Crystalllographic snapshot of the *Escherichia coli* EnvZ histidine kinase in an active conformation

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**Article info**

Article history:
Received 24 December 2013
Accepted 17 March 2014
Available online 26 March 2014

Keywords:
Two-component signal transduction system
HAMP domain
DHp domain
CA domain
Phosphoryl transfer

**Abstract**

Sensor histidine kinases are important sensors of the extracellular environment and relay signals via conformational changes that trigger autophosphorylation of the kinase and subsequent phosphorylation of a response regulator. The exact mechanism and the regulation of this protein family are a matter of ongoing investigation. Here we present a crystal structure of a functional chimeric protein encompassing the entire catalytic part of the *Escherichia coli* EnvZ histidine kinase, fused to the HAMP domain of the *Archaeoglobus fulgidus* Af1503 receptor. The construct is thus equivalent to the full cytosolic part of EnvZ. The structure shows a putatively active conformation of the catalytic domain and gives insight into how this conformation could be brought about in response to sensory input. Our analysis suggests a sequential flip-flop autokinase mechanism.

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1. Introduction

Two-component signal transduction (TCST) systems are key players in the adaptation of bacteria and fungi to changing environmental conditions (reviewed by Gao and Stock (2009)). The first component of these systems is a dimeric sensor histidine kinase; the second is its cognate target, termed the response regulator, which interacts directly with downstream elements, most commonly as a transcription factor. Sensor kinases have three obligate players in the adaptation of bacteria and fungi to changing environmental conditions (reviewed by Gao and Stock (2009)). The first is its cognate target, termed the response regulator, which interacts directly with downstream elements, most commonly as a transcription factor. Sensor kinases have three obligate components: an N-terminal sensor, a central dimerization/histidine phosphorylation (DHp) domain and a C-terminal catalytic/ATP-binding (CA) domain. In response to a signal generated in the sensor domain, phosphate from ATP bound to the CA domain is transferred to a histidine residue on the DHp domain. Depending on the architecture of the DHp domain (Ashenberg et al., 2013), this histidine is either on the same chain as the CA domain (cis preference) or on the other chain (trans preference). In a second step, the phosphate is transferred to an aspartate residue on the response regulator. The reverse reaction is also catalyzed – i.e. the dephosphorylation of the response regulator – via a mechanism potentially independent of the phospho-histidine intermediate (Hsing and Silhavy, 1997). Sensing events control the balance of these forward (kinase) and reverse (phosphatase) activities and thus the cellular levels of phosphorylated response regulator.

The segment linking the sensor domain to the catalytic core is highly variable, ranging from elements as simple as a single helix to linear arrays of multiple domains. The most common constellation is as a transmembrane receptor, where the sensor domain is extracellular, allowing for monitoring of the environment, and the linker includes the transmembrane helices. In this situation, HAMP, a small domain frequently encountered in linking segments, is often the first intracellular domain. We determined the first structure of a HAMP domain, that of the putative transmembrane receptor Af1503 from *Archaeoglobus fulgidus*, revealing a parallel, four-helical coiled-coil bundle (Hulko et al., 2006).

The amenability of Af1503 HAMP to structural studies led us to construct functional chimeras with various upstream and downstream domains from chemoreceptors, adenylyl cyclases and histidine kinases, including the catalytic core of EnvZ, the osmositivity-sensing histidine kinase from *Escherichia coli* (Hulko et al., 2006; Kanchan et al., 2010; Mondejar et al., 2012; Ferris et al., 2011; Ferris et al., 2012). Two recent examples of structural studies based on the Af1503 HAMP domain are found in this special issue (Ferris et al., 2014; Hartmann et al., 2014). In the course of our studies, we systematically introduced mutations at key positions in the HAMP domain, which modulate the activity of these chimeras. A particular focus of attention was the core residue A291, for which we showed a strong inverse correlation between sidechain size at this position and activity of the resulting chimera. Our studies of chimeras combining Af1503 HAMP with histidine kinase...
Table 1

| Data collection | P2₁ |
|-----------------|-----|
| Space group     |      |
| Unit cell parameters | a = 71.1 Å, b = 76.6 Å, c = 97.4 Å, β = 107.0° |
| Resolution range (Å) | 38.8–2.85 (3.02–2.85) |
| Completeness (%) | 99.2 (97.1) |
| Redundancy (%)   | 3.32 (3.23) |
| I/σ (I)         | 8.87 (2.09) |
| Rmerge (%)      | 9.0 (39.2) |
| Refinement      |      |
| Resolution range (Å) | 38.8–2.85 (2.98–2.85) |
| Rmerge (%)      | 25.8 (31.0) |
| Rfree (%)       | 27.7 (35.9) |
| Residues in core/allowed/generously allowed regions in Ramachandran plot (%) | 89.7/9.6/0.4 |

Values in parenthesis refer to the highest resolution shell; Ramachandran statistics were determined using PROCHECK (Laskowski et al., 1993).

