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Structure of the Cytoplasmic Segment of Histidine Kinase Receptor QseC, a Key Player in Bacterial Virulence†

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Abstract

QseC is a histidine kinase (HK) receptor involved in quorum sensing, a mechanism by which bacteria respond to fluctuations in cell population. We conducted a structural study of the cytoplasmic domain of QseC (QseC-CD) using X-ray crystallography. The 2.5 Å structure of the apo-enzyme revealed that the kinase domain of QseC retains the overall fold of the typical HK kinase domain. The construct that we used is inactive in the autokinase reaction and its inactivity is most likely caused by its atypical dimerization interface, as compared to that observed in the *T.maritima* HK cytoplasmic domain structure. Restoration of the activity may require that the entire dimerization domain be present in the protein construct. QseC, which plays an important role in bacterial pathogenesis, is a promising drug target and the structure of QseCCD provides a platform for developing more potent inhibitors of pathogen virulence.

Keywords

Cytoplasmic domain; histidine kinase; phosphorylation; quorum sensing; response regulator; two-component regulatory systems

INTRODUCTION

Two-component regulatory systems involving the His-Asp phosphotransfer are commonly utilized for signal transduction in prokaryotes and are also found in fungi and plants [1]. The systems typically consist of a histidine kinase (HK) receptor and a response regulator (RR). HKs function as signal-transducing receptors and have an autokinase activity with a signal-induced self-phosphorylation of a conserved histidine residue. Subsequently, the phosphoryl group is transferred to a conserved aspartate residue of an RR. Most RRs are transcription factors that, once phosphorylated, bind to DNA to activate or repress gene transcription. Besides the autokinase and phosphotransferase activities, HKs also possess a phosphatase activity as part of their self-regulatory mechanism.

A large majority of HKs are class I HKs [2]. A typical HK topology consists of a short cytoplasmic fragment followed by a membrane-spanning α helix (TM1), an extracellular (or periplasmic) sensor domain, the second transmembrane helix (TM2), and finally the C-terminal cytosolic kinase domain [1]. While the extracellular or periplasmic domain varies in sequence as well as length and can respond to different environmental stimuli, the cytoplasmic portion of HKs usually comprises an array of conserved modular structures Fig.

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(1A). The cytosolic region contains two distinct domain-ns: an N-terminal dimerization/histidine phosphotransfer (DHp) domain and a C-terminal catalytic/ATP-binding (CA) domain. The DHp domain is responsible for the dimerization of HKs and contains the conserved phospho-accepting histidine. The CA domain is composed of several conserved boxes (N, G1, F and G2), which are responsible for the molecular recognition and hydrolysis of ATP. Additionally, there typically exists a domain called HAMP domain, a variable-length linker that connects TM2 to the cytosolic domain. This domain has been hypothesized to transmit signals between the signal-sensing and the cytoplasmic domains and therefore is predicted to be highly flexible [3, 4]. The downstream RRs usually comprise two domains: the receiver domain that interacts with HKs and accepts the incoming phosphoryl group, and the effector domain that outputs the signal, usually by binding to DNA to activate or repress gene transcription. HK/RRs are the most commonly used sensory system for signal transduction in bacteria.

Quorum sensing is a mechanism utilized by bacteria to respond to changes in cell density by altering gene expression when a hormone-like compound or autoinducer (AI) reaches a threshold concentration [5, 6]. QseC, the Quorum Sensing histidine receptor from *E. coli*, autophosphorylates upon the detection of epinephrine/norepinephrine (EPI/NEI) or AI3 and subsequently donates the phosphoryl group to the aspartate residue on its response regulator QseB, as the start of a complex signaling pathway [7]. Through the QseC/QseB two-component system, quorum sensing is responsible for the regulation of *flhDC*, the master regulator of the flagella and its motility [8]. Previous studies have demonstrated that quorum-sensing via the QseC/QseB pathway is involved in bacterial pathogenesis in which the phosphorylation event leads to the expression of the key virulence genes in many human pathogens. It has also been shown that the *qseC*-null strain, rabbit enteropathogenic *E. coli* E22 (REPEC) is unable to trigger expression of these virulence genes and displays decreased growth in animal infection models [9–11].

