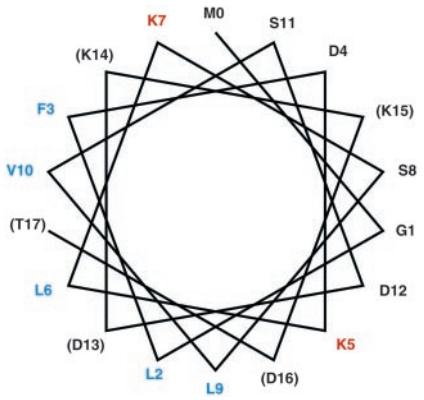

FIG. 1. **Helical wheel projection of the sequence corresponding to the N-terminal 18 residues of *E. coli* IIA^{Glc}.** In accordance with the nomenclature in the literature (1, 4–8), the N-terminal Met of IIA^{Glc} is numbered as zero. Hydrophobic residues are colored in *blue*, interfacial cationic residues in *red*, and hydrophilic residues in *black*. Residues Asp^{13}-Lys^{14}-Lys^{15}-Asp^{16}-Thr^{17} *in parentheses*, which do not fit the helical wheel projection, are proposed to be in the linker region between the two domains of IIA^{Glc} when associated with the membrane (see Fig. 3).

eter (calibrated using 0.06% *d*-(+)-10-camphorsulfonic acid at 290.5 nm) from 190 to 250 nm using a 2-mm path length cell, with a scan rate of 2 nm/min, a time constant of 0.5 s, a bandwidth of 1 nm, and a sensitivity of 20 millidegrees. Each spectrum is the average of 10 scans. After smoothing and background subtraction, the spectrum was expressed in molar ellipticity.

RESULTS AND DISCUSSION

The amino acid sequence of the N-terminal 18 residues of *E. coli* IIA^{Glc} is Met-Gly-*Leu*-*Phe*-Asp-Lys-*Leu*-Lys-Ser-*Leu*-*Val*-Ser-Asp-Asp-Lys-Lys-Asp-Thr. According to the convention adopted for *E. coli* IIA^{Glc} in the literature (1, 4–8), the N-terminal methionine, which can be hydrolyzed, is numbered as zero. An interesting feature of this sequence is the periodical occurrence of hydrophobic (italicized) and hydrophilic amino acid residues. Such periodicity, previously reported in human apolipoproteins (13, 14), is correlated with the potential to form an amphipathic helix. When the sequence is represented on a helical wheel projection (Fig. 1), it is apparent that there is a cluster of hydrophobic residues on one side of the wheel (Leu^2, Phe^3, Leu^6, Leu^9, and Val^{10}) and a cluster of hydrophilic residues on the other (Asp^4, Ser^8, Ser^{11}, and Asp^{12}). Located at the boundary of the hydrophobic and hydrophilic faces are two lysine residues (Lys^5 and Lys^7). Such features are reminiscent of the class A amphipathic helix in apolipoproteins (14). Because apolipoproteins bind lipids, we reasoned that the N-terminal sequence of IIA^{Glc} may interact with lipids in the *E. coli* membrane.

To test this hypothesis, we investigated the interaction of a synthetic peptide comprising the N-terminal 18 amino acid residues of IIA^{Glc} (referred to as Pep18) with a variety of phospholipid vesicles. Fig. 2*a* presents the CD spectra of Pep18 in the presence or absence of anionic lipids. In the absence of lipid (*green*), there is a strong negative band at 198–200 nm, indicative of a random coil (15), consistent with its disordered structure in intact IIA^{Glc} (4–8). In the presence of the anionic lipid PG (*red*), however, there is a dramatic change in the CD spectrum of Pep18, which displays double minima at ~208 and ~222 nm, characteristic of an α-helix (12, 16). The helicity was estimated to be ~50% based on 222-nm band analysis (17). In the context of the helical wheel projection shown in Fig. 1, this suggests that the helical segment comprises residues 2–10. In contrast, essentially no change was seen in the CD spectrum of Pep18 upon addition of the zwitterionic lipid dioleolyl-PC under the same conditions (data not shown). The observation that

