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The International Journal of Biochemistry & Cell Biology xxx (2005) xxx–xxx
IJBCB
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Molecules in focus

Bacterial histidine kinase as signal sensor and transducer

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Received 27 May 2005; received in revised form 9 August 2005; accepted 16 August 2005

Abstract

Adaptation to an environmental stress is essential for cell survival in all organisms, from *E. coli* to human. To respond to changes in their surroundings, bacteria utilize two-component systems (TCSs), also known as histidyl-aspartyl phosphorelay (HAP) systems that consist of a histidine kinase (HK) sensor and a cognate response regulator (RR). While mammals developed complex signaling systems involving serine/threonine/tyrosine kinases in stress response mechanisms, bacterial TCS/HAP systems represent a simple but elegant prototype of signal transduction machineries. HKs are known as a seductive target for anti-bacterial therapeutic development, because of their significance in pathological virulence in some bacteria such as *Salmonella enterica*. Recent molecular and structural studies have shed light on the molecular basis of the signaling mechanism of HK sensor kinases. This review will focus on recent advancements in structural investigation of signal sensing and transducing mechanisms by HKs, which is critical to our understanding of bacterial biology and pathology.

Keywords: Two-component system; Bacterial signal transduction; Stress response; Membrane receptors

1. Introduction

Two-component systems are required for innumerable adaptive responses in bacteria. These systems are widespread and exist not only in nearly all prokaryotes and many Archaea but also in eukaryotes such as plants, fungi and yeasts. A typical TCS consists of a histidine kinase sensor (HK), containing a conserved kinase core, and a cognate response regulator (RR), containing a conserved regulatory domain. The common feature among this large family of signaling proteins is that a dimeric histidine kinase undergoes ATP-dependent autophosphorylation on a specific histidine residue and subsequently transfers the phosphoryl group to an aspartate residue on a cognate response regulator, altering the latter's transcriptional, enzymatic or mechanistic properties (for a review see Stock, Robinson, & Goudreau, 2000). Several such His-Asp couples, including hybrid histidine kinases that contain additional response regulators or histidine-containing phosphotransfer domains, can be conjugated to constitute a phosphorelay pathway.

2. Structure

Thus far 30 *E. coli* HKs have been identified, and have been shown to contain common structural features, as well as a kinase catalytic motif topologically similar

Abbreviations: HK, histidine kinase; RR, response regulator; HAP, histidyl-aspartyl phosphorelay; TCS, two-component system; TM, trans-membrane

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doi:10.1016/j.biocel.2005.08.018