A handful of HK kinase domain structures have been solved [12–17]. In particular, the complete cytoplasmic domain of the *T. maritima* histidine kinase (HK853-CD), as well as the cytoplasmic catalytic core of *G. stearothermophilus* sporulation kinase (KinB) in complex with its inhibitor Sda, have been determined at 1.9 Å and 2.0 Å, respectively [15, 17]. This paper describes the expression, crystallization, and structural determination of the cytoplasmic domain of QseC, QseC-CD.

MATERIALS AND METHODS

Cloning, Protein Expression and Purification

The cytoplasmic domain of the original QseC (Swiss-Prot accession No. P40719) was amplified from the *E. coli* genomic DNA using two following primers: 5'-TAGGGCC-ATATGCGTGAACGACGCTTTACCT CCG-3' and 5'-TTGCACTTCTCGAGCCAGC TTACC-TTCGCCTCAAATC-3'. The amplified fragment was cloned into a pET-21b (+) vector (EMD Biosciences) using the restriction sites NdeI and XhoI. The new construct comprises the residues 236–449 of the native QseC linked to the His₆ tag by a dipeptide spacer (Leu-Glu). The mutant T338C was created by quick-change site-directed mutagenesis (Strata-gene) using the two quick-change primers 5'-TGCCTCAAT GCCACAGCATCAAACG -3' and 5'-CAGTCGCACGTCAATTTTCGC -3'. The plasmids of wild-type and mutants were transformed into *E. coli* strain BL21 (DE3) (Invitrogen).

The culture was grown in Luria–Bertani broth containing 100 µg/ml ampicillin. Fresh culture medium was inoculated with a 5 ml overnight culture of the wild-type enzyme or the mutant T338C and grown at 37°C to an O.D.600 of 0.5–0.6 where 0.4 mM IPTG was added.

The temperature was then reduced to 20°C and the culture was allowed to grow overnight before being harvested by centrifugation at 5000×g for 20 minutes. Cell pellet from 1L culture was resuspended with 45 ml Ni-NTA buffer A containing 40 mM Tris-HCl (pH 8.0), 150 mM NaCl, 10 mM imidazole, 1 mM PMSF. The cells were disrupted by sonication and the lysate was centrifuged at 20 000×g for 30 minutes to remove cell debris. The supernatant was bound with Ni-NTA resin (Qiagen) at 4 °C for one hour, washed with buffer A and eluted with buffer B containing 40 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1 mM PMSF, 250 mM imidazole. The QseC-CD-containing fractions were pooled, concentrated, applied onto a gel-filtration column (Sephadex 200 HR 16/30) (GE healthcare) and eluted with a buffer containing 20 mM Tris-HCl (pH 8.0), 150 mM NaCl. The selenomethionine (SeMet) culture was grown by the inhibition-pathway method [18–21]. The SeMet-incorporated protein was purified by the same protocol to the native protein except 2mM DTT was added.

The protein was concentrated to 8–10 mg/ml using a Vivaspin 20 centrifugal filter (MWCO=30KDa, Millipore) and crystallized by the vapor diffusion method of 2 µl hanging drops with 27–30% (NH₄)₂SO₄, 0.1 M HEPES (pH 7.5) at 15 degrees. Crystals were soaked for 3–5 minutes in a freshly-made cryoprotective solution containing all the components of the reservoir solution plus 20% (v/v) glycerol. The soaked crystals were mounted on a nylon loop and flash-frozen in liquid nitrogen.

