Crystal structure of the histidine-containing phosphotransfer protein ZmHP2 from maize

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Abstract

In higher plants, histidine-aspartate phosphorelays (two-component system) are involved in hormone signaling and stress responses. In these systems, histidine-containing phosphotransfer (HPt) proteins mediate the signal transmission from sensory histidine kinases to response regulators, including integration of several signaling pathways or branching into different pathways. We have determined the crystal structure of a maize HPt protein, ZmHP2, at 2.2 Å resolution. ZmHP2 has six α -helices with a four-helix bundle at the C-terminus, a feature commonly found in HPt domains. In ZmHP2, almost all of the conserved residues among plant HPt proteins surround this histidine, probably forming the docking interface for the receiver domain of histidine kinase or the response regulator. Arg102 of ZmHP2 is conserved as a basic residue in plant HPt proteins. In bacteria, it is replaced by glutamine or glutamate that form a hydrogen bond to N δ atoms of the phospho-accepting histidine. It may play a key role in the complex formation of ZmHP2 with receiver domains.

Keywords: histidine-containing phosphotransfer protein; crystal structure; four-helix bundle; two-component system; *Zea mays*

The histidine-aspartate (His-Asp) phosphorelay, also known as the two-component system, is a signal transduction mechanism originally identified in prokaryotes (Stock et al. 2000). Today, it is known to function also in eukaryotes such as fungi and plants (Saito 2001). In plants, the signaling cascade generally consists of three protein elements; a sensory histidine kinase (HK), a histidine-containing phosphotransfer (HPt) protein, and a response regulator (RR). Typical plant HKs contain an input domain, an HK domain,

and a receiver domain. In response to extra- or intra-cellular signals, HK autophosphorylates a His residue in the HK domain. Subsequently, the phosphoryl group is transferred to an Asp residue of the internal receiver domain. It is then successively passed on to a His residue of the HPt protein and an Asp residue of the receiver domain of RR, resulting in a signal output by interaction with target proteins or specific DNA motifs (Stock et al. 2000; Saito 2001).

Phosphorelay systems in plants function in signal transduction pathways triggered by the phytohormones ethylene and cytokinin as well as by environmental stresses (Hwang et al. 2002). In *Arabidopsis thaliana*, 11 HK genes have been identified (Kakimoto 2003). ETR1, ETR2, ERS1, ERS2, and EIN4 are involved in ethylene signaling (Hua and Meyerowitz 1998), whereas AHK2, AHK3, and AHK4/CRE1/WOL play a role in cytokinin signaling (Inoue et al. 2001; Yamada et al. 2001). AtHK1 has been implicated in osmotic responses (Urao et al. 1999), and CK1I was dem-

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Abbreviations: HPt, histidine-containing phosphotransfer protein; HK, histidine kinase; RR, response regulator; RMSD, root mean square deviation; SeMet, selenomethionine-labeled; NCS, noncrystallographic symmetry.

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onstrated to be essential for megagametophyte development (Pischke et al. 2002). In maize, a similar number of genes for HKs are found in the EST database (<http://www.maizgedb.org/est.php>), and ZmHK1 to ZmHK3 have been identified as functional cytokinin receptors (Yonekura-Sakakibara et al. 2004).

In *A. thaliana* and maize, 22 and 10 RR genes, respectively, have been identified (Sakakibara et al. 1998, 1999; Asakura et al. 2003; Kakimoto 2003). The RRs can be classified into two subtypes, type-A and type-B, as judged from their structural designs (Imamura et al. 1999). Type-B RRs have a Myb-related B (or GARP) motif which functions as a transcription factor (Riechmann et al. 2000); type-A RRs lack such motifs. Most of the genes for type-A RRs (ARR3 to ARR9, ARR15 to ARR17 in *A. thaliana*, and ZmRR1, ZmRR2, and ZmRR4 to ZmRR7 in maize) are cytokinin-inducible (Brandstatter and Kieber 1998; Taniguchi et al. 1998; D'Agostino et al. 2000; Asakura et al. 2003), whereas genes for type-B are not (Imamura et al. 1999; Kiba et al. 1999; Asakura et al. 2003). In *A. thaliana*, some type-A RRs have been characterized as negative feedback regulators of cytokinin signaling (Kiba et al. 2003; To et al. 2004).

