Journal of Cellular Biochemistry 51:75–82 (1993)

Three-Dimensional Structures of the Central Regulatory Proteins of the Bacterial Phosphotransferase System, HPr and Enzyme IIA^{glc}

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Abstract Enzyme IIA and HPr are central regulatory proteins of the bacterial phosphoenolpyruvate:sugar phosphotransferase (PTS) system. Three-dimensional structures of the glucose enzyme IIA domain (IIA^{glc}) and HPr of *Bacillus subtilis* and *Escherichia coli* have been studied by both X-ray crystallography and Nuclear Magnetic Resonance (NMR) Spectroscopy. Phosphorylation of HPr of *B. subtilis* and IIA^{glc} of *E. coli* have also been characterized by NMR spectroscopy. In addition, the binding interfaces of *B. subtilis* HPr and IIA^{glc} have been identified from backbone chemical shift changes. This paper reviews these recent advances in the understanding of the three-dimensional structures of HPr and IIA^{glc} and their interaction with each other. © 1993 Wiley-Liss, Inc.

Key words: IIA^{glc}, HPr, phosphotransferase system, 3D structures

Bacterial phosphoenolpyruvate:sugar phosphotransferase systems (PTS) mediate the concomitant transmembrane transport and phosphorylation of a number of simple carbohydrates. The PTS consists of two non-specific energy-coupling proteins, enzyme I and HPr, and a sugar-specific permease complex known as enzyme II [for reviews see Saier and Reizer, 1992; Meadow et al., 1990; Reizer et al., 1988; Saier, 1989]. The enzyme II complexes comprise three or four functional domains: two hydrophilic domains, enzyme IIA (IIA) and enzyme IIB (IIB), which both possess phosphorylation sites, and one or two hydrophobic transmembrane domains, IIC and IID. The permease complex may consist of one to four distinct polypeptide chains [Saier and Reizer, 1992; Geerse et al., 1989; Saier et al., 1988; Wu et al., 1990]. In the translocation and phosphorylation of glucose, a phosphoryl moiety is transferred sequentially from phosphoenolpyruvate (PEP) to enzyme I, HPr, glucose-specific enzyme IIA (IIA^{glc}), membrane-bound IIB (IIB^{glc}), and finally to the sugar. The IIC^{glc} domain forms the glucose-specific transmembrane channel [for a recent review, see Saier and Reizer, 1992].

Received September 11, 1992; accepted September 11, 1992.

IIA^{glc} and HPr are central regulatory proteins of the PTS. IIA^{glc} regulates not only the uptake and phosphorylation of glucose, but also the uptake and phosphorylation of other PTS and non-PTS sugars by transcriptional and post-transcriptional mechanisms; regulation is effected by phosphorylation of a histidine residue [Saier and Reizer, 1992; Meadow et al., 1990; Reizer et al., 1988; Saier, 1989]. HPr is a non-sugar specific protein which transfers a phosphoryl group from enzyme I to different sugar-specific IIA domains via a phospho-histidine HPr intermediate. HPrs in Gram-positive bacteria may also be phosphorylated at a serine residue by an ATP-dependent protein kinase. Phosphorylation of Ser-46 in the *Bacillus subtilis* system has an inhibitory effect on the activity of the protein and thus regulates the uptake and phosphorylation of the PTS sugars [for recent reviews, see Meadow et al., Reizer et al., 1988; Saier, 1989].

The solution structures of IIA^{glc} and HPr from both *Escherichia coli* and *B. subtilis* have recently been investigated by two- (2D) and three-dimensional (3D) heteronuclear NMR spectroscopy using ^{15}N labeled and/or $^{15}N/^{13}C$ doubly labeled protein samples [Pelton et al., 1991a,b; 1992; Fairbrother et al., 1991, 1992a,b; Stone et

