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STRUCTURE NOTE

X-Ray Structure of a Domain-Swapped Dimer of Ser46-Phosphorylated Crh From *Bacillus subtilis*

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Introduction. In *Bacillus subtilis*, approximately 10% of the genome is regulated by adenosine 5′-triphosphate (ATP)-dependent phosphorylation of Ser46 of HPr and its homolog Crh (for catabolite repression HPr).[1] The two proteins exhibit 45% sequence identity but residue 15 is a phosphorylable histidine in HPr and a glutamine in Crh. PEP-dependent phosphorylation of HPr His15 by enzyme I is the first step of the sugar phosphotransferase system called PTS in bacteria.[2] Crh does not participate in the PTS. In contrary, the ATP-dependent HPr kinase/phosphorylase (HprK/P) efficiently phosphorylates the conserved Ser46 of Crh.[3] The seryl-phosphorylated PserHPr and PserCrh act as alternate corepressors of the catabolite control protein A (CcpA).[4] CcpA is the central regulator of a fundamental bacterial signal transduction pathway called carbon catabolite repression.[5]

The X-ray structure of unphosphorylated Crh is a domain-swapped dimer.[6] Nuclear magnetic resonance (NMR) studies showed that Crh and PserCrh are both in slow monomer/dimer equilibrium.[7] The X-ray structure of complexed HprK/P with HPr or PserHPr[8] shows that the hexameric enzyme binds six molecules of monomeric protein substrate. The structure of CcpA in complex with PserHPr[9] reveals a PserHPr monomer bound to each subunit of the CcpA dimer.

Crystallization and Structure Determination. To elucidate whether the domain-swapped dimer is the active form of PserCrh protein that binds CcpA, we started cocrystallization experiments. *B. subtilis* Crh protein was produced in *Escherichia coli* with a C-terminal polyhistidine tag[3] and ATP-dependent seryl-phosphorylation was performed as described[10] using *B. subtilis* HprK/P. Cloning of the *B. subtilis* CcpA gene deleted from the N-terminal binding domain and production of the recombinant truncated protein, ΔCcpA, will be described elsewhere (Chaptal et al., in preparation). Crystals appeared in hanging drops containing 51 μM PserCrh, 49 μM ΔCcpA, 17.5% (w/v) polyethylene glycol (PEG) 4000, and 100 mM ammonium sulfate over pits containing 35% (w/v) PEG 4000 and 200 mM ammonium sulfate. The crystals were flash-frozen in liquid nitrogen after addition of 20% (v/v) glycerol to the mother liquor. They belong to the tetragonal space group P 4_322 with unit cell a = b = 67.7 Å and c = 117.1 Å. The asymmetric unit contains a dimer of PserCrh.

X-ray diffraction data were collected from a single crystal at 100 K at the ESRF (Grenoble, France) on beamline ID14-H2 with an ADSC Quantum Q4 detector. The data, evaluated using DENZO and SCALEPACK,[11] were 100% complete at 2.0 Å resolution, with I/σ = 4.8. Molecular replacement was performed with AMoRe[12] by using the dimeric Crh X-ray structure [Protein Data Bank (PDB) code 1MU4] as a search model. Refinement by CNS[13] yielded a final model with R_{cryst} = 19.9% and R_{free} = 21.4%, and good geometry. The final model contains a domain-swapped dimer of PserCrh (residues 1–86), two sulfate ions, and solvent atoms. Coordinates have been deposited in the RCSB under accession code 2AK7.

Structure Analysis. The two phosphorylated Ser46 residues of the dimer are well defined in the electron density and have B factors equivalent to the rest of the protein in the final model. As in the unphosphorylated form of Crh,[6] PserCrh dimerizes by domain swapping (Fig. 1). The first strand of one subunit completes the antiparallel β-sheet of the other subunit forming a tight dimer. Structural comparison of Crh and PserCrh dimers leads to a root-mean-square deviation value of 0.4 Å between all Cα excluding the three last residues of each subunit that are very flexible. Thus, as observed for HPr,[14] Ser46 phosphorylation does not induce any conformational changes in Crh.