Although the physiological function of HKs appears clear, the functional differentiation of HPt proteins in these pathways has not been well characterized. Five genes (*AHP1* to *AHP5*) for HPt proteins have been identified in *A. thaliana* (Suzuki et al. 1998, 2000), compared to three genes (*ZmHP1* to *ZmHP3*) in maize (Sakakibara et al. 1999; Asakura et al. 2003). Differences in the numbers of genes for each element of the phosphorelay system (HK–HPt protein–RR) suggest that plant HPt proteins play an important role in signal integration and branching between pathways in which different HKs and RRs function. Such pathway branches must be strictly regulated. Physical interactions between specific HPt proteins and RRs have been demonstrated in *A. thaliana* and maize (Suzuki et al. 2001; Asakura et al. 2003). However, the discrimination mechanisms at the molecular level, which likely depend on differential tertiary structures of the isoforms, have not been characterized.

So far, structures of HPt proteins have been determined in YPD1 from *Saccharomyces cerevisiae* (Song et al. 1999; Xu and West 1999), the HPt domain of ArcB from *Escherichia coli* (Kato et al. 1997, 1999; Ikegami et al. 2001), and the P1 domain of CheA from *Salmonella typhimurium* (Mourey et al. 2001). All of them possess a four-helix bundle with the phosphorylated His residue located in the center of the second helix. However, the sequence similarities between HPt proteins from higher plants and microorganism are too low to allow functional interpretations of plant HPts on the basis of the known structures of microbial HPts. Here, we report the crystal structure of ZmHP2 at 2.2 Å resolution and discuss it in comparison with other HPt proteins.

Results and Discussion

Overall structure

The ZmHP2 (145 residues in total) crystals obtained contain four independent monomers in the asymmetric unit (molecules A1, A2, B1, and B2). Noncrystallographic twofold rotational symmetry within the unit is present. The two pairs of proteins (molecules A1 and A2, and B1 and B2) have similar structures (root means square deviations, RMSD, of 0.2 Å for identical Cα atoms between the molecules of any pair, and RMSD of 0.8–1.1 Å for identical Cα atoms between different pairs). The N-terminal (residues 1–8 of molecule A1, 1–10 of molecule A2, and 1–4 of molecules B1 and B2) and the C-terminal regions (residues 140–145 of molecule A1, 139–145 of molecule A2, and 143–145 of molecules B1 and B2) are disordered. Moreover, residues 35–38 of molecules A2 are nonvisible.

ZmHP2 consists of 6 α-helices (A, B, C, D, E, and F; Figs. 1, 2). The four C-terminal helices (C, D, E, and F) form an antiparallel four-helix bundle, as it is known from YPD1 (Song et al. 1999; Xu and West 1999), the HPt domain of ArcB (Kato et al. 1997), and the P1 domain of CheA (Mourey et al. 2001). Moreover, the histidine residue for phosphorylation (His80; Sakakibara et al. 1999) lies in the center of helix D; this position is also conserved in other HPt domains (Figs. 1, 2, 3). The four-helix bundle of ZmHP2 consists of two long helices, C and F (23 and 28–32 residues, respectively), and two shorter ones, D and E (17–18 and 15 residues, respectively). Although the four-bundle helices mostly are amphipathic, the interhelix interactions in the bundle predominantly are hydrophobic contacts. In the N-terminal region, there are two extra helices, A and B. Helix B extends to the surface on which the imidazole ring

Figure 1. Ribbon model of ZmHP2. As in all figures, molecule A1 is shown. The four-helix bundle, other α-helices, and loops are colored purple, light blue, and yellow, respectively. Side-chain carbon and nitrogen atoms of the active site histidine are shown in green and blue, respectively.

Figure 2. Structure-based sequence alignment and secondary structures of ZmHP2 and other HPT proteins. Colors of secondary structures are as in Figure 1. Conserved residues in plant HPT proteins are indicated in green. Residues conserved in plant HPT proteins, YPD1, the HPT domain of ArcB, and the P1 domain of CheA are colored red. The residues of other HPT proteins that do not correspond to amino acids of ZmHP2 are given in italics. Residues boxed in orange in YPD1 are involved in the interaction with the receiver domain of SLN1 (Xu et al. 2003).