al., 1992; Hammen et al., 1991; van Nuland et al., 1992]. HPr from *B. subtilis* and *E. coli* have also been studied using 2D homonuclear NMR methods [Wittekind, 1989, 1990; Klevit et al., 1986; Klevit and Drobny, 1986; Klevit and Waygood, 1986]. The conformation of all four proteins has been independently investigated using X-ray crystallography techniques [Worthylake et al., 1991; Kapadia et al., 1991; Herzberg et al., 1992; El-Kabbani et al., 1987]. Effects of phosphorylation of IIA^{glc} from *E. coli* and of Ser-46 of *B. subtilis* HPr have also been characterized by 3D heteronuclear NMR and 2D homonuclear NMR studies [Pelton et al., 1992; Wittekind et al., 1989], respectively. More recently, the binding interfaces of HPR and IIA^{glc} have been determined from mapping of chemical shift changes using ^{15}N-edited and ^{15}N-filtered NMR experiments [Chen et al., in press]. The purpose of this paper is to review these recent advances in our understanding of the 3D structures and dynamics of IIA^{glc} and HPr, and their interactions with each other.

THREE-DIMENSIONAL STRUCTURE AND DYNAMICS OF IIA^{glc}

The 3D structure of *B. subtilis* IIA^{glc} has recently been determined independently by both X-ray crystallography [Liao et al., 1991] and NMR [Fairbrother et al., 1991, 1992a,b]. Although we have been unable to make a direct comparison between the coordinates of the crystal structure, determined at 2.2 Å resolution, and the NMR solution structure, currently refined to an average RMS deviation of 0.9 Å for the backbone heavy atoms of residues 12–162, it is clear that the overall fold of the polypeptide chain obtained using the two methods is similar. A schematic illustration of the NMR solution structure of *B. subtilis* IIA^{glc} is given in Figure 1. The protein has an antiparallel β-barrel structure, with the main eight stranded β-sheet adopting a "hybrid" Greek-key/jellyroll topology. In addition to the β-sheet structures there are several long loops, the most prominent of which is found between residues 25–41 (on the right side of the protein in the view of Fig. 1) and was identified as being an Ω-loop in the crystal structure. Two other Ω-loops were also identified in the crystal structure, between residues 99–112 and residues 146–157. The X-ray crystal structure and NMR solution structure of *B. subtilis* IIA^{glc} do, however, exhibit minor differences. Four short regular helices have been identified in the crystal structure between residues 26–28 (3_{10}-helix), 32–35 (α-helix), 89–91 (3_{10}-helix), and 117–123 (α-helix + 3_{10}-helix). Three of these regions were identified as having irregular helical structures in solution, (33–36, 89–92, and 117–123). While $d_{NN}(i, i + 1)$ and $d_{\alpha N}(i, i + 3)$ NOE connectivities, which are characteristic of helical conformations, have been observed in solution for all four segments defined as regular helix in the crystal structure, the $d_{\alpha N}(i, i + 3)$ NOEs are relatively weak and the measured amide hydrogen exchange rates are fast, suggesting that the conformations are neither regular α-helices nor 3_{10}-helices. Additional support for the "helical regions" of *B. subtilis* IIA^{glc} being irregular in solution has come from analysis of the $^{13}C\alpha$ chemical shifts [Fairbrother et al., 1992]. A second difference between the two structures is in the N-terminal region of the protein. In the crystal structure, the N-terminal residues 4–12 form a unique hairpin loop conformation. NMR data, however, clearly shows these residues to be flexible in solution [Stone et al., 1992], and devoid of any specific conformation. These differences are relatively minor and may result from differences in the nature of the samples. In the NMR studies, the protein was dissolved in aqueous solution at pH 6.6, while in the X-ray studies, the protein was crystallized from an ammonium sulfate solution at pH 8.0. Crystal packing forces could also constrain the internal motions of a protein, resulting in an ordered conformation for regions that are disordered or more flexible in solution. In particular, the unique conformation observed for the N-terminal residues in the crystal structure is likely due to a crystallization artifact. The 13 N-terminal residues of the protein studied have been defined as part of the Q-linker which covalently connects the IIA^{glc} domain to the membrane-bound $IICB^{glc}$ domains. Q-linkers are typically found in proteins of prokaryotic two-component regulatory and signal-transduction systems [Weston and Dutton, 1989]. In the case of the *B. subtilis* glucose-permease, this flexible linker presumably allows the independent domains to interact during phosphoryl transfer.

