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A Common Interface on Histidine-containing Phosphocarrier Protein for Interaction with Its Partner Proteins*

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The bacterial phosphoenolpyruvate:sugar phosphotransferase system accomplishes both the transport and phosphorylation of sugars as well as the regulation of some cellular processes. An important component of this system is the histidine-containing phosphocarrier protein, HPr, which accepts a phosphoryl group from enzyme I, transfers a phosphoryl group to IIA proteins, and is an allosteric regulator of glycogen phosphorylase. Because the nature of the surface on HPr that interacts with this multiplicity of proteins from *Escherichia coli* was previously undefined, we investigated these interactions by nuclear magnetic resonance spectroscopy. The chemical shift changes of the backbone and side-chain amide ^{1}H and ^{15}N nuclei of uniformly ^{15}N-labeled HPr in the absence and presence of natural abundance glycogen phosphorylase, glucose-specific enzyme IIA, or the N-terminal domain of enzyme I have been determined. Mapping these chemical shift perturbations onto the three-dimensional structure of HPr permitted us to identify the binding surface(s) of HPr for interaction with these proteins. Here we show that the mapped interfaces on HPr are remarkably similar, indicating that HPr employs a similar surface in binding to its partners.

An important sugar transport mechanism in a variety of bacterial species involves coupling the transport and phosphorylation processes via the PTS[1] (1). In the PTS, there is an initial cascade of phosphoryl transfers from phosphoenolpyruvate to sugar-specific permeases through a common pathway. Phosphoenolpyruvate transfers a phosphoryl group to an active site histidine (His-189 in *Escherichia coli*) of Enzyme I (EI, ~63 kDa). The phosphoryl group is then transferred to an active site histidine (His-15 in *E. coli*) of HPr (9 kDa). The phosphoryl group associated with HPr is available for transfer to any available sugar-specific IIA protein. In addition, there is considerable evidence that the common proteins of the PTS (EI, HPr, and enzyme IIA^{Glc}) function not only as phosphocarriers for sugar transport but also as regulatory factors. HPr functions as a regulatory subunit of the *E. coli* glycogen phosphorylase (GP, ~92 kDa) complex, resulting in a stimulation of the enzyme activity when the subunit is in the dephospho-form (2).

The structures of HPr, IIA^{Glc} (~18 kDa), and the N-terminal domain of EI (EIN, ~30 kDa), but not GP, have been solved by x-ray crystallography and/or NMR spectroscopy (3–5). In this communication, we report the elucidation, by NMR, of the nature of the surface on HPr that interacts with this collection of proteins from *E. coli*. The approach employed makes use of differential chemical shift mapping and is similar in spirit to that recently described by Medek *et al.* (6) to map binding site locations and orientation of protein-bound ligands.

MATERIALS AND METHODS

Expression and Purification of Proteins—IIA^{Glc} was expressed and purified as described previously (7). *E. coli* strain GI698, transformed with the plasmids pSP100 (8), pGP (2), and pLP2 (9), was used to overexpress and purify HPr, GP, and EIN, respectively. ^{15}N-Labeled HPr was prepared as described (10).

NMR Spectroscopy—All NMR spectra were collected on a Bruker DMX600 spectrometer equipped with a triple resonance probe. A series of two-dimensional ^{1}H-^{15}N HSQC spectra for separate NMR samples at various protein:protein molar ratios containing 90% H_2O,10% D_2O in 10 mM phosphate buffer, pH 7.1, were recorded as described (11). Spectra were processed using the nmrPipe software (12) and analyzed using the programs PIPP, CAPP, and STAPP (13).

RESULTS AND DISCUSSION

Fig. 1 depicts two-dimensional ^{1}H-^{15}N heteronuclear correlation (HSQC) spectra of ^{15}N-labeled HPr in the presence of unlabeled GP (*a*), IIA^{Glc} (*b*), and EIN (*c*) superimposed on the same spectral region of HPr in the free state. All three proteins have an effect on the spectrum of HPr: there is selective spectral line broadening from ~20 Hz (for free HPr) to 28–56 Hz for Ala-19, Phe-48, Lys-49, and Gln-51 (GP > EIN > IIA^{Glc}) and 23–29 Hz for Gln-3 and Glu-85 (for bound HPr) in the ^{1}H dimension, and cross-peak shifts, indicative of specific protein-protein interactions (8, 14–16). Further, addition of GP to HPr leads to a global decrease in signal intensity and eventually to cross-peak disappearance at a molar ratio of 1:1, due to the formation of a large protein complex of ≥100 kDa. For IIA^{Glc} or EIN, free and bound HPr are in fast exchange on the chemical shift time scale, and the largest chemical shift deviations were observed at a protein-protein molar ratio of 1:1 (Fig. 1, *b* and *c*) with no significant change after further addition of either EIN or IIA^{Glc} to HPr.

