Mapping of the Binding Interfaces of the Proteins of the Bacterial Phosphotransferase System, HPr and IIA^{glc} †

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ABSTRACT: Enzyme IIA^{glc} and HPr are central regulatory and phosphocarrier proteins of the phosphoenolpyruvate:sugar phosphotransferase system (PTS) of bacteria. During phosphoryl transfer from phosphoenolpyruvate to glucose, phosphate is transferred from HPr to enzyme IIA^{glc}. In order to characterize the binding interfaces of the two proteins during phosphate transfer, ^{15}N-edited and ^{15}N-filtered NMR experiments have been recorded for the complex of enzyme IIA^{glc} and HPr from *Bacillus subtilis*. Uniformly ^{15}N-labeled enzyme IIA^{glc} and nonlabeled HPr were used in these studies. Residues which undergo significant chemical shift changes upon complex formation have been identified for both proteins. The binding interfaces of the two proteins, suggested by the observed chemical shift changes, involve predominantly hydrophobic surfaces near the active site His-15 of HPr and the phosphoryl acceptor His-83 of IIA^{glc}.

The phosphoenolpyruvate:sugar phosphotransferase system (PTS)[1] of *Bacillus subtilis* transports sugars into the cell concomitantly with their phosphorylation. The PTS contains two nonsugar-specific energy-coupling proteins, enzyme I and HPr. The PTS also contains sugar-specific permease complexes known as enzyme II complexes [for recent reviews, see Saier and Reizer (1992), Meadow et al. (1990), Reizer et al (1988), and Saier (1989)]. The enzyme II complexes contain three functional domains: two hydrophilic domains (IIA and IIB) and one transmembrane domain (IIC). These three domains may be present within one, two, or three distinct polypeptide chains (Saier & Reizer, 1992; Geerse et al., 1989; Saier et al., 1988; Wu et al., 1990). In the PTS, a phosphoryl group is sequentially transferred from phosphoenolpyruvate (PEP) first to enzyme I, second to HPr, third to enzyme IIA, fourth to enzyme IIB, and finally to a sugar (Saier & Reizer, 1992).

The hydrophilic permease domain IIA contains the first phosphorylation site of the enzyme II complex (Saier & Reizer, 1992, and references therein). The glucose-specific IIA domain (IIA^{glc}) not only functions in the uptake and phosphorylation of glucose but also regulates the uptake of other sugars (Osumi & Saier, 1982; Nelson et al., 1983; Saier et al., 1983). *B. subtilis* IIA^{glc} consists of 162 amino acid residues covalently linked to the C-terminus of the membrane-bound IICB^{glc} domain (Sutrina et al., 1990). From biochemical and site-directed mutagenesis studies it has been found that His-83 is the site of phosphorylation by phospho-HPr. In addition, substitution of His-68 by site-directed mutagenesis produces a protein which is capable of accepting but not transferring phosphate to IICB^{glc} (Reizer et al., 1992). The three-dimensional structure of the *B. subtilis* IIA^{glc}, determined by both X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy, consists predominantly of 13 β-strands forming an antiparallel β-barrel with three β-sheets. The main eight-stranded β-sheet has a hybrid "Greek key"-"jelly roll" topology (Liao et al., 1991; Fairbrother et al., 1991, 1992a,b). The structure of the *Escherichia coli* IIA^{glc} has also been studied by X-ray crystallography and NMR and is found to be similar to that of the *B. subtilus* (Worthylake et al., 1991; Pelton et al., 1991a,b).

The phosphocarrier protein HPr is a nonsugar-specific protein that phosphorylates many PTS sugar permeases. *B. subtilis* HPr consists of a single chain of 88 amino acids (Reizer et al., 1988; Reizer, 1989). The three-dimensional structure of *B. subtilis* HPr has been determined by X-ray crystallography (Herzberg et al., 1992) and is similar to the structure suggested from NMR studies (Wittekind et al., 1990). The protein consists of four antiparallel β-strands with three α-helices superimposed onto the strands. It has been shown that phosphorylation of HPr by enzyme I occurs at His-15 which is located on the surface of the molecule (Reizer et al., 1988, and references therein; Herzberg et al., 1992). NMR studies show that the structure of the *E. coli* HPr is similar to that of the *B. subtilis* HPr, although an X-ray crystal structure of the *E. coli* protein (El-Kabbani et al., 1987) is quite different from the NMR structure (Klevit et al., 1986; Klevit & Drobny, 1986; Klevit & Waygood, 1986; Hammen et al., 1991; van Nuland et al., 1992). Recent results suggest that the NMR structure of the *E. coli* HPr is the relevant solution structure (Sharma et al., 1991).

Even though the three-dimensional structures of HPr and IIA^{glc} have been solved, the mechanism by which the phosphoryl group is transferred between these two proteins is not understood. It appears that complex formation between HPr and IIA^{glc} is independent of phosphorylation (Reizer et al., 1992). In order to characterize the regions of the two molecules involved at the binding interface during phosphate transfer, ^{15}N-edited (Bax et al., 1990; Norwood et al., 1990) and ^{15}N-filtered NMR experiments (Otting & Wüthrich, 1990, and references therein; Ikura & Bax, 1992; Gemmecker

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[1] Abbreviations: IIA^{glc}, glucose enzyme IIA domain; PTS, bacterial phosphotransferase system; NMR, nuclear magnetic resonance; 2D, two dimensional; 3D, three dimensional; TPPI, time-proportional phase incrementation; FID, free induction decay; TOCSY, total correlation spectroscopy; HSQC, heteronuclear single-quantum correlation spectroscopy.

