J. Biochem. 129, 343–350 (2001)

The OmpR-Family of Proteins: Insight into the Tertiary Structure and Functions of Two-Component Regulator Proteins

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Received December 6, 2000; accepted January 30, 2001

The *Escherichia coli* DNA-binding protein OmpR is the best characterized of those regulator proteins making up "two-component system," the simplest known form of bacterial signal transduction systems. Previous inspections of the *E. coli* genome DNA sequences have revealed that there are 15 proteins whose amino acid sequences show extensive similarities to that of OmpR (the OmpR-family of proteins). The three-dimensional structures of several OmpR-family proteins have been determined. In this review, we investigated the structures and amino acid sequences of this family of proteins. The results reveal several notable conservative varieties in their tertiary structures and functions.

Key words: bacterial signal transduction, DNA-binding domain, OmpR-family of proteins, transcriptional regulator, two-component system.

Bacteria have signal transduction systems through protein phosphorylation to respond to abrupt environmental changes. The simplest prototype of such systems involves two protein components, a sensor His-kinase and a response regulator (*1*). The sensor protein, which is often located in the cytoplasmic membrane, constantly monitors environmental change by detecting environmental stimuli, and relays the information to a response regulator in the cytoplasm by His-to-Asp phosphorelay (*2*). The response regulator, in turn, mediates changes in gene expression or locomotion in response to a given signal (*1*). This type of signal transduction system is referred to as a "two-component system."

In *Escherichia coli*, expression of the major outer membrane porin proteins, OmpC and OmpF, is regulated at the transcriptional level in response to medium osmolarity (*3*). Two regulatory factors, OmpR and EnvZ, are involved in this osmoregulation (*4*, *5*). EnvZ is the osmosensor that senses the osmolarity outside the cell and exhibits OmpR-specific kinase and phosphatase activity. OmpR is the transcriptional activator protein that binds to the recognition sequence in both the *ompC* and *ompF* promoters (*6*, *7*). as dimer (*8*). This DNA-binding protein is one of the best characterized of the bacterial positive regulators that enhance the transcriptional ability of RNA-polymerase (*9–12*). It consists of 239 amino acids with molecular weight of 27,400. It has two distinctive domains: the receiver domain (phospho-acceptor domain) at the N-terminal portion (residues 1–125), and the C-terminal DNA-binding domain (residues 137–239). The phosphorylation of Asp55 in the N-terminal domain of OmpR results in remarkable enhancement of its DNA-binding ability (*13–15*).

OmpR is one of the members of a large family of response regulators. In *E. coli* alone, there exist at least 15 proteins whose amino acid sequences show extensive similarities to that of OmpR (*16*). They include OmpR, PhoB, PhoP, KdpE, ArcA, and CreB. In addition, members of the OmpR-family have been reported to occur frequently in other bacterial species (*17–20*) as well as in some eukaryotes (*21*). These facts suggest that the OmpR-like proteins are one of the most widespread transcriptional regulators.

In recent years, the three-dimensional structures of a number of proteins which belong to two-component system have been determined (the kinase-domain of sensor proteins (*22–25*), the N-terminal receiver domain (*26–29*) and C-terminal DNA-binding domain (*30–32*) of regulator proteins) and they help us understand how these proteins work in the two-component system. In this short review, we focus on the DNA-binding domain of the OmpR-family of proteins and discuss their functions based on the amino acid sequences alignment and three-dimensional structure of the C-terminal DNA-binding domain of OmpR (OmpR-C), which has been solved by X-ray crystallography (*30*, *31*).

Outline of the structure of the DNA-binding domain

The structure of OmpR-C has been determined by two independent groups (*30*, *31*). Although the proteins used by the two groups have slightly different peptide-chain lengths (residues 122–239 (*31*) and residues 130–239 (*30*), respectively), the two crystals are more or less isomorphous, containing molecules that bear identical conformation with the disordered N-terminus. As shown in Fig. 1, at the N-terminus, there is a four-stranded β-sheet that is directly followed by an α-helical domain. The α-helical domain of OmpR-C consists of three α-helices with a β-hairpin at the C-terminus. The two helices, α2 and α3 and a loop between them create a structure similar to the helix-turn-helix (HTH) motif. Unlike the canonical HTH motif, in which four residues are involved in making a compact turn between two helices (*33*), the HTH of the OmpR-C has 11 residues at the corresponding region. These eleven residues protrude from the molecular body and form a loop structure.

