Activation of the Hetero-octameric ATP Phosphoribosyl Transferase through Subunit Interface Rearrangement by a tRNA Synthetase Paralog*

Karen S. Champagne†§, Marie Sissler†§, Yuna Larrabee‡, Sylvie Doublie‡§, and Christopher S. Franklyn*†§

From the Departments of †Microbiology and Molecular Genetics and §Biochemistry, University of Vermont, Burlington, Vermont 05405

ATP phosphoribosyl transferase (ATP-PRT) joins ATP and 5-phosphoribosyl-1-pyrophosphate (PRPP) in a highly regulated reaction that initiates histidine biosynthesis. The unusual hetero-octameric version of ATP-PRT includes four HisG₈ catalytic subunits based on the periplasmic binding protein fold and four HisZ regulatory subunits that resemble histidyl-tRNA synthetases. Here, we present the first structure of a PRPP-bound ATP-PRT at 2.9 Å and provide a structural model for allosteric activation based on comparisons with other inhibited and activated ATP-PRTs from both the hetero-octameric and hexameric families. The activated state of the octameric enzyme is characterized by an interstitial phosphate ion in the HisZ-HisG interface and new contacts between the HisZ motif 2 loop and the HisG₈ dimer interface. These contacts restructure the interface to recruit conserved residues to the active site, where they activate pyrophosphate to promote catalysis. Additionally, mutational analysis identifies the histidine binding sites within a region highly conserved between HisZ and the functional HisRS. Through the oligomerization and functional re-assignment of protein domains associated with aminoaacetylation and phosphate binding, the HisZ-HisG₈ octameric ATP-PRT acquired the ability to initiate the synthesis of a key metabolic intermediate in an allosterically regulated fashion.

Phosphoribosyl transferases (PRTs)§ catalyze the attack of a nitroreagent and/or aromatic base on 5-phosphoribosyl-1-pyrophosphate (PRPP), and thereby participate in essential reactions in the biosynthesis of nucleotides and the amino acids tryptophan and histidine (1, 2). The largest family (type I) of these structurally diverse enzymes includes many nucleotide salvage enzymes that share a five-stranded parallel β sheet fold with a substrate binding hood domain (3, 4). The folds of type II (5) and type III enzymes (6) are distinct from the class I enzymes, and the type III enzymes resemble nucleoside phosphorylase. Among the PRTs with complex quaternary structures and sophisticated regulation are the glutamine PRPP amidotransferase, which catalyzes the first committed step of purine biosynthesis (7), and ATP phosphoribosyl transferase (ATP-PRT), which joins ATP and PRPP to initiate synthesis of histidine (8, 9). Glutamine PRPP amidotransferase and ATP-PRT both exhibit pathway end product inhibition, and regulation by cellular energy levels (7, 10). ATP-PRT is competitively inhibited by AMP and ADP (8, 11–13) and non-competitively inhibited by histidine (Fig. 1) (8, 13). Despite decades of investigation, the structural basis of this regulation, and for PRTs in general, is not well understood.

ATP-PRTs constitute two distinct subfamilies with different quaternary structures but share a conserved catalytic domain that currently represents the sole member of a fourth (type IV) PRT family. The nominally hexameric "long form," (or HisG₇₈) enzymes (14–16) were characterized during early efforts to understand amino acid biosynthesis regulation in enteric bacteria (10, 11). Structures of the HisG₇₈ subfamily recently determined include apo and histidine-AMP complexes from Mycobacterium tuberculosis ATP-PRT (17), and AMP-bound and N-1-(5′-phosphoribosyl)-ATP (PR-ATP product) bound complexes of HisG₈ ATP-PRT from Escherichia coli (18). The hexameric HisG₇₈ enzymes possesses a bi-loval catalytic domain reminiscent of periplasmic binding proteins that bind sulfate, phosphate, and other small ligands (19, 20). The second ATP-PRT subfamily ("short form"; HisG₅₈) is defined by hetero-octameric enzymes composed of two subunit types (21). The catalytic subunit is denoted HisG₅₈ to reflect its relationship with HisG₉ (~25% sequence identity), as well as the absence of 85–100 residues that comprise the C-terminal regulatory region in the long form (21, 22). Hetero-octameric ATP-PRTs possess a second subunit type, HisZ, which is related to the catalytic domain of functional histidyl-tRNA synthetases (HisRSs), but is inactive on its own (21–23). The hetero-octamer is assembled from two dimers each of HisG₅₈ and HisZ (24), and both are required to reconstitute catalytic activity (21). The recent structure of the histidine-bound ATP-PRT complex from Thermotoga maritima provided the first view of the short form variant subfamily, and confirmed predicted structural relationships between HisG₅₈ and HisG₉, and between HisZ and HisRS (25). However, no conclusions could be drawn about the structural basis of PRT function and its activation.

Here, we present phosphate-bound and PRPP-bound structures of the hetero-octameric ATP-PRTs that represent the first illustrations of
the activated state. Comparison of these structures to the apo-, histidine/AMP, and PR-ATP-bound hexameric complexes of HisGΔ, and the histidine-bound complex of the hetero-octameric subfamily reveals a common activation mechanism resembling those of glycosyltransferases in general. These new structures define a specific structural role of the aminoacyl-tRNA synthetase-like subunit in the allosteric activation of the PRT reaction, and thereby illustrate how catalytic function emerged from the collaborative adaptation of two functionally distinct ancestral protein domains.