To identify the interacting residues on HPr, the cross-peak combined ^{1}H and ^{15}N chemical shift differences between the bound and free states of HPr, defined as $\delta = (\delta_H^2 + \delta_N^2)^{1/2}$, in

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[1] The abbreviations used are: PTS, phosphoenolpyruvate:sugar phosphotransferase system; HPr, histidine-containing phosphocarrier protein; GP, glycogen phosphorylase; EI, enzyme I; EIN, N-terminal domain of enzyme I; IIA^{Glc}, glucose-specific enyzme IIA; IIA^{Mtl}, mannitol-specific enzyme IIA.

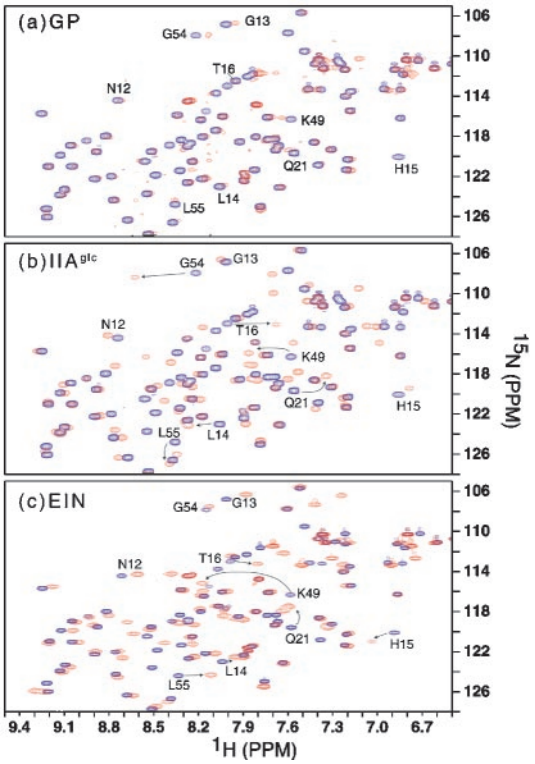

FIG. 1. **Perturbations of HPr NMR spectra by its partner proteins.** Portion of the ^{1}H-^{15}N HSQC spectra of 0.3 mM HPr in the presence and absence of 0.15 mM glycogen phosphorylase, at pH 7.1, 33.5 °C (*a*), 1.0 mM HPr in the presence and absence of 1 mM enzyme IIA^{Glc}, at pH 7.1, 35 °C (*b*), and 1.0 mM HPr in the presence and absence of 1.0 mM EIN, at pH 7.1, 40 °C (*c*), recorded at a ^{1}H frequency of 600 MHz. Cross-peaks for HPr in the bound state were assigned by following the shifts of cross-peaks from the free state during titration. Cross-peaks that showed significant shifts between free (*blue contours*) and bound (*red contours*) states are selectively labeled.

