Note
Mutational Analysis of the Histidine-containing Phosphotransfer (HPt) Signaling Domain of the ArcB Sensor in Escherichia coli

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Received April 28, 1998; Accepted July 2, 1998

The Escherichia coli Arc sensor is involved in anaerobic phosphotransfer signal transduction. ArcB is a hybrid sensor that contains three types of phosphotransfer signaling domains in its primary amino acid sequence, namely, transmitter (or His-kinase), receiver, and histidine-containing phosphotransfer (HPt) domains. However, examination of the function of the newly-discovered HPt domain (named ArcB*) is still at a very early stage. To gain a general insight into the structure and function of the widespread HPt domains, on the basis of its three-dimensional crystal structure, in this study we constructed a certain set of mutants each having a single amino acid substitution in the HPt domain of ArcB. These ArcB* mutants were characterized and evaluated, based on the in vivo ability to signal the OmpR receiver via trans-phosphorylation.

Key words: Escherichia coli; phosphotransfer signal transduction; ArcB sensor; HPt domain

Bacteria have devised two-component phosphotransfer signaling mechanisms for eliciting a variety of adaptive responses to their environment. A typical two-component system consists of two types of signal transducers, a "sensor kinase" and a "response regulator". These signal transduction proteins contain one or both of the following common phosphotransfer signaling domains, a "transmitter (or His-kinase)" and a "receiver". Besides these, some signal transducers were recently found to have another common signaling domain, termed a "histidine-containing phosphotransfer (HPt) domain". A typical HPt domain was first discovered in the Escherichia coli ArcB sensor, which is responsible for anaerobic responses. ArcB contains both a transmitter and a receiver in its primary amino acid sequence, followed by the HPt domain (previously designated as ArcB, see Fig. 1A). Then, it has generally been proposed that the HPt domains, found in a number of signal transducers, function as a common device involved in phosphotransfer signal transduction. However, examination of the structure and function of this newly-emerging phosphotransfer signaling domain is still at a very early stage. In this study, we address the relevant issue with special reference to the HPt domain of ArcB, for which the crystal structure has recently been analyzed.

On the bases of previous intensive in vitro studies from our and other laboratories, one can propose a scheme as to the phosphotransfer circuitry in ArcB (Fig. 1A). First of all, His-292 in the ArcB transmitter acquires a γ-phosphoryl group from ATP through its own catalytic function. This reaction is essential for the subsequent phosphotransfer, and in fact the phosphoryl group on His-292 moves to its intrinsic phospho-accepting aspartate (Asp-576) in the ArcB receiver domain. His-717 in the HPt domain can also be modified by phosphorylation through the function of ArcB itself (probably through Asp-576). The final destination of the phosphoryl group on His-717 appears to be Asp-54 in the cognate ArcA receiver. These processes are generally referred to as a "multistep His to Asp phosphotransfer". It is also known that the HPt domain of ArcB is capable of functioning as an alternative phospho-transmitter for the OmpR response regulator, provided that a multicyclop-lasmid (e.g., plasmid pAM5) carrying a gene specifying the ArcB* domain was introduced into an appropriate E. coli strain (e.g., strain YAC2). In YAC2 (ΔenvZ, Δlac), the resulting phospho-OmpR can activate the ompF-lacZ gene (see Fig. 1A), as demonstrated previously. This is because of the phosphotransfer from His-717 of ArcB* to Asp-55 of OmpR. Consequently, YAC2 harboring pAM5 has a Lac phenotype. This particular event thus can be used as a convenient (or practical) hallmark of the in vivo function of the HPt domain itself. Based on this experimental rationale, here we characterized a set of ArcB* mutants, each having a single amino acid substitution, in order to gain a general insight into the structure and function of the HPt domain.

The crystal structure of ArcB*, consisting of 125 amino acid residues, was identified recently. The X-ray analysis showed an all-α structure of ArcB*, consisting of six helices (helix-A to helix-F) (Fig. 1B and Fig. 2A). The four helices (C to F) make a bundle structure, in which the crucial His-717 site is located on the surface of helix-D, which lies in the internal curvature of the kidney-shaped molecule (Fig. 2A). As pointed out previously, E. coli has four other signal transducers that contain a presumed HPt domain (BarA, EvgS, TorS, and YojN). The structural view as to ArcB* allowed us to align the sequences of these E. coli HPt domains, as shown in Fig. 1B. Although they are highly variable in their primary amino acid sequences, helix-D containing the crucial histidine residue (His-717) appears to be the most highly conserved motif. In fact, we previously

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Fig. 1. Scheme for the Multistep Phosphotransfer Circuitry in ArcB, and Alignment of the Amino Acid Sequences Corresponding to the HPt Domain of the E. coli Hybrid Sensors.

(A) ArcB contains a transmitter, a receiver, and a C-terminal HPt domain, which are all involved in His to Asp phosphotransfer reactions, as schematically shown. Arrows indicate flows of phosphoryl groups. The OmpR protein is the non-cognate response regulator containing a receiver domain. Nonetheless, as shown in the box, OmpR can acquire a phosphoryl group through the phospho-HPt domain of ArcB (named ArcB'). If this polypeptide was overexpressed form plasmid pAM5 in E. coli. This event can be monitored in vivo by measuring increased expression of ompF in an envZ- and arcB'' background (host YAC2). (B) The amino acid sequence of the HPt domain of ArcB was aligned with those of the HPt domains of other E. coli hybrid sensors. Among them, the highly conserved amino acids including the crucial histidine residue (His-717) are boxed, and also the highly conserved hydrophobic amino acids are shaded. Note that the numbers given at the top are those for the amino acid sequence of ArcB. The amino acid substitutions, characterized in this study and previous studies, are indicated by arrows or arrowheads, respectively. At the bottom, the secondary structure of the HPt domain of ArcB is schematically shown. Other details are given in the text.