Data Collection and Structural Determination

2.5 Å two-wavelength MAD data sets were collected at Advanced Light Source Beamline 8.2.1 (Lawrence Berkeley National Laboratory, Berkeley, California, United States). The data were processed with DENZO and scaled with SCALEPACK from the HKL-2000 package [22]. Four Se-Met positions were located except for the N-terminal SeMet with the program SOLVE [23] using the MAD protocol and the phases were subsequently improved by RESOLVE [24]. The auto-build option of the program PHENIX [25] was used to trace the polypeptide chains from the electron-density map calculated to 2.5 Å resolution. The resulting model was completed manually and the amino-acid sequence was fitted to the electron-density maps using Coot [26]. The crystallographic refinement was performed with Refmac [27] using medium noncrystallographic symmetry (NCS) restraints and isotropic B factors. The stereochemistry of the final model was analyzed by PROCHECK from the CCP4 program suite [28] and MolProbity [29]. The final model has an R_{cryst} factor of 22.8% and an R_{free} of 26.2%. The molecular images were prepared using PyMOL [30]. The atomic coordinates and structure factors (code 3JZ3) were deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (<http://www.rcsb.org/>).

Autokinase Activity Assay

1 µM enzyme was incubated with 10 µM norepinephrine in a 10.5 µl buffer containing 75 mM Tris-HCl (pH 8.0), 75 mM KCl, 15 mM MgCl₂, 1.5 mM DTT and kept on ice for 1 h. The autophosphorylation reaction was allowed to start by adding 1.5 µl 50 µM non-labeled ATP and 3 µl [γ -³²P]ATP (10 µCi/µl at 3000 Ci/mmol). 5.0 µl aliquots were removed at 10-minute intervals and the reaction was stopped by addition of SDS-PAGE sample buffer containing 20 mM EDTA and subsequent heating at 80 °C for 3 minutes. The samples were analyzed on a 12.5% SDS-PAGE gel by electrophoresis. The gel was dried and exposed on KODAK BioMax XAR film using Lightning Plus intensifying screens (Dupont).

RESULTS

Overall Structure

The QseC-CD construct, which contains a part of the C-terminal domain of QseC, starts with Arg236 (the 54th residue from the C-terminus of TM2 as predicted by the SMART server [31, 32]) and includes the phospho-accepting histidine H246 Fig. (1A). The crystal structure revealed two molecules in the asymmetric unit Fig. (1B), which are related to each other by a two-fold non-crystallographic symmetry (NCS). In the structure, residues 236-274, 281 and 391-416 of chain A and 236-261, 271-275 and 392-417 of chain B are not observed. The electron density for the C-terminal his₆-tags is also absent except for the very first histidine residue in chain A. The refined model for the two chains contains a total of 310 amino acids, 44 water molecules and one sulfate ion with $R_{\text{cryst}} = 0.228$, $R_{\text{free}} = 0.262$. The statistics are summarized in Table 1.

The final structure is composed of a short N-terminal β strand ($\beta 1$) and an α helix ($\alpha 1$), in addition to the catalytic domain. The N-terminal region of QseC-CD is mostly disordered, probably resulting from the unfolding of the four-helix bundle and the formation of the short unexpected β sheet. The average r.m.s.d. value for the pairwise-superimposed subunits is 1.4 Å over 147 C α s with an alignment similarity Z-score of 26.1. The average temperature factors were 43.7 and 47.9 Å² for chains A and B, respectively. The two largest structural differences between the two chains reside at the $\alpha 2$ - $\beta 3$ junction and at the starting β strands, where a small β sheet is formed. Since chain B is more structured, its structure will be described unless otherwise specified.

The $\alpha 1$ helix is connected to the $\beta 2$ strand through a 12-residue loop see Fig. (2) for secondary structure designation-ns). The catalytic domain has the typical fold of a five-stranded β sheet mixed with three α helices. This two-layered sandwich fold resembles that of HK853-CD, a close homologue which shares about 30% sequence identity and 50% sequence similarity with QseC-CD. A β strand ($\beta 1$) is observed at the N-termini of both chains A and B in the electron density map, but some of the side-chains were not evident due to the weak density. The $\beta 1$ strands of each monomer form a parallel β sheet Fig. (1C) that specifically interacts with neighboring water molecules and residues to form an extensive interaction network. Particularly, the guanidino group of Arg285 in chain B forms a hydrogen bond with the backbones of Gln263, Leu264, as well as Ser265 in chain B; the guanidino group of Arg358 and the carboxyl oxygen of Asp323 in chain A each form a hydrogen bond to the Asp268 backbone of chain B. These interactions anchor the two β strands in place, even though their termini are disordered.

