Solution Structure of the IIA\textsuperscript{Chitobiose}-HPr Complex of the N,N\textquotesingle-Diacetylchitobiose Branch of the \textit{Escherichia coli} Phosphotransferase System* 

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Background: The bacterial phosphoryl transfer system (PTS) couples phosphoryl transfer to sugar transport. Results: The structure of the IIA\textsuperscript{Chitobiose}-HPr complex completes the structure elucidation of representative cytoplasmic complexes for all four sugar branches of the PTS. Conclusion: Phosphoryl transfer occurs without any significant backbone conformational changes. Significance: Recognition of multiple, structurally diverse partners is facilitated by complementary interaction surfaces and side chain conformational plasticity.

The solution structure of the complex of enzyme IIA of the N,N\textquotesingle-diacetylchitobiose (Chb) transporter with the histidine phosphocarrier protein HPr has been solved by NMR. The IIA\textsuperscript{Chb}-HPr complex completes the structure elucidation of representative cytoplasmic complexes for all four sugar branches of the bacterial phosphoryl transfer system (PTS). The active site His-89 of IIA\textsuperscript{Chb} was mutated to Glu to mimic the phosphorylated state. IIA\textsuperscript{Chb}(H89E) and HPr form a weak complex with a $K_D$ of $\sim 0.7$ mM. The interacting binding surfaces, concave for IIA\textsuperscript{Chb} and convex for HPr, complement each other in terms of shape, residue type, and charge distribution, with predominantly hydrophobic residues, interspersed by some uncharged polar residues, located centrally, and polar and charged residues at the periphery. The active site histidine of HPr, His-15, is buried within the active site cleft of IIA\textsuperscript{Chb} formed at the interface of two adjacent subunits of the IIA\textsuperscript{Chb} trimer, thereby coming into close proximity with the active site residue, H89E, of IIA\textsuperscript{Chb}. A His89-P-His-15 pentacoordinate phosphoryl transition state can readily be modeled without necessitating any significant conformational changes, thereby facilitating rapid phosphoryl transfer. Comparison of the IIA\textsuperscript{Chb}-HPr complex with the IIA\textsuperscript{Chb}-IIB\textsuperscript{Chb} complex, as well as with other cytoplasmic complexes of the PTS, highlights a unifying mechanism for recognition of structurally diverse partners. This involves generating similar binding surfaces from entirely different underlying structural elements, large interaction surfaces coupled with extensive redundancy, and side chain conformational plasticity to optimize diverse sets of intermolecular interactions.

The phosphoenolpyruvate:sugar phosphotransferase system (PTS) is a central bacterial signal transduction pathway in which phosphoryl transfer, via a series of bimolecular protein complexes, is coupled to both sugar transport across the membrane and the regulation of many cellular processes, including catabolite repression (1–6). The first component of the PTS, enzyme I, is autophosphorylated by phosphoenolpyruvate and subsequently transfers the phosphoryl group to the histidine phosphocarrier protein (HPr). HPr then transfers the phosphoryl group to the A domain of the sugar-specific enzymes II, which are divided into four structurally distinct families corresponding to the glucose, mannose, mannitol, and lactose/chitobiose branches of the PTS. All enzymes II have similar organizations comprising A and B cytoplasmic domains, and a membrane bound sugar transporter comprising the C domain, and sometimes a D domain as well. In some instances the domains are expressed as a contiguous protein, in others as separate proteins. From IIA, the phosphoryl group is transferred to IIB, and finally onto the incoming sugar molecule bound to the transmembrane IIC domain. Despite the similar domain organization of the enzymes II, the A and B cytoplasmic domains from the different branches of the PTS bear no sequence similarity to one another, and with the exception of IIB\textsuperscript{Mdt} (7, 8) and IIB\textsuperscript{Chb} (9–11), no similarity in either ternary or quaternary structures either.

Structures of the individual cytoplasmic components of the PTS have been solved by either NMR (7, 8, 10–20) or crystal-
Solution Structure of the IIA^Chb-HPr Complex

lography (9, 21–35). Structures of the cytoplasmic protein–protein complexes of the PTS, however, have been intractable to crystallography, presumably due to their weak affinity making successful co-crystallization difficult. Weak binding, however, is not an impediment to NMR, and we have solved the solution structures of all the cytoplasmic binary protein complexes of the PTS (15, 16, 18, 36–43) with the exception of the IIA^Chb-HPr complex. These complexes provide a wealth of information for understanding the unifying mechanism whereby a common interface, coupled with side chain conformational plasticity, can be used to recognize multiple, structurally dissimilar partners, and in addition, have yielded the first direct experimental evidence for the existence of highly transient, sparsely populated encounter complexes (44–46).

