An Atomic Model for Protein-Protein Phosphoryl Group Transfer*

Osnat Herzberg
From the Center for Advanced Research in Biotechnology, Maryland Biotechnology Institute, University of Maryland, Shady Grove Campus, Rockville, Maryland 20850

The high resolution crystal structures of two interacting proteins from the phosphoenolpyruvate:sugar phosphotransferase system, the histidine-containing phosphocarrier protein (HPr) and the IIA domain of glucose permease (IIA<sup>Glu</sup>) from Bacillus subtilis, provide the basis for modeling the transient binary complex formed during the phosphoryl group transfer. The complementarity of the interacting surfaces implies that no major conformational transition is required. The negatively charged phosphoryl group is buried in the interface, suggesting a key role for electrostatic interactions. It is proposed that the phosphoryl transfer is triggered by a switch between two salt bridges involving Arg-17 of the HPr. The first, prior to phosphoryl group transfer, is intramolecular, with the phosphorylated His-15. The second, during the transfer, is intermolecular, with 2 aspartate residues associated with the active site of IIA<sup>Glu</sup>. Such alternating ion pairs may be mechanistically important in other protein-protein phosphotransfer reactions.

Protein phosphorylation and phosphoryl group transfers are central to a wide range of processes such as metabolism, gene regulation and protein synthesis, muscle contraction, membrane transport, and signal transduction (1-4). Isotopic labeling experiments are consistent with the interpretation that a single or odd number of phosphotransfer steps proceeds with inversion of the configuration at phosphorus, while an even number of steps results in retention of configuration (5). This is consistent with the donor and acceptor molecules forming a complex with a pentacoordinated phosphoryl group in a trigonal bipyramidal geometry, such that the donor and acceptor are in apical positions and the oxygen atoms are equatorial. Presumably, the protein-protein interface that accommodates the negatively charged phosphoryl group must be exquisitely designed, including specific machinery to trigger the transfer. Until recently, such an interface could not be visualized because of lack of detailed three-dimensional structures of the protein pairs involved. With the aid of the recently determined crystal structures of such a pair: HPr<sup>i</sup> (6) and the IIA domain of the glucose permease (7) from the bacterial phosphoenolpyruvate:sugar phosphotransferase system (PTS), it is now possible to address mechanistic issues at the atomic level.

The PTS transports some carbohydrates across the cytoplasmic membrane and simultaneously phosphorylates them to initiate their metabolism and prevent leakage out of the cytoplasm (8). It also plays a role in chemotaxis toward PTS sugars, and in the regulation of the uptake of several non-PTS sugars. The system consists of two general energy-coupling components (Enzyme I and HPr), and of sugar-specific permeases (Enzymes II). An Enzyme II typically consists of three domains, IIA (also designated Enzyme III or Factor III), IIIB, and IIC (9). A total of five phosphoryl group transfers occur along the pathway, three of which involve protein-protein interactions.

Enzyme I-(His)<sup>+</sup> + PEP → Enzyme I-(His)~P + pyruvate
Enzyme I-(His)<sup>+</sup>~P + HPr → Enzyme I + HPr-(His)~P
HPr-(His)~P + Enzyme IIA → HPr + Enzyme IIA-(His)~P
Enzyme IIA-(His)~P + Enzyme IIIB →
Enzyme IIIB~P + Enzyme IIA

Enzyme IIIB~P + sugar(out) → Enzyme IIB + sugar ~ P(in)

**REACTIONS 1-5**

Phosphorylations of histidyl residues, such as occur in the PTS, are not unusual. Many bacterial histidine protein kinases have been identified (4).