Two conserved histidine residues (His-83 and His-68), which are involved in phosphoryl transfer activity [Reizer et al., 1992], are adjacent to each other in the 3D structure of IIA^{glc}. His-83, the site of phosphorylation by phospho-HPr, is located at the C-terminus of strand VI (using the numbering system of Fairbrother et al.,

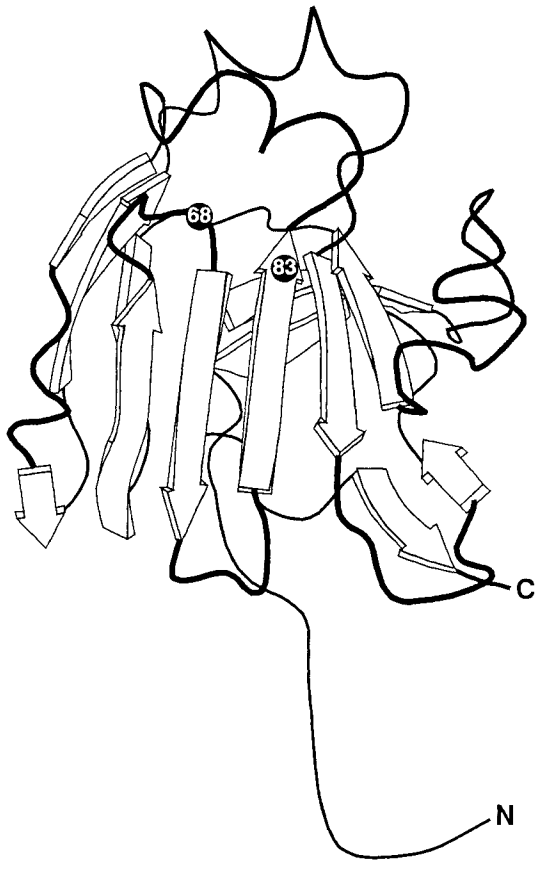

Fig. 1. A schematic illustration of the NMR solution structure of *B. subtilis* IIA^{glc}. The diagram was produced with a newly refined NMR structure and the view is similar to Figure 2, FEBS Lett 296:148–152, 1992.

1992) in the eight-stranded β-sheet (see Fig. 1). The active site histidines are surrounded by mainly hydrophobic residues, with the exception of two conserved aspartyl residues, Asp-31 and Asp-87, which are situated about 7 Å from His-83, at the edge of the hydrophobic patch. In the crystal structure [Liao et al., 1991], N-3 of His-83 is partially exposed to solvent, consistent with it being the site of phosphorylation, and is only 3.2 Å from N-3 of His-68. The N-1 atom of His-83 appears to be protonated and hydrogen bonded to the main chain carbonyl oxygen atom of Gly 85 (Fig. 2). The N-1 atom of His-68 also appears to be hydrogen bonded to the Oγ atom of the conserved residue, Thr-66. A number of other conserved residues have been found clustered around the active site, and possible structural and functional roles have been suggested for them by Liao et al. [1991].

In addition to the above structural studies, the backbone dynamics of *B. subtilis* IIA^{glc} have been characterized using ^{15}N NMR relaxation measurements [Stone et al., 1992]. Transverse and longitudinal relaxation time constants, T_1 and T_2, and steady-state [^1H]-^{15}N NOEs were measured for 137 (91%) of the 151 protonated backbone nitrogens. These relaxation data were analyzed by using the model-free dynamics approach of Lipari and Szabo [Lipari and Szabo, 1992a,b]. In this approach, two parameters describe the internal motion; a generalized order