Helical-Hairpin Domain

Class I HKs initiate signal transduction upon sensing ligands or other stimuli with their extracellular signal-sensor domain. Induced conformational changes are then transduced through the transmembrane four-helix bundle into the cytoplasmic domain of the dimeric receptor [3, 4]. In HK853-CD, the helical-hairpin DHp domain comprises two antiparallel helices connected by a turn, and in the dimer, two of these two-helix hairpin domains form a four-helix bundle packed in the coiled-coil arrangement Fig. (3A). In contrast, due to its disorder, the crystal structure of QseC-CD completely lacks the first hairpin helix, only retaining the second helix Fig. (3B). Gel-electrophoresis of the dissolved crystals followed by silver-staining indicated that the protein was still intact, supporting the notion that the lack of electron density for the N-termini is a result of protein flexibility, likely due to a choice of the construct. Additionally, the second helix ($\alpha 1$ in QseC-CD) consists of only 20 residues, and is substantially shorter compared to that of HK853-CD (residues 288-317). However, the loop that leads to the catalytic domain is longer in QseC-CD than the 7-

residue linker in HK853-CD. A major difference between the two structures is that the orientation of the $\alpha 1$ is nearly parallel to the β sheet in the kinase domain of QseC-CD, whereas it is practically perpendicular in HK853-CD. This unusual orientation of $\alpha 1$ may be a result of a partial disorder of the N-terminus. Consequently, $\alpha 1$ makes contact with the helices $\alpha 3$ and $\alpha 4$ in QseC-CD and creates a significantly buried inter-domain surface area of about 1600 Å². Therefore, $\alpha 1$ looks more like an integral part of the kinase domain than a part of a separate dimerization domain as in HK853-CD. Furthermore, the $\alpha 1$ helices from the two chains do not directly interact with each other as they do in HK853-CD. The two helices are closest at their membrane-proximal ends, diverging at ~ 35° (Fig. (3B)).

Nucleotide Binding Domain

The overall structure of the QseC-CD's kinase domain is similar to that of HK853-CD. In HK853-CD, residues corresponding to 433–441 of the so-called ATP lid are partially disordered in the presence of a hydrolyzed ATP analog. In the presence of ATP, this intrinsically flexible loop undergoes large conformational changes to accommodate the binding of ligands [13, 14]. The ATP lid is not observable in QseC-CD, possibly due to the fact that the structure does not contain any ligands. Therefore, the nucleotide-binding domain of QseC-CD could not be structurally defined based on electron density maps because of the disordered residues comprising the ATP lid of QseC-CD, the conformation of which might be similar to the conformation of a 26-residue loop also undefined in the putative ATP-binding pocket of PhoQ (PDB entry 3CGY [33]).