EXPERIMENTAL PROCEDURES

Construction of Mutant Proteins—Mutant versions (E130A and Y268F/Y269F) of the HisG-HisZ ATP-PRT were derived from a Lactococcus lactis pQE30 expression construct (21) by use of the QuickChange® procedure (Stratagene). The double-stranded primers were 45 nucleotides in length, and the mutations were verified by DNA sequencing.

Overexpression and Purification of Native and Selenomethionyl HisZ-HisG ATP-PRT—The octameric ATP-PRT complex from L. lactis was purified from an E. coli overexpression strain according to a previously published protocol (24). Selenomethionyl-octameric ATP-PRT was overexpressed by inhibiting the methionine pathway (26), and purified in the same fashion as the wild type enzyme. Pooled fractions from the final hydroxyapatite column were concentrated to 15–17 mg/ml, dialyzed into 50 mM Na2PO4 (pH 6.0), 300 mM NaCl, 10% (v/v) glycerol, 10 mM β-mercaptoethanol, and stored at 4 °C for crystallization experiments.

Crystallization and Data Collection—Crystals of the L. lactis complex were obtained by incubation at 12 °C by the hanging drop method using 2 μl of well solution (15–25% (v/v) polyethylene glycol 400, 0.1 M Tris-HCl, pH 7.5, 0.2 mM MgCl2) and 2 μl of protein sample at 10–16 mg/ml and 10 mM ATP (or 10 mM N-1-methyl-ATP and 5 mM PRPP) in the drop. The growth of well ordered crystals was dependent on ATP or N-1-methyl-ATP, although the ATP molecule was not visible in the final electron density maps. Initial selenomethionyl crystals were obtained by essentially the same procedures, and then improved by microseeding. Crystals grown in the presence of ATP were derivatized with 2.5 mM sodium tungstate dihydrate (Na2WO4·2H2O). Cryoprotection was performed by addition of 17–18% glycerol before flash cooling in liquid nitrogen. All crystals grew to ~0.4 × 0.2 × 0.03 mm3 in an orthorhombic space group (P21212) with unit cell parameters of a = 151.68 Å, b = 222.93 Å, c = 86.38 Å. There are four molecules each of HisG and HisZ per asymmetric unit, with an estimated solvent content of 57%. X-ray data were collected at 100 K on a Brandeis B1.2, ADSC Q315, or an ADSC Q4 CCD detector on beamlines X12C, X25, or X26C, respectively, at the National Synchrotron Light Source (Upton, NY). Complete selenomethionyl MAD data sets to 3.1 Å were collected for peak and remote wavelengths on X25, and another remote data set at X26C. The PO4-bound dataset served as the reference for refinement, and was collected at 1.2124 Å on beamline X26C from a tungstate-derivatized crystal that diffracted to 2.9 Å. An additional 3.2-Å dataset was collected from a crystal grown in the presence of N-1-ATP and PRPP. All datasets were processed and scaled using DENZO and SCALEPACK (27), and the data statistics are summarized in TABLE ONE.

Structure Determination and Refinement—Twenty of the 48 possible selenium sites in the asymmetric unit were initially determined with SHELXD (28). These sites were refined with SOLVE (29), allowing identification of 24 additional selenium atoms. Further heavy atom refinement and phasing of the resulting 44 selenium atom substructure, followed by density modification using the 4-fold non-crystallographic symmetry with RESOLVE (30), resulted in an interpretable electron density map. Model building was accomplished with the program O (31). Subsequent rounds of refinement were performed using CNS (32). Simulated annealing with a 4-fold NCS restraint was alternated with manual rebuilding. TLS refinement using REFMAC within the CCP4 suite (33) proved valuable in reducing the free R factor. The PRPP bound model was refined using rigid body refinement followed by simulated annealing in CNS (32). The PRPP-bound and PO4-bound structures contained a total of 2064 and 2056 residues of 2116, respectively. In the final models all non-glycine residues fell into the allowed regions of the Ramachandran plot. Refinement statistics are reported in TABLE ONE.

Histidine Inhibition Assays—ATP phosphoribosyl transferase activity was measured by following the formation of PR-ATP at 22 °C over time (9). Reactions included ATP-PRT at a concentration of 100 nM, and were initiated with PRPP. The absorbance at 290 nm was detected every 9 s for 10 min using a BioMate5 spectrophotometer. End product inhibition was examined by the inclusion of 1 mM histidine. The baseline absorbance was established by setting A340 to zero for the reaction...
mixture before adding PRPP. Each experiment was repeated at least five times.