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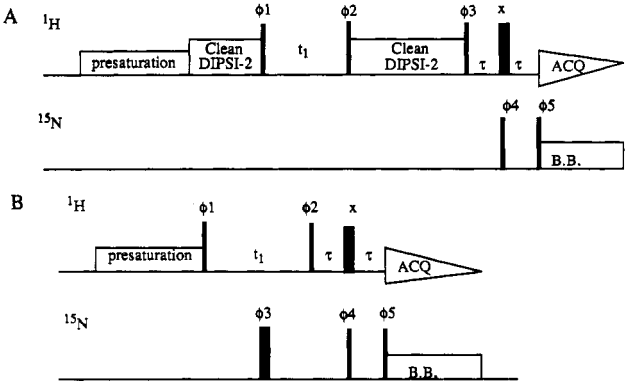

FIGURE 1: Pulse schemes of (A) ω_2-half-filtered pre-TOCSY TOCSY and (B) ω_2-half-filtered COSY. In the diagrams, wide vertical bars represent 180° pulses and narrow bars represent 90° pulses. The phase cycling is as follows: (A) $\phi_1 = x, -x, -x, x, y, -y, -y, y, -x, x, x, -x, -y, y, y, -y$; $\phi_2 = x, x, -x, -x, y, y, -y, -y, -x, -x, x, x, -y, -y, y, y$; $\phi_3 = 4(x), 4(y), 4(-x), 4(-y)$; $\phi_4 = 16(x), 16(-x)$; $\phi_5 = 32(x), 32(-x)$; receiver = $2(x, -x), 2(-y, y), 2(-x, x), 2(y, -y)$; (B) $\phi_1 = x, x, y, y, -x, -x, -y, -y$; $\phi_2 = x, -x, y, -y, -x, x, -y, y$; $\phi_3 = x$; $\phi_4 = 8(x), 8(-x)$; $\phi_5 = 16(x), 16(-x)$; receiver = $x, x, -y, -y, -x, -x, y, y$. The delay τ was set to 5.3 ms. Quadrature detection in t_1 was achieved using the TPPI method by changing ϕ_1 in both experiments. Broad-band decoupling (B.B.) was achieved by GARP-1 phase modulation.

et al., 1992) have been recorded with uniformly ^{15}N-labeled IIA^{glc} and nonlabeled HPr. Resonances of both proteins that undergo chemical shift changes upon complex formation have been identified. These resonances are localized to well-defined surfaces of the proteins and thus allow identification of the binding interface.

MATERIALS AND METHODS

Sample Preparation. Overproduction of both *B. subtilis* IIA^{glc} and HPr in *E. coli*, purification, and ^{15}N-labeling of IIA^{glc} was as described previously (Reizer et al., 1989, 1992; Fairbrother et al., 1991). For ^{15}N-edited experiments, excess HPr (approximately 1.8:1) was added in order to saturate IIA^{glc}. The NMR sample contained 10 mM potassium phosphate, pH 6.7, in 90% H_2O/10% D_2O with the IIA^{glc} concentration at approximately 0.5 mM. A sample of free IIA^{glc} of approximately 0.8 mM concentration in 10 mM potassium phosphate, pH 6.7 (90% H_2O/10% D_2O), was also prepared. For ^{15}N-filtered experiments, the sample consisted of an approximate 1:1.6 ratio of HPr and IIA^{glc}. The sample contained 10 mM potassium phosphate, pH 6.7, in 90% H_2O/10% D_2O with IIA^{glc} and HPr concentrations at approximately 1.4 and 2.2 mM, respectively. A sample of free HPr [approximately 1 mM in 10 mM potassium phosphate, pH 6.7 (90% H_2O/10% D_2O)] was also prepared.

NMR Experiments. NMR spectra were recorded on Bruker AMX-500 and AMX-600 spectrometers, equipped for multichannel operation. All experiments were run at 308 K. Quadrature detection was used in the acquisition dimension, and time-proportional phase incrementation (TPPI) (Redfield & Kunz, 1975; Bodenhausen et al., 1980; Marion & Wüthrich, 1983) was used in t_1 to obtain sign discrimination.

Two-dimensional ^{1}H–^{15}N HSQC and HSQC-TOCSY spectra of the IIA^{glc}–HPr complex were recorded using published pulse sequences (Bax et al., 1990; Norwood et al., 1990). ^{15}N decoupling during acquisition was achieved by GARP-1 phase modulation (Shaka et al., 1985). The HSQC-TOCSY spectrum was acquired using the sensitivity-enhanced method which gives approximately $2^{1/2}$ higher sensitivity relative to the conventional method (Cavanagh et al., 1991). In the HSQC-TOCSY experiment, a DIPSI-2 spin lock period (Shaka et al., 1988) of 40 ms was used for isotropic mixing, and a 2-ms homospoil pulse followed by a 7-ms delay was applied in order to reduce artifacts. Suppression of the water