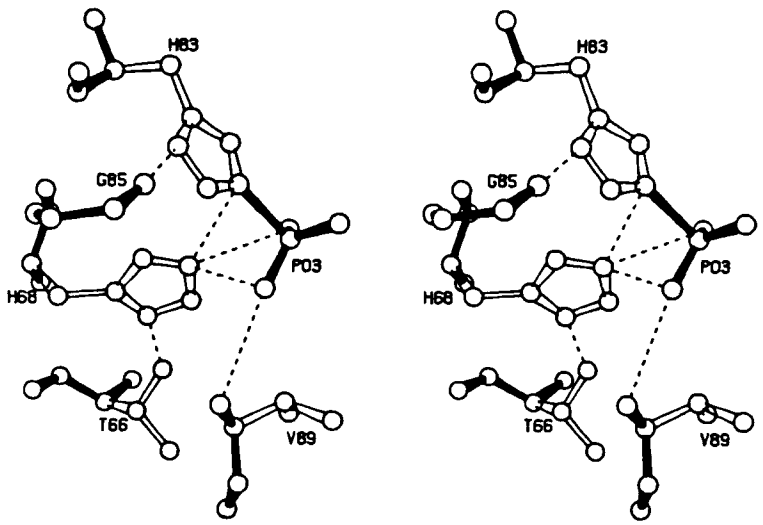

Fig. 2. Stereo diagram of the model of phosphorylated histidine of IIA^{glc}. Filled bars represent bonds between main-chain atoms, and open bars represent bonds between side chain atoms. In the model, two of the oxygen atoms of the negatively charged phosphoryl group interact with the N3 atom of His-68 and the main chain nitrogen of Val-89. (Reproduced Liao et al., 1991 with permission.)

parameter, S^2, and an effective internal correlation time, τ_e. The value of S^2 can range from zero, indicating completely isotropic internal motions, to 1 for internal motions that are completely restricted, relative to a fixed molecular frame of reference. Most of the residues in *B. subtilis* IIA^{glc} exhibit relatively restricted internal motions with order parameters in the range of 0.75–0.90 and effective correlation times, τ_e, less than ca. 25ps. These values are typical for well-defined globular proteins. Two areas of the molecule, however, were found to be significantly more flexible, as characterized by smaller S^2 values (< 0.5) and τ_e with time scales of approximately 100 ps; the N-terminal 12 residues, as discussed above, and the middle (around residue 32) of the Ω-loop which packs adjacent to the active site. In addition, residues 146–149 which are located in another loop region are more flexible than the average. As discussed above, flexibility of the N-terminal region was anticipated because the first 13 residues are part of the Q-linker which joins the IIA^{glc} domain to the membrane bound $IICB^{glc}$ domains. The high mobility of the Ω-loop comprising residues 25–41, together with its proximity to the active site, has led to the suggestion that it may form part of the binding surface for HPr and/or IIB^{glc}.

The 3D structure of *E. coli* IIA^{glc}, which shares approximately 42% sequence identity with the *B. subtilis* IIA^{glc} domain, has also been determined recently by X-ray crystallography at 2.1 Å resolution [Worthylake et al., 1991]. In addition, the structure has been investigated using 3D heteronuclear NMR spectroscopy [Pelton et al., 1991a,b], although a solution structure has not yet been reported. The X-ray crystal structure determination shows that *E. coli* IIA^{glc} is comprised primarily of antiparallel β-sheets forming a β-sandwich (or β-barrel) with six strands on either face. While this structure shares many common features with those determined for *B. subtilis* IIA^{glc}, there are several significant differences including the number, lengths and topology of the β-strands. The secondary structure of *E. coli* IIA^{glc} as determined by solution NMR studies is, however, in good agreement with that found in the X-ray crystal structure. The same is true of the X-ray crystal structure of *B. subtilis* IIA^{glc} relative to the NMR solution structure, suggesting that crystallization has not resulted in any major structural perturbations in either protein and that the observed structural differences between the two homologues is not artifactual. Despite the differences, the active site regions of the *E. coli* IIA^{glc} appears remarkably similar to that of the *B. subtilis* protein, with the two conserved histidines (His-75 and His-90 in *E. coli*) being found close to each other (the N-3 atoms are 3.3 Å apart), and surrounded by hydrophobic residues. The conserved aspartyl residues, Asp-38 and Asp-94 in *E. coli* IIA^{glc}, are also located on the edge of the hydrophobic region in analogous positions to Asp-31 and Asp-87 in *B. subtilis* IIA^{glc}. The N-3 atom of His-90 is exposed to solvent, while the N-1 atom of His-90 appears to be protonated and hydrogen bonded to the carbonyl oxygen of Gly 97. This is again consistent with the N-3 position of His-90 being the site of phosphorylation. The N-1 atom of His-75 appears to be hydrogen bonded to the Oγ atom of Thr-73. The position of the histidine rings are fixed by the hydrogen bonding and steric interactions. In contrast to the results found for *B. subtilis* IIA^{glc}, the N-terminal 18 residues of *E. coli* IIA^{glc} appear to be disordered in both the solution and crystal states. Note that *E. coli* IIA^{glc} has a seven residue N-terminal extension relative to the *B. subtilis* IIA^{glc} domain discussed above. The two techniques also indicate that the only helical structures present in *E. coli* IIA^{glc} are irregular or distorted.