RESULTS

Overall Structure and Monomer Architecture—The 2.9-Å crystal structure of the phosphate-bound (ATP activated) \textit{L. lactis} hetero-octameric ATP-PRT was solved by multilength anomalous diffraction, and a PRPP-bound (N-1-methyl-ATP activated) form was refined to 3.2 Å (TABLE ONE). Because of the fact that these forms were crystallized in the presence of ATP or N-1-methyl-ATP (which sedimentation velocity experiments suggest stabilize the 10.7 S R-form of the complex (24)), they presumably represent the active form of the complex. The overall architecture of the \textit{L. lactis} complex features an X-shaped central core composed of the HisRS-like HisZ subunits, with dimers of HisG\textsubscript{c} catalytic subunits inserted into either end (Fig. 2a), and is generally similar to the histidine-bound complex from \textit{T. maritima} (25). The HisG\textsubscript{c} subunits closely resemble HisG\textsubscript{c} subunits from the hexameric ATP-PRTs (17, 18), but lack the C-terminal histidine binding domain (Fig. 2b). The HisG\textsubscript{c} fold consists of two \(\alpha/\beta\) domains connected by a twisted \(\beta\) ribbon. Domain I (residues 1–90 and 178–205) folds into a six-stranded mixed \(\beta\) sheet flanked by five \(\alpha\) helices, whereas domain II (residues 91–177) comprises a five-stranded \(\beta\) sheet with one helical crossover connection above and below. The active site lies in the crevice between these two domains. The HisZ subunits (Fig. 2c) feature the seven-stranded antiparallel \(\beta\) sheet flanked by 3–4 \(\alpha\) helices and class defining motifs found in HisRS (34) and class II aminocyl-tRNA synthetase paralogs (35, 36), but possess an \(\alpha/\beta\)-helical insertion domain with a different topology.

The HisG\textsubscript{c} and HisZ monomers from the PRPP-bound (\textit{L. lactis}) and histidine-bound (\textit{T. maritima}) complexes can be readily superimposed, but there are notable structural differences. In the phosphate-bound and PRPP-bound \textit{L. lactis} complexes, residues 30–35 of HisG\textsubscript{c} form a poorly structured loop, whereas these constitute a well ordered \(\beta\) strand in the histidine-bound \textit{T. maritima} complex (Fig. 3a, arrow) (25). Conversely, residues 142–147 comprise helix Ga6 in the phosphate-bound and PRPP-bound complexes, but lose substantial helical character in the histidine-bound complex. In the \textit{L. lactis} complexes, the loop and Ga6 are critical components of the HisG\textsubscript{c} dimer interface that undergo conformational changes in response to activation.

Dimeric Interactions and the HisZ-HisG Interface—The two monomers in each HisG\textsubscript{c} dimer pack in an antiparallel fashion, creating a hydrophobic interface defined by contacts between Ile-47, Phe-57, and Ile-62 from domain I with Leu-143’ and Val-147’ from domain II (Fig. 4a). Hydrogen bonds between Asn-52 and Glu-138’, and between Asp-53 and the main chain NH of Glu-138’ also strengthen the dimer. The HisZ dimer is stabilized by the symmetrical interaction of Zα1 and Zα1’ in the bottom of the interface, and extensive hydrophobic interactions from aromatic residues that lie under the antiparallel \(\beta\) ribbons that arch over the interface (Fig. 4b). Notably, the histidine-bound \textit{T. maritima} and \textit{L. lactis} PRPP-bound complexes differ with respect to the motif 2 loop. Whereas all four motif 2 loops are ordered in the \textit{T. maritima} complex, only one motif 2 loop is ordered in each of the HisZ dimers in the \textit{L. lactis} complexes.

The four HisZ-HisG interfaces in the phosphate-bound and PRPP-bound \textit{L. lactis} complexes feature the interaction of HisG\textsubscript{c} domain I (especially Gβ5 and Ga6–α9) with the insertion domain and antipar-
allel β sheet of HisZ (Fig. 5a). In the two HisZ subunits with ordered motif 2 loops, the loop interacts directly with helix Ga2 in the HisGdimer interface (Fig. 5b). Principal contacts include salt bridges between Lys-118 and Arg-120 in the motif 2 loop with Glu-59 and Asp-77, which are located at the C termini of Ga2 and Ga3 in HisGd, respectively (Fig. 5b). Notably, this motif 2 loop/HisGdimer interaction is conspicuously absent in the T. maritima histidine-bound complex (25). The PRPP- and histidine-bound complexes are also distinguished by an interstitial phosphate ion seen in the two L. lactis HisZ-HisG interfaces with ordered motif 2 loops, but not in the T. maritima complex (Fig. 5a).
position of the phosphate is fixed by Tyr-82 and Lys-189 in HisGS and Lys-275 and Tyr-277 from HisZ. The association of the interstitial phosphate with the two HisZ subunits possessing ordered motif 2 loops, as well as the high degree of conservation of the residues serving as its ligands (supplemental materials Fig. S1a and S1b) suggests that its presence is not merely a crystallization artifact. By virtue of its location in the HisZ-HisG interface, and its absence in the T. maritima histidine-bound complex, the interstitial phosphate may work in concert with the HisZ motif 2 loop to alter the structure of the HisG dimer interface, thereby promoting transition to the activated state.

