

SIGNALING PATHWAY IN TWO-COMPONENT REGULATORY SYSTEM REVEALED BY COMBINATION OF X-RAY CRYSTALLOGRAPHY AND SOLUTION SCATTERING TECHNIQUE

A sensor histidine kinase (HK) and a cognate response regulator (RR) are protein elements of a two-component regulatory system, which are ubiquitously present in prokaryotes, fungi and plants. The system is involved in cellular signal transduction for homeostasis in response to sudden changes in the environment. HK is a single peptide consisting of three domains, i.e., a sensor, a dimerization/histidine-containing phosphotransfer (DHP), and ATP-binding catalytic (CA) domains from N- to C-termini (Fig. 1). HK functionally acts as a dimer through the interaction of the DHP domain between each subunit. The sensor domain of HK senses an environmental change as an external signal, and then the conformational change induced upon signal receipt would be transmitted to either activate or inactivate the CA domain. The activated CA domain catalyzes the phosphorylation of the special His residue at the DHP domain using ATP. The phospho-group is then transferred to the special Asp in RR, and the resultant phospho-RR can act as a transcriptional factor for promoting the expression of target proteins.

Depending on external signals such as metal ions, amino acids, nitrogen compounds, light, and osmotic pressure among others, the sensor domains display a variety of structures. For example, the oxygen sensor FixL contains a heme iron as an O₂ binding site in the sensor domain, while the ethylene sensor ETR1 has a transmembrane sensor domain containing Cu as an ethylene binding site. On the other hand, the

structures of the DHP and CA domains in HK and of RR are essentially the same among a variety of two-component systems. This fact implies that the interdomain or intermolecular signal transduction can be achieved using a similar mechanism. However, there has been no report on the structures of the HK/RR complex, although the structures of each separated domain have been reported for many HKs and RRs in the two-component regulatory system.

The structure of the HK/RR complex of the thermophilic bacterium *Thermotoga martina* was determined by the combination of X-ray crystallography (BL44B2) and solution scattering (BL45XU) technique [1]. The solution scattering for HK was measured with and without the sensor domain, and in the presence and absence of RR (titration experiment). A comparison of the SAXS parameters thus obtained (Table 1) showed that the sensor domain of HK is located on the molecular edge of the HK dimer giving the longest dimension, and that two molecules of RR can associate with the central portion of the HK dimer with a dissociation constant (K_d) of 8 × 10⁻¹¹ M⁻². On the other hand, an electron density map of the HK/RR complex was obtained at 4.2 Å resolution. On the basis of structural information obtained from the SAXS analysis, the domains of each subunit of HK and RR could be unambiguously assigned, as shown in Fig. 2. To our knowledge, this is the first report on spatial arrangement in the two-component regulatory system.

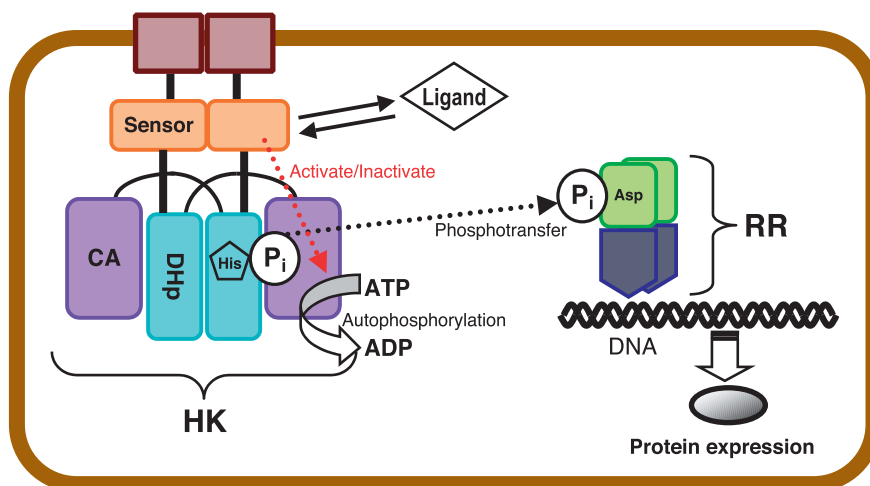


Fig. 1. Schematic drawing of two-component regulatory system.