The crystal structures of HPr (~9 kDa) and of the IIA<sup>Glu</sup> (~17 kDa) from the Bacillus subtilis PTS have been determined (6, 7) and refined at high resolution (2.0 and 1.9 A, respectively). The HPr phosphorylation site is the N<sup>ε</sup> atom of His-15. The crystal structure shows the side chain of the evolutionarily invariant Arg-17 to be in close proximity to the side chain of His-15, with a sulfate anion in between. We proposed that this crystal structure resembles the phosphorylated state of the protein, and modeled it such that the phosphoryl group on the N<sup>ε</sup> atom of His-15 is close to the position of the sulfate. The phosphorylation site of IIA<sup>Glu</sup> is the N<sup>ε</sup> atom of His-83 (equivalent to His-90 of Enzyme III<sup>Glu</sup> from Escherichia coli). The crystal structure reveals an intriguing interaction between His-83 and a second invariant histidine, His-68 (equivalent to His-75 in the E. coli Enzyme III). A model of the phosphorylated protein indicates that the phosphoryl group interacts with His-68. The structure of IIA<sup>Glu</sup> also highlights 2 conserved aspartate residues that are located close to the active site, which may have a functional role. A clustering of hydrophobic residues is associated with each of the active sites. This is particularly striking in the case of IIA<sup>Glu</sup>. Such clusters are interpreted as indicating the region of protein-protein contacts during the phosphoryl group transfer.

With the above structural information in mind, the modeling of the transient complex formed between HPr and IIA<sup>Glu</sup> has been undertaken. The results of these studies are presented below.
The crystal structures of HPr and of IIA Glc from B. subtilis were determined using the multiple isomorphous replacement method (6, 7). The two structures have been refined by a combination of molecular dynamics refinement (10) and re-}

strain-parameter least-squares methods (11, 12). HPr (accession number 1HPR in the Protein Data Bank; Ref. 13) has been refined at 2.0Å resolution to a crystallographic R-factor of 0.145 (R = Σ|Fo| - |Fc| |Σ|Fo|, where |Fc| and |Fo| are the observed and calculated structure factor amplitudes, respectively). The IIA domain has been refined at 1.9Å resolution to a crystallographic R-factor of 0.155 (1GPR in the Protein Data Bank). The stereochemical parameters are well within the range known from crystal structures of small peptides. Details of the refinement will be published elsewhere.

The complex was modeled on a Silicon Graphics Iris 4D workstation, using the Polygen software package QUANTA. A phospho-histidine was built on the N atom of His-15 of HPr, with a planar phosphoryl group, and geometrical parameters consistent with small molecule crystal structures and with quantum mechanical calculations. The dihedral angle of His-15 was rotated by 10° to avoid close contacts between one of the phosphoryl oxygen atoms and main-chain atoms of the following helix. No other modification have been made to either protein.

The HPr-P model was docked to the IIA domain, with the phosphorus atom positioned 2.0 Å away from the N atom of the active site His-83, such that a trigonal bipyramidal geometry at phosphorus was formed with the N atom of His-15 of HPr and the N atom of His-83 of the IIA Glc in apical positions. The HPr molecule was then rotated around the apical axis to minimize close contacts between the two proteins. The relative orientation was selected by visual inspection and should not be considered precise. Nevertheless, only a narrow range of axial rotations avoids substantial interpenetration of the two molecules.

Short contacts between side-chains created by the above procedure (there were no clashes between main-chain atoms) were relieved by the CHARMM energy minimization (14) available in QUANTA. Prior to minimization, a planar PO₃ group was defined in the dictionaries, and key distances that maintain the trigonal bipyramidal geometry of the phosphoryl group transition state were restrained. The purpose of the minimization was to demonstrate that formation of the complex does not impose major steric clashes rather than to accurately evaluate the electrostatic energy of the system. The latter would require accurate potentials and knowledge of the protonation states of the different groups, including that of the pentacoordinated phosphorus. Thus, the phosphoryl group and the histidine residues were treated as uncharged. All other charges were included. Four hundred steps of steepest descent and 400 steps of conjugate gradient energy minimization reduced the energy from +8.5 × 10⁶ kcal/mol to −11,466 kcal/mol. The minimized complex demonstrates that the interface is acceptable by van der Waals criteria.

A model of a productive complex HPr-P-IIAGlc can be formed without a requirement for any gross conformational transition (Fig. 1). Those short contacts created by the manual docking procedure were readily relieved during the energy minimization, while the rest of the structure was not significantly altered. Superposition of atomic coordinates showed that the r.m.s. difference before and after minimization was 0.6 Å for a-carbons and 0.8 Å for all atoms. Note that a shift of 0.5 Å is commonly observed in any energy minimization of protein crystal structures.