More recently the solution structure of PhoB-C (the

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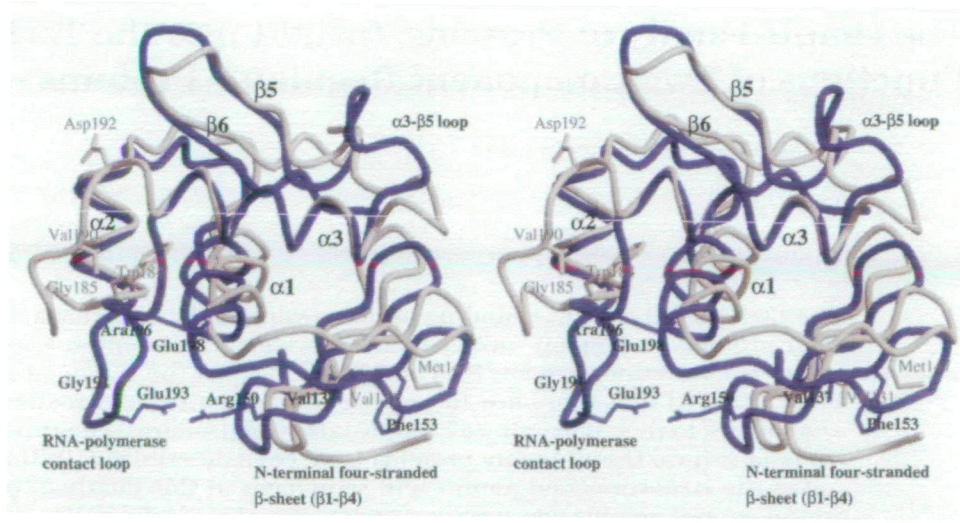


Fig. 1. **A superposition stereo-view of the three-dimensional structure of the C-terminal DNA-binding domain of OmpR (OmpR-C: blue) and PhoB (PhoB-C: gray).** They contain an N-terminal four-stranded β-sheet and a C-terminal α-helix bundle with a β-hairpin. A putative RNA-polymerase contact loop and amino acid residues that affect the activation ability for RNA-polymerase (*36*, *37*, *39*) are indicated. The most notable structural difference between these two molecules is in the RNA-polymerase contact loop, and may reflect the fact that they make contact with different subunit of RNA-polymerase. In OmpR, Glu193 makes a salt-bridge with Arg150 of the N-terminal four-stranded β-sheet. Two hydrophobic residues Val137 and Phe153 (OmpR), or Val131 and Met147 (PhoB) are exposed to surface. These residues may be involved in the interaction with the N-terminal receiver domain. [All of the three-dimensional structure presentations were generated by the program MOLSCRIPT (*54*) and RASTER3D (*55*).]