In this paper we present the solution structure of the IIA^Chb-HPr complex, the remaining outstanding cytoplasmic complex of the PTS, thereby completing our long term goal of solving all the cytoplasmic complexes of the PTS.

EXPERIMENTAL PROCEDURES

Protein Expression and Mutagenesis—Genes encoding IIA^Chb* (corresponding to a N113/D92L mutant of wild-type IIA^Chb) (20) and HPr (39, 47) were cloned into the pET-11 vector. H89E and H15D mutations of the active site histidines of IIA^Chb* and HPr, respectively, were introduced using the QuikChange mutagenesis kit (Stratagene, La Jolla, CA). (Residues of HPr are denoted in italics throughout.) Both mutations were designed to mimic the charge effect of phosphorylation of the active site histidines.

The IIA^Chb*, IIA^Chb*(H89E), HPr, and HPr(H15D) plasmids were introduced into Escherichia coli BL21(DE3) (Novagen) cells for protein expression and induced at an A600 ~ 0.8 with 1 mM isopropyl β-D-thiogalactopyranoside at 37 °C. Cells were grown in either Luria-Bertani medium or minimal medium (in either H2O or D2O with 15NH4Cl or 14NH4Cl as the sole nitrogen source, and U-[13C/1H]-, U-[12C/1H]-, U-[13C/2H]-, or U-[12C/3H]-glucose as the main carbon source. Because Leu, Val, Ile, Met, Gly, Thr, Ser, Phe, and Ala residues are involved in the IIA^Chb*-HPr binding interface, selective amino acid labeling was also employed in the preparation of NMR samples. For H2/13C/15-N-(Ile/Leu/Val)-methyl-protonated (but otherwise fully deuterated) protein samples, 100 mg of α-[13C0,3-2H1]-ketosiovalerate and 50 mg of α-[13C0,3,3,3-2H2]ketobutyrate (Cambridge Isotopes) were added to 1 liter of D2O medium 1 h prior to induction (48). 2H/13C/14N-(Ile/Gly/Phe-protonated)-IIA^Chb*(H89E), 2H/13C/14N-(Leu/Met/Tyr-protonated)-IIA^Chb*(H89E), and 2H/13C/14N-(Val/Ala/His-protonated)-IIA^Chb*(H89E) samples were prepared by supplementing 1 liter of D2O medium with 300 mg of Ile/Gly/Phe/Leu/Met/Tyr/Val/Ala/His (Sigma) at natural isotopic abundance 1 h prior to induction. Cells were grown an additional 7 h after induction. Cells expressing IIA^Chb*(H89E) or HPr were harvested by centrifugation at 15,900 × g for 25 min. IIA^Chb* and IIA^Chb*(H89E), and HPr and HPr(H15D) were purified as described previously in Refs. 43 and 39, respectively.

NMR Data Collection and Analysis—All NMR samples were prepared in a buffer of 20 mM sodium phosphate, pH 7.4, 0.2 mM sodium azide, and either 90% H2O/10% D2O or 99.99% D2O. IIA^Chb* is a symmetric trimer with 3 eq binding sites for HPr. As in the case of the IIA^Chb*(H89E)-IIB^Chb*(C10S) complex (43), a 1:1 mixture of IIA^Chb*(H89E) trimer to HPr monomer was employed to achieve optimal line widths for NMR spectroscopy. NMR spectra were recorded at 20 and 35 °C on Bruker DMX500, DMX600, DRX600, DRX800, and DRX900 spectrometers equipped with z-shielded gradient triple resonance cryoprobes. Spectra were processed with the NMRPipe package (49) and analyzed using the programs PIPP (50) and XIPP (51). Sequential and side chain assignments of IIA^Chb*(H89E) and HPr were derived from the following three-dimensional double and triple resonance through-bond correlation experiments (51, 52): HNCA, HN(CO)CA, CBCA(CO)HN, HAHN, HNCA-TROSY, HN(CO)CA-TROSY, HNCB-TROSY, HN(CO)CB-TROSY, C(CCO)NH, H(CC)ONH, and HCH-TOSY. Three-dimensional 15N-separated, 13C-separated, and 13C/12C-separated nuclear Overhauser enhancement (NOE) experiments were used to facilitate side chain assignments (51).