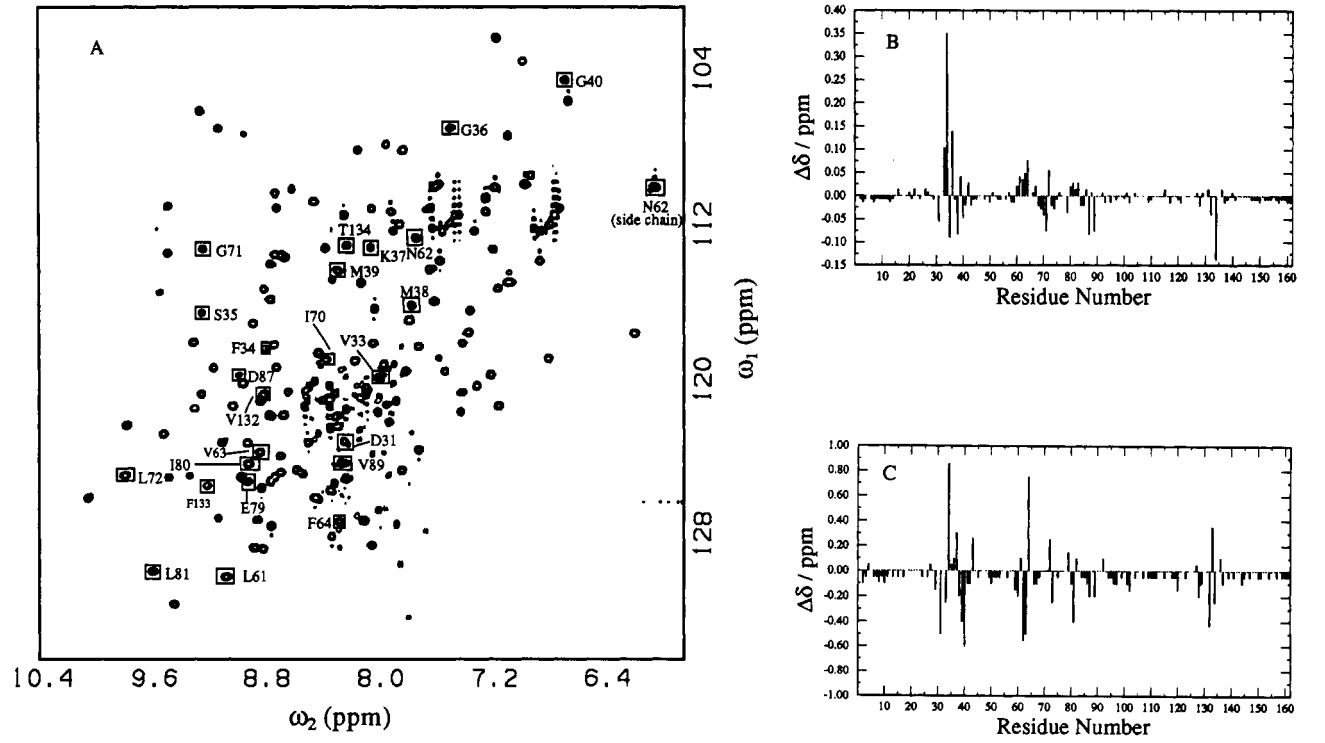

FIGURE 2: (A) HSQC spectra of the complex of HPr and IIA^{glc} at 308 K and pH 6.7. The sample of the IIA^{glc} and HPr complex contained approximately 0.5 mM IIA^{glc} and 0.9 mM HPr. The sample of the free IIA^{glc} contained 0.8 mM protein. Both samples were dissolved in 90% H_2O/10% D_2O with 10 mM potassium phosphate. Boxed cross peaks in (A) correspond to residues whose amide ^{15}N or ^{1}H chemical shifts changed significantly (>0.04 ppm in the ^{1}H dimension or >0.2 ppm in the ^{15}N dimension) when forming the complex with HPr. (B and C) Plots of chemical shift differences, $\Delta\delta$ (ppm), versus residue number of the amide ^{1}H (B) and ^{15}N (C) between the free IIA^{glc} and IIA^{glc} in the complex with HPr.

signal was carried out by low-power presaturation. With the ^1H carrier placed on the H_2O signal, a spectral width of 25 ppm was used in the ω_2 dimension to improve the dynamic range and the baseline (Delsuc & Lallemand, 1986). A total of 4096 complex points in t_2 with 256 t_1 increments were acquired. When the ^1H carrier was placed in the middle of the amide proton signals, a 7.5 ppm spectral width in the t_2 dimension was used, and 1024 complex data points with 256 t_1 increments were recorded. The ^{15}N carrier was placed in the middle of the amide ^{15}N signals, and the spectral width in the ^{15}N dimension was 37 ppm. Proton chemical shifts were referenced to internal dioxane; amide ^{15}N chemical shifts were indirectly referenced to liquid NH_3 by using the ^1H frequency of the H_2O signal (Bax & Subramanian, 1986; Live et al., 1984). An HSQC spectrum of free IIA^{glc} was also recorded for comparison.

The ω_2-^{15}N-half-filtered COSY and TOCSY spectra of the IIA^{glc}-HPr complex were recorded using the pulse sequences shown in Figure 1. The sequences combine the same isotope filtering scheme described recently by Ikura and Bax (1992) with COSY (Aue et al., 1976; Bax & Freeman, 1981; Nagayama et al., 1980) and TOCSY (Cavanagh & Rance, 1992) sequences. A clean DIPSI-2 (Cavanagh & Rance, 1992) spin lock period of 35 ms was used in the ^{15}N-half-filtered TOCSY spectrum. Half-filtered COSY and TOCSY spectra were recorded with 4096 complex data points in t_2 and 1024 and 512 t_1 increments, respectively. Spectral widths of 25 ppm in t_2 and 12.5 ppm in t_1 were used. Water suppression was achieved by low-power presaturation. In the half-filtered TOCSY experiment, a 15-ms DIPSI-2 pre-TOCSY period was used in order to recover CαH signals close to or underneath the water signal (Otting & Wüthrich, 1987). A pre-TOCSY TOCSY spectrum of free HPr was also recorded for comparison.