The molecular surfaces at the interface traced out by the edge of a probe of radius 1.4 Å were calculated using the Connolly algorithm (15) as available in QUANTA. The interface is formed by two complementary surfaces, such that no large cavities are introduced (Fig. 2). Almost the same extent of surface area of each molecule has been buried: 536 Å² of HPr and 563 Å² of IIA Glc. These values are about 20–30% lower than those reported for complexes between antibodies and antigens (16, 17). Note that although antibodies associate tightly with antigens, the PTS protein complexes are transient, since once a phosphoryl group transfer has occurred, the complex should dissociate to allow for the next step. The extent of buried surface area is also lower (10–20%) than those found for protease-inhibitor complexes (17). The number of residues that form intermolecular contacts is of the
The crystal structures of HPr and of IIA^Glc reveal hydrophobic patches associated with each of the active sites (6, 7). That of IIA^Glc is particularly extensive. In the modeled complex, these patches form part of the interface, as can be seen from the compilation of hydrophobic contacts in Fig. 3. There are only five inter-protein electrostatic interactions that do not involve key catalytic residues (Fig. 3). The most striking electrostatic interactions other than those involving the phosphoryl moiety occur in the active site between the guanidinium group of Arg-17 of HPr and the 2 aspartate residues of IIA^Glc, Asp-31 and Asp-87 (Fig. 4).

The HPr histidyl residue with the phosphoryl transfer function, His-15, is located on a convex surface of the protein, at the N-terminus of the first α-helix (6). By slightly adjusting the side-chain conformations of His-15 and Arg-17, we have proposed a model for HPr-P, with a tetrahedral phosphoryl group covalently bonded to the N^α atom of His-15. The negative charge is stabilized by a salt bridge to Arg-17 and by electrostatic interactions with the two main-chain nitrogen atoms of Ala-16 and Arg-17 of the following helix. On the other hand, the crystallographically observed conformation of Arg-17 is ideal for forming a salt bridge with Asp-31 and Asp-64 on the IIA^Glc domain of the modeled complex (Fig. 4). Similarly to the putative tetrahedral phosphoryl group of HPr-P, the pentacoordinated phosphoryl group in the modeled complex maintains its interactions with Ala-16 and Arg-17 main-chain nitrogen atoms.

For IIA^Glc-P we also proposed a tetrahedral phosphoryl group, this time covalently bonded to the N^α atom of His-83, located in a shallow depression on the surface of the molecule (7). This modeled phosphoryl moiety forms an electrostatic
interaction with the side chain of the adjacent His-68. In addition, it also interacts with the main chain nitrogen atoms of Thr-88 and Val-89. As noticed by Worthylake et al. (18), the disposition of these two nitrogen atoms is reminiscent of the oxy anion hole environment seen in the serine proteases, indicating a role for these atoms in stabilizing a negatively charged species. In the modeled complex, the interaction of the phosphoryl group with His-68 is maintained (Fig. 4), but

The negatively charged phosphoryl group is buried in the interface of the transient complex; thus, electrostatic interactions must play an extremely important role. Indeed the model shows that a switch between two salt bridges is central to the exchange of the phosphoryl group between HPr and IIA. In free HPr-P Arg-17 is available to form an ion pair with the phosphoryl group, whereas upon formation of HPr-P-IIA the two carboxylates of Asp-31 and Asp-87 of IIA are exquisitely positioned to form an alternate ion pair with Arg-17 of HPr. Evaluation of the relative energetics of
the two phosphorylated proteins that define the preferred direction of the process awaits detailed computational analysis. It is interesting to note that the crystal structure of the catalytic subunit of the cyclic AMP-dependent protein kinase bound to a peptide inhibitor also reveals several salt bridge interactions between arginine residues on the inhibitor and carboxylate groups on the enzyme (22).

Finally, forming a multimolecular complex in which more than one phosphoryl transfer step can occur at a time is improbable, considering the nature of the surfaces. Such a complex would require gross unfolding of the proteins involved, to accommodate the various phosphoryl group donors and acceptors in an appropriate spatial arrangement that allows several transfers, each via a trigonal bipyramidal phosphorus, to occur. This would be unprecedented.

Acknowledgment—I thank Walt Stevens for many helpful discussions.

REFERENCES