Intermolecular NOEs were observed on the IIA^Chb*(H89E)-HPr complex in D2O buffer using three-dimensional 12C-filtered(F1)/13C-separated(F2) or 13C-separated(F2)/12C-filtered(F1) NOE experiments, and in H2O buffer using two-dimensional 15N-separated/13C-edited and 13C-separated/15N-edited NOE experiments (53, 54). Nine different combinations of isotope-labeled complexes were used for analysis of intermolecular NOEs (Table 1).

Structure Calculations—NOE-derived interproton distance restraints were classified into loose approximate distance ranges of 1.8–2.7, 1.8–3.5, 1.8–5.0, and 1.8–6.0 Å corresponding to strong, medium, weak, and very weak NOE cross-peak intensities, respectively (55). An empirical correction of 0.5 Å was added to the upper distance bounds of distance restraints involving methyl groups to account for the higher apparent intensity of methyl resonances (56), and NOEs involving nonspecifically assigned methyl, methylene, and aromatic protons were represented by a (3/2−r−6)−1/6 sum (57). Backbone torsion angle restraints for the active site region (residues 13–17) of HPr were derived from backbone 1H/15N/13C chemical shifts using the program TALOS+ (58) and used in the calculations of the phosphorylation transition state. The current experiments yielded interproton distance restraints and interfacial side chain torsion angle restraints.

Structures were calculated using conjoint rigid body/torsion angle-simulated annealing (59, 60) with the program Xplor-NIH (61). The target function that is minimized comprises NOE-derived interproton distance restraints, torsion angle restraints, residual dipolar coupling restraints, 13Cα/13Cβ chemical shift restraints, a quartic van der Waals repulsion term for the nonbonded contacts, a multidimensional torsion angle data base potential of mean force (62), and a gyration volume potential to ensure optimal packing (63). Structure figures were generated using the programs VMD-XPLOR (64) and GRASP (65). Reweighted atomic probability density maps were calculated as described previously (66). The atomic coordinates

5 G. S. Garrett and G. M. Clore, unpublished data.
and NMR experimental restraints (accession codes 2lrk and 2lrl for the unphosphorylated and phosphoryl transition state complexes, respectively) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ.

RESULTS AND DISCUSSION

Equilibrium Binding of IIA\textsuperscript{Chb}* (H89E) and HPr—At concentrations used in NMR experiments, wild type IIA\textsuperscript{Chb} is highly prone to nonspecific aggregation promoted by the presence of a disordered 13-residue N-terminal tail and divalent cations required to neutralize and coordinate three symmetry-related, buried aspartate side chains (Asp-92) located at the center of the trimer interface (20). As in previous work (20, 43), we therefore made use of the IIA\textsuperscript{Chb}* construct throughout the current study. IIA\textsuperscript{Chb}* forms a stable monodisperse trimer, and comprises a deletion of the disordered 13-residue N-terminal tail and mutation of the buried Asp-92 to Leu (20). Leu and Asp constitute well packed hydrophobic methyl-methyl interactions at the trimer interface in place of the role fulfilled by the metal cation. These mutations do not affect phosphoryl transfer activity.

The interaction between IIA\textsuperscript{Chb} and HPr was assessed by monitoring \(^1\text{H}_\text{H}^{15}\text{N}\) chemical shift perturbations of \(^{15}\text{N}\)-labeled HPr upon addition of unlabeled IIA\textsuperscript{Chb} (Fig. 1). Studies were carried out with HPr, IIA\textsuperscript{Chb}, and HPr(H15D). (Note that throughout the text, residues of HPr are printed in italics to distinguish them from residues of IIA\textsuperscript{Chbs}.)

The latter two mutations are designed to mimic the charge effects of phosphorylation of the active site histidines at the Nε2 (H89E) and Nδ1 (H15D) positions. At pH 7.4 and 20 °C we could not detect any significant chemical shift perturbations upon addition of IIA\textsuperscript{Chbs} to either HPr or HPr(H15D) at the concentrations employed (up to \(\sim 1.2 \text{mM}\) in subunits of IIA\textsuperscript{Chbs} with \(\sim 0.4 \text{mM}\) HPr). However, measurable chemical shift perturbations were obtained upon addition of IIA\textsuperscript{Chb*(H89E)} to HPr yielding a \(K_d\) of \(0.7 \pm 0.1 \text{mM}\) (Fig. 1). Therefore all structural studies were carried out with the IIA\textsuperscript{Chb*(H89E)} phosphomimetic mutant.

