Structural Evidence for Ammonia Tunneling across the ($\beta\alpha)_8$ Barrel of the Imidazole Glycerol Phosphate Synthase Bienzyme Complex

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Summary
Since reactive ammonia is not available under physiological conditions, glutamine is used as a source for the incorporation of nitrogen in a number of metabolic pathway intermediates. The heterodimeric ImGP synthase that links histidine and purine biosynthesis belongs to the family of glutamine amidotransferases in which the glutaminase activity is coupled with a subsequent synthase activity specific for each member of the enzyme family. Its X-ray structure from the hyperthermophile Thermotoga maritima shows that the glutaminase subunit is associated with the N-terminal face of the ($\beta\alpha)_8$ barrel cyclase subunit. The complex reveals a putative tunnel for the transfer of ammonia over a distance of 25 Å. Although ammonia tunneling has been reported for glutamine amidotransferases, the ImGP synthase has evolved a novel mechanism, which extends the known functional properties of the versatile ($\beta\alpha)_8$ barrel fold.

Introduction
There are a large number of metabolic pathways, such as amino acid and nucleotide biosynthesis, in which nitrogen is incorporated into pathway intermediates. Since free ammonia is mainly present as nonreactive ammonium ion under physiological pH conditions, glutamine is used as the amide donor in these reactions, which are catalyzed by the family of glutamine-dependent amidotransferases (GATases) [1, 2]. GATases are modular biotransferases that catalyze two reactions at separate active sites. In the glutaminase reaction, the hydrolysis of glutamine yields ammonia, which, in the subsequent synthase reaction, is added to an acceptor substrate that is specific for each GATase. According to specific patterns of the active site residues in their glutaminase domains, GATases are categorized into two unrelated classes: class I GATases contain a conserved cysteine-histidine-glutamate catalytic triad, whereas class II GATases are identified by a conserved N-terminal cysteine residue [1]. The reactions at the two active sites of GATases are coupled 2-fold: (1) the glutaminase activity is stimulated by the binding of the acceptor substrate to the synthase active site, which avoids wasteful hydrolysis of glutamine, and (2) by the transfer of nascent ammonia, which is sequestered from the solvent, thus avoiding its protonation.

Recent structural and functional studies on several members of the GATase family have identified ammonia tunnels that connect the glutaminase and synthase active sites [3–7]. In some GATases, the tunnel is constitutively present, as in carbamoyl phosphate synthetase (CPS) [8] and asparagine synthetase B [9]. In contrast, tunnel formation in the glutamine phosphoribosylpyrophosphate (PRPP) amidotransferase is dependent on binding of the substrate PRPP to the synthase active site [10]. In other GATases, ammonia tunnel formation was postulated but has not yet been proven experimentally. Examples of the last group are anthranilate synthase [11–13] and GMP synthetase [14].

The imidazole glycerol phosphate (ImGP) synthase is a key metabolic enzyme, which links amino acid and nucleotide biosynthesis. It uses the substrates glutamine and N'[(5'-phosphoribulosyl)formimino]-5-aminoimidazole-4-carboxamide-ribonucleotide (PRFAR) to catalyze the closure of the imidazole ring within histidine biosynthesis and additionally provides 5-aminoimidazole-4-carboxamide ribotide (AICAR) for use in the de novo synthesis of purines [15] (Figure 1). In yeast, ImGP synthase bears both the glutaminase and the cyclase catalytic activities on a single polypeptide chain [16], while bacterial ImGP synthases form heterodimeric biotransferase complexes that consist of one glutaminase (HisH) and one cyclase (HisF) subunit. Biochemical data on the ImGP synthases from Escherichia coli and the hyperthermophile Thermotoga maritima have demonstrated that, under in vitro conditions, HisF is able to catalyze ImGP formation in the absence of HisH using externally added ammonium salts that can replace glutamine at basic pH values [17, 18].