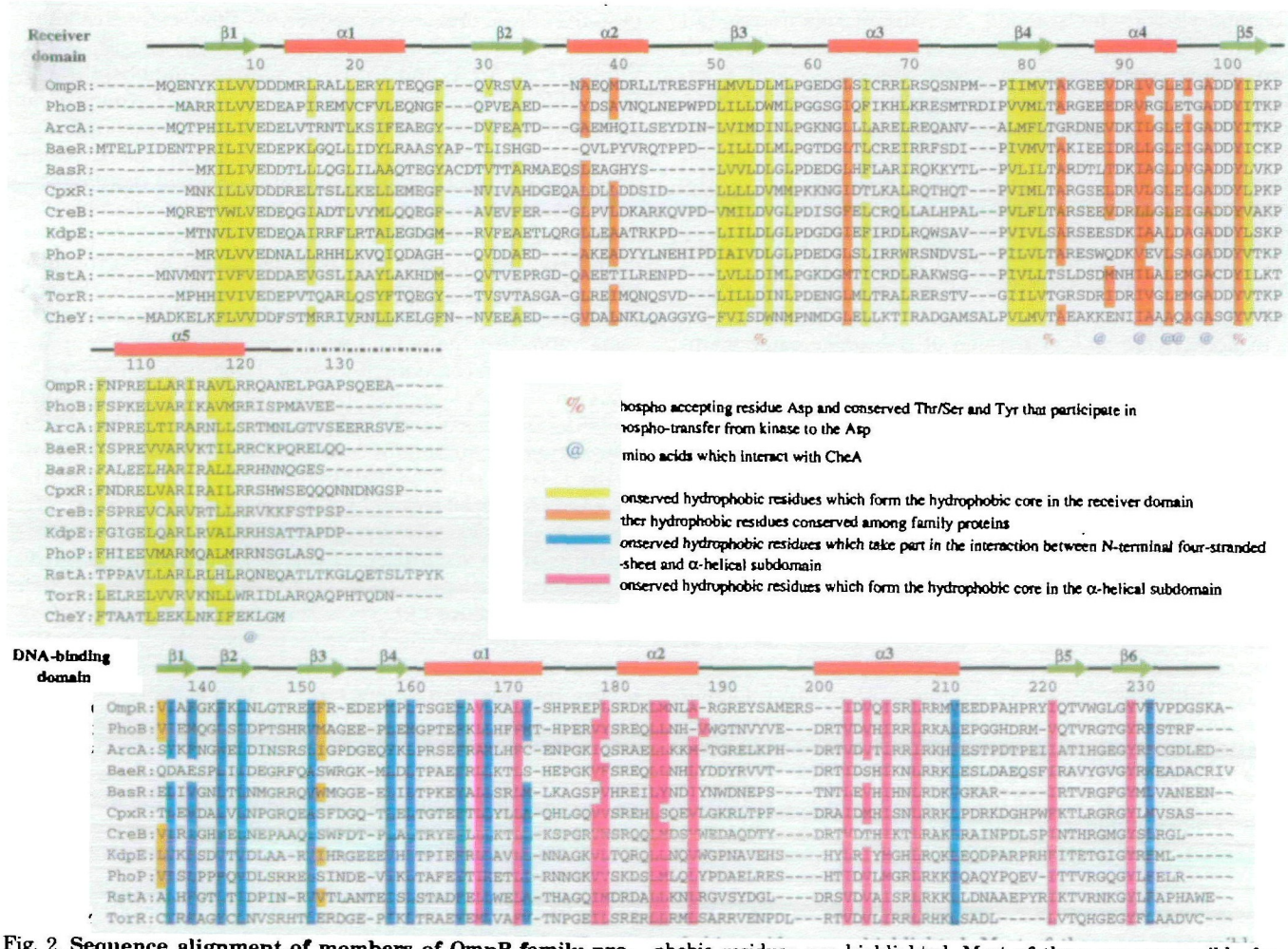


Fig. 2. **Sequence alignment of members of OmpR-family proteins from *Escherichia coli*.** The residue number is that of the OmpR. The secondary structure as determined by the X-ray structure analysis is presented [N-terminal receiver domain: CheY (*26*); C-terminal DNA-binding domain: OmpR (*30*, *31*)]. Highly conserved hydrophobic residues are highlighted. Most of them are responsible for forming a hydrophobic core structure. Asp as a phospho-accepting residue and Ser/Thr and Tyr, which play an important role in receiving phosphate (*28*), are indicated. Amino acids which play a role in contact with CheA (*44*) are also mapped in.

PhoB DNA-binding domain) has been determined (*32*). PhoB-C has a tertiary structure similar to that of OmpR-C except for the loop region. The largest difference between the two molecules is observed in the α2-α3 loop, though both the α2 and α3 helices maintain the same orientation with respect to other part of the molecule. The α2-α3 loop of OmpR-C contacts the N-terminal β-sheet subdomain *via* a salt-bridge. On the other hand, the loop of PhoB-C is shorter than that of OmpR-C, and no interaction between its N-terminal portion is observed (Fig. 1).

Correlation between OmpR-family protein structures and their functions

Structure of the OmpR-family of proteins. Conservative variety. An inspection of the *E. coli* genome DNA (*34*) revealed that *E. coli* has at least 15 proteins whose amino acid sequences show extensive similarities to OmpR in their C-terminal DNA-binding domains as well as in the N-terminal receiver domains (OmpR-family of proteins). To gain insight into the structure and function of these proteins, we examined amino acid sequences of the family protein with reference to the three-dimensional structures of the member proteins whose structures are available.