All spectra were processed on a Silicon Graphics IRIS 4D/25 work station using a modified version of the FTNMR software (Hare Research, Inc.). Skewed and phase-shifted sine-bell weighting functions or a cosine function followed by a Lorentzian-to-Gaussian transformation was applied before Fourier transformation. A simple spline baseline correction was applied to the ω_2 dimension after Fourier transformation.

RESULTS

Mapping the Chemical Shift Changes for IIA^{glc}. Figure 2A shows the HSQC (Bax et al., 1990; Norwood et al., 1990) spectrum of the IIA^{glc}–HPr complex. In this spectrum, the cross peaks correspond to the amide ^{15}N and amide proton chemical shifts of each amino acid in the IIA^{glc} sequence. The amide ^{15}N or ^1H chemical shifts of some residues in IIA^{glc} are perturbed by complex formation, while those of the majority of the residues are not significantly affected. The boxed cross peaks in Figure 2A correspond to residues for which the amide ^{15}N or ^1H chemical shift changes are significantly greater than the average chemical shift changes (0.018 or 0.088 ppm in the ^1H or ^{15}N dimensions, respectively) upon formation of the complex with HPr. The assignments of shifted cross peaks other than T134 and E79 were obtained by following resonance shifts (in fast exchange) in the HSQC spectra during titration of IIA^{glc} with increasing amounts of HPr. The cross peaks of T134 and E79 overlap with other resonances in the HSQC spectrum of free IIA^{glc}; assignments of these two residues were therefore obtained from an HSQC-TOCSY spectrum.

The changes in amide ^{15}N and ^1H chemical shifts for IIA^{glc} upon complexation with HPr are plotted in panels B and C of Figure 2, respectively. The location of residues undergoing

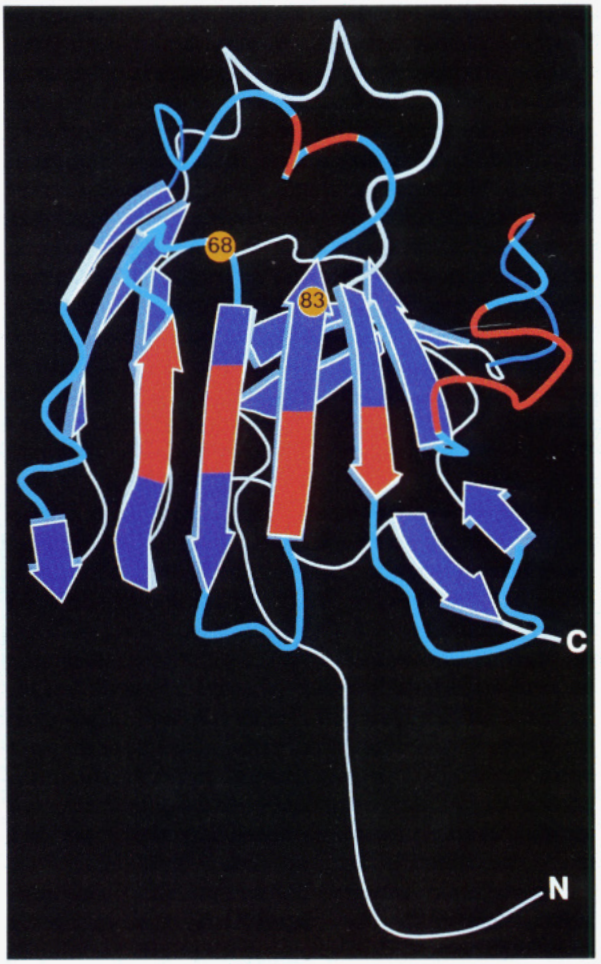

FIGURE 3: Summary of the chemical shift changes observed for IIA^{glc}. Residues undergoing significant chemical shift changes (>0.04 ppm in the ^1H dimension or >0.2 ppm in the ^{15}N dimension) are indicated in red in the three-dimensional structure of IIA^{glc}. The diagram was produced by modifying a drawing created using the program MOLSCRIPT (Kraulis, 1991).

chemical shift changes significantly greater than average is indicated in the three-dimensional structure of IIA^{glc} shown in Figure 3. The red region corresponds to residues for which chemical shift changes greater than 0.04 or 0.2 ppm in the ^1H or ^{15}N dimensions, respectively, have been observed. These regions include a segment (residues 31–40) of the loop whose apex is close to the active site, a few segments of β-strands (residues 132–134, 79–81, 61–64, and 70–72) forming the eight-stranded β-sheet, and residues 87 and 89, also found on a loop (Liao et al., 1991; Fairbrother et al. 1992a). As illustrated in Figure 3, these regions are located in the vicinity of the site of phosphorylation, His-83, as well as the catalytic His-68. The backbone amide ^{15}N and proton chemical shifts of the active site residues 83 and 68 are, however, essentially unchanged.