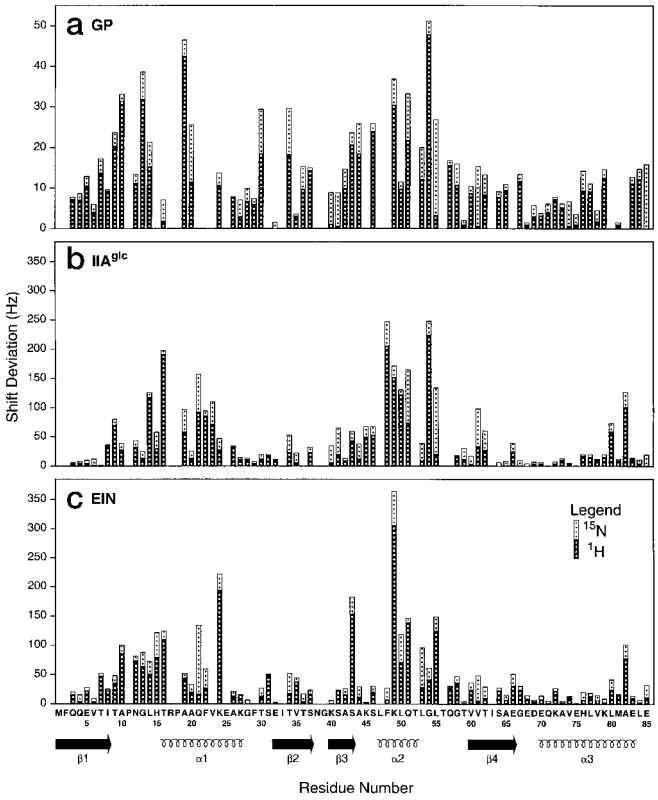

FIG. 2. **Bar plots of chemical shift deviations between free HPr and HPr bound to GP (*a*), IIA^{Glc} (*b*), and EIN (*c*).** The total cross-peak chemical shift deviation (in Hz) is defined as $\delta = (\delta_H^2 + \delta_N^2)^{1/2}$, where δ_H and δ_N are amide ^{1}H and ^{15}N chemical shift differences between the bound and free states of HPr (see Fig. 1) measured under the same conditions. The weighted contributions of δ_H and δ_N are represented by *open* and *filled bars*, respectively. The weighting factors for ^{1}H and ^{15}N are defined as $f(\delta_H) = \delta_H^2/(\delta_H^2 + \delta_N^2)$ and $f(\delta_N) = \delta_N^2/(\delta_H^2 + \delta_N^2)$. The sequence and secondary structure of HPr is shown at the *bottom* of the figure.

Hz, are plotted as a function of the residue number (Fig. 2). In the presence of GP, the backbone amide resonances of residues Ala-19 and Gly-54 shift by ~50 Hz while residues Ala-10, Gly-13, Ala-20, Thr-30, Thr-34, Ala-44, Ser-46, Lys-49, Gln-51, and Leu-55 display shifts of 25–40 Hz (Fig. 2*a*). In addition, the lower field side-chain amide proton of Gln-51 shifts by 78 Hz. In the presence of enzyme IIA^{Glc}, the backbone amide signals of residues Leu-14, Thr-16, Gln-21, Val-23, Phe-48, Lys-49, Leu-50, Gln-51, Gly-54, Leu-55, and Ala-82 shift by more than 100 Hz (Fig. 2*b*). A similar shift perturbation pattern for HPr can be seen in Fig. 2*c* in the case of EIN, where the cross-peaks of residues His-15, Thr-16, Gln-21, Lys-24, Ser-43, Lys-49, Leu-50, Gln-51, Leu-55, and Ala-82 show chemical shift perturbations greater than 100 Hz. Further, at least one of the side-chain amide proton resonances of Asn-12, Gln-21, and Gln-51 of HPr is shifted by 50–490 Hz, suggesting their importance in interacting with both IIA^{Glc} and EIN. Clearly, the residues of HPr most influenced by interaction with these proteins are very similar, although not identical.

To visualize the interaction surface(s) on HPr, Fig. 3 shows the chemical shift perturbations of HPr induced by GP, IIA^{Glc}, and EIN mapped onto ribbon diagram (*left*) and accessible surface (*right*) representations of the three-dimensional structure of HPr. The color varies from *blue* (no chemical shift change) through *yellow* (intermediate change) to *red* (maximum change). It is apparent that, in all three cases, the most perturbed residues are clustered in the three-dimensional structure, forming a contiguous surface composed of helix 1, helix 2, the C terminus of helix 3, and the loops between β-strand 1 and helix 1, β-strand 3 and helix 2, and helix 2 and β-strand 4. Whereas IIA^{Glc} and EIN perturb the same narrow region on the surface of HPr, GP appears to interact with a somewhat wider region on the surface of HPr (Fig. 3, *right-hand panels*). The narrower HPr surface associated with the interactions with EIN and IIA^{Glc} may be necessary for rapid on and off reactions required by the cascade for sugar transport. The wider surface associated with the GP interaction is consistent with the higher binding affinity (2–3 orders of magnitude) of HPr for GP than for either EIN or IIA^{Glc} (2, 8).