Table 1. Structural parameters obtained from solution scattering experiments

	M_w (kDa) ^(b)	R_g (Å) ^(c)	D_{max} (Å) ^(d)	Association state
HK/RR	98 (54)	38	105	2:2 Dimer
HK	76 (41)	37	105	Dimer
ΔHK^(a)	51 (28)	31	88	Dimer
RR	11 (13)	16	42	Monomer

(a) HK without sensor domain.

(b) Molecular mass estimated by the experiments. The values in the parentheses represent theoretical mass of the monomeric protein.

(c) Radii of gyration.

(d) Longest linear distances.

The structure of the HK/RR complex, even at a low resolution, can provide us clues to unveiling interdomain and intermolecular signal transductions in the two-component regulatory system. (i) The sensor domain of one subunit comes in contact with the CA domain of the same subunit, suggesting that the signal could directly transfer from the sensor to the CA domains in the *cis* manner. (ii) In the complex structure, the active site of the CA domain of one subunit is located about 25 Å away from the phosphorylation target His in the DHp domain of

another subunit, because His imidazole can be phosphorylated in the *trans* manner. Therefore, in the course of the catalytic reaction, the CA domain would move by 25 Å to directly come in contact with the DHp domain in the absence of RR [2]. (iii) RR interacts with the HK sensor domain as well as its DHp domain, implying that RR might affect the sensing capability of the sensor domain. These findings significantly support previous experimental results for the oxygen sensor system [3].

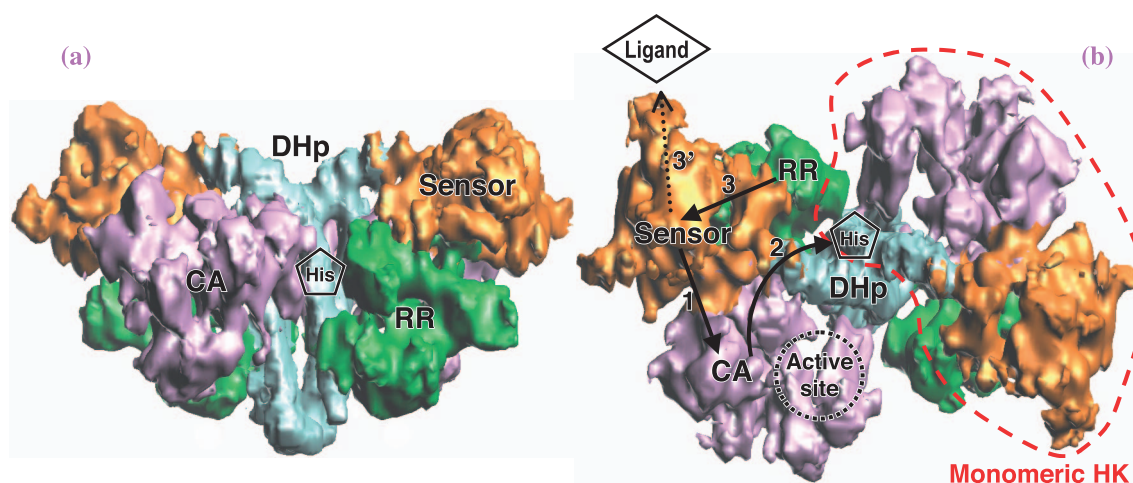


Fig. 2. Electron density map of HK/RR complex and assignment of domains. (a) side view and (b) top view along dimerization axis. The arrows indicate the pathway of the signal transduction we proposed.

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References

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