Mapping the Chemical Shift Changes for HPr. Figure 4A shows the fingerprint region of the ω_2-^{15}N-half-filtered TOCSY spectrum of the IIA^{glc}–HPr complex. The cross peaks correspond to CαH–NH connectivities for individual amino acids of HPr, although the CβH to NH cross peaks of Thr and Ser are also observed in this region. The resonances of the ^{15}N-labeled IIA^{glc} are eliminated by the ^{15}N-half-filter. CαH resonances beneath the solvent water resonance have been recovered by using a pre-TOCSY (Otting & Wüthrich, 1987) sequence. Boxed cross peaks correspond to amino acid residues of HPr for which chemical shift changes of CαH or NH resonances significantly greater than the average chemical

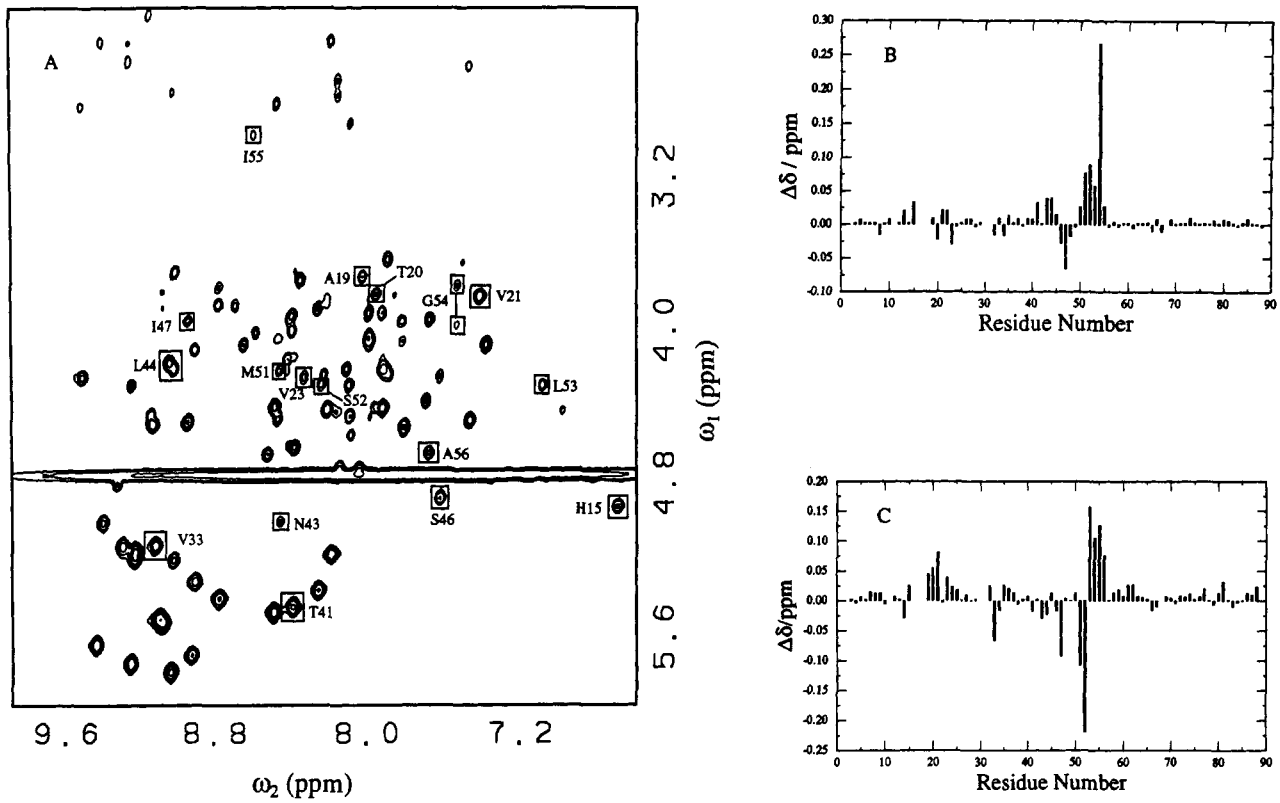

FIGURE 4: (A) Fingerprint regions of the ω_2-^{15}N-half-filtered pre-TOCSY TOCSY spectrum of the IIA^{glc}–HPr complex. Boxed cross peaks in (A) correspond to residues for which CαH or NH chemical shift changes greater than 0.027 or 0.04 ppm in the CαH or NH dimension, respectively, were observed. (B and C) Plots of chemical shift changes, $\Delta\delta$ (ppm), of CαH (B) and NH (C) versus residue number after HPr forms a complex with IIA^{glc}.

shift changes (0.016 or 0.024 ppm in the CαH or NH dimensions, respectively) are observed upon complex formation with IIA^{glc}. Assignments of resonances undergoing chemical shift changes, with the exception of S52, were made on the basis of connectivities observed to side-chain protons (Wittekind et al., 1990) in the ω_2-^{15}N-half-filtered TOCSY spectrum. The CβH resonances of S52 were not observed in the ω_2-^{15}N-half-filtered TOCSY spectrum. S52 was assigned for the free HPr according to Wittekind et al. (1990), and the shift of the NH–CαH cross peak upon complexation was followed during titration of HPr with IIA^{glc}. Also, the assignments of all neighboring cross peaks, which might potentially be confused with S52, could be made unambiguously from connectivities observed in the ^{15}N-filtered TOCSY spectrum of the complex.