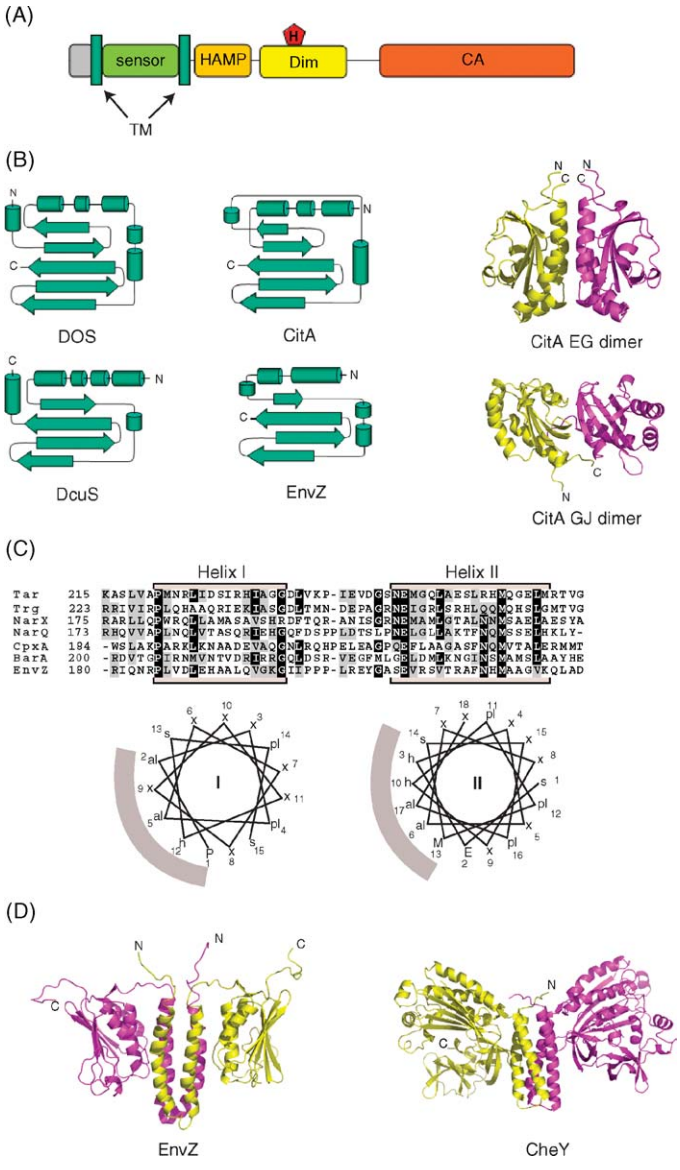

Fig. 1. Histidine kinase domain structure. (A) Schematic representation of Class I histidine kinase structure. This classification is based on the position of the conserved histidine-containing region with respect to the catalysis and ATP-binding (CA) domain (which contains the N, G1, F and G2 conserved boxes) in the primary sequence. The conserved His residue (H) is located in the H box of the dimerization (Dim) domain adjacent to the catalysis domain. (B) Representative secondary and ribbon diagrams of sensor PAS domains. Schematic representation of the secondary structures of DOS, CitA, DcuS, and EnvZ are shown. All secondary structures presented here are based on solved structures, except for that of EnvZ, which is based on secondary structure determinations and biophysical analyses (Khorchid, Inouye, & Ikura, 2005). Ribbon diagrams of two dimeric forms of CitA periplasmic domain observed in the crystal lattice are represented with individual monomers shown in *yellow* or *magenta*. (C) Multiple alignments of representative HAMP linkers in sensory proteins. For the purposes of comparison amino acid code sequences, immediately following TM2, for seven different sensory proteins (MCPs, first 2 rows; HK sensors, last 5 rows) were aligned. Numbers in the rightmost column denote the positions within the full-length protein sequences. The two predicted amphipathic helices are boxed. Helical representation of the two amphipathic helices, I and II, shows the consensus sequence calculated: h indicates hydrophobic residues (A, C, F, I, L M, V, W, Y), al indicates aliphatic residues (I, L, V), p indicates polar residues (D, E, H, K, N, Q, R, S, T), s indicates small residues (A, C, S, T, D, N, V, G, P), while x indicates non-conserved residues. The hydrophobic face of the helices is shaded in grey. (D) *Ribbon diagrams* of the dimeric kinase core domains of EnvZ and CheY. Within each dimer, individual monomers are *yellow* or *magenta*.

to the ATP-binding domains of a few ATPases. There are also significant differences in domain organization between them, possibly reflecting their distinct locations, functions and regulatory mechanisms. The typical HK, as exemplified by *E. coli* EnvZ, functions as a periplasmic membrane receptor. EnvZ is a 450 residue inner membrane histidine kinase with typical domain architecture: an N-terminal cytoplasmic tail (residues 1–15), two transmembrane domains (TM1, residues 16–34 and TM2, residues 163–179) flanking a periplasmic domain (residues 48–162) which has been thought to function as an osmosensor domain, and a cytoplasmic domain (residues 180–450) (Fig. 1A) (Forst, Comeau, Norioka, & Inouye, 1987). The cytoplasmic domain can be further dissected into a linker or HAMP domain (residues 180–222), domain A (dimerization and histidine containing domain, residues 223–289) and domain B (catalysis assisting and ATP binding domain, residues 290–450). Whereas EnvZ represents the most common membrane topology, some HKs have multiple TM segments. *Rhizobium meliloti* FixL and *E. coli* UhpB contain four and eight TM segments, respectively. Moreover, all known HKs are known or presumed to be membrane bound except for CheA and NtrB, which are soluble cytoplasmic proteins.

3. Activation

Although much is known about the structure and function of the catalytic core of HKs, the mechanisms underlying signal propagation and modulation of their catalytic activity remain elusive. It is believed that signal detection by the periplasmic sensor domain is propagated through the TM helices and the linker domain to the catalytic kinase core, which then undergoes conformational changes required to align the conserved His residue facing the ATP-binding pocket and form the kinase active site. The hinge region between domains A and B of the catalytic core has been implicated in mediating these conformational changes. Recent studies on these structural domains involved in signal propagation from the extracellular input, across the cell membrane, to the conserved kinase core of typical HKs are summarized below.