Catalytic Domain for ATP hydrolysis

A structure-based sequence alignment with several other HK kinase domains is shown in Fig. (2). Not surprisingly, the aligned residues primarily cluster in the N, G1, F, and G2 boxes. Unfortunately, the entire F box and parts of the G1 and G2 boxes in QseC-CD fall into the disordered region. A search against the protein databank for structural homologues of the catalytic domain retrieved two closest structures: the kinase domains of HK853-CD (PDB entry 2C2A) and of PhoQ (PDB entry 3CGY). The r.m.s.d of the superposition of 117 C α s from QseC-CD and HK853-CD is 2.1 Å and the similarity Z-score is 16.5. Similarly, the r.m.s.d of the superposition of 113 C α s from QseC-CD and PhoQ is 1.9 Å and the similarity Z-score is 15.9 Fig. (4). Therefore, despite the unstructured regions and differences in the dimerization domains, the general CA fold is preserved in the catalytic domain of QseC-CD. The small local variations between QseC-CD and HK853-CD are mainly found at residues 323-325, 340-345, 369-373, 380-382, 388-391, 418-419, 438-442 and 450-451, most of which are from non-conserved regions such as β -turns or loops. On the other hand, the N, G1, F, G2, and G3 boxes, which form the ATP binding pocket, are preserved in QseC-CD Figs. (2 and 5). It is known that in HK853-CD, Asp411 in the G1 box makes a hydrogen bond with N6 of the adenosine moiety while the side chain of Tyr384 in the N-box makes hydrogen bonds with O3' of the sugar, as well as the β phosphate and also makes stacking interactions with the base ring Fig. (5A). These two residues help to anchor the nucleoside and lock it in place. Additionally, Lys383 from the N box forms a salt bridge with the β phosphate while the backbone of Leu446 from the G2 box donates a hydrogen bond to the α phosphate Fig. (5A). Sequence alignment revealed that these essential residues are highly conserved and structural overlay shows that their orientations are approximately the same Fig. (5B and C). A basic/aromatic pair is preferred at positions 366/367 in the major class of HKs. Fifty-eight percent of the HKs have either a lysine or an arginine at position 366 and nearly all have an aromatic residue at position 367 (86% cases) [15]. As described above, the role of Tyr367 in QseC-CD (Tyr384 in HK853-CD) is to make stacking interactions with the adenine base of the nucleotide while the role of Arg366 is to neutralize the negative charge of the phosphate leaving group.

Crystal Packing

The crystal packing reveals two possible dimer interfaces one involving the $\beta 3$ strand and another involving the $\alpha 1$ helix Fig. (6). The intermolecular interactions involving $\alpha 1$ also involve the $\beta 1$ strand and the $\alpha 1$ - $\beta 2$ linker (they are summarized in Table 2). The other interface is created by $\beta 3$, which forms a continuous β -sheet with $\beta 3'$ of its symmetry mate Fig. (6A). In the latter case, Leu337 and Leu339 form backbone hydrogen bonds with their symmetry-related residues Leu339' and Leu337' at the interface. Side chains of the four leucines point to a similar direction and stack against each other to form part of the hydrophobic core. Usually, HKs dimerize through the helical hairpin that includes helix $\alpha 1$. However, the QseC-CD construct does not possess the entire predicted DHp domain. Constructs that contained the entire DHp domain yielded proteins that formed aggregates. To further test the $\beta 3$ interface, we generated the T338C mutant whose cysteine residues should easily form a disulfide bond if the $\beta 3$ interface exists in solution. Our studies show that the T338C mutation has no perceptible impact on the oligomeric state, failing to form a cross-linked dimer in a non-reducing SDS-PAGE gel. Furthermore, the buried surface area of the intermolecular contacts involving $\beta 3$ is only 1219.2 Å² and the ΔG^{int} calculated by the PISA server is -4.3 kcal/mol, while the buried interface involving $\alpha 1$ is 3225.8 Å² with a ΔG^{int} of -19.6 kcal/mol [34] (the surface representations of the two different dimers are shown in Fig. (6B)). Therefore, it seems unlikely that the dimerization interface involving $\beta 3$ has any biological relevance.

In the structure of QseC-CD, the first hairpin helix and an N-terminal part of the second helix are disordered. When we performed an autokinase reaction activity assay with 1 mM, 2 mM, 5 mM and 10 mM MgCl₂ at neutral to basic pHs, we found that QseC-CD remained inactive under all tested conditions. In contrast, the full-length enzyme retained its activity while solubilized in detergents (data not shown). Therefore, our structure is of an inactive conformer.