The PRPP Binding Site and Activation of PRT Function—Prior sedimentation analysis of the hetero-octameric L. lactis complex revealed that ATP stabilizes a 10.7 S activated R state, whereas histidine and AMP in combination promote transition to a 9.5 S inhibited T state (24). The phosphate-bound and PRPP-bound L. lactis complexes were crystallized in the presence of ATP and N-1-ATP, respectively. Even though no density was seen for ATP, the sedimentation analysis supports the assignment of these L. lactis complexes to the R state. In contrast, the T. maritima histidine-bound hetero-octamer represents a putative histidine-inhibited T state complex (25). Similarly, the hexameric M. tuberculosis histidine/AMP bound HisG2 (17) and PR-ATP bound E. coli HisG2 complexes (18) represent, respectively, inhibited and activated forms. The structural alignment of HisG monomers from all published ATP-PRT structures revealed that pairs of monomers representing the same state (i.e. "activated" or "inhibited") exhibited lower root mean square deviations (normalized to 100 Cα) than pairs representing different states (TABLE TWO). Thus, the identity of the bound ligand was a more reliable indicator of structural similarity than whether or not the two proteins belonged to the same subclass (i.e. HisG2 or HisGα). The observed structural differences between active and inhibited states are therefore not merely artifacts of comparing enzymes from different organisms.

Crystals of the L. lactis enzyme grown in the presence of N-1-methyl-ATP and PRPP provided the first data on PRPP binding in either the hexameric or octameric ATP-PRT subfamilies. Whereas no density for ATP was observed in the refined maps, there was a clear density peak for PRPP (Fig. 6). The PRPP binding site is located on the C-terminal edge of the domain II β sheet, and is principally defined by interactions with helices Ga6–α7 and strand Gβ9 (Fig. 2b). Recognition of the 5′ phosphate is provided by the conserved PRPP binding loop (18), particularly the main chain amides of Thr-159 and Gly-160, and the γ-OH groups of Thr-159 and Thr-162 (Fig. 6). The 2′ and 3′ ribose hydroxyls of PRPP are similarly recognized by Asp-155 and Glu-142, respectively. Notably, structurally equivalent conserved carboxylates make identical interactions with the phosphoribosyl moiety of PR-ATP in the hexameric E. coli HisG2 complex (18). In crystals grown in the presence of ATP but in the absence of PRPP, a phosphate ion was observed to bind to the
Allosteric Activation of ATP-PRTase

TABLE TWO

| Normalized root mean square deviations (rmsd) calculated for pairwise superpositions of HisG<sub>s</sub> and HisG<sub>k</sub> monomers from various ATP-PRT structures |
|-------------------------------------------------------------------------------------------------------------|
| Alignments were created using the Fast force subroutine of LSQMAN from the O package (31). The runs were performed using a fragment size of 50, a step size of 25, and 100 as the minimum number of residues to match. The choice of the algorithm was dictated by the uneven number of Ca carbons in the comparison of HisG<sub>s</sub> and HisG<sub>k</sub> monomers. The values to the left of the hyphen in the parentheses represent the raw root mean square deviation in Å, which was calculated over the number Ca carbons given to the right of the hyphen. All Ca atoms from the individual HisG<sub>s</sub> monomers were used as input in the search for the best alignment. The values in bold type represent root mean square deviations normalized to 100 Ca atoms for comparison, according to the formula: rmsd<sub>n<sub>s</sub>=rmsd<sub>s</sub>/√n<sub>s</sub>/100, where n<sub>s</sub> is the number of amino acid residues in the raw calculation. The normalized values therefore provide a statistical basis on which to compare superpositions within and across the HisG<sub>s</sub> and HisG<sub>k</sub> families. |
| MtHisG<sub>s</sub>, apo (1NH7) | MtHisG<sub>s</sub>, inhibited His/AMP (1NH8) | EcHisG<sub>s</sub>, active PR-ATP (1Q1K) | EcHisG<sub>s</sub>, inhibited AMP (1H3D) | TmHisG<sub>s</sub>, apo (1O64) | TmHisG<sub>s</sub>, inhibited His/AMP (1USY) | L1HisG<sub>s</sub>, active PO<sub>4</sub> (1Z7M) | L1HisG<sub>s</sub>, active PRPP (1Z7N) |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| 1NH7 | 1NH8 | 1Q1K | 1H3D | 1O64 | 1USY | 1Z7M | 1Z7N |
| 0.76 | 0.76 | 1.20 | 1.21 | 1.35 | 1.37 | 1.24 | 1.26 |
| (1.06/205) | (1.77/251) | (1.54/200) | (1.76/249) | (1.60/200) | (0.32/288) | (1.59/142) | (1.58/163) |
| 1.15 | 1.19 | 1.14 | 0.21 | 1.15 | 1.08 | 1.11 | 1.24 |
| (1.54/200) | (0.32/288) | (1.42/181) | (1.43/180) | (1.40/179) | (1.69/167) | (1.44/183) | (1.30/183) |
| 1.08 | 1.35 | 1.08 | 1.37 | 1.11 | 1.10 | 1.11 | 1.27 |
| (1.42/181) | (1.43/180) | (1.74/170) | (1.29/166) | (1.65/181) | (1.40/183) | (1.44/183) | (1.40/183) |
| 1.04 | 0.99 | 1.04 | 1.26 | 1.01 | 0.98 | 1.26 | 1.23 |
| (1.30/183) | (1.33/104) | (1.32/183) | (1.38/124) | (1.30/183) | (1.23/182) | (1.38/124) | (0.52/201) |

PRPP binding loop in the same fashion as the ribose 5'-phosphate in the PRPP complex.