Structure Determination—The IIA\textsuperscript{Chb}–HPr complex is in fast exchange on the chemical shift time scale (\(i.e.\) only a single set of population weighted average resonances are observed). The \(^1\text{H}_\text{H}^{13}\text{N}\) chemical shift perturbations upon complex formation are small indicative of no significant change in backbone conformation (within the limits of the NMR method). The \(^1\text{H}_\text{H}^{13}\text{N}\) chemical shift perturbations span residues 18–34 and 53–102 of IIA\textsuperscript{Chb}, and residues 8–24, 52–53, 61–62, 80, and 85 of HPr, thereby providing an approximate delineation of the interaction surfaces.

Given that three molecules of HPr can bind to the IIA\textsuperscript{Chb} symmetric trimer and binding is weak, all NMR experiments were carried on samples comprising 1 mM IIA\textsuperscript{Chb} (in trimer) and 1 mM HPr. Under these conditions, there is 24% free HPr and 42, 29, and 5%, singly, doubly and triply bound HPr; and 42, 29, and 5%, singly, doubly and triply bound IIA\textsuperscript{Chb} with one, two, and three HPr molecules bound. Given molecular masses of \(\sim 34\) and 9.5 kDa for free IIA\textsuperscript{Chb} and HPr, respectively, the population weighted average masses of IIA\textsuperscript{Chb} and HPr, which determine the line widths in the NMR experiments, are \(\sim 40 \text{kDa}\) each. Note that the existence of multiple bound states, as well as the presence of a significant fraction of free proteins, precludes the use of residual dipolar couplings for determining the relative orientation of the two proteins in the complex, because the apparent alignment tensor can no longer be deconvoluted into individual alignment tensors for each component in the system (43).

The structure of the IIA\textsuperscript{Chb}–HPr complex was largely based on intermolecular NOE data derived from three-dimensional \(^1\text{H}\)-filtered/\(^1\text{C}\)-separated three-dimensional NOE experiments in which NOEs are exclusively observed between protons attached to \(^1\text{H}\) and protons attached to \(^1\text{C}\). An array of different combinations of isotopically labeled samples, com-

| Table 1: Labeling schemes for samples used for intermolecular NOE measurements on the IIA\textsuperscript{Chb*(H89E)}–HPr complex |
|---|---|---|
| Isotope labeling | Sample | HPr |
| 1 | \([^{1}\text{CH}_3-\text{ILV}][H^{13}/C^{15}/N]\) | \([^{1}\text{H}-\text{Leu, Gly, Phe}][H^{13}/C^{15}/N]\) |
| 2 | \([^{1}\text{CH}_3-\text{ILV}][H^{13}/C^{15}/N]\) | \([^{1}\text{H}-\text{Leu, Met, Tyr}][H^{13}/C^{15}/N]\) |
| 3 | \([^{1}\text{CH}_3-\text{ILV}][H^{13}/C^{15}/N]\) | \([^{1}\text{H}-\text{Val, Ala, His}][H^{13}/C^{15}/N]\) |
| 4 | \([^{1}\text{H}-\text{Leu, Gly, Phe}][H^{13}/C^{15}/N]\) | \([^{1}\text{CH}_3-\text{ILV}][H^{13}/C^{15}/N]\) |
| 5 | \([^{1}\text{H}-\text{Leu, Met, Tyr}][H^{13}/C^{15}/N]\) | \([^{1}\text{CH}_3-\text{ILV}][H^{13}/C^{15}/N]\) |
| 6 | \([^{1}\text{H}-\text{Val, Ala, His}][H^{13}/C^{15}/N]\) | \([^{1}\text{CH}_3-\text{ILV}][H^{13}/C^{15}/N]\) |
| 7 | \([^{1}\text{H}-\text{Leu, Gly, Phe}][H^{13}/C^{15}/N]\) | \([^{1}\text{H}-U^{13}/C^{15}/N]\) |
| 8 | \([^{1}\text{H}-\text{Leu, Met, Tyr}][H^{13}/C^{15}/N]\) | \([^{1}\text{H}-U^{13}/C^{15}/N]\) |
| 9 | \([^{1}\text{H}-U^{13}/C^{15}/N]\) | \([^{1}\text{H}-U^{13}/C^{15}/N]\) |
prising both uniform and residue-specific labeling (Table 1),
was employed to remove any ambiguities in assignment of
intermolecular NOEs. An example of the quality of the inter-
molecular data is provided in Fig. 2.