DISCUSSION

HKs are multifunctional enzymes that participate in auto-kinase, phosphotransferase, and phosphatase reactions, all three of which involve the phospho-accepting histidine [35]. To better understand the phosphorelay mechanism between the histidine kinase QseC and its response regulator QseB, we determined the crystal structure of the cytoplasmic domain of QseC (QseC-CD). Like HK853, QseC-CD is predicted to contain the conserved long helical hairpin region following TM2. Several domain boundaries were tested along this region in order to search for the best-behaving construct. The resulting QseC-CD construct starts with Arg236, which included most of the sequence that corresponds to the four-helix bundle in the HK853 structure. The structure was determined from a selenomethionine-labeled protein by the MAD phasing method at the resolution of 2.5 Å. The Ca r.m.s.d between the catalytic domains of QseC-CD and HK-853-CD is 2.1 Å for the 117 Ca's that lie outside of the four-helix bundle motif.

QseC-CD protein is dimeric in solution, as determined by gel filtration chromatography. On a calibrated Sephadex 200 gel filtration column, the estimated molecular weight of QseC-CD is 65 kDa. The crystal structure revealed that two different dimerization interfaces are possible: one involving the $\alpha 1$ helix and the other involving the $\beta 3$ strand. However, the $\alpha 1$ interface appears to be more likely because of its larger buried surface area and more negative ΔG^{int} energy associated with its formation. Further, the results obtained from the interface mutant T338C also indirectly suggested that dimerization through $\beta 3$ is unlikely.

Apart from the N-terminal region, there is another disordered area in the catalytic domain between $\beta 6$ and $\beta 7$ that is a part of the nucleotide-binding pocket. The structure of HK853-

CD in complex with AMPPNP also has about ten unstructured residues surrounding this pocket. We could not locate any ligands bound to QseC-CD even though both Mg^{2+} /AMPPNP and AMPPCP were individually added to the crystallization sample. Additionally, crystal-soaking experiments did not reveal the presence of the substrates even though up to 10 mM ATP analog had been added. The auto-kinase assay of QseC-CD indicates that this construct was not able to autophosphorylate itself although the full-length enzyme was active. The lack of autokinase activity of the QseC-CD construct was consistent with the failure of the soaking and co-crystallization experiments. The non-productivity of QseC-CD most likely results from the distorted dimer interface, which is due to the N-terminal truncation of the first hairpin helix. Restoration of the activity may require the presence of the entire dimerization domain in the protein construct.

QseC is a bacterial adrenergic receptor which links cross-kingdom signaling by sensing a bacterial hormone-like compound (AI-3) as well as the host hormones EPI or NE. QseC homologues are present in at least 25 important human and plant pathogens, such as EHEC and *S. typhimurium* [36], and they mediate the expression of virulence genes. Indeed, the *qseC* mutant is attenuated for virulence in animal models [9]. A recent study demonstrated that a lead molecule, LED209, is able to very effectively block QseC signaling in *E. coli*, *S. typhimurium*, and *F. tularensis* (with a picomolar concentration inhibition) by preventing its autophosphorylation and inhibiting virulence factor gene expression [36]. Crystallization of the complex of the periplasmic domain of QseC bound with LED209 is underway and the resulting structure will be an excellent starting point for structure-guided drug design.

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Fig. 1. Construct and overall structure of QseC-CD. (A) Modular domain diagram of the full-length QseC receptor and QseC-CD construct. (B) Overall structure of QseC-CD in ribbons. The two subunits are colored in cyan and green. A 90° rotation shows the dimer interface. (C) Local environment involving the $\beta 1$ sheets. Specific hydrogen-bonding interactions and their distances are shown.

Fig. 2. Structure-based sequence alignment of QseC-CD, TM0853, PhoQ and KinB HKs. The conserved motifs N, G1, F, G2, G3 boxes and ATP lid are labeled. Residues identical in all four sequences are highlighted in red.

Fig. 3. Interface close-up views. **(A)** The HK853-CD dimer structure. The two subunits are colored in green and cyan and the four-helix bundle is approximately perpendicular to the membrane plane. The second helix in the DHp domain of each subunit is colored in red. **(B)** The QseC-CD dimer structure. The two subunits are colored in green and cyan and are shown in a similar orientation relative the membrane plane to HK853-CD. The $\alpha 1$ helices, corresponding to the second helix in the DHp domain of HK853-CD, are also colored in red and they form an angle ~ 35 degree.