The amino acid sequence alignments of the C-terminal domain of the OmpR-family of proteins are shown in Fig. 2 (these proteins exhibit 20 to 30% sequence homology to OmpR). Investigation into the structures of OmpR-C and PhoB-C shows that the internal hydrophobic core of these molecules can be divided into two regions: one participates in the interaction between the N-terminal four-stranded β-sheet and C-terminal α-helical subdomain, and the other occurs in the interior of the α-helical subdomain (Fig. 3a). Hydrophobic residues that are involved in the former hydrophobic core, which maintains the spatial arrangement of the N-terminal four-stranded β-sheet with respect to the α-helical subdomain, are well conserved among them (Fig. 2). Some of the conserved hydrophobic residues at α3 and β6 also take part in these hydrophobic interactions (Fig. 3a). Other conserved hydrophobic residues that locate on the α-helical region form the latter hydrophobic core (Figs. 2 and 3a). The three-dimensional arrangement of these three helices is maintained by these hydrophobic interactions. Other conserved residues of OmpR, Arg182 in α2, Asp202 in α3, and Tyr230 in β6 and their corresponding residues Arg176, Asp196, and Tyr223 in PhoB are involved in a hydrogen-bonding network connecting these three structural units. In the tertiary structure of OmpR-C and PhoB-C, the amino acids that maintain the three-dimensional arrangement of their secondary structural unit are well conserved.

As shown in Fig. 2, amino acids that are responsible for making the internal hydrophobic core in the OmpR-C and PhoB-C are well conserved among the OmpR-family of proteins. Most of the substitutions in the buried regions of the OmpR-C and PhoB-C are conservative ones and are unlikely to destroy the protein hydrophobic core. The sites at which the largest differences occur map either to the surface of the molecule or to the linker region. Among the family of proteins, some of the amino acids that locate at the site of conserved hydrophobic residues are occasionally substituted. For example, at the site of residue number 147 in OmpR, amino acid substitution occurs with relatively high frequencies (Fig. 2). The position is located at the edge of the hydrophobic core region, and it is not likely that this substitution causes serious conformational change. We therefore conclude that all members of the family have a similar three-dimensional structure with OmpR-C and PhoB-C.

Though OmpR-C and PhoB-C exhibit similar tertiary structures, a comparison of the two structures reveals some significant differences as well. The most notable difference between OmpR-C and PhoB-C occurs in the α2-α3 loop. Biological data suggest that OmpR interacts with α-subunit of RNA-polymerase (*35*) *via* this loop region (*36*, *37*), while PhoB interacts with the σ-subunit of RNA-polymerase (*38*). An inspection of the amino acid sequences in this loop region suggests that there are some structural varieties in this region among family-proteins. This difference may be responsible for the diversity of the means of transcriptional activation among proteins of the OmpR-family. The length of α3 helix, the putative DNA-binding helix, also differs among these proteins. The α3 of OmpR contains 12 amino acids, and that of PhoB contains 15. In OmpR, three residues, Glu198, Arg199, and Ser200, which correspond to Asp192, Arg193, and Thr194, which are located at the N-terminal end portion of α3 in PhoB, are involved in the former linker region. PhoB recognizes a specific DNA sequence called the phosphate box (*39*). In contrast, OmpR alters its recognition sequences on DNA (*ompF* or *ompC*) depending on their phosphorylated level of the N-terminal receiver domain. This large difference may be derived from some differences in the DNA recognition mechanism among OmpR-family proteins. Furthermore, the structural difference in the α3-β5 loop between OmpR-C and PhoB-C is obvious. Mutation experiments show that a residue in this loop region is necessary to interact with DNA for OmpR (*36*), and another DNA-cleaving study also shows that this region is included in DNA binding (*40*). Structural differences in this region may also suggest different manners of interacting with DNA to recognize the specific binding-sequence of OmpR-family proteins on the DNA.