Superposition of the HisG Ca atoms from histidine-bound and PRPP-bound complexes suggests that the aforementioned structural elements responsible for 5' phosphate and 2' and 3' hydroxyl recognition do not undergo significant conformational changes upon activation. In contrast, side chains that contact the pyrophosphate moiety of PRPP do undergo such changes, providing a structural basis for the switch from repression to activation. In the PRPP-bound complex, strictly conserved Ser-140 is suitably positioned (~<2.5 Å) to provide leaving group activation of the pyrophosphate moiety (Fig. 6). Lys-8' is also well positioned (albeit at a distance of ~4 Å), but must reach across the dimer interface from the other monomer (Figs. 6 and 7a). In the histidine-bound T. maritima structure, this interaction is blocked by a hydrogen bond between the amino group of lysine and the main chain carbonyl of Ser-31', effectively sequestering Lys-8' within its own subunit (Fig. 7, a and b). The interaction between Ser-140 and the pyrophosphate group is also blocked in the histidine-bound complex, because of a hydrogen bond between the seryl γ-OH and the main chain carbonyl of Met-43' in the other subunit (Fig. 7b). Thus, the apparent switch from an inhibited to activated state is dictated by structural changes in the HisG<sub>s</sub> dimer interface that involve the reversible mobilization of catalytic residues that are highly conserved in ATP-PRTs (supplemental materials Fig. S1a).

Movement of Lys-8' across the dimer interface is also constrained by a conserved basic residue (Lys-50' in L. lactis and Arg-45' in T. maritima) located in the loop between Gβ3 and Gα2. In the histidine-bound complex, electrostatic repulsion between Arg-45' and Lys-8' would discourage movement of the latter across the dimer interface. In the PRPP-bound L. lactis complex, Lys-50' shifts down and away from Lys-8', alleviating this repulsive interaction (compare Fig. 7, a and b). Although there is currently no direct information about the specific binding sites for ATP in either the PRPP- or histidine-bound octameric complexes, the re-orientation of Lys-50' may be promoted by a potential contact with the γ-PO<sub>4</sub> of ATP. This hypothesis gains support from the comparative analysis of the histidine/AMP-bound HisG<sub>s</sub> M. tuberculosis and PR-ATP bound HisG<sub>k</sub> E. coli hexameric complexes, which indicates that a similar recruitment of catalytic residues across the dimer interface provides activation of PRT function in the hexameric subfamily (Fig. 7, c and d).

In the octameric ATP-PRTs, the re-arrangement of Ser-140 and Lys-8' during the activation process is coupled to additional changes in the HisG<sub>s</sub> dimer interface. In the histidine-bound T. maritima complex, strands β2-β3-β4-β1 in domain I of HisG<sub>s</sub> comprise a mixed β sheet that is stabilized by extensive inter-strand hydrogen bonds (Figs. 3a and 7b). In the PRPP-bound L. lactis complex, strands β2 and β3 are no longer paired to β4, and effectively undergo a strand to coil transition. Although density for residues 32–36 is either poor or missing in three of
Allosteric Activation of ATP-PRTase

FIGURE 7. Switch between active and inactive conformations in HisG and HisG. a, the active site of the L. lactis activated HisGdimer. The bound PRPP in the active site is stick rendered and colored by atom type. A subset of the conserved side chains (Ser-140, Glu-142, Thr-159, and Thr-162) positioned to contact the PRPP are indicated, as are side chains (Lys-K8, Arg-34, and Lys-50) that are predicted to be important in cross-subunit interactions. b, the active site of the T. maritima inhibited HisGdimer. In the inhibited structure, Ser-132 and Lys-K8 are sequestered by hydrogen bonds to the backbone oxygens of Met-43 and Ser-31, respectively, preventing the former from interacting with PRPP. Structural transitions between repression and activation in αb and β2 are denoted by changes in the helical and strand representations, respectively. c, the active site of the hexameric E. coli PR-ATP activated HisGdimer. Note the orientation of the loop from the gold subunit approaching Glu-156 in the PR-ATP binding site. d, the active site of the hexameric M. tuberculosis histidine/AMP-inhibited HisGdimer. In this structure, loop movements reposition Lys-9 (Lys-15 in E. coli) to prevent its participation in catalysis.

the four HisG subunits, the α1/β2 loop in subunit “F” can be seen to pivot toward the PRPP binding site. Arg-34 is located at the tip of this loop, and reaches toward the 5’ phosphate group of PRPP (Figs. 6 and 7a). This large loop motion could provide additional contacts to PRPP or, more likely, serve to exclude solvent molecules from the active site.