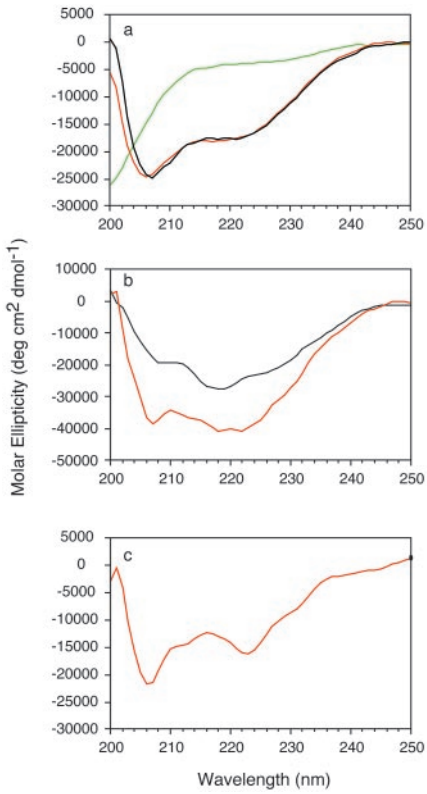

FIG. 2. CD spectra of (*a*) 52 μM Pep18 and (*b*) 13 μM IIA^{Glc} in the presence and absence of 2 mM PG, pH 7, and 22 °C are shown. The difference CD spectrum of IIA^{Glc} in the presence and absence of PG is shown in *c*. Pep18 is a synthetic peptide corresponding to the N-terminal 18 residues of IIA^{Glc} (see Fig. 1). In *a*, *green* and *red lines* represent the CD spectra of Pep18 in phosphate buffer alone and in the presence of the anionic lipid PG, respectively. The *black line* is the CD spectrum of Pep18 in a mixture of the anionic *E. coli* lipids PG (25%) and PE (75%) (w/w) (total lipid concentration, 2 mM). In *b*, the *black* and *red lines* are the spectra of IIA^{Glc} in the absence and presence of PG, respectively.

Pep18 interacts with an anionic but not zwitterionic lipid indicates that electrostatic interactions between the anionic lipid head groups and cationic lysine side chains of Pep18 play a significant role in α-helix stabilization (12, 18, 19). In this regard, it is noteworthy that the predominant lipids in the membrane of *E. coli* are anionic, comprising ~25% PG and ~75% PE (20). Indeed, Pep18 also forms an α-helix in a 1:3 PG:PE mixture (Fig. 2*a*, *black*) with near identical helicity to that observed with PG alone.

The interaction of intact IIA^{Glc} with PG was also investigated. The CD spectrum of IIA^{Glc} in phosphate buffer has a negative band at ~218 nm with shoulders at ~210 and ~224 nm (Fig. 2*b*, *black*), consistent with the known secondary structure of IIA^{Glc}, which comprises predominantly β-strands with a few short helices (4–8). In the presence of PG, the CD spectrum of IIA^{Glc} became more negative with double minima at ~208 and ~222 nm (Fig. 2*b*, *red*), indicating α-helix formation. The difference in the CD spectra of IIA^{Glc} in the presence and absence of PG (Fig. 2*c*) resembles the CD spectrum of Pep18 in the presence of PG (Fig. 2*a*, *red*) and can therefore be attributed to the conformational change of only the N-terminal segment of the protein. This interpretation is further supported by the observation that the migration of IIA^{Glc} in a nondenaturing gel is only slightly different in the presence or absence of PG, indicating that the overall shape of IIA^{Glc} remains essentially unaltered, and no global conformational change has taken place. Moreover, because the CD spectrum of IIA^{Glc} in the presence of PG is given by approximately the sum of the spectra of IIA^{Glc} without PG and Pep18 with PG, the amphipathic

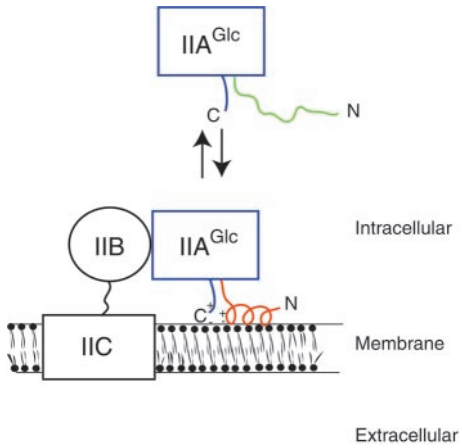

FIG. 3. **A two-state model for the functional role of the N-terminal domain of *E. coli* IIA^{Glc}.** The folded core domain of IIA^{Glc} (residues 19–168) is represented by the *blue box*. The disordered and helical states of the N-terminal segment (residues 1–18) of IIA^{Glc} are colored in *green* and *red*, respectively. Phosphoryl transfer involves formation of protein-protein complexes via contact between active sites (8, 26) as depicted here for IIA^{Glc}-IIBC^{Glc}. Attachment of IIA^{Glc} to the *E. coli* membrane surface stabilizes the complex and consequently enhances its ability to donate the phosphoryl group to the IIB domain (*circle*), which is also on the membrane surface and covalently linked to the transmembrane domain IIC (*rectangle*).

helical domain and the folded domain of IIA^{Glc} are independent of each other. Collectively, these data strongly suggest that the N-terminal segment of IIA^{Glc} is capable of binding to a negatively charged *E. coli* membrane surface.