Although the tertiary structure of the N-terminal receiver domain of OmpR is not known, the three-dimensional structures of several other regulator proteins have already been determined (*26–29*, *41–43*) (in amino acid sequence, these structures show 24–41% identity with OmpR), and have all been found to be similar. Based on the structures of the CheY (*26*) and N-terminal receiver domain of PhoB (PhoB-N) (*27*), the amino acid sequences of the N-terminal receiver domain of OmpR-family proteins were examined. As aligned in Fig. 2, many hydrophobic residues are highly conserved among family proteins, and structural investigation into the CheY and PhoB-N revealed that most of these residues form a hydrophobic core in the interior of the molecule (Fig. 3b). Not only these hydrophobic amino acids, but also hydrophilic amino acids which stabilize the structure by forming a hydrogen-bond network, and such amino acids as Gly and Pro which affect the secondary structure formation are well-conserved. Based on these facts, it is likely that the N-terminal receiver domain of all OmpR-family proteins also has a tertiary structure similar to that of CheY and PhoB-N. Other conserved hydrophobic residues that do not participate in the hydrophobic core (Ile96, Ala99, and Ala103 in CheY) are exposed to the exterior of the molecule. In the case of CheY, these

residues interact with the CheA a kinase-domain of the sensor protein (*44*), and this may be also the case with the other receiver domain. These residues may thus take part in interaction with the kinase-domain of the sensor protein.

The importance of the N-terminal four-stranded β-sheet plane. When the N-terminal receiver domain receives the information from its sensor protein *via* by phosphorylation, the DNA-binding activity of the C-terminal DNA-binding domain is altered. In the case of OmpR, the DNA-binding ability of phosphorylated OmpR is increased 10- to 30-fold depending on the binding site (*15*). On the other hand, the N-terminal receiver domain of DNA-bound state OmpR is more easily phosphorylated than that of the DNA-free state OmpR (*45*). Furthermore, single amino acid substitution in the C-terminal DNA-binding domain of OmpR (V203M) also affects the phosphorylation level of the N-terminal receiver domain (*46*). These results suggest there is a bi-directional signal transduction pathway between the N-terminal receiver domain and C-terminal DNA-binding domain. These domains are linked by a linker region containing about 10 amino acid residues. The linker region of OmpR has twelve residues (125–136) (*30*), and structural changes of this region between the phosphorylated state and non-phosphorylated state have been reported (*45*, *47*). This may indicate that the linker region plays an important role in the domain-domain signal transduction mechanism.

The three-dimensional structure of OmpR-C and PhoB-C reveals that the OmpR-family proteins have a large β-sheet plane in the N-terminal portion of their DNA-binding domain. It may be worthwhile to note that although quite a few transcription factors are known to have a similar α-helical domain similar to that of OmpR-C (so called the winged helix-turn-helix motif), such an N-terminal β-sheet is not observed in other classes of two-component regulator protein, such as NalL (*48*) and NtrC (*49*). Only OmpR-family proteins have a four-stranded β-sheet as an integral part of the domain. Thus, investigation into the features of the N-terminal four-stranded β-sheet of OmpR-C may provide some clue to the possible interaction between the DNA-binding domain and the receiver domain.

The N-terminal four-stranded β-sheet plane of OmpR-C and PhoB-C is located almost parallel to the α1 helix axis at the opposite side of the RNA-polymerase binding site (Fig. 1). The β-sheet plane has contacts with the α1, the C-terminus of α3 and the C-terminal end of the DNA-binding