The calculation strategy used to determine the structure of
the complex made use of conjoined rigid body/torsion angle
dynamics simulated annealing (60). In this instance, the back-
bone and noninterfacial side chains of the 2.0-Å resolution
x-ray coordinates of free HPr (30) were treated as a rigid body
with rotational and translational degrees of freedom, whereas
interfacial side chains were given torsional degrees of freedom.
The only coordinates of free IIAChb* available are NMR coor-
dinates (20), which are inherently less accurate than x-ray coor-
dinates (especially in terms of translation and packing). Thus
for IIAChb full torsional, rotational, and translational degrees
of freedom were allowed with the coordinates restrained by the
experimental NMR restraints (NOEs, torsion angles, dipolar
couplings) obtained for free IIAChb (20). This approach, rather
than using the restrained regularized mean coordinates of free
IIAChb (20) as a rigid body, was employed for the following
reasons: the interface of both partners is largely helical and
structurally rigid; the active site residue (H89E) is located
within a deep cleft at the interface of adjacent subunits; and
therefore small errors in the backbone coordinates of the free
NMR structure of IIAChb* can readily propagate and distort
the docking of HPr onto IIAChb*. The backbone coordinate
shifts relative to the free IIAChb* coordinates, however, are
small (<1 Å) and well within the uncertainties of the NMR
coordinates. In the case of the IIAChb*-IIBChb complex, on
the other hand, the IIBChb interaction site comprises a loop
so that uncertainties in the IIAChb coordinates could be
assimilated by simply giving the backbone of the active site
loop of IIBChb torsional degrees of freedom, while treating
the remaining backbone of IIBChb as well as the backbone of

FIGURE 2. Intermolecular NOEs in the IIAChb*(H89E)-HPr complex. NOEs in a three-dimensional 12C-filtered/13C-separated NOE experiment recorded in D2O
are specifically observed from protons attached to 1C (in the F1 dimension) to methyl protons attached to 13C (in the F3 dimension). Strips are shown for NOEs
involving the 13C methyl group of Ile-72 (at 12.48 ppm) and one of the 13C methyl groups of Val-21 (at 23.63 ppm) of IIAChb (H89E). The amino acid (AA)
specific labeling schemes used for [1H-AA]/[2H,12C,14N]HPr are shown above each strip.
IIAChb* (excluding the disordered loop from residues 75–84) as rigid bodies (43).

As in the case of the weak IIAChb–IIBChb complex, a heuristic approach was employed for interfacing side chains since the samples comprised a mixture of free and bound states (43). Thus, the interfacing side chains were given torsion angle degrees of freedom within the X1 and where appropriate X2 rotamers of the free structures, unless contradicted by the intermolecular NOE data. A summary of the structural statistics is provided in Table 2, a best fit superposition of the final ensemble of 100 simulated annealing structures of the complex is displayed in Fig. 3A, and a reweighted atomic probability density map for some interfacing side chains is shown in Fig. 3C.

The Overall Structure of the IIAChb*–HPr Complex—A ribbon diagram of the overall complex showing two and three molecules of HPr bound per trimer is displayed in Fig. 3B. Each HPr molecule interacts with two adjacent subunits of IIAChb*: specifically subunits A and C, C and B, and B and A, where the first subunit in each pair contributes the active site residue at position 89. For the purposes of describing intermolecular contacts between HPr and IIAChb*, we will restrict ourselves to the interaction surface formed at the interface of the A and C subunits of IIAChb*.