The binding of the N-terminal segment of IIA^{Glc} to anionic phospholipids present in the membrane of *E. coli* suggests a two-state model for the structure of IIA^{Glc} (Fig. 3). In the cytosol, IIA^{Glc} is composed of the previously described globular core (residues 19–168, *blue rectangle*) and a disordered N terminus (residues 1–18, *green tail*) (4–8). In the second state, the N-terminal tail of IIA^{Glc} (residues 2–10) assumes a helical conformation (*red*) upon binding to the *E. coli* membrane via hydrophobic and electrostatic interactions, whereas the folded core domain interacts with the peripheral IIB domain of the membrane protein IIBC^{Glc}. Residues Asp^{13}-Lys^{14}-Lys^{15}-Asp^{16}-Thr^{17}-Gly^{18} act as a linker between the two domains in the membrane-associated form of IIA^{Glc}. The proposed model provides a biological role for the amphipathic helical domain of cytoplasmic IIA^{Glc}. We suggest that effective phosphoryl transfer from IIA^{Glc} to IIBC^{Glc} requires the formation of a stable complex between the two proteins; the combination of the interaction of the folded domain (residues 19–168) with the IIB domain of IIBC^{Glc} and the helical domain (residues 2–10) with the membrane achieve this stability.

It was previously shown that IIA^{Glc} could be cleaved at Lys^{7} by a membrane protease (21), consistent with the membrane association of the N-terminal domain of IIA^{Glc}. The clipped IIA^{Glc} (referred to as the "fast" form on account of its behavior during gel electrophoresis) has the same structured domain and ability to accept a phosphoryl group from HPr as does full-length IIA^{Glc} (known as the "slow" form) (6, 10, 21). However, the fast form of IIA^{Glc} is only 2–3% as active as the slow form in donating a phosphoryl group to the membrane protein IIBC^{Glc} (10). In light of the present data, we suggest that removal of the first seven N-terminal residues of IIA^{Glc} disrupts the amphipathic helix, making it a very poor membrane anchor.

Interestingly, IIA^{Glc} of *Mycoplasma capricolum* is cytoplasmic and contains a similar short N-terminal sequence, Met-*Trp-Phe-Phe*-Asn-Lys-Asn, which is rich in aromatic residues (italicized). Because aromatic residues can also play an important role in lipid binding (22), we propose that this segment of *M. capricolum* IIA^{Glc} has a similar membrane-anchoring role. Indeed, the N-terminal segment of *M. capricolum* IIA^{Glc} adopts an L-shaped structure (with the Trp sidechain disordered) in the crystal structure (23), which may be relevant for membrane binding as depicted in Fig. 3 for *E. coli* IIA^{Glc}. The difference in amino acid sequence, composition, and structure between the N-terminal membrane anchors of *E. coli* and *M. capricolum* IIA^{Glc} may reflect the lipid composition of the respective membranes. For example, cholesterol is one of the major lipid components of the membrane of *M. capricolum* (24).

Because the C terminus of IIA^{Glc} is adjacent to the N terminus with both ends being some 30 Å away from the active site (see Fig. 3 and Refs. 4, 5, and 8), the two positively charged residues at the C terminus (Lys^{167} and Lys^{168}) of IIA^{Glc} may also participate in electrostatic interactions with the negatively charged *E. coli* membrane, further stabilizing the membrane-bound state (Fig. 3). Indeed, C-terminal truncation or mutation of the C-terminal basic residue to an acidic residue in the β-glucoside permease from *E. coli* results in a 10-fold decrease in the catalytic rate of phosphoryl transfer (25).

The domain structure of the *E. coli* glucose transport system is IIA^{Glc} + IIBC^{Glc}, whereas that of some other sugar transport systems, for example that of mannitol, is IIABC, where all the domains are covalently linked and membrane-bound (1). Hence, those covalently linked IIAs are efficient phosphocarriers but are not available for other functions. The two states of IIA^{Glc} allow it to play multiple roles; it is a phosphocarrier in the PTS, as well as a regulator of a variety of other metabolic systems. The anchor function for the N-terminal helical domain defined here permits IIA^{Glc} to overcome the inherent defect in phosphotransfer capacity associated with its free-floating presence in the cytoplasm.

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