The recently determined X-ray structure of the isolated HisF subunit from T. maritima has identified it as a ($\beta\alpha)_8$ barrel [19], which is probably the most frequent fold among soluble enzymes in metabolic pathways [20, 21]. HisF shares its ($\beta\alpha)_8$ barrel fold and a 2-fold repeat structure with another enzyme of the histidine biosynthesis pathway, HisA, catalyzing an isomerization reaction that precedes the ImGP synthase reaction [19]. Two conserved aspartate residues in symmetrical positions at the C-terminal face of the central $\beta$ barrel of HisF, D11 and D130, are essential for catalysis of the cyclase reaction [18]. The amino acid sequence of HisH contains the catalytic cysteine-histidine-glutamate triad, identifying it as a class I glutaminase [1]. Binding of a substrate analog to the active site of HisF stimulates the glutami-

Key words: bienzyme complex; glutamine amidotransferase; imidazole glycerol phosphate synthase; ($\beta\alpha)_8$ barrel; ammonia tunnel; X-ray structure
nase activity of HisH, both in the *E. coli* and *T. maritima* enzymes, indicating tight coupling of the two reactions [17, 18]. Furthermore, several conserved residues in HisF were shown to be crucial for the glutamine-dependent reaction of the bi-enzyme complex but not for the ammonia-dependent reaction of the isolated cyclase subunit [18, 22].

In order to investigate the structural basis of the coupling of these two reactions, we solved the X-ray structure of the HisH-HisF heterodimer from *T. maritima*. The HisH subunit associates with the N-terminal face of the β barrel of the HisF subunit, leaving its active site exposed at the C-terminal face of the β barrel. The structure shows that ammonia needs to be transferred from the glutaminase active site to the cyclase active site over a distance of about 25 Å. We suggest a model in which ammonia travels through a bipartite tunnel, the larger part of which is formed by the interior of the HisF β barrel. In the presented structure, which does not contain physiological ligands, the postulated tunnel is closed by a conserved salt bridge cluster at the HisH-HisF interface. Binding of the substrate PRFAR to the active site of HisF probably induces a conformational transition, which results in the stimulation of the HisH glutaminase activity and the opening of the ammonia tunnel.

**Results**

**Overall Structure**

We have solved the crystal structure of the heterodimeric bi-enzyme ImGP synthase from the hyperthermophile organism *T. maritima*, comprising the glutaminase subunit HisH and the cyclase subunit HisF, at 2.4 Å resolution (Figures 2 and 3; Table 1). The HisF subunit within the complex contains the amino acid exchange D11N. The structures of the isolated subunits, HisH and HisF, have been determined as well at 2.4 Å (presented in this contribution) and 1.4 Å resolution [19], respectively, thus permitting insight into the conformational changes that occur upon complex formation. The structure of the bi-enzyme complex was solved by molecular replacement, using the HisF structure [19] as a structural template. Since the crystals contained three HisH-HisF heterodimers per asymmetric unit, 3-fold noncrystallographic symmetry (NCS) averaging allowed a straightforward interpretation of the HisH electron density, for which no phases were provided by the structural template. The three heterodimers have practically the same structure, except for some active site loops in HisF (see below). The separate HisH structure was determined using the glutaminase subunit of the bi-enzyme complex as a structural template.

The HisF cyclase subunit folds as a β-barrel with extensive loops at the C-terminal face of the central β barrel, harbouring the active site, and a rather flat surface at the N-terminal face, to which the HisH subunit is bound (Figure 3). The overall HisF structure within the bi-enzyme complex is similar to that of isolated HisF, with rmsds for all main chain atoms between 0.4 and 0.6 Å, depending on which of the three bi-enzyme complexes is used for comparison. Nevertheless, there are considerable structural differences between the active site loops of the three HisF molecules of the bi-enzyme complexes when compared to each other and to the separate HisF structure [19]. The largest differences are found within the first two active site loops, connecting strand β1 to helix α1 and strand β2 to helix α2 (Figure 3). Two of the three HisF subunits bind two phosphate ions, each mimicking the two phosphate groups of the substrate PRFAR, as observed for isolated HisF [19]. In these two HisF subunits, the long loop connecting strand β1 and helix α1 contains a small two-stranded β sheet (Figures 3A and 3B). However, the HisF subunit of the third heterodimeric complex within the asymmetric unit (Figure 3C) contains only one active site phosphate ion, which is bound to the loops connecting strands β7 and β8 with the subsequent helices, respectively. Comparison of the two different HisF active site conformations demonstrates that, in the HisF subunit of Figure 3C, the β1-α1 loop is flipped toward the outer α-helical barrel ring, leading to a partial opening of the HisF active site (Figure 3D), as observed for isolated HisF [19]. In this subunit, there is no additional β sheet within the loop β1-α1, and three residues (20-22) were not visible in the final electron density. Furthermore, the corresponding HisH-HisF complex is considerably more flexible, as judged by the average atom displacement values, than the other two heterodimers (Table 1). These findings suggest that this loop region is capable of adopting more than a single conformation and that it is likely to be involved in coupling the catalytic activities of HisF and HisH.