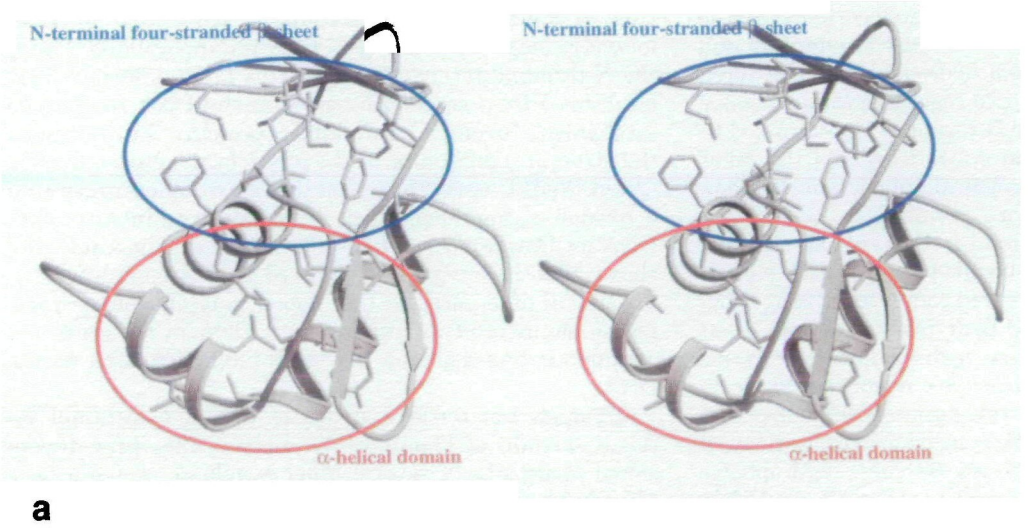


Fig. 3. **a: A stereoview of the hydrophobic core in the OmpR-C molecule.** The hydrophobic core is divided into two clusters. One is formed between the N-terminal four-stranded β-sheet and α-helical subdomain, and the other is formed in the α-helix bundle. Hydrophobic residues in these cores maintain the arrangement of secondary structure by hydrophobic interaction. **b: A stereoview of the hydrophobic core in the CheY molecule.** As in the case of OmpR, well conserved hydrophobic residues maintain the arrangement of secondary structure by forming a hydrophobic core.

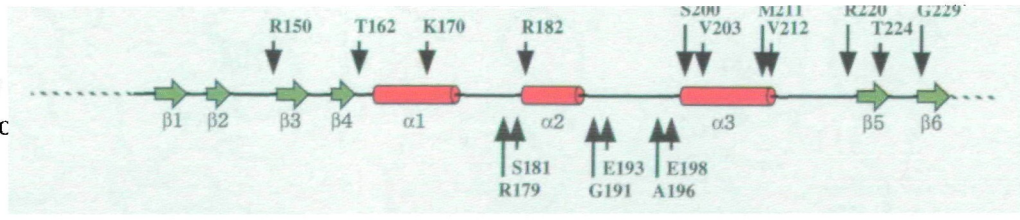
domain. In the contact region the hydrophobic residues are clustered. In the case of OmpR-C, the side chain of Arg150 in the N-terminal β-sheet is directed toward Glu193 in the RNA-polymerase binding loop and makes a salt-bridge (Fig. 1). The polar nature of the N-terminal four-stranded β-sheet is also obvious. Most of the hydrophobic residues with the exceptions of Val137 and Phe153 in OmpR-C, and Val131 and Met147 in PhoB-C face the C-terminal α-helical domain. These two residues are exposed to the molecular surface in this truncated molecule (Fig. 1), and might be used to contact the N-terminal receiver domain in the intact molecule. They are only partly conserved in the family proteins, which suggests that the interactions between the N- and C-domains do not all occur similarly within the family. The sequential shortening of the C-terminal portion of OmpR indicates that the DNA-binding domain of OmpR lies between residues 123 to 239 of OmpR (117 residues) (*50*), and further shortening of the C-terminal portion of OmpR (*e.g.*, the C-terminal 82 residues) results in lack of binding ability to the *omp* gene (*50*). Thus, the N-terminal four-stranded β-sheet is indispensable for the integrity of the C-terminal DNA-binding domain. This region may also take part in the communication between the N-terminal receiver domain and C-terminal DNA-binding domain.

Correlation with mutation experiments. OmpR is a member of a family of bacterial positive transcriptional regulators of which many mutants have been investigated (*36*, *37*, *51–53*). These mutants are classified into two groups: mutants which are defective in DNA-binding ability (DNA-binding mutants) and mutants which lack transcription activating ability but retain DNA-binding ability (positive control-like or PC-like mutants). Figure 4 shows their mutation points and structural characteristics. Most of the mutation points that affect the DNA-binding ability of OmpR are located either on the molecular surface or in the internal hydrophobic core. The former include residues Ser200 and Val203 at α3, the putative DNA-recognition helix (*36*, *51*, *53*). These residues may be directly involved in the DNA-binding or sequence recognition. Thr162 at the loop between β4 and α1 is also exposed to the molecular surface and affects DNA-binding. This residue is on the same side of the molecule as the former two residues and may also interact directly with DNA. The mutation sites in the internal hydrophobic core, which cause lack of DNA-binding, include residues Met211 and Val212. The side-chains of these residues are directed toward the interior of the molecule and form a hydrophobic core, which is important for maintaining the intact structure of the DNA-binding domain by properly aligning the secondary structural units. Substitutions of these residues may change the molecular conformation, which in turn would affect the DNA-binding ability of the molecule. The side-chain of Thr224 at β5 interacts with the side-chain of Arg209 through the hydrogen-bonding network. Substitution of residue Thr224 may cause a lack of DNA-binding ability of OmpR by breaking the hydrogen-bond. The residue of Arg209 is directed toward the molecular exterior and is likely to play an important role for DNA-binding. Taking these facts together, it is tempting to speculate that substitution in the position of Thr224 may affect the orientation of the side-chain of Arg209 (Fig. 5a), thereby affecting the DNA-binding ability of OmpR.