Difference electron density maps between native *E. coli* IIA^{glc} and a chloroplatinate ($PtCl_4^{-2}$) heavy atom derivative revealed an anion binding site involving the N3 positions of both His-75 and His-90 [Liao et al., 1991]. This interaction does not cause large structural changes, implying that a phosphate moiety could form a monovalent bond with His-90, or a divalent transition state intermediate with both histidines, and cause minimal structural change. Similar conclusions were drawn from modeling the phosphorylated state of *B. subtilis* IIA^{glc}. In the model two of the oxygen atoms of the negatively charged phosphoryl group interact with the N3 atom of His-68 and the main chain nitrogen of Val-89 (see Fig. 2). The environment of the modeled phosphate appears very similar to the experimentally determined environment of the chloroplatinate anion in *E. coli* IIA^{glc}.

Mutation of His-75 to glutamine in *E. coli* IIA^{glc} [Presper et al., 1989] and His-68 to alanine in *B. subtilis* IIA^{glc} [Reizer et al., 1991] results in a protein that can accept but cannot transfer phosphoryl groups. In view of the current structural information, these observations can be interpreted in two ways. Either this histidine is

directly involved in the phosphoryl transfer, possibly with the phosphoryl group migrating from the catalytic histidine (i.e., His-90 in *E. coli* IIA^{glc} or His-83 and *B. subtilis* IIA^{glc}) to the adjacent histidine, as proposed by Presper et al. [1989], or it is required for interaction with IIB^{glc} but not with HPr. Liao et al. [1991] favor the latter and suggest that His-68 (or His-75 in *E. coli* IIA^{glc}) must be protonated for a productive IIA–IIB interaction to occur. Clearly further structural work is required to distinguish between these possibilities.

The phosphorylated state of the *E. coli* IIA^{glc} has been studied directly in solution using NMR techniques [Pelton et al., 1992; Dörschung et al., 1984]. Early 1D NMR work [Dörschung et al., 1984] observed ^{1}H chemical shift changes in the aromatic region of the spectrum upon phosphorylation; it was not possible, however, to interpret these changes in structural terms. More recently, with the use of 3D heteronuclear NMR techniques and ^{15}N or $^{15}N/^{13}C$ doubly labeled proteins, the phosphorylated form of *E. coli* IIA^{glc} has been characterized in detail [Pelton et al., 1992]. Upon phosphorylation, backbone chemical shift changes were observed only for residues within four segments; 87–100, for which the largest changes were seen, 36–46, 75–78, and 131–138. All four of these segments are in the vicinity of the phosphorylation active site. The chemical shifts of other residues in the protein were not observed to change significantly upon phosphorylation, indicating that IIA^{glc} does not undergo any large structural changes following phosphorylation of the catalytic histidine. The chemical shift changes which were observed could result from either local conformation changes or from differences in magnetic shielding due to phosphate binding. Details of the conformation in the region experiencing chemical shift perturbations were investigated by comparing NOE cross peak patterns of phospho-IIA^{glc} and free IIA^{glc}. Except for residues Ala-76, Asp-94, and Val-96, NOEs observed in the phosphorylated protein are almost identical to those observed in the unphosphorylated form, indicating that there are no large structural changes in the active site region as a consequence of phosphorylation. The differences in the NOE connectivities observed for the above residues are also minor and correspond to changes of less than 1.5 Å in interproton distances. One particularly interesting structural change, however, is that the amide proton of residue Asp-94 appears to move slightly towards the C-2 ring proton of His-90 and away from the amide proton of Thr-95. This observation suggests that the amide proton of Asp-94 may be involved in the interaction with the phosphate group. This is consistent with model building studies using the crystal structure of the *E. coli* IIA^{glc}, in which the amide protons of Asp-94 and Thr-95 help to stabilize the phosphate through interaction with the oxygen atoms of the phosphate group. Observation of characteristic backbone NOEs involving residues Ala-76, Asp-94, and Val-96 indicates that secondary structures at these three residues remain intact in the phosphorylated form of *E. coli* IIA^{glc}. The conclusions reached from this solution study support the modeling results, which were based entirely upon the crystal structures of the unphosphorylated forms of IIA^{glc}.

