CHAPTER IV

INTERACTION OF ENVZ AND OMPR

IV-1. INTRODUCTION

Phosphorylated EnvZ transfer its phosphoryl group to the cognate response regulator OmpR by kinase activity. Asp-55 of OmpR receives the phosphate and the phospho-OmpR activates and modulates the transcription expression of *ompF* and *ompC*. In reverse, EnvZ can dephosphorylate the phospho-OmpR and inactivates the transcription activity of *ompF*. These two opposite enzymatic activities of EnvZ regulate the level of phosphorylated OmpR and, as the result, modulate the affinity of phospho-OmpR to promoter regions of the porin genes. OmpR is a cytoplasmic phosphotransferase of 239 amino acids which contains the phospho receiving site, Asp-55. In consideration of the interaction with EnvZ, its N-terminal half should be involved in this interaction because it possesses the Asp-55. The structure of the C terminal half of OmpR has already been determined and it revealed to have a winged-helix motif (Kondo et al., 1997). On the other hand, the structure of the N terminal half has not been determined yet. The N-terminal domain contains the regulatory domain that has highly conserved amino acid sequence common to other response regulators, and thus probably share the same fold (Volz, 1993). Indeed, structures of three regulatory domains of CheY (Stock et al., 1993), NtrC (Volkman et al., 1995) and Spo0f (Feher et al., 1997; Madhusudan et al., 1997; Madhusudan et al., 1996) were found to have the same fold.

To address the question how EnvZ interacts with OmpR in transferring the phosphate, NMR titration experiments were performed. The results suggest that OmpR likely to move towards the EnvZ from the bottom half of the four-helix bundle. In addition, a structure prediction on OmpR (residues 6-120) using homology modeling methods of ProMODII produced a structure which superposes well according to the template protein backbones. The resulting molecular surface shows the high potency of complementary electrostatic interaction with molecular surface of domain A (Chapter III).

IV-2. MATERIALS AND METHODS

IV-2-1. Titration experiments

OmpR sample is provided by Dr. M. Inouye (UMDNJ, USA). A series of ^{1}H-^{15}N HSQC spectra were recorded on 1.0 mM of ^{15}N-labeled EnvZ domain A at titration points (0.2, 0.4, 0.6,

0.8, and 1.0 mM) while adding the non-labeled OmpR sample. A relative intensity ratio was calculated for each peak with/without OmpR by normalizing the maximum to a value of 1.0.

IV-2-2. Homology modeling

The model structure of OmpR phosphorylation domain (6-120) was deduced by the ProMODII, an automated cooperative protein modeling program in SWISS-MODEL (http://expasy.hcuge.ch/swissmod), using the structures of CheY [Protein Data Bank accession number: 2CHE (Stock et al., 1993)], NtrC [1NTR; (Volkman et al., 1995)], and Spo0f [1FSP, and 1SRR; (Feher et al., 1997; Madhusudan et al., 1997)] as the template structures. The evaluated Model-B factors for the main-chain atoms of the resulted model structure against those of templates were less than 13, indicating the relatively low deviation of the model structure from the templates [SWISS-MODEL algorithm: (Guex and Peitsch, 1997; Peitsch, 1996; Peitsch, 1995)].

IV-3. RESULTS

IV-3-1. Titration

The ^{1}H-^{15}N HSQC spectra of EnvZ domain A were recorded with successive additions of OmpR regulatory domain (residues 1-124). During the titration, many backbone NH peaks underwent broadening and eventually disappeared below the noise level. This intermediate exchange phenomenon is consistent with the moderate affinity of OmpR for EnvZ. Interestingly, the residues whose NH signals deonstrate peak broadening are mapped on the bottom half of the four-helix bundle structure (Figure IV-1).