Each subunit of IIAChb* comprises 3 helices in an up, down, up topology comprising residues 17–43 (helix 1), 47–74 (helix 2), and 85–114 (helix 3) (20). HPr has three helices formed by residues 16–28 (helix 1), 47–52 (helix 2), and 70–83 (helix 3), as well as a four-stranded antiparallel β-sheet (30). The active site histidine at position 89, as well as His-93, of the A subunit of IIAChb* are located deep within a cleft formed at the interface of the A and C subunits of IIAChb*.
The predominant intermolecular contacts between HPr and II\textsubscript{AChb\textsuperscript{*}} involve helices. The N-terminal halves of helices 1 (residues 16–27) and 2 (residues 347–348) of HPr interact with the N-terminal halves of helices 1 (residues 18–33) and 3 (residues 89 and 93) of subunit A of II\textsubscript{AChb\textsuperscript{*}}; while the loop preceding helix 1 (residues 11–15), helix 2 (residues 47–53), and a stretch of extended strand (residues 55–57) of HPr interact with the C-terminal half of helix 2 (residues 58–73), the loop connecting helices 2 and 3 (residues 76–82), and the middle half of helix 3 (residues 91–98) of the C subunit of II\textsubscript{AChb\textsuperscript{*}}. The total accessible surface area buried upon complex formation is \(11011\) \(580\) \(\AA\textsuperscript{2}, comprising \(350\) \(\AA\textsuperscript{2} and \(450\) \(\AA\textsuperscript{2} for subunits A and C, respectively, of II\textsubscript{AChb\textsuperscript{*}}, and \(780\) \(\AA\textsuperscript{2} for HPr (subdivided into \(350\) and \(430\) \(\AA\textsuperscript{2} for contacts with the A and C subunits of II\textsubscript{AChb\textsuperscript{*}}, respectively). The binding site on II\textsubscript{AChb\textsuperscript{*}} for both subunits A and C comprises \(45\%\) hydrophobic residues, with the remainder equally divided between polar and charged residues (Fig. 4A); for HPr, the portion of the binding surface that interacts with the A subunit of II\textsubscript{AChb\textsuperscript{*}} is \(40\%\) hydrophobic, with the remainder equally divided between polar and charged residues (Fig. 4B, left half), while the portion of the HPr binding surface that interacts with the C subunit of II\textsubscript{AChb\textsuperscript{*}} is composed of \(55\%\) hydrophobic and \(45\%\) uncharged polar residues (Fig. 4B, right half). As in the other complexes of the PTS (15, 16, ...
At the interface of HPr and the C subunit of IIA\textsuperscript{Chb}, the side chain carboxyl of Gln-57 has electrostatic interactions with the guanidino group of Arg-58\textsuperscript{c} and the side chain amide group of Asn-62\textsuperscript{c}; the side chain amide group of Asn-12 forms a potential hydrogen bond with the S8 atom of Met-98\textsuperscript{d}; the backbone amide of Leu-53 donates a potential hydrogen bond to the carboxylate of Glu-73\textsuperscript{d}; and the carboxamide group of Gln-S1 forms potential hydrogen bonds with the carboxylate of Glu-73\textsuperscript{d} and the side chain amino group of Lys-82\textsuperscript{c} (Fig. 5B). Given that the interaction surfaces of HPr and IIA\textsuperscript{Chb} are complementary both in terms of shape and distribution of residue type, it is likely that many of the above intermolecular electrostatic interactions are rather weak and transient, thereby accounting for the high equilibrium dissociation constant ($K_d \sim 0.7$ mM; cf. Fig. 1) for the complex.

**The Phosphoryl Transition State**—It is known from isotope labeling experiments that the phosphoryl transition state in complexes of the PTS comprises a pentacoordinate phosphoryl group in a trigonal bipyramidal geometry, with donor and acceptor atoms at apical positions and the oxygen atoms of the phosphoryl group lying in an equatorial plane (67, 68). The His-89\textsuperscript{a}(Ne2)-P and His-15(NB1)-P distances can lie anywhere between 1.8 and 3.5 Å corresponding to pure associative and pure dissociative transition states, respectively, and the phosphorus atom lies in the plane of the imidazole group of both active site histidines.

To model the transition state, we therefore carried out conjoined rigid body/torsion angle-simulated annealing calculations using exactly the same protocol and experimental restraints as those used for the unphosphorylated complex but with the addition of covalent geometry restraints for the pentacoordinate phosphoryl group and the introduction of backbone torsional degrees of freedom for residues 13–17 of HPr encompassing the active site. The overall backbone r.m.s. shift between the restrained regularized mean structures of the transition state and unphosphorylated complexes is 0.5 Å overall, and 0.3 Å for the interface (Fig. 6B), which is well within the errors of the NMR coordinates. In addition, there are only minor perturbations in side chain positions (Fig. 6B). Thus, one can conclude that the transition state can be readily accommodated without any significant perturbation in backbone conformation. Furthermore, agreement with the experimental restraints and indicators of structural quality are unaffected by the introduction of the phosphoryl transition state (Table 1).