of the phospho-acceptor His80 is exposed (Fig. 4A,B). On the contrary, helix A points away from this surface due to the connection to helix B by a β -turn (residues 21–24) at an angle of -144° to -165° . These two helices are connected with the N-terminal side of the four-helix bundle by a β -turn (residues 39–42). Around the connecting loop (residues 31–41), there are structural differences between the pairs of ZmHP2 molecules in the asymmetric unit (RMSD of 1.9–2.5 Å for identical C α atoms on the loop between different pairs) (Fig. 5). In this region, there are three glycine residues (37, 38, and 41; Figs. 2, 5). Therefore, these regions in the molecules A1 and A2 are highly flexible or disordered (average B-factors = 65 Å² in this region, compared to 46 Å² and 48 Å², respectively, for the whole protein). However, the corresponding regions in the molecules B1 and B2 appear less flexible (average B-factors for this region are 48 Å² and 51 Å², respectively, compared to 40 Å² and 44 Å², respectively, for the whole protein). In the crystal, the loops of the molecules B1 and B2 interact with two residues of the molecules A1 and A2 which seems to cause the structural differences discussed (Fig. 5).

Active site features

Compared to bacterial HPT proteins, the most conspicuous structural difference close to the phospho-accepting His

residue is Arg102 in ZmHP2. In bacterial HPT proteins, the corresponding residue is glutamine (Gln86 and Gln739 in YPD1 and the HPT domain of ArcB, respectively) or glutamate (Glu70 in the P1 domain of CheA), whose side chains form hydrogen bonds to N δ atoms of the active site histidine (Fig. 3). Mutational analyses in YPD1, the HPT domain of ArcB, and the P1 domain of CheA have shown that these residues are not essential for the phospho-transfer reaction (Matsushika and Mizuno 1998a; Janiak-Spens and West 2000; Mourey et al. 2001). In contrast to bacterial HPT proteins, the position corresponding to Arg102 in ZmHP2 is always occupied by basic residues (arginine or lysine) in plant HPT proteins (ZmHP1 to ZmHP3, AHP1 to AHP5, OsHP1 (GenBank accession no. AK072521) and OsHP2 (AK061111) from *Oryza sativa*, and CrHP1 from *Catharanthus roseus* (Papon et al. 2002). Noteworthy, the side chain of Arg102 does not interact with the imidazole ring of the phospho-acceptor His80 in ZmHP2, in contrast to the glutamine or glutamate residues found at the corresponding position in bacterial HPT proteins. In the yeast YPD1, the side chain of Gln86 which corresponds to Arg102 in ZmHP2 forms a hydrogen bond to the receiver domain of SLN1 (Xu et al. 2003), suggesting that the ZmHP2 Arg102 might have a similar function. On the other hand, in the bacterial phosphoenolpyruvate:sugar phosphotransfer system, the conserved Arg17 of histidine-containing phosphocarrier protein (in *E. coli*) is situated close to the phosphorylation site, His15. Arg17 is essential for the complex formation with its phospho-donor, enzyme I, and the phospho-

Figure 3. Superimposition of active site structures of ZmHP2 and YPD1. Helices D and E, their connecting loops of ZmHP2 (all in purple), and the corresponding regions of YPD1 (pink) are shown. Oxygen, nitrogen, and sulfur atoms of selected residues are shown in red, blue, and green, respectively. Hydrogen bonds and salt bridges are indicated by dots.

Figure 4. Positions of conserved residues and comparison with the receiver-domain docking interface of YPD1. Colors of residues are as in Figure 2, and colors of secondary structures are as in Figure 1. (A) Conserved residues in plant HPT proteins. Gly90 is not shown. (B,C) Conserved residues shown on the molecular surface of ZmHP2. C is rotated by 180° around the vertical axis. (D) YPD1 interface docking with the receiver domain of SLN1.

acceptor, enzyme IIA^{Glucose}, as revealed by mutational (Anderson et al. 1993; Kruse et al. 1993) and structural analyses (Garrett et al. 1999; Wang et al. 2000). Similarly, Arg102 may contribute to the complex formation of ZmHP2 with receiver domains.