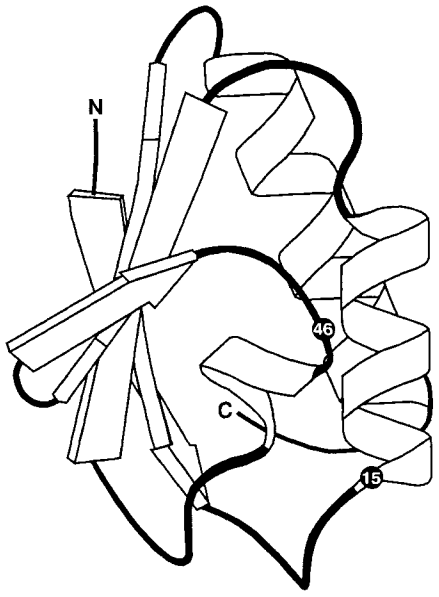

Fig. 3. Schematic diagram of the three-dimensional structure of *B. subtilis* HPr determined by X-ray crystallography at 2.0 Å resolution. Adapted from Herzberg et al., 1992.

THREE-DIMENSIONAL STRUCTURE OF HPr

The three-dimensional structure of *B. subtilis* HPr has been determined by X-ray crystallography at 2.0 Å resolution [Herzberg et al., 1992] and is illustrated schematically in Figure 3. Protein crystals were obtained of an inactive mutant, H15A, and of an active mutant, S83C. The latter mutant was made in order to prepare heavy-atom derivatives, a necessary step for solv-

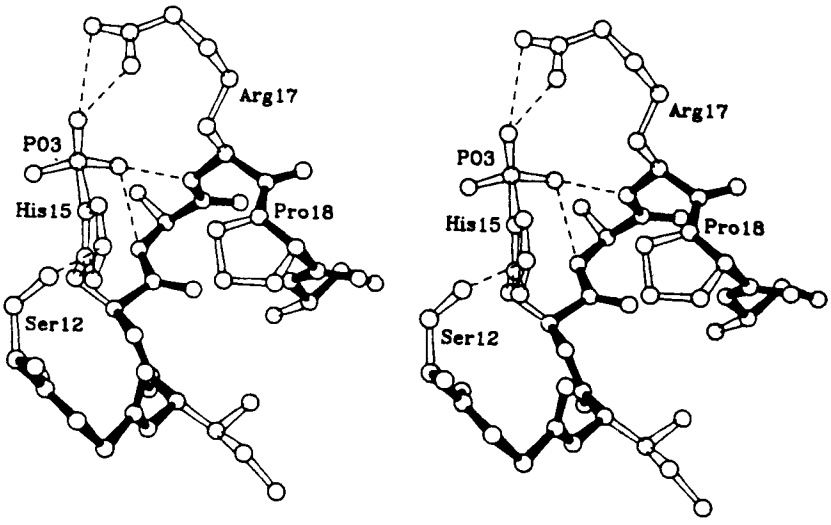

Fig. 4. A model of phospho-His-15 HPr. The bound phosphate moiety interacts directly with the side chain guanidinium group of Arg-17 and the main chain amide groups of Ala-16 and Arg-17. Reproduced from Herzberg et al., 1992.