The changes in CαH and NH chemical shifts of HPr upon complex formation with IIA^{glc} are shown in panels B and C of Figure 4, respectively. Residues undergoing chemical shift perturbation cluster on the surface in the vicinity of the active site (indicated in red in Figure 5). Significant chemical shift perturbations (>0.027 or 0.04 ppm in the CαH or NH dimensions, respectively) are observed for several residues in a region of the protein spanned by residues 15–23, which comprises the active site (His-15) followed by a short segment of α-helix. Significant chemical shift changes have also been observed for two neighboring regions spanned by residues 51–56, which form a loop connecting an α-helix and a strand of the β-sheet, and residues 46–47, which contain the site of phosphorylation by ATP-dependent kinase (residue 46). A few residues in the middle of the β-sheets (residues 33 and 41–44) (Herzberg et al., 1992; Wittekind et al., 1990) are also affected by complex formation. These regions are indicated in the structure of HPr shown in Figure 5. The red area in Figure 5 forms a continuous surface including the active site and an area spatially close to the active site.

DISCUSSION

It is well established that the chemical shift is exquisitely sensitive to local nuclear environment. The chemical shift of a nucleus in a protein is the result of magnetic shielding of its environment, including aromatic ring current effects, peptide bond anisotropy, electrostatic interactions, and hydrogen bonding. When two proteins form a complex, the various interactions between them will inevitably cause changes in the environments of nuclei in amino acid residues at the interface, resulting in chemical shift changes. Any additional conformational changes resulting from complex formation will cause further chemical shift perturbations. Indeed, while chemical shift effects gave good initial estimates into the location of the site of binding of the peptide cyclosporin A to cyclophilin, it was noted that these effects extended slightly beyond the direct contact area as defined in a model of the complex determined using experimental intermolecular NOE distance restraints (Spitzfaden et al., 1992).

The chemical shift changes observed on formation of a complex between IIA^{glc} and HPr are most likely due to direct contact between the two proteins, although local structural changes at or near the interface cannot be completely ruled out. For both IIA^{glc} and HPr, the largest chemical shift changes occur for residues clustered on continuous surfaces in the immediate vicinity of the phosphorylation active site. If the overall conformation of either protein were to change significantly upon formation of the protein–protein complex, the chemical shift changes would be expected to be distributed more widely than is observed in the present experiments. Also, in the known cases of protein–ligand interaction, significant

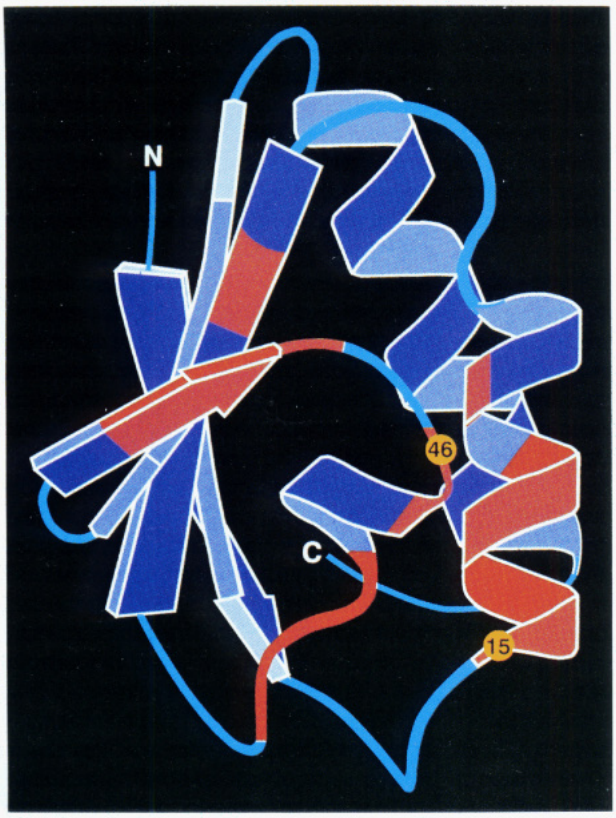

FIGURE 5: Summary of chemical shift changes observed for HPr. The schematic diagram of the three-dimensional structure of HPr was adapted from Figure 2a of Herzberg et al. [Reprinted with permission from Herzberg et al. (1992). Copyright 1992 National Academy of Sciences.] The red color indicates regions where chemical shift changes greater than 0.027 or 0.04 ppm in the CαH or NH dimension, respectively, were observed.

overall structural changes due to ligand binding usually involve proteins or ligands that have flexible conformations. The available data suggest that HPr and IIA^{glc} have relatively rigid conformations. Thus significant overall structural changes are not likely to occur upon complex formation. Inspection of the three-dimensional structures of the two proteins shows that the suggested interface on IIA^{glc} is concave while the proposed binding surface on HPr is convex (Fairbrother et al., 1992a; Liao et al., 1991; Herzberg et al., 1992). In addition, the surface areas over which chemical shift changes are observed on the two proteins are comparable. It is highly probable, therefore, that the observed areas of chemical shift change on IIA^{glc} and HPr represent, or at least include, the areas involved at the binding interface of these proteins.