PC-like mutation points are rather localized on one side of the molecule. Especially important is the localization in

Mutation sites which affect to DNA-binding ability.

- R150[51] : form salt-bridge with E193
- T 162[51] : residues at loop between β4 and α1
- K170[51] : interaction with L147
- R182[51] : hydrogen-bonding network with D202
- S200[51] : exposed to the molecular surface
- V203[36] : exposed to the molecular surface
- M211[53] : hydrophobic interaction with internal hydrophobic core
- V212[53] : hydrophobic interaction with internal hydrophobic core
- R220[36] : interaction with Y221
- T224[51] : hydrogen-bonding network with R209
- G229[51] : first residues in β6



Mutation sites which affect RNA-polymerase activation ability of OmpR with retaining its DNA-binding ability (PC-like mutation-site)

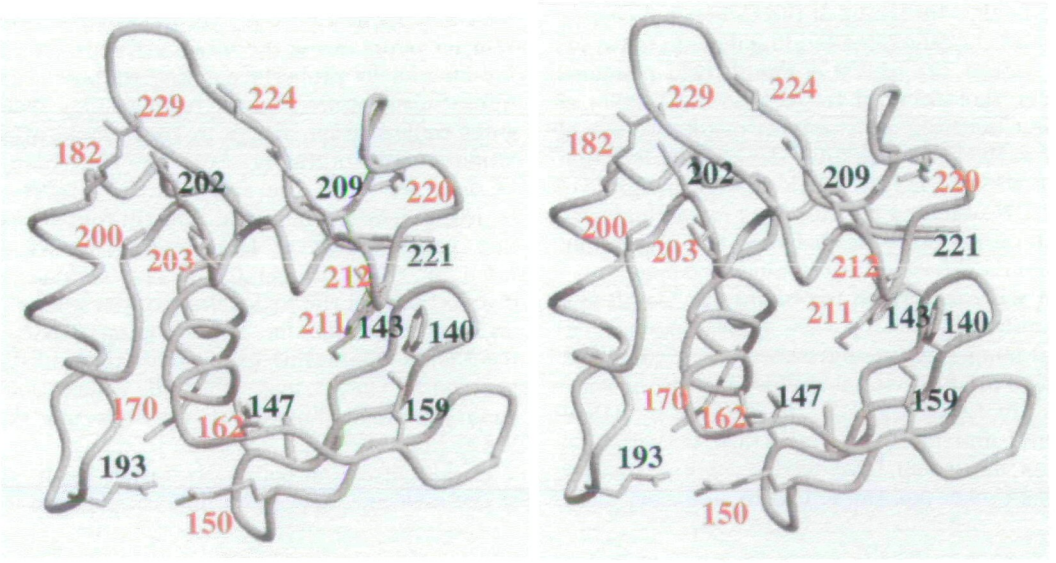
- P179[37] : hydrophobic interaction with V225, L228 and V231
- S181[51] : exposed to the molecular surface
- G191[36] : exposed to the molecular surface
- E193[36] : form salt-bridge with R150
- A196[37] : exposed to the molecular surface
- E198[37] : interaction with T162, T164 and Q204

Fig. 4. **Classification of OmpR mutants and their structural circumstances.** Mutation points that affect the function of OmpR-C are shown with their structural characteristics.

the loop between α2 and α3 (*36*, *37*) (Figs. 1 and 5b). Most of the mutations which affect interaction with RNA-polymerase are mapped on this loop, which suggests that this loop is an RNA-polymerase contact site. These residues (Glu193, Ala196, and Glu198) are involved in either the hydrophobic or the ionic interactions with neighboring residues (Figs. 4 and 5b). Substitutions at these points may cause structural changes of the molecular surface, which in turn would affect contacts with RNA-polymerase. The rather large *B*-factors of the atoms in this loop suggests the loop's flexible nature. The loop may change its conformation by interacting with RNA-polymerase. Two other mutation sites that cause lack of transcription activation ability while retaining DNA-binding ability are Pro179 and Ser181. Substitution at these points may also alter the RNA-polymerase contact loop by eliminating the β-sheet type hydrogen bond with the α-helical domain.