The HisH glutaminase subunit has a similar fold to that in other members of the class I family [1], containing the catalytic triad C84-H178-E180 (Figure 2). The closest structural similarity (rmsd of ~1.8 Å for all main chain atoms) is shared with the TrpG glutaminase subunit of the anthranilate synthase [11–13]. The structures of isolated and complexed HisH are basically identical (rmsd for all main chain atoms is ~0.4 Å), indicating that HisH does not undergo significant conformational changes.
Structure of the ImGP Synthase Bienzyme Complex

Figure 2. Topography of Secondary Structural Elements, Mapped on the Sequences of HisF and HisH from T. maritima (Tm)

The positions of secondary structural elements were taken from the ImGP synthase heterodimer shown in Figure 3C. The α helices and β strands are indicated by cylinders and arrows, respectively (HisF [A], yellow and red; HisH [B], blue and cyan). Extensions of secondary structural elements that are present in the other two heterodimeric complexes within the asymmetric unit (Figure 3A) as well as in the separate HisF cyclase [19] are marked by secondary structure symbols in faint colors. Cons: invariant residues (upper case characters) and conserved residues (identical in at least 90% of the known sequences, lower case characters) were identified by aligning all available HisF and HisH sequences [25]. Residues that contribute to the HisH-HisF interface are in green. Essential catalytic residues of the HisF cyclase subunit (D11 and D130) [18] and the HisH glutaminase subunit (C84, H178, and E180) [1] are highlighted in red. See text for further details.

upon binding to HisF. Interestingly, the conserved P119, which is located at the N terminus of strand β7, is in cis conformation in all three HisH molecules. As in other class I glutaminase structures [11–14], the essential C84β of the three different HisH molecules within the HisH-HisF complex shows unusual main chain conformations, with angles of $\phi = +55^\circ$ and $\Psi = -120^\circ$. The spatial arrangement of the catalytic triad residues C84-H178-E180 is virtually identical to the equivalent residues of its closest relative with known structure, the TrpG glutaminase subunit of the anthranilate synthase from Sulfolobus solfataricus [11].

There is additional electron density within the active site of isolated HisH, which has been interpreted as an acetate anion, originating from the crystallization buffer. This acetate ion is located at a similar position to that of the carboxylate group of the glutamyl thioester reaction intermediate that was detected within the TrpG active site of the anthranilate synthase structure from Serratia marcescens [13].

The HisH Glutaminase-HisF Cyclase Interface

The HisH subunit docks onto the N-terminal face of the β barrel of the HisF subunit, which is located opposite the C-terminal active site face (Figures 3 and 4). Thus, the HisF active site is not blocked or directly affected upon complex formation with the HisH subunit. This function of the N-terminal barrel face of HisF is reflected by a remarkable amount of sequence conservation (Figure 5C), which is comparable to that of the C-terminal active site face of the HisF (β5) barrel. The N-terminal face of the β barrel from the closely related but monomeric HisA from T. maritima, for example, is not more conserved than its overall sequence [19].

This HisH-HisF interface extends over an area of approximately 1100 Å², as calculated with a Web-based server [23]. The closest contacts are formed between the N and C termini of the HisF subunit and several residues of strands β7 and β8 of the HisH subunit (Figures 3 and 4). In particular, the N terminus of HisF is accommodated by a deep pocket of HisH, which is formed...
Table 1. X-Ray Data and Structure Refinement Statistics

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Numbers in parentheses refer to highest resolution shell.

a HisH-HisF dimers A, B, and C.

b Rms differences for superimposed complexes A-B, A-C, and B-C.

by residues from strand β7 and the loop that connects strands β9 and β10. An analysis of specific intersubunit interactions has identified ten protein-protein hydrogen bonds, which are distributed almost uniformly over the entire interface (Figure 4). On the basis of sequence conservation, two of these hydrogen bonds are expected to be present in all known HisH-HisF complexes from different organisms (Figures 2 and 4). One of them is formed between the N-terminal amino group of M1 from HisF and the side chain of the invariant N124 from HisH. The N-terminal M1 of HisF also forms a salt bridge with the side chain of E157 of HisH, which is, however, not conserved. The second conserved hydrogen bond is formed between the main chain carboxyl group of D45 from HisF and the indole side chain of W123 from HisH. Both residues are invariant. W123, representing the only tryptophan in the HisH glutaminase, is in an ideal position to monitor the ImGP synthase complex formation by fluorescence spectroscopy [18]. Another solvent-mediated interaction is observed between the side chain hydroxyl group of the conserved Y138 from HisH and the side chains of R5 and E46 from the HisF subunit, which both belong to the conserved salt bridge cluster of HisF (see below).