The contacts between the two proteins may involve different types of interactions, including van der Waals forces, hydrogen bonding, and salt bridges. The residues that show the largest chemical shift changes on IIA^{glc} include D31, V33, F34, S35, G36, K37, M38, M39, G40, F43, L61, N62, V63, F64, I70, G71, L72, E79, I80, L81, D87, V89, V132, F133, and T134. The residues that show the largest chemical shift changes on HPr include H15, A19, T20, V21, V23, T41, N43, L44, S46, I47, M51, S52, L53, G54, I55, A56, and V33. Since most of the residues proposed to be involved in the interaction have hydrophobic side chains, it is likely that hydrophobic interactions play an important role in the protein–protein interaction. It has been noted earlier that surface residues surrounding the phosphorylation active sites of both HPr and IIA^{glc} are predominantly hydrophobic, and it was suggested that these hydrophobic residues may be important for protein–protein interactions (Herzberg et al., 1992; Liao et al., 1991; Worthylake et al., 1991), although hydrogen bonding and salt bridge formation might also play a role. It has also been found that ATP-dependent phosphorylation of HPr at S46 reduces (approximately 10-fold) the affinity of HPr for IIA^{glc} (Reizer et al., 1992). Since S46 forms part of the putative binding surface identified for HPr (Figure 5), it is possible that phosphorylation of S46 disrupts some contact between S46 and IIA^{glc}.

Interaction between HPr and IIA^{glc} is necessary for the transfer of a phosphoryl group between the two proteins. The above NMR results provide direct evidence pertaining to the binding interface between the two proteins through observation of chemical shift changes among predominantly hydrophobic residues surrounding the active site histidines. On the basis of X-ray crystal structures, Herzberg et al. (1992) have proposed a mechanism for phosphoryl group transfer in which the proteins first interact by way of complementary surfaces near the active sites. A pentacoordinate intermediate or transition state is then formed in which N-1 of His-15 and N-3 of His-83 of HPr and IIA^{glc}, respectively, occupy the apical positions of a trigonal-bipyramidal phosphorus moiety. Phosphoryl transfer is accompanied by inversion of configuration at the phosphorus (Begley et al., 1982). 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 a 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} (Herzberg et al., 1992). The NMR data are consistent with this proposed mechanism, and we note that both Asp-31 and Asp-87 of IIA^{glc} undergo significant shifts of their backbone resonances upon complex formation with HPr.

In summary, the binding interfaces involved in complex formation between IIA^{glc} and unphosphorylated HPr have been identified through mapping of chemical shift changes. Further characterization of the complex must await detailed analysis of NOESY spectra obtained with ^{13}C-labeled proteins and identification of specific intermolecular interactions. The current results, however, provide a starting point from which modeling studies can be initiated. The isotope-edited NMR methods used in the present study should be generally applicable to a wide range of interacting protein system.

ADDED IN PROOF

Since acceptance of the present paper, corrected assignments have been reported for several resonances of HPR (Wittekind et al., 1992). The only change significant to the present work is that the spin system of Met-51 has been reassigned to Met-48. Thus the perturbation reported in the present paper for Met-51 must be reassigned to Met-48. Both residues 48 and 51 lie on the surface of HPr that contacts IIA^{glc} in the complex.

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REFERENCES

Aue, W. P., Bartholdi, E., & Ernst, R. R. (1976) *J. Chem. Phys. 64*, 2229–2246.

Bax, A., & Freeman, R. (1981) *J. Magn. Reson. 44*, 542–561.

Bax, A., & Subramanian, S. (1986) *J. Magn. Reson. 67*, 565–569.