The crystal structure of Af1503-EnvZ. (A) The asymmetric unit contains two dimers, yellow/green and red/blue, which both have the same conformation. (B) A coiled coil of DHp domains show HAMP to be connected to the following four-helical continuous helices. These connecting helices resemble a loosely packed, two-helical coiled coil and contain a stutter in their sequence repeat pattern, corresponding to the insertion of four residues into the underlying heptad period. A conserved sequence motif – DRT in EnvZ – is located at the stutter and resembles a loosely packed, two-helical coiled coil and contain a stutter in their sequence repeat pattern, corresponding to the insertion of four residues into the underlying heptad period. A conserved sequence motif – DRT in EnvZ – is located at the stutter and forms the locus of conformational changes induced by A291 mutations (Ferris et al., 2012).

Building on our analyses of HAMP–DHp tandems combining Af1503 HAMP with EnvZ DHp, we now extend our studies to constructs that include the full histidine kinase catalytic core, wherein the Af1503 HAMP serves as a stable replacement for the native EnvZ HAMP domain. In a previous study we have shown this chimera to be fully functional in an in vivo assay (Ferris et al., 2012). Now, based on a crystal structure, we derive a model for the activation of the EnvZ sensor histidine kinase.

### 2. Purification, crystallization and structure determination

We devised the constructs Af1503 HAMP (wt) – EnvZ DHp-CA and Af1503 HAMP (A291F) – EnvZ DHp-CA as extensions of the HAMP–DHp fusion constructs in Ferris et al. (2012) to include the whole C-terminus of the EnvZ protein. Expression and purification were performed as previously described with a final gel-sizing step in a buffer containing 30 mM MOPS, pH 7.0 and 50 mM NaCl for the wild-type construct and a buffer containing 20 mM MOPS, pH 7.0 and 100 mM NaCl for the A291F variant. For subsequent crystallization, the proteins were concentrated to 2 and 20 mg/ml, respectively.

Crystallization trials were performed via the sitting-drop vapor diffusion method at 295 K. Drops containing 400 nl of protein solution and 400 nl of reservoir solution were prepared on 96-well plates with a reservoir volume of 50 μl. The best-diffracting crystals where obtained in conditions containing 0.1 M MMT buffer pH 4.0 and 25% (w/v) PEG 1500 for the wild-type fusion and 0.2 M lithium acetate and 20% (w/v) PEG 3350 for the A291F variant. The crystals were transferred into a drop containing 10% (v/v) PEG 400 in addition to the respective reservoir solution, loop-mounted, and flash-cooled in liquid nitrogen. Diffraction data were collected at 100 K and a wavelength of 1 Å on a PILATUS 6 M detector at beamline PXII of the Swiss Light Source (PSI, Villigen, Switzerland). Data were indexed, integrated and scaled using XDS (Kabsch, 1993). We first solved the structure of the A291F variant with data to 2.85 Å in space group P2₁. Molecular replacement was carried out with MOLREP (Vagin and Teplyakov, 2000) and the HAMP–Dhp fusion structure 3ZRV as a search model, locating residues unstructured in at least one chain are grayed out. These belong – apart from the termini – to loop regions in the CA domains. The structural representations in this and the next figure were prepared using MolScript (Kraulis, 1991) and Raster3D (Merritt and Bacon, 1997).
two dimers in the asymmetric unit (ASU). After initial rigid body refinement using REFMACS (Murshudov et al., 1999), electron density for all four CA domains became apparent, which could be traced extensively using Buccaneer (Cowtan, 2006). The structure was finalized by cyclic manual modeling with Coot (Emsley and Cowtan, 2004) and refinement with Phenix (Adams et al., 2010). Residues were numbered as previously in Ferris et al. (2012), according to the Af1503 HAMP domain. Data collection and refinement statistics are summarized in Table 1. Structure solution of the wild-type fusion was subsequently done by molecular replacement using the A291F variant structure with twinned data to 3.6 Å resolution in space group \( P2_1 \) with \( \beta \approx 90^\circ \) and a twinning fraction close to 50%, locating four dimers in the ASU. After initial rigid body refinement and several rounds of restrained refinement with REFMACS it became apparent that the conformation of the four dimers in the ASU was identical to the conformation of the two dimers in the A291F variant structure. At that point the wild-type dataset was abandoned. In the rest of this report we will refer to the A291F variant structure if not stated otherwise. It was deposited in the Protein Data Bank (PDB) under accession code 4CTI.