Structure of the Putative Ammonia Tunnel across the HisF (βα)₅ Barrel

The two active sites, marked by the key catalytic residues (Figures 2 and 3), are separated by about 25 Å in the HisF-HisH bienzyme complex. The only plausible path for ammonia between these two active sites is through the HisH-HisF interface (about 10 Å) and across the central β barrel of HisF (about 15 Å; Figure 3B). As in other (βα)₅ barrel structures, the interior of the central eight-stranded, parallel β barrel is built up by four side chain layers [24]. Each layer is either formed by the side chains from residues of the four odd- or even-numbered β strands, leading to a 4-fold symmetric layer arrangement (Figure 5A). Typically, these layers form the hydrophobic core of the (βα)₅ barrel, providing the platform for the active site at its C-terminal face. In comparison with the related HisA structure [19] and most other known (βα)₅ barrels [24], the layers in the β barrel of HisF show two distinct differences. Within the first three upper layers, the side chains do not pack as tightly as they do in other (βα)₅ barrels, leaving significant holes in the center that, when combined, form a tunnel. Moreover, the fourth layer, which is located closest to the interface between HisF and HisH, differs from the other three layers in that it is formed by four charged residues (R5, E46, K99, and E167). One of these residues, R5, does not fit into the 4-fold symmetry pattern but locates the guanidinium group of its long side chain in front of the small side chain of A220, which is the regular member of layer 4. Each of the two positively and two negatively charged side chains forms two salt bridges with its neighbors, resulting in a ring of salt bridges, which closes the putative ammonia tunnel (Figure 5B). These four residues are invariant within the known HisF sequences (Figure 2).
Figure 3. Overall Structure of the ImGP Synthase Bienzyme Complex, Showing Ribbon Diagrams of Two of the Three Heterodimers Found in the Solved Crystal Form (A and C). The most significant differences between (A) and (C) are within the HisF active site. In (A), the active site is in a closed conformation, and two phosphate ions (in CPK presentation) are bound to the active site. In (C), the active site of HisF is in an open conformation, and only the C-terminal phosphate ion is bound. The colors of the secondary structural elements are as in Figure 2. Essential catalytic residues are depicted in ball and stick presentation in red (cp. Figure 2). The positions of the N and C termini of each subunit are indicated.

(B) The putative path for ammonia between the active sites of HisF and HisH is indicated by a number of red spheres, using the coordinates of the HisF subunit of (A).

(D) Superposition of the Cα backbones of the three HisF subunits (subunit from [A], original colors; subunit from [C], blue; HisF subunit from the third HisF-HisH complex, red). The break between residues 19 and 23 of the blue subunit from (C) is indicated by a dashed line.

[18, 19, 25], implying that the salt bridge ring is crucial for functional interactions between HisF and HisH. In support of this hypothesis, the single amino acid exchanges R5H and E46G impair the glutamine-dependent reaction of the HisH-HisF complex from *E. coli*, while having little effect on the ammonia-dependent reaction of isolated HisF [22].

Discussion

Coupling of the Glutaminase and Synthase Activities

The structure of the ImGP synthase complex from *T. maritima* provides insight into the coupling of the two catalytic activities of this bienzyme. The HisH glutaminase reaction is activated by the binding of the substrate PRFAR (or a substrate analog) to HisF [18]. The presented structure shows that this activation signal needs to be transmitted over a distance of about 25 Å (Figure 3). The HisF active site loop β1-α1, which is found in two different conformations (Figure 3), could be involved in the transmission of the HisH activation signal from the HisF active site. In support of this hypothesis, R27, which is located within this loop (Figure 2), displays increased sensitivity toward proteolysis upon HisH-HisF complex formation. Furthermore, replacement of the con-
Figure 5. Putative Ammonia Tunnel within the Central β Barrel of HisF
(A) Schematic presentation of the four layers of the central β barrel of the HisF cyclase. The layers are numbered from the C-terminal to the N-terminal face of the barrel. Within each layer, the side chains are provided either by odd-numbered or even-numbered β strands, leading to a 4-fold symmetric arrangement. While the upper three layers are formed mostly by small hydrophobic residues, layer 4, which is close to the HisH-HisF interface, is formed by invariant amino acids with charged side chains that form a salt bridge cluster. R5 does not fit into the 4-fold symmetry pattern but locates the guanidinium group of its long side chain in front of the small side chain of A220, which is the regular member of layer 4. The approximate positions of the eight β strands are indicated within each layer.
(B) View onto layer 4 from the HisH-HisF interface. The salt bridge ring, formed by R5, E46, K99, and E167, is shown in ball and stick presentation. The positions of the N and C termini are indicated.
(C) Surface presentation of the HisF cyclase, with the same view as in (B). Conserved and invariant residue patches are colored in red and orange (cp. Figure 2, upper case and lower case characters, respectively), demonstrating strong conservation of residues involved in the HisH-HisF interface and of the entrance into the postulated ammonia tunnel across the HisF (ββββ)8 barrel. The salt bridge ring formed by R5, E46, K99, and E167 is shown in ball and stick presentation, as in (B). The hole in the center is indicted by the cyan background.
(D) Stereo presentation of the 2Fo–Fc electron density map of layer 4 of the most mobile HisH-HisF heterodimer (Figure 3C; Table 1) using the final model, contoured at 1.0σ.

