ABSTRACT

Sensor histidine kinases (SHKs) or histidine protein kinases (HPKs) are widely distributed stimuli-sensing components of two-component signaling systems (TCSs) present in bacteria, archaea, and lower eukaryotes. SHKs are known to play a vital role in detecting environmental stimuli such as temperature, light, pressure, osmolytes, gases, nutrients, metals, antibiotics, and antimicrobial peptides. They allow organisms to sustain through changes by regulating gene expression and modifying physiological functions by undergoing reversible phosphorylation upon signal detection. Studies on SHKs have been constrained to structural and biochemical information on truncated domains of a few representative members while lacking information on full-length membrane-embedded forms. To uncover the mechanism of intramolecular signal transfer in metal-sensing SHKs, we used X-ray crystallography to determine the crystal structure of the complete cytoplasmic portion of a zinc-sensing SHK ZraS from *Escherichia coli* (EcZraS-CD). The EcZraS-CD structure is resolved at a resolution of 2.49Å. For the first time, we could trap two successive nucleotide-bound intermediate conformers that provide insights into the dynamics of SHK signaling. Our analysis of the conformers reveals reorganization of the dimerization interface in the cytoplasmic four-helix bundle (DHp) caused by segmental helical bending, sliding, and rotation that leads to kinase activation. The findings emphasize on the kink-forming bundle residues that generate helical bending, polar and apolar residue interactions that facilitate sliding motions, and aromatic residue interactions that regulate the directionality of rotation. We also identified a unique linker that undergoes helical bending, aiding the transfer of signal to the cytoplasmic kinase region and the multi-step internal transitions through which the catalytic domain (CA) approaches the DHp for phosphorylation. To further investigate the rate of ATPase activity, we conducted an in vitro real-time kinetics using the pyruvate kinase-lactate dehydrogenase coupled assay. Collectively,

our work provides several important mechanistic insights into the signaling process of metal-sensing SHKs.

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