3.1. Periplasmic sensor domain

To date the structures of only two periplasmic domains of HKs have been solved, namely CitA (Reinelt, Hofmann, Gerharz, Bott, & Madden, 2003) and DcuS (Pappalardo et al., 2003). Both of these domains were shown to possess a Per-Ant-Sim (PAS) domain fold (Fig. 1B). Although this fold has been identified for the cytoplasmic sensing domain of oxygen sensing HK FixL, what is unique to CitA and DcuS is that PAS domains had not been previously observed outside the cytoplasm or been implicated in conformational signals across the membrane. PAS domains have been identified in over 1100 proteins and have been shown to convert various input stimuli into signals that propagate to downstream components by altering intra- and inter-molecular protein–protein interactions. The most strongly conserved feature of the PAS domain is the presence of a central β-sheet. Although the periplasmic domains of CitA, DcuS, and EnvZ show very little homology (pairwise sequence identity varying between 10% for CitA–EnvZ to 24% for Cita–DcuS), secondary structure analyses show that these domains have a very similar topology (Fig. 1B). Interestingly, the crystal structure of CitA periplasmic domain shows two different dimer interfaces. In the EG-dimer the dimer interface is formed mainly by the strands S3 and S4, the helical connector H5, with an additional but small interaction in the N-terminal region. In the GJ-dimer, the dimer interface is formed by the parallel association of the N-terminal helices H1 and H3 (Fig. 1B) (Reinelt et al., 2003).

Recent crystallographic studies on the phosphodiesterase DOS as well as NMR studies on the light-oxygen-voltage (LOV2) domain of phototropin have shown that the FG loop and Iβ of PAS domains play an important role in signal propagation by converting local conformational changes sensed by the ligand–binding motif into global subunit–subunit interactions. These observed changes provide important insight into signal transduction mechanisms mediated by PAS domain sensors. In one mechanism, light induced structural changes in the Hβ and Iβ strands of LOV2 lead to the disruption of their interaction with a C-terminal amphipathic alpha helix (αJ) and its release from the PAS domain core of the protein, resulting in the switch from a ‘closed’ dark state into an ‘open’ lit state of the protein (Harper, Neil, & Gardner, 2003). Based on the crystal structures of the Fe^{3+} and Fe^{2+} forms of *E. coli* DOS, a novel heme-redox switch mechanism was proposed, in which heme iron reduction is accompanied by heme-ligand switching from the His-77 side chain to a side chain of Met-95 in the FG loop, significantly rigidifying the flexible FG loop thereby leading to a change in the hydrogen bonding pattern and the rotation of subunits relative to each other. This locks the dimer into a tighter complex with each subunit moving in a ‘scissor-type’ motion as a rigid body by a 3° rotation around the rotation axis (Kurokawa et al., 2004).

3.2. Transmembrane domain

Most of our knowledge of transmembrane signaling comes from studies on the aspartate receptor (Tar) and the ribose and galactose receptor (Trg). Many studies have employed site-directed Cys residues and sulfhydryl chemistry to probe the molecular details of the helix movements and made use of engineered inter-helix disulfide bonds within these chemoreceptors. In vitro studies of Tar found that engineered disulfides in chemoreceptors block signaling if placed across TM1–TM2 helical interfaces (Chervitz & Falke, 1995) but not TM1–TM1′ interfaces (Chervitz, Lin, & Falke, 1995). An in vivo study of Trg in intact, functional cells found that four α1/TM1–α1′/TM1′ disulfides that constrain the subunit interface each allowed normal receptor signaling, but two TM1–TM2 disulfides each eliminated cellular responses to attractant stimulation (Lee, Lebert, Lilly, & Hazelbauer, 1995). In another in vivo study of the effects of signaling on the formation of disulfides tested 67 transmembrane Cys pairs spanning neighbouring helices of Trg. In the absence and presence of ligand, the same 19 pairs exhibited disulfide crosslinking indicating that conformational signaling did not produce large movements between transmembrane helices (Hughson & Hazelbauer, 1996). Among Cys pairs for which accurate rates of disulfide formation could be determined in vivo, ligand occupancy did not have a significant effect on any of four TM1–TM1′ intersubunit pairs but changed the rates for all four TM1–TM2 pairs, increasing two and decreasing two.

These collective observations lead to the common conclusion that in the periplasmic and transmembrane domains the α1/TM1–α4/TM2 interface within a receptor subunit is the locus of conformational signaling. The TM1–TM1′ subunit interface, which could be immobilized without affecting signaling, exhibited no movement detectable by diagnostic crosslinking. By contrast, the TM1–TM2 interface, at which immobilization eliminated signaling, was the same interface at which signaling altered the crosslinking rate of diagnostic Cys pairs. The simplest conformational change consistent with these constraints is a displacement of helix α4/TM2, refered to as the signaling helix, relative to the static subunit interface in the periplasmic and transmembrane domains.