Further perspectives

The three-dimensional structures of some of the domains in the OmpR-family of proteins are now available. Careful investigation into these structures and amino acid sequences of the family proteins suggests that the tertiary structures of these proteins are moderately varied and cor-



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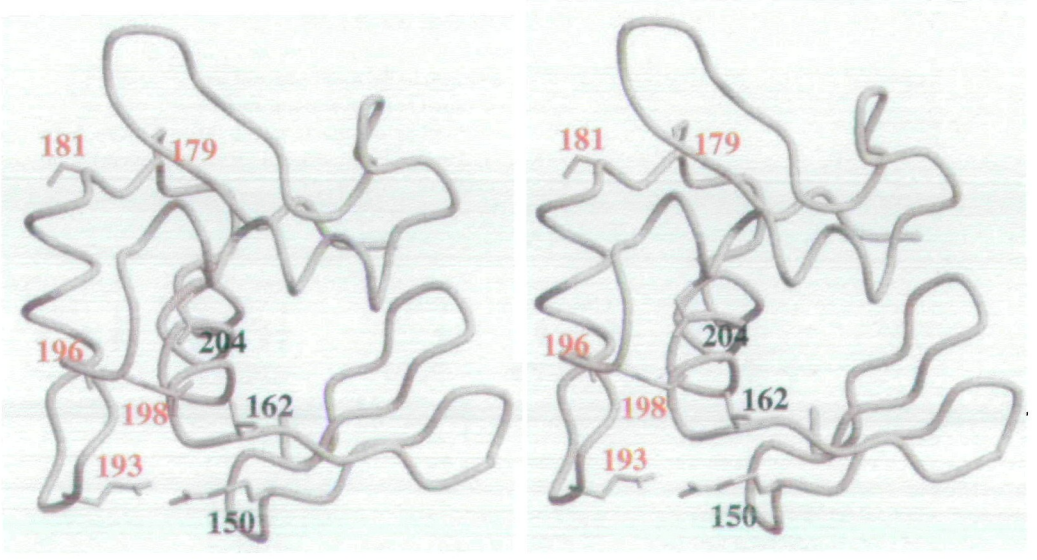

b

Fig. 5. **A stereo presentation of OmpR-C showing residues which affect molecular functions (Red) and their direct neighbors (Black).** All figures are shown viewed from the same direction as in Fig. 1. a: Residues which affect DNA-binding ability. Substitutions at these points interfere with DNA-binding either directly or indirectly by changing the molecular conformation. b: Positive control-like mutation residues. All residues are located at one side of the molecule. Most of these residues are located in the loop between α2 and α3.

relate with their function from a structural point of view. The present review pointed out that the tertiary structure of OmpR-family proteins is very similar not only with respect to the N-terminal receiver domain but also the C-terminal DNA-binding domain. It may be important to note that, although these proteins have highly similar structures, they receive signals from specific sensor proteins and recognize their own specific binding sequences on DNA. Of course, proteins of the OmpR-family have sufficient flexibility to exhibit their functional specificity while retaining common tertiary structures through amino acid substitution and variable loop regions. The tertiary structure of each domain suggests to us that each protein has several functional mechanisms, but cannot adequately clarify the whole mechanism of the two-component regulatory protein. How is the signal transferred from a kinase-domain of a sensor protein to an N-terminal receiver domain of a regulator protein? How is the signal transduced from the receiver domain to a C-terminal DNA-binding domain? How does the DNA-binding domain recognize the binding sequence on DNA? And what about inter-molecular interaction during dimerization? To answer these questions, more biological experiments will be needed, in addition to structural analyses of intact OmpR-family proteins. No whole structure has yet been determined for any protein in this family, although the structure of intact NalL, a regulator protein belonging to another subfamily of the two-component system in *E. coli*, has been reported (*48*). Furthermore, the tertiary structure of the DNA-bound form of OmpR-family proteins has been required. Today the structure of not only regulator proteins, but also of some of sensor proteins are also available (*22–25*). From integration of these structures we can define the features of the two-component signal transduction system at the "component" level, but the most important question remains to be answered. That is, how will these components work together? Our goal is to understand this series of signal transduction mechanisms as a "system."

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