The imidazole ring of the phospho-accepting His80 protrudes from the molecule surface since residues with shorter side chains such as Ala77 and Gly84 are positioned around it (Fig. 3). Gly84 is conserved in HPT proteins (Kato et al. 1999; Song et al. 1999; Xu and West 1999). In YPD1 and the HPT domain of ArcB, phosphorylation efficiencies of mutants whose corresponding glycine residues were replaced by Gln or Asp were reduced to 10%–20% of the wild-type level (Matsushika and Mizuno 1998b; Janiak-Spens and West 2000), suggesting that the exposure of His80 is important for phosphate transfer. Moreover, Ala77 may also play a similar role, while corresponding residues in other HPT proteins are long-chain ones. Another conserved residue in the vicinity of His80 is Lys83 (Fig. 2). In YPD1, the phosphorylation efficiency of a mutant in which Lys67 was replaced by Ala was reduced to about 20% and 10% of the phospho-transfer level from the receiver domains of SLN1 and RR CheY as compared to the wild type

(Janiak-Spens and West 2000). On the contrary, in the P1 domain of CheA, the phosphorylation efficiency remained unchanged if Lys51 was replaced by Ala (Mourey et al. 2001). The terminal amino groups of lysine residues at positions corresponding to Lys83 in ZmHP2 take part in electrostatic interactions in all known HPT proteins (Kato et al. 1999; Xu and West 1999; Mourey et al. 2001) except for ZmHP2 (Fig. 3).

Conserved residues and phosphorelay function

Conserved residues of plant HPT proteins are localized on helices C, D, and E of the four-helix bundle, and on helix B (Figs. 2, 4A). They surround the His80 phosphorylation site (Fig. 4B). In contrast, there is no conserved residue on helix A and only one (Cys113) on helix F. On the protein molecule, helix A and F are located at the opposite side as His80. Only Leu65 which is conserved in HPT proteins exposes a part of its C δ 1 atom on the side of the molecule opposite to His80 (Fig. 4C). In YPD1, the docking surface for SLN1 is formed by the helices B, C, and D of the four-helix bundle, and by helix A (Xu et al. 2003). The structural similarity of these four helices to the corresponding ones in ZmHP2 (helices B, C, D, E) is higher (RMSD of 1.0–1.2 Å for 65 C α atoms) than the similarity between the whole proteins (RMSD of 1.6 Å for 114 C α atoms). The *Arabidopsis* genes AHP1 to AHP3 rescue YPD1-defective yeast mutants (Miyata et al. 1998; Suzuki et al. 1998), sug-

Figure 5. Structural differences between ZmHP2 molecules in the asymmetric unit around the loop connecting the four-helix bundle with the N-terminal helices. Compared to Figure 1, this figure is rotated by 90 degrees around the horizontal axis. Since the structures of the monomers of each pair of ZmHP2 molecules in the asymmetric unit are essentially the same, only molecules A1 (green) and B1 (purple) are shown. Oxygen, nitrogen and sulfur atoms are shown in red, blue and green, respectively. Hydrogen bonds and salt bridges are indicated by dots. In molecule A1, residues 35–38 are indicated in yellow. Gly41 in molecule A1 and three glycine residues (37, 38, and 41) in molecule B1 are colored pink. The residues of molecule A1 involved in packing interactions with molecule B1 in the crystal are shown in cyan.

gesting that these AHPs can accept phosphate groups from the receiver domain of SLN1 and transfer them to RR SSK1 or SKN7. The primary structures of ZmHP2 and AHPs are homologous with 40%–47% identical residues. Thus, the binding mode between HPT proteins and receiver domains seems to be essentially the same in yeast and plants. If so, the conserved amino acids on the surface close to the protruding His80 would probably function as a docking interface for the receiver domains in plants.

On the other hand, nonconserved residues in the isoforms of HPT proteins in each plant species would appear important for the recognition of specific molecular partners. Ala87 and Ser88 which are surrounded by conserved residues (Fig. 4B) are likely candidates for such specificity-mediating elements. The side chains of the corresponding residues in YPD1, Ala71, and Ala72, form intermolecular contacts with the receiver domain of SLN1 in the complex (Xu et al. 2003). Ala87 is conserved in maize, but the corresponding residues in *A. thaliana* are threonine (AHP4) or serine (all other AHPs). Specific physiological functions of AHP4 therefore might depend on this residue. Similarly, Ser88 is conserved in all known plant HPTs except ZmHP1 where it is replaced by Asn84. Asn84 might therefore be crucial for the specific activity of ZmHP1. In addition, Met99 of ZmHP2 might be also the candidate (Fig. 4B). It is close to fully conserved Lys83. In the yeast complex, the corresponding residue in YPD1, Glu83, also makes intermolecular contact with the receiver domain (Xu et al. 2003). In maize, Met99 is replaced by Ile in ZmHP1 and ZmHP3. In *A. thaliana*, the corresponding residues are Val (AHP1 to AHP3), Thr (AHP4) or Ile (AHP5). Further biochemical characterization of the interactions of specific plant HPT proteins and receiver domains will help us to identify the key residues involved in discriminating the physiological partner.