Bax, A., Ikura, M., Kay, L. E., Torchia, D. A., & Tschudin, R. (1990) *J. Magn. Reson. 86*, 304–318.
Begly, G. S., Hansen, D. E., Jacobson, G. R., & Knowles, J. R. (1982) *Biochemistry 21*, 5552–5556.
Bodenhausen, G., Vold, R. L., & Vold, R. R. (1980) *J. Magn. Reson. 37*, 93–106.
Cavanagh, J., & Rance, M. (1992) *J. Magn. Reson. 96*, 670–678.
Cavanagh, J., Palmer, A. G., III, Wright, P. E., & Rance, M. (1991) *J. Magn. Reson. 91*, 429–436.
Delsuc, M. A., & Lallemand, J. Y. (1986) *J. Magn. Reson. 69*, 504–507.
El-Kabbani, O. A. L., Waygood, E. B., & Delbaere, L. T. J. (1987) *J. Biol. Chem. 262*, 12926–12929.
Fairbrother, W. J., Cavanagh, J., Dyson, H. J., Palmer, A. G., III, Sutrina, S. L., Reizer, J., Saier, M. H., Jr., & Wright, P. E. (1991) *Biochemistry 30*, 6896–6907.
Fairbrother, W. J., Gippert, G. P., Reizer, J., Saier, M. H., Jr., & Wright, P. E. (1992a) *FEBS Lett. 296*, 148–152.
Fairbrother, W. J., Palmer, A. G., III, Rance, M., Reizer, J., Saier, M. H., Jr., & Wright, P. E. (1992b) *Biochemistry 31*, 4413–4425.
Geerse, R. H., Izzo, F., & Postma, P. W. (1989) *Mol. Gen. Genet. 216*, 517–525.
Gemmecker, G., Olejnczak, E. T., & Fesik, S. W. (1992) *J. Magn. Reson. 96*, 199–204.
Hammen, P. K., Waygood, E. B., & Klevit, R. E. (1991) *Biochemistry 30*, 11842–11850.
Herzberg, O., Reddy, P., Sutrina, S., Saier, M. H., Jr., Reizer, J., & Kapadia, G. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*, 2499–2503.
Ikura, M., & Bax, A. (1992) *J. Am. Chem. Soc. 114*, 2433–2440.
Klevit, R. E., & Drobny, G. P. (1986) *Biochemistry 25*, 7770–7773.
Klevit, R. E., & Waygood, E. B. (1986) *Biochemistry 25*, 7774–7778.
Klevit, R. E., Drobny, G. P., & Waygood, E. B. (1986) *Biochemistry 25*, 7760–7769.
Kraulis, P. J. (1991) *J. Appl. Crystallogr. 24*, 946–950.
Liao, D.-I., Kapadia, G., Reddy, P., Saier, M. H., Jr., Reizer, J., & Herzberg, O. (1991) *Biochemistry 30*, 9583–9594.
Live, D. H., Davis, D. G., Agosta, W. C., & Cowburn, D. (1984) *J. Am. Chem. Soc. 106*, 6104–6105.
Marion, D., & Wüthrich, K. (1983) *Biochem. Biophys. Res. Commun. 113*, 967–974.
Meadow, N. D., Fox, D. K., & Roseman, S. (1990) *Annu. Rev. Biochem. 59*, 497–542.
Nagayama, K., Anil-Kumar, K., Wüthrich, K., & Ernst, R. R. (1980) *J. Magn. Reson. 40*, 321–334.
Nelson, S. O., Wright, J. K., & Postma, P. W. (1983) *EMBO J. 2*, 715–720.
Norwood, T. J., Boyd, J., Heritage, J. E., Soffe, N., & Campbell, I. D. (1990) *J. Magn. Reson. 87*, 488–501.
Osumi, T., & Saier, M. H., Jr. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 1457–1461.
Otting, G., & Wüthrich, K. (1987) *J. Magn. Reson. 75*, 546–549.
Otting, G., & Wüthrich, K. (1990) *Q. Rev. Biophys. 23*, 39–96.
Pelton, J. G., Torchia, D. A., Meadow, N. D., Wong, C.-Y., & Roseman, S. (1991a) *Biochemistry 30*, 10043–10057.
Pelton, J. G., Torchia, D. Z., Meadow, N. D., Wong, C.-Y., & Roseman, S. (1991b) *Proc. Natl. Acad. Sci. U.S.A. 88*, 3479–3483.
Redfield, A. G., & Kunz, S. D. (1975) *J. Magn. Reson. 19*, 250–254.
Reizer, J. (1989) *FEMS Microbiol. Rev. 63*, 149–156.
Reizer, J., Saier, M. H., Jr., Deutscher, J., Grenier, F., Thompson, J., & Hengstenberg, W. (1988) *CRC Crit. Rev. Microbiol. 15*, 297–338.
Reizer, J., Sutrina, S. L., Saier, M. H., Jr., Steward, G. C., Peterkofsky, A., & Reddy, P. (1989) *EMBO J. 8*, 2111–2120.
Reizer, J., Sutrina, S. L., Wu, L.-F., Deutscher, J., Reddy, P., & Saier, M. H., Jr. (1992) *J. Biol. Chem. 267*, 9158–9169.
Saier, M. H., Jr. (1989) *Microbiol. Rev. 53*, 109–120.
Saier, M. H., Jr., & Reizer, J. (1992) *J. Bacteriol. 174*, 1433–1438.
Saier, M. H., Jr., Novotny, M. J., Comeau-Fuhrman, D., Osumi, T., & Desai, J. D. (1983) *J. Bacteriol. 155*, 1351–1357.
Saier, M. H., Jr., Yamada, M., Lengeler, J., Erni, B., Suda, K., Argos, P., Schnetz, K., Rak, B., Lee, C. A., Steward, G. C., Peri, K. G., & Waygood, E. B. (1988) *FASEB J. 2*, 199–208.
Shaka, A. J., Barker, P. B., & Freeman, R. (1985) *J. Magn. Reson. 64*, 547–552.
Shaka, A. J., Lee, C. J., & Pines, A. (1988) *J. Magn. Reson. 77*, 274.
Sharma, S., Georges, F., Delbaere, L. T. J., Lee, J. S., Klevit, R. E., & Waygood, E. B. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 4877–4881.
Spitzfaden, C., Weber, H.-P., Braun, W., Kallen, J., Wider, G., Widmer, H., Walkinshaw, M. D., & Wüthrich, K. (1992) *FEBS Lett. 300*, 291–300.
Sutrina, S. L., Reddy, P., Saier, M. H., Jr., & Reizer, J. (1990) *J. Biol. Chem. 265*, 18581–18589.
van Nuland, N. A. J., van Dijk, A. A., Dijkstra, K., van Hoesel, F. H. J., Scheek, R. M., & Robillard, G. T. (1992) *Eur. J. Biochem. 203*, 483–491.
Wittekind, M. G., Reizer, J., & Klevit, R. E. (1990) *Biochemistry 29*, 7191–7200.
Wittekind et al. (1992) *Protein Sci. 1*, 1363–1376.
Worthylake, D., Meadow, N. D., Roseman, S., Liao, D.-I., Herzberg, O., & Remington, S. J. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 10382–10386.
Wu, L.-F., Tomich, J. M., & Saier, M. H., Jr. (1990) *J. Mol. Biol. 213*, 687–703.
Wüthrich, K. (1986) *NMR of Proteins and Nucleic Acids*, J. Wiley and Sons, New York.

Registry No. His, 71-00-1; Asp, 56-84-8; phosphoenolpyruvate–sugar phosphotransferase enzyme II, 37278-09-4.