ing the crystal structure. The conformation of *B. subtilis* HPr has also been studied by NMR [Wittekind et al., 1990], although a detailed tertiary structure has not yet been published. The three-dimensional structure of HPr derived from X-ray crystallography consists of a four stranded β-sheet with α-helices packed on top of the β-strands. This is a classical topology which has been described as an "open-face β-sandwich" [Richardson, 1981]. While the overall topology is similar to that described by NMR, there are some differences regarding identification of α-helices. The first α-helix was assigned to residues 16–26 in the crystal structure but was assigned to residues 19–29 in NMR studies. The second short α-helix defined by X-ray crystallography as residues 47–51 has not been identified in the NMR studies of *B. subtilis* HPr, although an analogous helix has been identified in NMR studies of *E. coli* HPr. A putative one-turn α-helix was, however, identified between residues 50–53 in the NMR studies of *B. subtilis* HPr. The third α-helix spanning residues 70 and 84 was assigned both by NMR and X-ray crystallography.

In the crystal structure [Herzberg et al., 1992], His-15, the residue with the phosphoryl transfer function, and Ser-46, the "regulatory" phosphorylation site, are both located on the surface of the protein with their side chains capping the N-termini of the first and second α-helices, respectively. The N-1 atom of His-15, the target of phosphorylation, was modeled so as to point towards Arg-17, another conserved residue. In the chosen model the N-1 atom of His-15 is also close to the amides of both Ala-16 and Arg-17, consistent with it being deprotonated as required for a phosphoryl acceptor. The N-3 atom then forms a hydrogen bond to the Oγ of Ser-12. Interaction between a sulfate anion and the guanidinium group of Arg-17 has been observed in the crystal structure. It was proposed that crystal packing may have trapped the Arg-17 side chain in a conformation that resembles the phosphorylated state of HPr, since in the absence of sulfate the electrostatic interactions between Arg-17 and the N-terminus of the α-helix would be repulsive. A model of phospho-His-15 HPr was built in which the bound phosphate moiety interacts directly with the side chain guanidinium group of Arg-17 and the main chain amide groups of Ala-16 and Arg-17 (Fig. 4). In this configuration the negatively charged phosphoryl group may also be stabilized by the N-terminal dipole of the first α-helix. Three hydrophobic residues were noted to be close to the active site on the surface of the protein between His-15 and Ser-46; it was suggested that these residues, Ile-47, Met-48, and Met-51, may play an important role in interactions between HPr and enzyme I and/or IIA^{glc}. The side chain of Ser-46 also caps the N-terminus of an α-helix with its side chain Oγ atom hydrogen bonded to the main chain amide nitrogen of Gly-49. As with phospho-His-15, the negative

charge of the phosphorylated serine could be stabilized by the helix dipole.

The secondary structure and folding topology of the *E. coli* HPr determined by NMR methods is very similar to that determined by X-ray crystallography for *B. subtilis* HPr [Hammen et al., 1991; van Nuland et al., 1992; Klevit et al., 1986; Klevit and Drobny, 1986; Klevit and Waygood, 1986]; three α-helices pack against an anti-parallel β-sheet made of four strands. The *E. coli* HPr has about 33% sequence identity to the *B. subtilis* HPr. High resolution NMR structures, however, have yet to be published for either protein. Unlike *B. subtilis* HPr, the published crystal structure of *E. coli* HPr [El-Kabbani et al., 1987] is substantially different from the NMR structure. Epitope mapping studies, however, suggest that the NMR derived topology is the physiological relevant one [Sharma et al., 1991]. It is possible that structural changes in the crystal form occurred as a result of the crystallization process.

Phosphorylation of the regulatory Ser-46 of *B. subtilis* HPr has been investigated by two-dimensional homonuclear NMR spectroscopy [Wittekind et al., 1989]. Upon phosphorylation a number of main chain chemical shift changes were observed for residues both close to and remote from Ser-46 in the three-dimensional structure. These observations indicate that phosphorylation of Ser-46 may result in conformational changes in regions of the protein which are distant from the site of phosphorylation. Although the main chain chemical shifts of proteins in general are extremely sensitive to conformational perturbations they cannot be interpreted in terms of magnitude or type of conformational change. Full characterization of the conformational changes accompanying phosphorylation of HPr will require detailed comparisons between NOESY spectra of both the phosphorylated and unphosphorylated forms, as has been carried out for *E. coli* IIA^{glc} (see above); the results of such studies have not yet been published. Whatever the nature of the conformational change, it is apparent that it results in a form of the protein which cannot form an active complex with IIA^{glc}. NMR spectra of the inactive S46D mutant were also acquired and were found to be very similar to the phospho-Ser-46 form of HPr, indicating that the inactivation of the mutant results from the same structural changes that occur upon phosphorylation of Ser-46.