In principle, the transmembrane signal could be carried by helix sliding, tilting or rotation, or altered helix dynamics. Essentially, all data indicate that the signaling movement is subtle and almost all relevant observations of signaling movement in the periplasmic and cytoplasmic domains are consistent with either a modest 1–2 Å piston displacement (Hughson & Hazelbauer, 1996; Bass & Falke, 1999), or a ∼4° rotation of the signaling helix (Cochran & Kim, 1996; Kwon, Georgellis, & Lin, 2003).

3.3. Linker HAMP domain

The linker domain connects TM2 to the conserved catalytic core of HKs. This domain represents an approximately 50-amino acid conserved α helix-turn-α helix motif that is present in multiple signaling contexts and is accordingly also refered to as a HAMP domain (domain present in histidine kinases, adenylyl cyclases, methyl-accepting proteins and phosphatases) (Aravind & Ponting, 1999) (Fig. 1C). Fifteen of the thirty known *E. coli* histidine kinases and all five methyl-accepting chemotaxis proteins contain a single HAMP domain. The two helices in the HAMP domain can be modelled as amphipathic α helices (Fig. 2). A number of studies have shown that mutations within the linker HAMP domain block the osmosensing function of EnvZ suggesting its important role in signal transduction. These studies suggested an important role for the amphipathic nature of the predicted HAMP domain helices in signal transduction, since only substitution of hydrophobic residue to charged residue, but not to hydrophobic residue, disturbed normal osmoregulation. The effects of these linker mutations on the function of the catalytic domain could only be observed when EnvZ is associated with the membrane indicating that the linker has to be fixed on the membrane to transmit the mutational effects to the catalytic domain (Park & Inouye, 1997).

Functional hybrid histidine kinases have been constructed by fusing the sensor module of one histidine kinase with the transmitter module of another histidine kinase at the HAMP linker region (NarQ–NarX, NarX–NarQ, and NarX–CpxA fusions) or by replacing the HAMP linker of one histidine kinase (NarX) with the HAMP linker of another histidine kinase (CpxA) (Appleman & Stewart, 2003). Other hybrid histidine kinases have also been constructed by fusing the sensor module of chemoreceptors to the transmitter module of histidine kinase EnvZ using the HAMP domain from either protein (Utsumi et al., 1989). The ligand responsiveness of all these hybrid sensor proteins indicates that a common mechanism may be shared for signal transduction. It also implies that the HAMP linkers of different sensor proteins function in a similar manner for signal propagation.

The exact mechanism of signal relay via the HAMP domain remains unknown. However, based on the requirement of the amphipathic nature of the HAMP