When superimposing one subunit of the PserCrh dimer with the PserHPr molecule bound to one CcpA subunit (PDB code 1RZR[9]), the other PserCrh subunit overlaps

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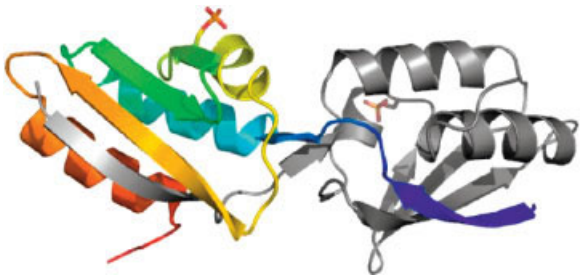

Fig. 1. Cartoon representation of the domain-swapped dimer of PserCrh. One subunit is colored in rainbow and the other in gray. The two Pser46 are shown in sticks.

with the C-subdomain of this CcpA subunit, suggesting that only the monomeric form of PserCrh can act as CcpA corepressor. The NMR structure of monomeric Crh (PDB code 1K1C[15]) is therefore the best model to analyze its CcpA binding and activation modes. The differences observed between PserHPr and PserCrh mediated activations of CcpA[16] cannot therefore be explained by PserCrh dimerization.

Modeling suggested that the His to Gln mutation of Crh residue 15 should not perturb the interaction with CcpA.[9] Contrariwise, the Thr20Ala mutation observed in Crh (and HPr from Gram-negative bacteria that are devoid of CcpA) will lead to the loss of the interaction with CcpA Tyr295. The Thr20–Tyr295 interaction has been shown to be crucial for the corepressor binding. Indeed, mutating the conserved CcpA Tyr295 into an arginine decreased its affinity for PserHPr and increased its ability to bind DNA *cre* sites in absence of the corepressor.[17] The absence of this interaction in the modeled PserCrh–CcpA complex suggests that the activation mode of CcpA may be different when PserCrh is used as corepressor instead of PserHPr.

When exploring the biological relevance of Crh and PserCrh dimers, one should consider their interactions with the bifunctional HprK/P. Superimposition of one subunit of the PserCrh dimer on a PserHPr molecule bound to HprK/P (PDB code 1KKM[8]) revealed a clash of the second subunit of PserCrh with the HprK/P C-terminal helix. Further unfavorable contacts are observed between two Crh dimers bound to opposite faces of the HprK/P hexamer. This analysis suggests that only the monomeric form of Crh or PserCrh can be substrate of the bifunctional HprK/P. HPr residues implicated in HprK/P interaction are conserved in Crh. Phosphorylation of HPr His15 is unfavorable to Ser46 phosphorylation by HprK/P.[18] In contrast, replacement of His15 by a glutamine in Crh should not prevent its interaction with the kinase.

Domain-Swapped Dimer. Crh and PserCrh crystallize as dimers whereas HPr and PserHPr crystallize as monomers. Solution studies showed that Crh and PserCrh form a mixture of monomers and dimers in very slow equilibrium, and that the percentage of dimers increases with protein concentration.[7] HPr is known to be a monomer. A recent publication[19] showed nevertheless that variants of HPr from *Bacillus stearothermophilus* could also form dimers via domain swapping. Interestingly, the structure of the domain-swapped dimer of HPr is very different from that observed with Crh. Domain swapping has been proposed as a mechanism for amyloid fibril formation[20] and HPr has been reported to form amyloid fibrils at acidic pH.[21]

The cellular protein concentrations are more consistent with the monomer and Crh synthesis has been shown to be even 100-fold lower than HPr synthesis.[22] It is conceivable that conformational changes between monomers and domain-swapped dimers could be of physiological importance under some conditions as a regulatory mechanism of signal transduction.

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