Fig. 4.

α trace representation of the HK kinase domains with stereo view: QseC-CD (*green*), HK853-CD (*cyan*, Protein Data Bank code 2c2a) inactive PhoQ (*magenta*, Protein Data Bank code 3cgy), PhoQ bound with radicicol (*grey*, Protein Data Bank code 3cgz), and KinB-CC (*orange*, Protein Data Bank code 3d36).

Fig. 5. Comparison and superposition of kinase domain of four HKs. **(A)** The stick representation of the active site of HK853-CD and the essential residues are labeled. The hydrogen bonding distances are shown. **(B)** The hypothetical Qsec-CD'-ADP complex. The ADP coordinate is from the HK853-CD structure (Protein Data Bank code 2c2a) and is shown in grey. **(C)** The overlay of conserved essential residues at the active site of the four HKs.

Fig. 6. Crystal packing of QseC-CD. **(A)** Ribbon diagrams of the two possible dimers in the cell. Other symmetry mates are omitted from the view. Inset: the enlarged view of the new dimer. Dimer interface residues are shown in sticks. **(B).** The surface representations of the two dimers. Left: dimer interface involving $\alpha 1$; right: dimer interface involving $\beta 3$.

Table 1

Statistics of Diffraction Data and Structure Determination

Diffraction		
	Peak	Inflection
Wavelength (Å)	0.96789	0.9794
Resolution	50–2.5 (2.59–2.5)	50–2.5 (2.59–2.5)
Unique reflections	16317	16362
Completeness (%)	99.9 (100)	99.9 (100)
Redundancy	13.5 (13.8)	13.4 (13.7)
I/σ^a	29.9 (10.4)	25.1 (4.6)
Rmerge (%) ^b	6.3 (29.4)	8.2 (70.3)
Refinement		
Resolution	34.7–2.5	
$R_{\text{crystal}}/R_{\text{free}} (\%)^d$	0.228/0.262	
Average B factor (Å ²)	45.9	
R.m.s. bonds (Å)	0.006	
R.m.s. angles (deg)	0.909	
Ramachandran analysis Favored/outlier (%) ^e	99.3 (0)	

Mean figure-of-merit (FOM) for phasing = 0.51 from solve and 0.64 after Resolve.

^a I/σ (I) is the mean reflection and intensity/estimated error.

^b $R_{\text{sym}} = \sum |I - \langle I \rangle| / \sum I$, where I is the intensity of an individual reflection and $\langle I \rangle$ is the average intensity over symmetry equivalents.

^c $R_{\text{crystal}} = \sum ||F_o| - |F_c|| / \sum |F_o|$, where F_o and F_c are the observed and calculated structure factor amplitudes.

^d R_{free} is equivalent to R_{crystal} but calculated for a randomly chosen set of reflections that were omitted from the refinement process.

^e Analysis from <http://kinemage.biochem.duke.edu>.

Table 2

The Interactions at the Dimer Interface

Residues in Chain B	Residues in Chain A	Distance (Å)
ASP 267[N]	LEU 276[O]	2.72
ASP 268[N]	ASP 323[OD2]	2.94
ARG 288[NE]	ASP 307[OD2]	3.22
ARG 288[NH2]	ASP 307[OD1]	2.78
GLN 292[NE2]	ASN 304[O]	2.99
GLN 306[N]	GLN 292[OE1]	2.84
SER 265[O]	LEU 276[N]	3.41
ASP 267[O]	GLN 278[N]	2.97
ASP 268[O]	ARG 358[NH1]	2.91
PRO 269[O]	HIS 280[N]	3.10
GLN 278[O]	LEU 277[N]	3.08
GLN 292[OE1]	GLN 306[N]	2.68
ASN 304[O]	GLN 292[NE2]	2.72
ASP 307[OD1]	ARG 288[NH2]	3.21