The activated form of the octameric ATP-PRT is stabilized by ATP (24), which also binds first in the mechanism determined for the hexameric enzymes (12). ATP binding may therefore promote assembly of the PRT active site, and/or conformational changes in the HisG dimer interface. Because of the absence of electron density for ATP, contacts to ATP in the L. lactis complex were inferred from the PR-ATP bound HisG, E. coli complex (18), which suggests that recognition of the ATP ribose is accomplished by conserved Asp-71. Potential ligands to the triphosphate include Lys-50, Lys-116, and Tyr-118, which are also highly conserved. These predicted functions for Lys-50 and Asp-71 are noteworthy, as these two residues are located in the N termini of helices Ga2 and Ga3, whose roles in the dimer interface and contacts to motif 2 loop were noted above (Fig. 5b). In addition, the random coil segment that immediately follows Ga3 terminates with Tyr-82, which is coordinated to the interstitial phosphate (Fig. 5a). Thus, direct structural pathways linking the HisG interface to the binding of ATP and the interstitial PO4 involving conserved residues can be readily identified, supporting the model that effector binding leads to re-arrangement of the HisG dimer interface.

The HisZ Active Site as a Potential Allosteric Binding Site for Histidine—These observations underscore the role of the HisZ-HisG interface in ATP-PRT regulation. Previously, Vega et al. (25) identified eight histidine binding sites in the octamer on the basis of difference density calculated from crystals grown in the presence of histidine. These sites do not involve conserved residues in HisZ, or those in the histidine binding pocket predicted by comparison to HisRS (supplemental materials Fig. S1b). Notably, the histidine binding loop between β8 and β9 and selected residues in motif 2 represent sequence elements that are particularly well conserved between the two families (Fig. 8, and supplemental materials Fig. S1b). To test this relationship, a single mutant was

FIGURE 8. Feedback inhibition of ATP-PRTase from L. lactis by histidine in WT and mutant enzymes. The plot indicates the formation of PR-ATP product formation over time, as described “Experimental Procedures.” Activity of the wild type enzyme is indicated by an open red circle, and a closed red circle in the presence of 1 mM histidine. The Y268F/Y269F double mutant is indicated by open diamonds, and closed blue diamonds with 1 mM histidine. The E130A mutant is represented by an open square, and a closed green square with 1 mM histidine. Each assay was performed reproducibly five times. The conserved residues in the histidine binding pocket of histidine bound HisRS from T. thermophilus (green) with HisZ from L. lactis (blue) are aligned to illustrate their potential capability to bind histidine and thereby regulate the activity of the hetero-octamer.
created by substitution of Glu-130 with alanine (E130A), and a double mutant was created by substitution of Tyr-268 and Tyr-269 with phenylalanine (Y268F/Y269F). All three residues are predicted to contact the imidazolium group of histidine. In accord with our predictions, both mutant proteins were resistant to 1 mM histidine, a concentration sufficient to completely inhibit wild type PRT activity (Fig. 8). Thus, sequences in HisZ corresponding to the histidine binding site in functional histidyl-tRNA synthetase appear to serve as a regulatory site in the octamer, providing functional replacement of the C-terminal domain in the hexameric ATP-PRT enzymes (17).

**DISCUSSION**

On the basis of the structural comparison presented here, activation of both hexameric and octameric ATP-PRTs involves a conformational change in the HisG dimer interface that recruits conserved residues to the active site where they activate the pyrophosphate leaving group. Despite the similarity in activation mechanism, the two subfamilies possess different quaternary structures, and likely transition to inhibited states through different mechanisms. In the hexameric enzymes, histidine binding alters the orientation of the C-terminal domain relative to the two catalytic domains, strengthening interactions defined by the 3-fold trimer axis at the expense of the dimeric interface. Access of substrates to the active site becomes blocked (17, 18), and catalytic residues are redirected away from the active site (this work). For the octameric subfamily, the switch between inhibited and active states is more accurately described as a re-arrangement of dimer interactions, which we propose is driven by the dissociation of histidine and the binding of the interstitial phosphate. Notably, inorganic phosphate activates bacterial and mammalian PRPP synthetases, which are structurally similar to type I PRTases (37, 38). The potential role of phosphate in stimulating both the synthesis of PRPP and histidine remains to be explored further, but may represent a cellular signal to stimulate energy-requiring pathways like nucleotide and histidine biosynthesis.

By contrast, histidine binding likely modulates a conformational change at the HisZ-HisG interface leading to down-regulation. Here, we have shown that mutation of the highly conserved residues in a putative HisZ histidine binding pocket abolishes histidine regulation without eliminating PRT function. These residues are distinct from the non-conserved residues that make up the eight histidine binding sites identified in the histidine-bound T. maritima structure reported by Vega et al. (25). Our mutational study raises the possibility that these latter sites are non-biological and that the inhibited state may be fortuitously stabilized by lattice interactions. Notably, the interactions in the HisG dimer interface (Fig. 7) that define the inhibited state are seen both in the histidine-bound T. maritima heter-octamer, and the apo T. maritima HisG dimer determined independently (Protein Data Bank code 1O64, but not described in the literature). Under either model, histidine-dependent conformational changes that orient the insertion domain relative to the HisZ β sheet domain are likely to be critical in remodeling the HisZ-HisG interface during the activation process, just as they facilitate induced fit binding of histidine in the functional histidyl-tRNA synthetases (39, 40).