3. Structure description and analysis

The crystals contain two dimers in the asymmetric unit, which have a virtually identical conformation (Fig. 1). The HAMP and DHp domains form an elongated helical bundle as in the fusion structure 3ZRV, where the C-terminal helices of the HAMP domain connect to the N-terminal helices of the DHp domain. Within each dimer, HAMP and the tips of the DHp domain are in the same conformation as in 3ZRV, but the connecting helices are asymmetric, with one connector forming a continuous helix and the other showing a kink at the transition between the two domains. This kink is located at the DRT motif-containing stutter, where the helix is unwound and broken into two separate helices. We have previously observed similar asymmetric conformations at this location in the crystal structures of different HAMP–DHp constructs (Ferris et al., 2012). In the present structure, at the height of the kink, the two CA domains are attached to each side of the bundle. The CA domains have the expected \( \alpha/\beta \)-sandwich fold with three \( \alpha \)-helices packed on one side of a mixed five-stranded \( \beta \)-sheet. As the electron density of the CA domains was less well defined than for the HAMP and DHp domains, several connecting loops of the CA domains were not visible and thus not included in the final structure (Fig. 1F). Accordingly, compared to the HAMP and DHp domains, the CA domains have elevated temperature factors.

The CA domains of the two subunits of the bundle are found in different orientations. On one side, the CA domain is loosely packed with its C-terminal helix to the C-terminal helix of the DHp domain of its own chain. In contrast, on the other side, its C-terminal helix forms a tight helical bundle with the C-terminal DHp helix of its own chain and the N-terminal DHp helix of the other chain.

![Fig. 2. Analysis of the Af1503-EnvZ structure and comparison to VicK. For both kinases, the DHp and CA domains are colored by chain and other domains grayed out. As EnvZ phosphorylates in trans and VicK phosphorylates in cis, the connectivity of the helices at the tip of the DHp domains differs so that the colors of the CA domains appear to be swapped between the two kinases (Ashenberg et al., 2013). In both, the CA domain on the left side is in a putative active conformation and has an ATP molecule superimposed from the CA domain from PDB entry 3SL2. The ATP molecules and the catalytic histidines are shown in stick representation. The bottom panels show the association of the active CA domains with the DHp domains as viewed from the tip of the DHp. While the active CA domains are in similar conformations in both kinases, the orientations of the two different active CA domains are related by a \( \sim 90^\circ \) rotation. In EnvZ, the helices of the active CA domain are aligned with the helices of the DHp domain and the C-terminal helix forms a helical bundle with the DHp domain (highlighted grey). Right at the center of this helical bundle is the kink at the DRT motif (encircled red). In contrast, the helices of the active CA domain of VicK run orthogonal to the DHp domain (C-terminal helix highlighted grey).]
particular, this interface also spans the unwound DRT motif in the connector between the HAMP and DHp domains (Fig. 2). This conformation may therefore be dependent on the presence of the HAMP domain upstream of the DHp domain.

As the crystal structure does not contain ATP, we modeled ATP molecules into the CA domains by superposition with the ATP-bound CA domain from the Walk histidine kinase form Bacillus subtilis, PDB entry 3SL2 (Celikel et al., 2012). To our delight, in the tightly attached CA domain, the ATP molecule is positioned such that it is in the most direct vicinity of the catalytic histidine in the other chain of the DHp domain (Fig. 2). As EnvZ is known to phosphorylate in trans and only a subtly nuanced conformational change would be necessary to align the ATP and the histidine for phosphoryl transfer, we submit that this is an active conformation. The relevance of this conformation is further supported by the crystal form of the wild-type construct. Therein, all four dimers in the asymmetric unit exhibit the same conformation within a completely different crystal packing.