served K19 by serine, which is also located in this loop, impairs the glutamine-dependent reaction of the HisH-HisF complex but has little influence on the ammonia-dependent reaction of isolated HisF [18].

At present, it remains unclear what kind of structural adjustments within the HisH active site are required for its activation. The active sites of all known class I glutaminases have a virtually identical architecture, regardless of whether they are void of any ligands or contain a glutamyl thioester intermediate [13, 14, 26]. Therefore, we expect the required structural changes within the glutaminase active site to be small. It is noted, however, that the catalytic triad cysteine is in an unfavorable conformation in all available glutaminase class I structures. Release of this cysteine from its high-energy conformation could potentially be involved in the activation of glutaminase catalysis.

Structural Evidence for Ammonia Tunneling
The structure of the *T. maritima* ImGP synthase complex, together with steady-state enzyme kinetic data on the homologous enzyme from *E. coli* [22], suggests a
model explaining how the HisH product ammonia could be transferred to the active site of the HisF cyclase while being sequestered from the solvent. In this model, ammonia first crosses the HisH-HisF interface over a distance of about 10 Å toward the N-terminal face of the central β barrel of the HisF cyclase subunit, which is defined by the invariant salt bridge ring forming the fourth layer of the HisF β barrel interior. Upon opening of this salt bridge cluster by a still unknown mechanism, the second and longer part of the ammonia transfer, over a distance of about 15 Å, would then take place along the central axis of the HisF β barrel. Finally, ammonia would exit the tunnel at the C-terminal layer 1 of the central β barrel to become available as second substrate for the HisF cyclase reaction. While it is anticipated that HisF substrate binding is sufficient to sequester the interior of the HisF β barrel from the bulk solvent, the HisH-HisF interface, as observed in the present inactive ImGP synthase complex, would allow leakage of the HisH product ammonia. Therefore, it is plausible to assume that the HisH activation process is accompanied by a rearrangement of the HisH-HisF interface plugging the HisH active site from solvent. It remains to be seen whether this required interface plug- ging is paralleled by a breakage of the salt bridge cluster to open the postulated ammonia channel across the HisF β barrel. Removal or repositioning of the structured water molecule between Y138 from the HisH subunit and R5 and E46 from the HisF subunit (Figure 4) during HisH glutaminase catalysis could potentially play a role in changing the salt bridge cluster in layer 4 to permit ammonia to enter the interior of the HisF β barrel.

Figure 6 presents a working model of the coupling of the two separate activities within the ImGP synthase biocatalytic complex. In this model, the reaction is first coupled by an activation signal from HisF to HisH, followed by coupling of the two reactions of the ImGP synthase by ammonia transfer from HisH to HisF. While the second process requires the physical presence of a tunnel, it is not necessarily required for the activation of HisH, and the precise path of this signal remains to be elucidated.