The phosphoryl group in the transition state is hydrogen bonded to the hydroxyl group of Thr-16 of HPr, the His2 atom of His-93\textsuperscript{c}, the guanidino group of Arg-17 forms potential salt bridges with the hydroxyl group of Ser-33\textsuperscript{A} and the side chain carboxyl of Gln-30\textsuperscript{A}, with the orientation of the side chain of Arg-17 further stabilized by an intramolecular interaction between its guanidino group and the side chain carboxyl of Gln-21; and Lys-24 and Lys-73 form potential salt bridge and longer range electrostatic interactions with the carboxylate of Glu-19\textsuperscript{A} (Fig. 5A). In addition, the carboxylate of H89E\textsuperscript{A} is sufficiently close (<5 Å) to the hydroxyl group of Thr-16 to allow for an electrostatic interaction that may explain why the IIA\textsuperscript{Chb} (H89E)-HPr complex is of higher affinity than either the IIA\textsuperscript{Chb}-HPr or IIA\textsuperscript{Chb}-HPr(H15D) complexes.

![Figure 4. Interaction surfaces for the IIA\textsuperscript{Chb}(H89E)-HPr complex.](image-url)
The interaction surfaces share 10 residues in common for subunit A and 9 for subunit C. The residues that are not shared by the two interaction surfaces are located at the peripheries of the binding sites. In the view shown in Fig. 4, the binding surface for HPr extends slightly upwards to include Ser-33A of subunit A and Arg-58C, Asn-62C, and Met-98C of subunit C, whereas the binding surface for IIBChb extends slightly downwards to include Glu-15A of subunit A, and Gly-74C and Gly-77C of subunit C (43). These small differences can be readily appreciated by the superposition of the two complexes shown in Fig. 7, and probably reflect two factors: first, the slightly larger size of the binding site on IIBChb, which comprises 29 residues versus 19 for HPr; and second the slightly more peripheral location of His-15 relative to its counterpart on IIBChb. The active site residues, H89E of IIAChb*(H89E), and His-15 of HPr are shown in bold letters.

Although small, the above differences nicely illustrate the concept of redundancy in a system in which one partner, IIAChb*, recognizes multiple partners, while making use of the same active site residue (His-89) to effect phosphoryl transfer. Thus, the four additional residues at the top edge of the IIAChb binding surface for HPr that are not used in the interaction with IIBChb, namely Ser-33A, Arg-58C, Asn-62C, and Met-98C (Fig. 4A), are all involved in potential hydrogen bonding and electrostatic interactions with HPr (Fig. 5, A and B) that contribute to correctly orienting HPr relative to IIAChb*. The same is true of Glu-15A, located at the bottom edge of the IIAChb binding surface for IIBChb but absent from the interaction with HPr, which forms a salt bridge with Lys-86 of IIBChb (43).

At the same time, side chain conformational plasticity allows side chains to participate in similar interactions (cf. Fig. 5 of this paper and Fig. 4 of Ref. 43). For example, Gln-30A forms a hydrogen bond with Arg-17 of HPr and Tyr-62 of IIBChb, both located in rather similar positions relative to their respective active site residues. Likewise, Glu-19A is involved in a potential salt bridge with Lys-27 of HPr and a potential electrostatic interaction with the hydroxyl group of Ser-33 of IIBChb. Finally, Glu-73C is hydrogen bonded to both the backbone amide of Leu-53 and the side chain amide of Gln-51 of HPr, and to the side chain guanidino group of Arg-24 of IIBChb. As a final example, the interaction of Met-22A with Phe-48 of HPr (Fig. 5A) is
Solution Structure of the IIAChb-HPr Complex

FIGURE 6. The phosphoryl transition state of the IIAChb-HPr complex. A, environment surrounding the His-89^-P-His-15 pentacoordinate phosphoryl transition state. The backbone is displayed as transparent tubes with HPr in red, and the A and C subunits of IIAChb in blue and green, respectively. B, identical view to A showing a superposition of the structure of the IIAChb-(H89E)-HPr complex (transparent tubes and bonds) with the structure of the IIAChb^-P-HPr transition state (opaque tubes and bonds). Exactly the same experimental restraints are used to calculate the two structures, but, in addition, the calculations for the transition state include geometric restraints specifying the geometry of the phosphoryl transition state and backbone torsion angle degrees of freedom for residues 13–17 of HPr encompassing the active site His-15. Color coding: red, HPr; blue, A subunit of IIAChb^-; green, C subunit of IIAChb^-Side chains are displayed as stick diagrams with the atoms color coded according to type (carbon, gray; nitrogen, blue; oxygen, red; phosphorus, gold; sulfur, yellow). Residues of HPr are labeled in italics. Dashed black lines indicate hydrogen bonds to the phosphoryl group in the transition state, and the dashed gray line indicates a potential intermolecular hydrogen bond between the carboxamide group of Asn-12 of HPr and the Met-98(S8) atom of the C subunit of IIAChb^-.