**PRTase Mechanism across the Classes: A Case of Convergent Evolution?**—Our structures suggest that activation of the pyrophosphate leaving group contributes to rate acceleration by ATP-PRT, constituting an important mechanistic feature shared with other, structurally unrelated PRTs. A rate-limiting step involving cleavage of the C1′-pyrophosphate bond would be consistent with a dissociative mechanism whose features include ribooxocarbenium character at C1′, and variable bond orders to both the leaving group and the nucleophile (41, 42). Other important features of the type I PRT transition state include a change in sugar pucker from C2′ endo to C4′ endo, a substantial (1.0–2.0 Å) migration of C1′ over the reaction coordinate, and a tightly fixed nucleophile in the active site (43). Whereas the refined models of PRPP in our complex and PR-ATP in the HisG, E. coli complex appear to recapitulate some of these features, the current resolution of these structures precludes definitive conclusions about the sugar pucker. Tight binding transition state analogs may be necessary to address both this issue and the presence or absence of coordinated magnesium. An additional mechanistic feature is the requirement to protect the transition state from water, because of the high reactivity of ribooxocarbenium ions. The Gα1/Gβ2 loop reaches across the dimer interface in both the L. lactis PRPP-bound and E. coli PR-ATP complexes, and may ultimately serve to exclude solvent molecules from the active site.

**ATP-PRT as a Model for Complex Enzyme Evolution**—The nearest structural neighbors to HisG and HisZ are the periplasmic binding protein (PBP II) and histidyl-tRNA synthetase families, respectively, and these likely represent the ancestral domains of the octamer. Whereas neither enzyme family possesses PRT function, it is significant that the periplasmic binding protein family includes phosphate- and sulfate-binding proteins (44). HisG clearly exhibits an affinity for these anions (17, 25). On the basis of the mechanism proposed here, dimerization of the periplasmic binding protein fold may have served to recruit catalytically important residues into the active site in a reversible fashion, and was therefore a critical step in the evolution of this fourth PRT class. Notably, this functional re-assignment could not have been predicted on the basis of bioinformatics-based genome annotation, as it did not involve the duplication and specialization of an existing PRT, or the selection and amplification of secondary catalytic function from a related enzyme (45). The retention of structural and sequence elements in HisZ associated with histidine binding in HisRS argues that these elements likely provided selective pressure to recruit the tRNA synthetase-like paralog. Remarkably, motif 2 loop residues that bind ATP in HisRS (46, 47) have been re-programmed to activate PRT function, whereas other determinants for ATP and tRNA binding (including the anticodon binding domain) have been lost from HisZ over time. HisZ is a member of the HisRS-like family (HisRS, HisZ, and GCN2), all of whose members are implicated in the control of amino acid biosynthesis, albeit by remarkably distinct mechanisms (48). The versatility of this family as both catalytic and regulatory agents highlights the potential significance of the aminoacylation reaction in early biosynthetic reactions (49), and underscores the degree to which a simple protein scaffold can be reshaped to perform a wide variety of essential biological functions.

Acknowledgments—We thank Frank vonDelft, Annie Héroux, and Ralf Grosse-Kunstleve for help with data collection and processing at the RapiData 2003 course, and Alan Weiner for inspiration. We are grateful to Robert Sweet for access to beamlines X12C, X26C, and X25 of the National Synchrotron Light Source where the data for this study were measured.

**REFERENCES**

1. Musick, W. D. (1981) CRC Crit. Rev. Biochem. 11, 1–34
2. Schramm, V. L., and Grubmeyer, C. (2004) Prog. Nucleic Acids Res. Mol. Biol. 78, 261–304
3. Smith, J. L. (1999) Nat. Struct. Biol. 6, 502–504
4. Sinha, S. C., and Smith, J. L. (2001) Curr. Opin. Struct. Biol. 11, 733–739
5. Eads, J. C., Ozturk, D., Wexler, T. B., Grubmeyer, C., and Sacchettini, J. C. (1997) Structure 5, 47–58
6. Mayans, O., Ivens, A., Nissen, L. J., Kirschner, K., and Wilmanns, M. (2002) EMBO J. 21, 3245–3254
Allosteric Activation of ATP-PRTase