INTERACTION BETWEEN HPr AND IIA^{glc}

The interaction of *B. subtilis* HPr with IIA^{glc} has been characterized directly by ^{15}N-edited and ^{15}N-filtered NMR spectroscopy [Chen et al., submitted]. Uniformly ^{15}N-labeled IIA^{glc} and unlabeled HPr were used in these studies. Regions of the polypeptide backbone of both proteins which undergo significant chemical shift changes upon formation of the complex have been identified. Significant chemical shift changes were observed in several regions for IIA^{glc}, using ^{15}N-edited experiments; these include a segment (residues 33–40) of the Ω-loop whose apex is found close to the active site, several residues from the eight-stranded β-sheet (61–64, 70–72, 79–81, and 132–134), and residues 87 and 89 which are also found in a loop. The backbone amide ^{15}N and ^{1}H chemical shifts of the active site histidine residues 83 and 68, and the remainder of the protein, are essentially unchanged. All of the residues for which chemical shift changes are observed on binding of HPr are located in the vicinity of the phosphorylation active site, and for the most part form a continuous surface.

Chemical shift changes induced in HPr upon forming a complex with IIA^{glc} were also identified, using ^{15}N-filtered NMR experiments. Significant backbone chemical shift perturbations in HPr are observed for residues 13 to 23, which includes the active site His-15 and a short segment of α-helix. Significant chemical shift changes were also observed for residues 51–56, which form part of a loop between an α-helix and a strand of the β-sheet for residues 46 and 47 which contain the site of phosphorylation, and for a few residues in the middle of the β-sheet (residues 33 and 41–44). These residues form a continuous surface, including the active site and an area immediately adjacent to the active site.

It is notable that perturbed residues in both proteins are in regions that are predominantly hydrophobic in nature. Since the areas of observed chemical shift changes in both HPr and IIA^{glc} are in the vicinity of the known active sites and appear to be complimentary to each other, it is highly probable that they indicate residues involved in the binding interface. Further characterization of the HPr/IIA^{glc} complex must await detailed analysis of NOESY spectra and identification of specific intermolecular NOE connectivities, for both phosphorylated and unphosphorylated forms. Until then, the current

results provide a starting point for modeling of the IIA^{glc}-HPr interaction.

Interaction between HPr and IIA^{glc} is necessary for the transfer of a phosphoryl group between the two proteins. NMR provides direct evidence pertaining to the binding interfaces between the two proteins through observation of chemical shift changes among predominantly hydrophobic residues surrounding the active site histidines. On the basis of crystal structures, Herzberg et al. [1992] have proposed a mechanism for phosphoryl group transfer in which the proteins first interact by way of complimentary surfaces near the active sites. A pentacoordinate intermediate or transition state is then formed in which N-1 of His-15 in HPr and N-3 of His-83 in IIA^{glc}, respectively, occupy the apical positions of a trigonal bipyramidal phosphorus moiety. Phosphoryl transfer is accompanied by inversion of configuration at the phosphorus. Asp-31 and Asp-87 of IIA^{glc} and Arg-17 of HPr, all of which are near the active site histidines, may facilitate phosphoryl transfer by switching of the postulated salt bridge between Arg-17 and the phosphoryl group on HPr to a salt bridge between Arg-17 and the two aspartates of IIA^{glc}.

NOTE ADDED IN PROOF

Since acceptance of the present paper, the three-dimensional NMR structure of *B. subtilis* HPr has been published (Wittekind et al., Protein Science 1:1363–1376, 1992).

ACKNOWLEDGMENTS

This work was supported by grant number GM36643 from the National Institutes of Health.

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