FIGURE 7. Comparison of the IIAChb-HPr and IIAChb^-IIBChb complexes. A, overall stereoview with IIAChb^- from the two complexes best-fitted to one another, and, B, close up of the His-P-His and His-P-Cys phosphoryl transition states for the IIAChb^-HPr and IIAChb^-IIBChb complexes, respectively. The backbone is displayed as a ribbon diagram and the His-P-His and His-P-Cys transition states as stick diagrams with the atoms color coded according to type (carbon, gray; nitrogen, blue; oxygen, red; phosphorus, gold; sulfur, yellow). For the IIAChb^-HPr complex, IIAChb^- and HPr are shown in red and blue, respectively; for the IIAChb^-IIBChb complex, IIAChb^- and IIBChb are shown in gray and purple, respectively. The coordinates of the IIAChb^-IIBChb complex are taken from Ref. 43 (PDB code 2WWW). The small differences in the IIAChb^-coordinates from the two structures is within coordinate error. Also note that the region that displays the largest apparent differences is the loop from residues 77 to 84 of IIAChb*, which is disordered in solution. 

very similar to that with Tyr-84 of IIBChb, except that the hydrophobic contacts between these two pairs of residues is supplemented by a potential hydrogen bond between the S6 atom of Met-22^ and the hydroxyl group of Tyr-84.

Concluding Remarks—The structure of the IIAChb^-HPr complex in the present paper completes the structure elucidation of representative soluble complexes for all four sugar branches of the PTS (15, 16, 18, 36 – 40, 43). This collection of structures provides a paradigm of protein recognition in signal transduction pathways that allows for multiple recognition partners, transient interactions, and specificity.

Although the structures of the IIA components of the four sugar branches bear no sequence or structural similarity to one another, their recognition surfaces for HPr are remarkably similar in shape and residue composition. Moreover, each enzyme IIA makes use of highly overlapping surfaces to recognize both its upstream partner HPr and its downstream partner, enzyme IIB (this paper and Refs. 15, 16, 37 – 40, and 43).

The ability to recognize multiple different partners relies on a number of design features. First, similar surfaces are constructed from completely different underlying structural elements. Thus, the shape of the binding surfaces on HPr and the four classes of enzymes IIB are convex in shape and similar in size. Likewise, all four classes of enzymes IIA have a concave binding surface of similar size. Second, all the surfaces generally share similar features comprised of predominantly hydrophobic residues, interspersed by uncharged polar residues, at the center of the interface surrounded by polar and charged residues at the periphery. Third, the interactions surfaces are all large (600–1000 Å^2), thereby allowing considerable redundancy in the intermolecular interactions that have to be formed to achieve appropriate docking and orientation of the phosphoryl transfer complexes. A corollary to large surfaces and redundancy of specific intermolecular interactions is that all the complexes are transient and weak ranging from K_D values of 10 μM to the millimolar range (this paper and Refs. 15, 16, 36 – 40, 42, and 43)). Fourth, conformational plasticity of amino acids with long side chains (such as Arg, Lys, and Glu) permit similar types of intermolecular interactions to occur across complexes involving one shared partner. Finally, although HPr uses the same binding surface to recognize enzyme I and all four classes of enzyme IIA, and the binding surfaces on enzymes IIA used to interact with HPr and the corresponding enzymes IIB are highly overlapping, the absence of any detectable interaction between enzyme I and any of the enzymes IIB arises through electrostatic selection. The binding surface on HPr contains no negative charges, and the charged residues on the binding surface of enzyme I are predominantly negative. In contrast, the binding surfaces on enzymes IIA and IIB comprise a mixture of positively and negatively charged residues that largely complement one another. Thus these charged residues are either involved in intermolecular salt bridges, hydrogen-bonding interactions, or participate in van der Waals contacts. Intermolecular electrostatic repulsion, however, between like-charged residues is avoided. The positively charged residues located in the binding surface of the enzymes IIA are accommodated by the binding surface of HPr, either by making use of their long side chains in hydrophobic contacts, or by electrostatic interactions with polar groups (e.g. in the case of the IIAChb^-HPr complex, Arg-58^ and Lys-82^ of subunit C of IIAChb interact with the side chain carboxyls of Gln-57 and Gln-51 of HPr, respectively).

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Solution Structure of the IIAChb–HPr Complex

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