7. Smith, J. L., Zaluzec, E. J., Wery, J. P., Niu, L., Switzer, R. L., Zalkin, H., and Satow, Y. (1994) Science 264, 1427–1433
8. Martin, R. G. (1963) J. Biol. Chem. 238, 257–268
9. Vold, M. J., Appella, E., and Martin, R. G. (1967) J. Biol. Chem. 242, 1760–1767
10. Winkler, M. E. (1987) in Escherichia coli and Salmonella Typhimurium, Cellular and Molecular Biology (Neidhardt, F. C., ed) Vol. 1, pp. 395–411, American Society for Microbiology, Washington, D.C.
11. Brenner, M., and Ames, B. N. (1971) in Metabolic Regulation (Voge, H. J., ed) Vol. 5, pp. 349–387, Academic Press, New York
12. Morton, D. P., and Parsons, S. M. (1976) J. Biol. Chem. 251, 3903–3914
13. Morton, D. P., and Parsons, S. M. (1977) J. Biol. Chem. 252, 181–188
14. Parsons, S. M., and Koshland, D. E., Jr. (1974) J. Biol. Chem. 249, 4119–4126
15. Bell, R. M., Parsons, S. M., Dubravac, S. A., Redfield, A. G., and Koshland, D. E., Jr. (1974) J. Biol. Chem. 249, 4110–4118
16. Klungsoyr, L., and Kryvi, H. (1971) Biochim. Biophys. Acta 227, 327–336
17. Cho, Y., Sharma, V., and Sacchettini, J. C. (2003) J. Biol. Chem. 278, 8333–8339
18. Lohkamp, B., McDermott, G., Campbell, S. A., Coggins, J. R., and Lapthorn, A. J. (2004) J. Mol. Biol. 336, 131–144
19. Quirocho, F. A., and Ledvina, P. S. (1996) Mol. Microbiol. 20, 17–25
20. Wang, Z., Luecke, H., Yao, N., and Quirocho, F. A. (1997) Nat. Struct. Biol. 4, 519–522
21. Sissler, M., Delorme, C., Bond, J., Ehrlch, S. D., Renault, P., and Francklyn, C. (1999) Proc. Natl. Acad. Sci. U.S.A. 96, 8985–8990
22. Bond, J. P., and Francklyn, C. (2000) J. Mol. Biol. 300, 339–347
23. Delorme, C., Ehrlch, S. D., and Renault, P. (1992) J. Bacteriol. 174, 6571–6579
24. Bovee, M. L., Champagne, K. S., Demeler, B., and Francklyn, C. S. (2002) Biochemistry 41, 11838–11846
25. Vega, M. C., Zou, P., Fernandez, F. I., Murphy, G. E., Sterner, R., Popoz, A., and Wilmanns, M. (2005) Mol. Microbiol. 55, 675–686
26. Doublié, S. (1997) Methods Enzymol. 276, 533–530
27. Otwinowski, Z., and Minor, W. (1997) Methods Enzymol. 276, 307–326
28. Uson, I., and Sheldrick, G. M. (1999) Curr. Opin. Struct. Biol. 9, 643–648
29. Terwilliger, T. C., and Berendzen, J. (1999) Acta Crystallogr. D. Biol. Crystallogr. 55, 849–861
30. Terwilliger, T. C. (2000) Acta Crystallogr. D Biol. Crystallogr. 56, 965–972
31. Jones, T. A., Zou, J. Y., Cowan, S. W., and Kjeldgaard, M. (1991) Acta Crystallogr. Sect. A 47, 110–119
32. Brungger, A., Adams, P., Clore, G., DeLano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, J. S., Kuszelewski, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T., and Warren, G. L. (1998) Acta Crystallogr. D. Biol. Crystallogr. 54, 905–921
33. Collaborative Computational Project, N. (1994) Acta Crystallogr. D. Biol. Crystallogr. 50, 760–763
34. Arnez, J. G., Harris, D. C., Mitschler, A., Rees, B., Franklyn, C. S., and Moras, D. (1995) EMBO J. 14, 4143–4155
35. Nakatsu, T., Kato, H., and Oda, J. (1998) Nat. Struct. Biol. 5, 15–19
36. Carrodeguas, J. A., Theis, K., Bogenhagen, D. F., and Kisker, C. (2001) Mol. Cell 7, 43–54
37. Willemoes, M., Hove-Jensen, B., and Larsen, S. (2000) J. Biol. Chem. 275, 35408–35412
38. Erikson, T. A., Kadziola, A., Bensten, A., Harlow, K. W., and Larsen, S. (2000) Nat. Struct. Biol. 7, 303–308
39. Qiu, X., Janson, C. A., Blackburn, M. N., Chhohan, I. K., Hibbs, M., and Abdel-Meguid, S. S. (1999) Biochemistry 38, 12296–12304
40. Yaremchuk, A., Tukalo, M., Grotli, M., and Cusack, S. (2001) J. Mol. Biol. 309, 989–1002
41. Goitein, R. K., Chelsky, D., and Parsons, S. M. (1978) J. Biol. Chem. 253, 2963–2971
42. Schramm, V. L. (2003) Acc. Chem. Res. 36, 588–596
43. Fedorov, A., Shi, W., Kicska, G., Fedorov, E., Tyler, P. C., Furneaux, R. H., Hanson, J. C., Gainsford, G. J., Larese, J. Z., Schramm, V. L., and Almo, S. C. (2001) Biochemistry 40, 853–860
44. Quirocho, F. A. (1996) Kidney Int. 49, 943–946
45. O’Brien, P. J., and Herschlag, D. (1999) Chem. Biol. 6, R91–R105
46. Arnez, J. G., Augustine, J. G., Moras, D., and Franklyn, C. S. (1997) Proc. Natl. Acad. Sci. U.S.A. 94, 7144–7149
47. Hawko, S. A., and Franklyn, C. S. (2001) Biochemistry 40, 1930–1936
48. Franklyn, C., and Arnez, J. G. (2000) in Aminoacyl-tRNA Synthetases (Ibba, M., Franklyn, C., and Cusack, S., eds) pp. 135–148, Landes Publishing, Austin, TX
49. Franklyn, C. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 9650–9652
50. DeLano, W. (2002) The PyMOL Molecular Graphics System. DeLano Scientific, San Carlos, CA