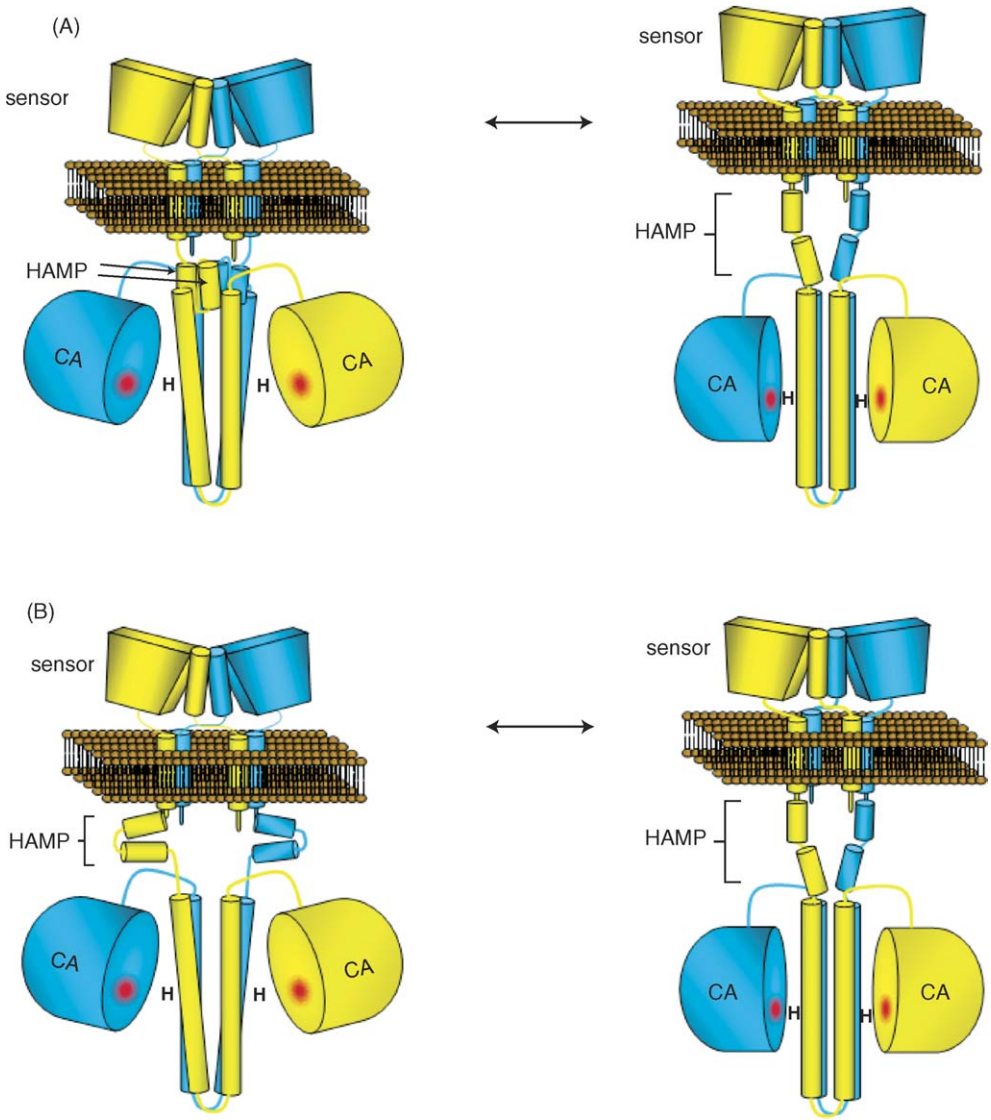

Fig. 2. Models for signal transduction via the HAMP domain. (A) In the inactivated state, HAMP domain helices bind intramolecularly to the anti-parallel helices of the dimerization domain forming a four-helix bundle. Ligand binding alters the conformation of HKs allowing dissociation of the HAMP domain helices and subsequent alignment of the four-helix bundle within the dimerization domain. This in turn leads to the alignment of the conserved histidine residue with the ATP binding site of the CA domain required for catalysis. The alignment could be achieved through the opening and closing of the kinase core as depicted in the illustration, or by sliding of the histidine containing helix of the dimerization domain relative to the CA domain. (B) In the inactivated state, the HAMP domain helices lie parallel to the plane of the membrane surface and transiently associate with one another. Ligand binding releases helix I from the membrane and changes HAMP helical association. This causes conformational changes that result in activation of the kinase core as discussed in (A).

domain helices, two models have been postulated. In the first model, the bihelical HAMP domain binds intramolecularly to the bihelical dimerization domain of HKs thereby forming a four-helix bundle. This internal association between the HAMP domain and the dimerization domain would prevent spontaneous signaling in the absence of cognate extracellular ligands by obstructing proper molecular alignment involving pairs of bihelical dimerization domains. (Fig. 2A) (Aravind & Ponting, 1999). The second model proposes that helix I of the HAMP domain lies parallel to the plane of the membrane surface and transiently interacts with helix II in the HAMP domain (Williams & Stewart, 1999) (Fig. 2B). Both models predict that ligand binding alters the conformation of HKs allowing dissociation of the HAMP domain, and subsequent alignment of the four-

helix bundle within histidine kinase dimers The two HAMP α-helices adopt two contrasting conformations depending on the ligand bound state of the HK, concomitantly altering the conformation of the catalytic core thereby modulating HK function.

4. Possible medical applications

The modular design of HKs has allowed for the high versatility and adaptability of bacterial TCSs. The necessity of bacterial adaptation to ever changing multifaceted environments has predicated the processing of multiple signals by different TCSs. This networking between different TCSs has been shown to occur when multiple sensors interact with a single response regulator, by phosphatases interrupting phosphoryl transfer in phosphorelays and through transcriptional and post-transcriptional mechanisms (Bijlsma & Groisman, 2003). HKs are of great medical interest because their involvement in multiple aspects of bacterial regulation and their absence in animals make them ideal targets for the development of new classes of antimicrobial drugs. Antimicrobials that interfere with a pathogen's HK signaling systems may expose it to destruction by the host immune system rather than being directly toxic. This form of indirect antibiotics is extremely important at a time when we are seeing increasing drug resistance by a number of bacterial pathogens.

Acknowledgements

We apologize for all the researchers whose work could not be adequately discussed and cited due to space limitations. We would like to thank Dr. Masayori Inouye and Takeshi Yoshida for their critical reading of this manuscript. This work was supported by a grant from the Canadian Institutes of Health Research (CIHR). MI is Canada Research Chair in Cancer Structural Biology. AK is supported by a grant from the American Foundation for AIDS Research.

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