Materials and methods

Construction of expression plasmid

For expression of ZmHP2, the coding region of ZmHP2 was amplified by polymerase chain reaction with the primers 5'-AG-GATCCATGGCTGCCGCCGCTGAG-3' and 5'-TAAGCT-TATCATGTGAGGCCACATGC-3'. The BamHI/HindIII fragment was ligated into pQE30 (Qiagen). To remove the His-tag extension by dipeptidyl peptidase I, the BamHI site (GGATCC) was replaced by a Gln codon (CAG) using QuikChange (Stratagene). The resulting plasmid was designated pQEZmHP2QM.

Preparation of ZmHP2

For the production of selenomethionine-labeled (SeMet) protein, the Met-auxotroph *E. coli* strain B834 (Novagen) harboring pREP4 (Qiagen) was used as the host cell. The pQEZmHP2QM transformants were cultured with shaking in LeMaster medium supplemented with 1 M sorbitol and 25 mg L⁻¹ seleno-L-methio-

nine (LeMaster and Richards 1985; Hendrickson et al. 1990), 100 µg mL⁻¹ ampicillin, and 25 µg mL⁻¹ kanamycin at 37°C until the A_{600} was 1.0. Temperature of the medium was shifted to 25°C and isopropyl-D-thiogalactopyranoside was added to a final concentration of 1 mM. The culture was grown overnight with shaking. Cells were harvested and lysed with CellLytic B-II (Sigma). After treatment with DNase I, cell debris was removed by centrifugation at 18,000 × *g* for 20 min. The supernatant was loaded onto a Ni-NTA superflow column (Qiagen) equilibrated with 50 mM Tris-HCl (pH 8.0), 300 mM NaCl, 10% (v/v) glycerol, and 3.5 mM 2-mercaptoethanol. Adsorbed proteins were eluted by 500 mM imidazole. Gel filtration was performed on a HiLoad Superdex 75 prep-grade column (Amersham Biosciences) equilibrated in 25 mM Tris-HCl (pH 7.5), 200 mM NaCl, and 3.5 mM 2-mercaptoethanol. The His-tag region was digested using dipeptidyl peptidase I (Qiagen), and the resulting N-terminal Gln residue was removed by glutamine cyclotransferase and pyroglutamyl aminopeptidase (Qiagen) according to the suppliers' protocols. The enzymes and unprocessed ZmHP2 proteins were removed using a Ni-NTA superflow column. SeMet samples were dialyzed against 10 mM Tris-HCl (pH 7.5) and 5 mM dithiothreitol.

The expression and purification conditions for native ZmHP2 were the same as for SeMet ZmHP2 except that Luria-Bertani medium was used instead of LeMaster medium.

Crystallization

Crystallization was performed by the hanging-drop vapor-diffusion method. The drops consisted of 2 µL of a protein solution and 2 µL of a precipitating solution. Plate-like crystals (0.15 × 0.15 × 0.2 mm³) were obtained at 20°C after a few days in 50 mM ammonium sulfate and 100 mM sodium acetate buffer (pH 4.0) when the protein solution was concentrated to 5 mg mL⁻¹. Protein crystals were also obtained with 20% (v/v) glycerol as a cryo-protectant in the solution. Crystallization conditions for native ZmHP2 protein were the same as for SeMet.

Data collection

SeMet and native crystals were flash-frozen in a nitrogen cold stream. Data collections were performed on a SPRING-8 beamline BL45XU (Yamamoto et al. 1998). X-ray diffraction data were processed using the program HKL2000 (Otwinowski and Minor 1997). The space group of native ZmHP2 crystals was determined to be monoclinic *C*2 and the unit cell parameters of native crystals were $a = 148.80$, $b = 81.41$, $c = 89.50$ Å and $\beta = 123.42^\circ$. Assuming four molecules of the protein in the asymmetric unit, the value of the Matthews constant, V_M (Matthews 1968), is 3.5 Å³ D⁻¹, corresponding to a solvent content of 65% (w/v). The final data for native ZmHP2 crystals comprised 42,708 independent reflections with 94.1% completeness for a range of 20–2.2 Å and an overall R_{merge} of 4.7%. Data collection statistics are listed in Table 1.