The absence of any shifts in the IIA^{Glc} ^{1}H-^{15}N correlation spectrum following addition of unlabeled GP (data not shown) agrees with previous "fishing" experiments, which identified HPr as the only ligand that interacts with GP (2). Further, we found that addition of a 2-fold excess of GP to the 1:1 complex of ^{15}N-labeled IIA^{Glc} and HPr leads to the restoration of the IIA^{Glc} ^{1}H-^{15}N correlation spectrum to its free state and the disappearance of all the HPr signals because essentially all of the added HPr is bound to GP and is thus not available to interact with IIA^{Glc}. This competition experiment provides further evidence that HPr uses a similar surface to interact with either IIA^{Glc} or GP. Because HPr is present in excess *in vivo*, it is not likely that its interaction with GP influences its participation in the phosphoryl transfer cascade.

In conclusion, we have characterized by NMR the interaction surface of HPr that binds to GP, IIA^{Glc}, and EIN in *E. coli*. The important finding is that HPr utilizes a similar surface to interact with all three enzymes. The key interacting residues are located in helices 1 and 2 and the loops preceding helix 1

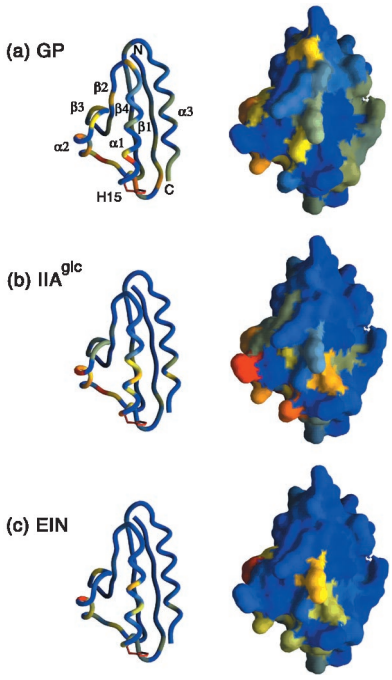

FIG. 3. **Binding surface on HPr for GP (*a*), IIA^{Glc} (*b*), and EIN (*c*).** The backbone chemical shift perturbations (Fig. 2) have been mapped onto a ribbon diagram (*left*) and the accessible surface (*right*) of HPr, where the magnitude of the shifts is depicted in colors varying from *blue* (no shifts) through *yellow* (intermediate shifts) to *red* (maximum shifts). The secondary structure elements are labeled in the ribbon diagram shown in *a*. The side chain of the active site His-15 is shown in *red* on the ribbon diagram. The figure was generated with the program GRASP (20) using the x-ray coordinates of HPr (PDB accession code 1POH; Ref. 21).

and following helix 2 (see Fig. 3). The mapped surface for HPr has been confirmed in the case of EIN by the structure of the HPr-EIN complex elucidated by NMR (9) and is consistent with mutagenesis data (2, 17). Further, there is no difference in the mapped surfaces on HPr contacting EIN (8) or EI (18), indicating that only the N-terminal region of EI is required for binding HPr. An important consequence of these data is that phosphotransfer in the phosphoryl transfer cascade is achieved by association and dissociation of bimolecular protein complexes rather than by formation of a ternary complex, *i.e.* the HPr-EI complex must dissociate in order for HPr to pass the phosphoryl group to IIA^{Glc}. Because HPr uses a similar surface to interact with the mannitol-specific enzyme IIA (IIA^{Mtl}) from *E. coli* (15), we conclude that phospho-HPr is equally competent to donate its phosphoryl group to various sugar-specific enzyme IIAs. The interaction surface of HPr with IIA^{Glc} from *Bacillus subtilis* (14) is remarkably similar, extending our conclusion to homologous proteins from other species. In other species, HPr performs additional functions (cofactor in catabolite repression in some Gram-positive organisms and substrate for an ATP-dependent kinase that phosphorylates Ser-46 from *Mycoplasma capricolum*). NMR and mutagenesis studies in these systems suggest a similar surface on HPr for these interactions (16, 19) as described above. Because the folding topology of HPr from different species has been demonstrated to be identical (3), it appears that HPr uses a consensus surface to interact with proteins of the sugar transport cascade as well as proteins that it regulates.

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