Independent Evolution of the Coupling of HisH-HisF Catalysis
While the folds of the two single subunits of the complex share structural and evolutionary relations with other metabolic enzymes [1, 19], the molecular basis of the postulated ammonia transfer in the ImGP synthase biocatalytic complex is dissimilar to that in other GATases [3, 4], suggesting its independent evolution. Among the complex structures of the glutamine-dependent amido- transferases currently available, ammonia tunnels have been localized in glutamine phosphoribosylpyrophosphate amidotransferase (GPATase) and CPS. In the activated conformation of GPATase, the active sites are connected by a tunnel that is predominantly hydrophobic and about 20 Å in length [10]. Remarkably, the structure of CPS has revealed a long tunnel with a length of about 96 Å, which is formed, in part, by hydrophilic residues [8]. Using these two tunnels as a reference, the activated tunnel in the ImGP synthase complex is expected to be more reminiscent of that of GPATase than that of CPS in terms of its hydrophobicity and distance between the active sites. Our structure of the ImGP synthase adds to the increasing evidence that tunneling of ammonia in GATases could present a paradigm of independent, convergent evolution of a biological transfer process that requires two separate active sites linked by a tunnel that, at least in the active state, is plugged from solvent.

Biological Implications
Substrate channeling describes a process in which the product of an enzymatic reaction is transferred without contact to bulk solvent to the active site of the subsequent enzyme in the metabolic pathway. The potential,
biological advantages of substrate channeling include protection of labile metabolites, the prevention of hydrophobic metabolites from diffusion through cell membranes, and the decreased transit time of intermediates [3, 27].

GATases catalyze two different reactions at two separate active sites. At the glutaminase active site, glutamine is hydrolyzed to glutamate and ammonia, whereas at the synthase active site, reacts with a specific acceptor substrate. Since, at physiological pH values, nascent ammonia is protonated to a large extent to yield the nonreactive ammonium ion, it was postulated that the channeling of ammonia between glutaminase and synthase active sites is a general phenomenon among GATases. Indeed, through a combination of X-ray crystallography [8–10] and mutational analysis [5–7], the existence of functional ammonia tunnels was proven for several GATases [3, 4].

The presented structure of the ImGP synthase (HisH-HisF bienzyme complex) provides a model of how ammonia is channeled over a distance of about 25 Å within this key metabolic GATase, which links amino acids with the de novo purine synthesis. The larger part of the putative ammonia tunnel is provided by the interior of the β barrel of HisF, which has a (βββ/α) barrel fold. Although the (βββ/α) barrel is the most frequently encountered fold among enzymes [21, 28], some of which display a tunnel across their central β barrels [29, 30], the transport of a metabolite across such a tunnel adds a new function to this versatile structural class of enzymes.

Experimental Procedures

Protein Production and Purification of ImGP Synthase from T. maritima

The HisF_D11N variant and HisH were produced separately in E. coli and purified as described [18, 31]. The complex HisH-HisF_D11N was prepared by mixing equimolar amounts of both proteins followed by gel filtration in order to remove any unintentional surplus of either of the components [18].

Crystallization and Structure Determination of the ImGP Synthase Bienzyme Complex

Sample solution containing 28.8 mg/ml purified ImGP synthase complex from T. maritima in 10 mM Tris-HCl (pH 8.0), 1 mM EDTA, and 1 mM DTT, was mixed stoichiometrically with the reservoir solution, containing 15 % (v/v) PEG 8000, 0.9 M ammonium nitrate, 0.1 M HEPES-NaCl (pH 8.5), 10 mM DTT, and 5 % (v/v) MPD, yielding drops of 1 μl volume, initially. The vapor diffusion method was used to obtain crystals with a rod-like morphology of a maximum size of about 0.4 × 0.2 × 0.2 mm³. The crystals were mounted onto a nylon loop and shock-frozen to 100 K, without adding any further cryoprotectants. A native data set was collected to 2.4 Å resolution, using synchrotron radiation from the endstation X11 at EMBL c/o DESY, Hamburg. In total, 107 images, each with a rotation of 1.0°, were recorded on a mar165 CCD detector. All subsequent steps were carried out using the same software packages employed for the structure solution of the ImGP synthase complex.

The structure was solved by molecular replacement, using the AmoRe package [38]. The coordinates of the HisH subunit from the ImGP synthase complex from T. maritima served as a structural template. The HisH molecule could be unambiguously placed into the asymmetric unit, yielding a correlation coefficient of 0.57 and an R factor of 0.44, as defined in AmoRe [36]. The computational step for the refinement of isolated HisH was, like that for the HisH-HisF complex. Since refinement of atomic displacement factors yielded an increase of the R_m, probably because of insufficient numbers of available reflection data, it was decided to apply the residue group protocol of CNS for B factor refinement. The final model includes 200 residues, 65 solvent molecules, and 1 acetate ion. Further statistics are provided in Table 1. The figures have been prepared using MOLSCRIPT [37], BOBSCRIPT [38], and GRASP [39].

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