4. Mechanistic implications and comparison to VicK

The structure suggests that the docking of the CA domain in the active conformation is correlated to the kinking of the stutter found at the junction between HAMP and DHp. As we have previously implicated this site in the response to up-stream signals (Ferris et al., 2012), it is conceivable that the conformational response of the DRT motif at the stutter regulates its tendency to adopt a kinked conformation and thereby regulates CA docking in the observed conformation.

Due to the asymmetric nature of the kink, we assume that only one CA domain can dock in an active conformation at one time. We deduce from this a sequential flip-flop autokinase mechanism, where the bundle alternately kinks upon docking of a CA domain on either side. In this model, the CA domains do not interact with the DHp domain in the region of the tip, but stay associated with it at the height of the DRT motif, above the catalytic histidine. As the response regulator binds at the tip below the catalytic histidine, it is conceivable that the kinase could be active while an interaction with the response regulator is already established.

Recently, a sequential autokinase activation model has also been described for the sensor histidine kinase VicK from Streptococcus mutans by Wang et al. (2013). Although structures of other histidine kinases have been reported, e.g. HKS3 (Marina et al., 2005), DesK (Albanesi et al., 2009) and YFI (Diensthuber et al., 2013), VicK is the only kinase for which a putative catalytic conformation of the CA domains has been observed. In the VicK model, the sequential mechanism is inferred from asymmetric helical bending in the DHp domain, which is analogous to the observed kinking in the EnvZ structure. In their article, the authors report the (almost) full-length structure of VicK and also find one of the CA domains docked in a putatively active conformation to the DHp domain. This structure, with ATP modeled into the active CA domain, is compared to our EnvZ structure in Fig. 2. The major differences to the EnvZ structure are (I) the orthogonal rather than parallel orientation of the active CA domain relative to the DHp domain, (II) its attachment towards the tip of DHp, below the catalytic histidine, and (III) its interaction with the histidine on its own chain (cis preference), rather than that of the other monomer. Despite these differences, the two models for EnvZ and VicK have the same underlying principle and similar mechanistic implications. While the different orientations of the CA domains and their cis vs. trans preference for phosphoryltransfer may reflect system-specific adaptations, the underlying asymmetry-based flip-flop mechanism could reflect a common mechanistic feature of histidine kinases.

Acknowledgments

We thank Kerstin Bär and Reinhard Albrecht for technical assistance and are grateful to the staff of beamline PX1 at the Swiss Light Source for excellent technical support. The work was supported by institutional funds from the Max Planck Society.