characterized a set of ArcB' mutants (see Fig. 1B), each of which has a single amino acid substitution in helix-D (e.g., Glu-714 to Lys, His-717 to Leu or Tyr, Lys-718 to Glu, Gly-721 to Asp, Gly-724 to Glu, see Fig. 1B). The results supported the idea that helix-D is structurally and/or functionally important for the HPt domain to function as a phospho-transmitter. It is also clear that a number of hydrophobic residues are conserved at certain positions (see the shaded residues in Fig. 1B). These hydrophobic residues mostly appear to play a role in formation of a hydrophobic core that allows association of the four-helix bundle. When the aligned sequences of the HPt domains were inspected more closely, it was noticed that several other amino acid residues, located outside of helix-D, are also highly conserved. They are Leu-681, Asp-684, Asp-708, and Gln-729 in ArcB (see the boxed residues in Fig. 1B). To gain a further insight into the structure and function of the HPt domain, in this study we wanted to assess the significance of these conserved amino acids. They were thus changed to alanine (Ala), one by one, by site-directed mutagenesis of pAM5. The results of analyses of these particular ArcB' mutants, expressed from the mutated AM5 plasmid, are shown in Fig. 2B.

YAC2 was transformed by each mutant plasmid, and then the cellular contents of the expressed ArcB' mutants were first examined by immunoblotting anaylysis.

Fig. 2. Three-dimensional Structure of the HPt Domain, Immunoblotting Analysis, and ß-Galactosidase Activity Expressed by the ompF-lacZ Transcriptional Fusion Gene.

(A) Three-dimensional structure of the ArcB' is shown, in which the locations of the relevant amino acid residues are highlighted. Note that the HPt-domain consists of six α-helices, as denoted (αA to αF). (B) YAC2 were transformed by pAM5 carrying the arcB' gene encoding the HPt domain. This strain was also transformed by a set of mutant plasmids, constructed in this study, as indicated at the bottom of this panel. These transformants were grown in Luria-broth under anaerobic conditions. The harvested cells were analyzed by immunoblotting with an anti-ArcB' antiserum (upper panel). They were also assayed for ß-galactosidase (lower panel), according to Miller's method.
sis with an antiserum against ArcB\(^+\) (Fig. 2B, upper panel). ArcB\(^-\)L681A and -Q739A were expressed in a stable form in the cells, as comparably as in the case of the wild-type ArcB\(^+\) and ArcB\(^-\)H717Y, while the amounts of ArcB\(^-\)D684A and -D708A in the cells were significantly lower. The function of these mutants were monitored by measuring the level of ompF-lacZ expression, as explained above. As a control, ArcB\(^-\)H717Y was shown to lack the ability to signal OmpR, although this particular mutant protein was produced in a stable form in the cells (Fig. 2B, the left panel). It was further seen that ArcB\(^-\)L681A, -D684A, and D708A had a partially reduced ability to activate the ompF-lacZ gene (Fig. 2B, the right panel). Surprisingly, on the other hand, it was found that ArcB\(^-\)Q739A appears to be normal with regard to both the stability and function.

Before discussing our results in this study, the following issue should be first addressed. Although one of the mutants characterized (ArcB\(^-\)L681A) appears to be defective in the ability to function as a phospho-transmitter, as demonstrated above, what remains unclear is whether this particular mutant is defective in the ability to acquire the phosphorl group from the ArcB receiver domain, or, whether it is defective in the ability to transfer the phosphorl group to the OmpR receiver domain. A plausible possibility is that ArcB\(^-\)L681A may be defective in both the interactions with the ArcB and OmpR receiver domains, because both the phosphotransfer interactions should be mechanistically the same, as one can easily envisage.\(^2\) To clarify this point, of course, an in vitro experiment using the purified ArcB\(^-\)L681A mutant should be done. Keeping this issue in mind, nevertheless, our results together with the ArcB three-dimensional structure provided with new insights into the structure and function of the ArcB-HPt domain, as discussed below.

(i) In this study, it was shown that the ArcB\(^-\) mutants, ArcB\(^-\)D684A and ArcB\(^-\)D708A, are unstable in the cells, and have a reduced ability to signal OmpR. It was thus suggested that these mutated amino acids have a structural role, to properly fold the HPt domain. (ii) In this respect, the side chain of Asp-684 is exposed to the solvent region, with buried hydrophobic residues Leu-681 and Leu-686 as anchors. It was found that even when alanine replaced Leu-681, the structural integrity of the HPt domain seems not to be affected markedly, although the ability to signal OmpR was partially impaired (see the result of ArcB\(^-\)L681A). (iii) The conserved Asp-708 at the N-terminus of helix-D makes a hydrogen bond with the main-chain amino group of Gly-711 (not shown) to form the N-terminal cap of the helix. Our result for ArcB\(^-\)D708A suggested that this cap may be important to fold the bundle-structure of the HPt domain. (iv) Finally, it should be noted that Gln-739 in helix-E is spatially adjacent to His-717 in helix-D, and a hydrogen-bonding network is formed that involves the side chains of these residues, suggesting that Gln-739 may be important for the activity of the HPt domain. Surprisingly, however, ArcB\(^-\)Q739A appears to be normal in both structure and function. As a whole, our results suggest that these apparently conserved four amino acids in a set of HPt domains may not absolutely be essential for the function. This is a rather unexpected finding, suggesting that the amino acid sequences of the common HPt domains are much more plastic than thought previously. This view will give us a clue to further understanding of the structure and function of the HPt domain in general.

Acknowledgment

This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan.

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