Structure determination and refinement

The crystal structure of SeMet ZmHP2 was solved by a multi-wavelength anomalous dispersion method using the program SOLVE (Terwilliger and Berendzen 1999). Density modification and automated model building were performed with the program RESOLVE (Terwilliger 2000). Main chains of the initial model were 70% complete. When the four molecules in the asymmetric

Table 1. Diffraction data statistics of native and SeMet ZmHP2 crystals

	Native	Peak	Edge	Remote
Wavelength (Å)	1.0000	0.9791	0.9793	0.982
Resolution (Å)	2.2	2.4	2.4	2.4
No. of reflections	149,189	254,527	253,337	127,761
No. of unique reflections	42,707	34,458	34,907	34,535
Completeness (%) ^a	94.1 (91.7)	99.0 (92.7)	98.7 (89.9)	98.7 (90.3)
I/sigma	11.6	12.9	10.6	13.3
R _{merge} (%) ^{a,b}	4.7 (21.4)	9.3 (21.9)	8.1 (51.0)	6.2 (27.0)

^a Values in brackets refer to the highest resolution shells (2.28 – 2.2 Å of native crystal, 2.49–2.4 Å of SeMet crystal).

^b $R_{\text{merge}} = \frac{\sum_{hkl} \sum_i |I(hkl)_i - \langle I(hkl) \rangle|}{\sum_{hkl} I(hkl)}$, where $I(hkl)_i$ is the i th measurement of the intensity of reflection hkl , and $\langle I(hkl) \rangle$ is the mean intensity of reflection hkl .

unit were superimposed after the identification of side chains, a 90% complete model of one ZmHP2 molecule could be constructed. Each of the four molecules was replaced with this model, and rigid-body refinement was carried out. The model was refined using the program CNS (Brünger et al. 1998) with noncrystallographic symmetry (NCS) restraints. Five percent of the reflections were set aside for R_{free} calculations (Brünger 1997). Manual fitting of the model was carried out with the program Xfit (McRee 1999). Finally, the model was refined using the program REFMAC (Mursudov et al. 1999) including TLS refinement (Winn et al. 2001) without NCS restraints. The crystal structure of native ZmHP2 was determined by the molecular replacement method using the program AMoRe (Navaza 1994). The search model was the SeMet ZmHP2 structure. The refinement procedure for the native ZmHP2 was the same as that for SeMet. The quality of the final model was assessed by Ramachandran plots and analysis of model geometry with the program PROCHECK (Laskowski et al. 1993). The plot indicated that 95.7% of the residues lay in favorable regions and 4.3% in allowed regions. The final R and R_{free} factors for all the reflections between 20.0 and 2.2 Å resolution were 0.211 and 0.248, respectively. The refinement statistics are listed in Table 2.

Table 2. Refinement statistics of native ZmHP2

	Native
Resolution (Å)	20–2.2
No. of protein molecules	4
No. of protein atoms	4,175
No. of water molecules	103
No. of reflections	
Working set	40,566
Test set for R_{free}	2,141
R_{free} (%) ^a	24.8
R_{work} (%) ^a	20.9
R_{cryst} (%) ^a	21.1
RMSD from standard geometry	
Bond length (Å)	0.041
Bond angle (degrees)	2.56
Ramachandran plot	
Residues in most favored regions (%)	95.7
Residues in additional allowed regions (%)	4.3
Residues in generously allowed regions (%)	0

^a R_{free} , R_{work} and $R_{\text{cryst}} = \frac{\sum_{hkl} |F_{\text{obs}}| - |F_{\text{calc}}|}{\sum_{hkl} |F_{\text{obs}}|}$, where F_{obs} is the observed structure factor amplitude and F_{calc} is calculated independently for the working set, the test set and both sets combined, respectively.

Secondary structure assignment was performed with the program PROMOTIF (Hutchinson and Thornton 1996). Figures were created with the program PyMOL (DeLano 2002).

Atomic coordinates

The coordinates have been deposited in the RCSB Protein Data Bank, accession code 1WN0.

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