References

Adams, P.D., Afonine, P.V., Bunkoczi, G., Chen, V.B., Davis, I.W., Echols, N., Headd, J.J., Hung, L.W., Kapral, G.J., Grosse-Kunstleve, R.W., McCoy, A.J., Moriarty, N.W., Oeffner, R., Read, R.J., Richardson, D.C., Richardson, J.S., Terwilliger, T.C., Zwart, P.H., 2010. PHENIX: a comprehensive python-based system for macromolecular structure solution. Acta Crystallogr. D Biol. Crystallogr. 66, 213–221.
Albanesi, D., Martin, M., Trajtenberg, F., Mansilla, M.C., Haouz, A., Alzari, P.M., de Mendoza, D., Buschiazzo, A., 2009. Structural plasticity and catalysis regulation of a thermosensor histidine kinase. Proc. Natl. Acad. Sci. USA 106, 16185–16190.
Ashenberg, O., Keating, A.E., Laub, M.T., 2013. Helix bundle loops determine whether histidine kinases autophosphorylate in cis or in trans. J. Mol. Biol. 425, 1198–1209.
Cowtan, K., 2006. The Buccaneer software for automated model building. 1. Tracing protein chains. Acta Crystallogr. D Biol. Crystallogr. 62, 1002–1011.
Celikel, R., Veldere, V.H., Mathews, I., Devine, K.M., Varughese, K.L., 2012. ATP forms a stable complex with the essential histidine kinase Walk (YycG) domain. Acta Crystallogr. D Biol. Crystallogr. 68, 839–845.
Diensthuber, R.P., Bonmer, M., Gleicchmann, T., Möglich, A., 2013. Full-length structure of a sensor histidine kinase pinpoints coiled coils as signal transducers and modulators. Structure 21, 1127–1136.
Emsley, P., Cowtan, K., 2004. Coot: model-building tools for molecular graphics. Acta Crystallogr. D Biol. Crystallogr. 60, 2126–2132.
Ferris, H.U., Dunn-Horkawicz, S., Mondejar, L.G., Hulko, M., Hantke, K., Martin, J., Schultz, J.E., Zeth, K., Lupas, A.N., Coles, M., 2011. The mechanisms of HAMP-mediated signaling in transmembrane receptors. Structure 19, 378–385.
Ferris, H.U., Dunn-Horkawicz, S., Horing, N., Hulko, M., Martin, J., Schultz, J.E., Zeth, K., Lupas, A.N., Coles, M., 2012. Mechanism of regulation of receptor histidine kinases. Structure 20, 56–66.
Ferris, H.U., Zeth, K., Hulko, M., Dunn-Horkawicz, S., Lupas, A.N., 2014. Axial helix rotation as a mechanism for signal regulation inferred from the crystallographic analysis of the E. coli serine chemoreceptor. J. Struct. Biol. 186, 349–356.
Gao, R., Stock, A.M., 2009. Biological insights from structures of two-component proteins. Annu. Rev. Microbiol. 63, 133–154.
Hartmann, M.D., Dunn-Horkawicz, S., Hulko, M., Martin, J., Coles, M., Lupas, A.N., 2014. A soluble mutant of the transmembrane receptor Af1503 features strong changes in coiled-coil periodicity. J. Struct. Biol. 186, 357–366.
Hsing, W., Silhavy, T.J., 1997. Function of conserved histidine-243 in phosphatase activity of EnvZ, the sensor for porin osmoregulation in Escherichia coli. J. Bacteriol. 179, 3729–3735.
Hulko, M., Berndt, F., Gruber, M., Linder, J.U., Trautlaff, V., Schultz, A., Martin, J., Schultz, J.E., Lupas, A.N., Coles, M., 2006. The HAMP domain structure implies helix rotation in transmembrane signaling. Cell 126, 929–940.
Kabsch, W., 1993. Automatized processing of rotation diffusion data from crystals of initially unknown symmetry and cell constants. J. Appl. Crystallogr. 26, 795–800.
Kanchan, K., Linder, J., Winkler, K., Hantke, K., Schultz, A., Schultz, J.E., 2010. Transmembrane signaling in chimeras of the Escherichia coli aspartate and serine chemotaxis receptors and bacterial class III adenylyl cyclases. J. Biol. Chem. 285, 2090–2099.
Kralik, P.J., 1991. MOLSCRIPT: a program to produce both detailed and schematic plots of protein structures. J. Appl. Crystallogr. 24, 946–950.
Laskowski, R.A., Macarthur, M.W., Moss, D.S., Thornton, J.M., 1993. Procheck - a program to check the stereochemical quality of protein structures. J. Appl. Crystallogr. 26, 283–291.
Marina, A., Waldburger, C.D., Hendrickson, W.A., 2005. Structure of the entire cytoplasmic portion of a sensor histidine-kinase protein. EMBO J. 24, 4247–4259.
Merritt, E.A., Bacon, D.J., 1997. Raster3D: photo-realistic molecular graphics. Methods Enzymol. 277, 505–524.
Mondejar, L.G., Lupas, A., Schultz, A., Schultz, J.E., 2012. HAMP domain-mediated signal transduction probed with a mycobacterial adenyl cyclase as a reporter. J. Biol. Chem. 287, 1022–1031.
Murchudov, G.N., Vagan, A.A., Lebedev, A., Wilson, K.S., Dodson, E.J., 1999. Efficient anisotropic refinement of macromolecular structures using FFT. Acta Crystallogr. D Biol. Crystallogr. 55, 247–255.
Vagan, A., Teplyakov, A., 2000. An approach to multi-copy search in molecular replacement. Acta Crystallogr. D Biol. Crystallogr. 56, 1622–1624.
Wang, C., Sang, J., Wang, J., Su, M., Downey, J.S., Wu, Q., Wang, S., Cai, Y., Xu, X., Wu, J., Senadheera, D.B., Civitkovitch, D.G., Chen, L., Goodman, S.D., Han, A., 2013. Mechanistic insights revealed by the crystal structure of a histidine kinase with signal transducer and sensor domains. PLoS Biol. 11, e1001493.