IV-3-2. Homology modeling of OmpR N-terminus domain (6-121)

At present, no X-ray or NMR structure is available for the N-terminal domain of OmpR. Despite of great diversity in their domain organization, response regulators contain a regulatory domain that is highly conserved in amino acid sequence and probably share the same fold (Volz, 1993). High sequence identity of OmpR (residues 6-121) with CheY (29.2 %), NtrC (30.6 %), and Spo0f (33.0 %) (Figure IV-2) makes it possible to build a model structure for the OmpR regulatory domain.

The predicted OmpR structure (Figure IV-3) reveals several interesting structural features. First, three acidic residues (Asp-12, Asp-13 and Asp-55) cluster to form a negatively charged patch at the phosphorylation site (Asp-55). Secondly, a cluster with basic residues (Lys-85, Lys-105 and Arg-15) is formed near Asp-55.

IV-4. DISCUSSION

The result of titration experiments is a potent evidence for the interaction between domain A and OmpR, and indicates that OmpR interacts primary with the bottom half of the four-helix bundle of EnvZ domain A (Figure IV-1). Interestingly, this region contains not only His-243 but also the aforementioned (in Chapter III) acidic patch and two conserved residues, Thr-247 and Pro-248.

According to the predicted structure of OmpR [OmpR(N): residues 6-121], the basic cluster on the surface of OmpR(N) could provide a complementary surface to the acidic patch nearby His-243 of the domain A. Of the three residues in the acidic cluster, Asp-273 and Glu-276 of helix II are the specific residues to EnvZ that are not conserved among members of the histidine kinase family (Figure III-6). In addition, Lys-85 and Arg-15 are specific to OmpR and not conserved among members of response regulators. Moreover, substitution of Arg-15 to cysteine abolish the ability of the dephosphorylation of phospho-OmpR (Aiba et al., 1989). Taken together, these putative electrostatic interactions possibly play a role in the interaction between EnvZ and OmpR. Additional binding energy may be provided by a number of hydrophobic residues surrounding Asp-55, the phosphorylation site of OmpR.

Genetic and biochemical analyses have shown that residues important in phosphorylation and activation of the response regulators are Asp-55 (OmpR), His-243 (EnvZ domain A), Thr-247 (EnvZ domain A), and Asn-347 (EnvZ domain B) (Dutta and Inouye, 1996). With these residues combined, the EnvZ-OmpR signal transduction pathway is simulated as follows. In response to the osmolarity changes, activated EnvZ autophosphorylate His-243 using ATP as a phosphate donor, followed by the EnvZ kinase activity, in which, involving Asn-347 and Thr-247, the phosphoryl group is transferred to Asp-55 of its cognate response regulator OmpR. After the phospho-OmpR regulates the transcription of OmpF or OmpC, the phospho-OmpR is dephosphorylated by the phosphatase

activity of EnvZ. For such schemes to be carried out eficiently, the side chains of all the residues listed above should be in close spacial proximity to one another (Figure IV-4).

Several reports have indicated that EnvZ functions in a trans manner, that means, His-Asp phosphotransfer system is performed within domain A of one subunit and domain B of another subunit. The results of ^{1}H-^{15}N HSQC measurements on ^{15}N-labeled domain A and unlabeled domain B mixtures by titration method show no dramatic change in cross peaks (data not shown). Therefore, the interaction between domains A and B can be weak and in fast exchange state. In Figure IV-5, the general architecture of EnvZ is postulated using the structure obtained in this study. Functionally, domain A, domain B, OmpR and ATP should all be in close vicinity around H243 (suggested in Figure IV-4), and the movement should occur in a msec period considering the phospho-His-243 lifetime (Egger et al., 1997). To make this succeed, the loosened structure of helix I of domain A will play an important role to provide an enough space within a compact environment for the autophosphorylation and phosphotransfer mechanism, and the helix II of domain A will orientate OmpR towards the proper position in receiving the phosphoryl group from phospho-His-243. The obtained results in this study suggest further structural studies probing into the relative orientations between EnvZ domains A and B, and between domain A and OmpR to provide more concrete insight into the EnvZ-OmpR signal transduction mechanism.

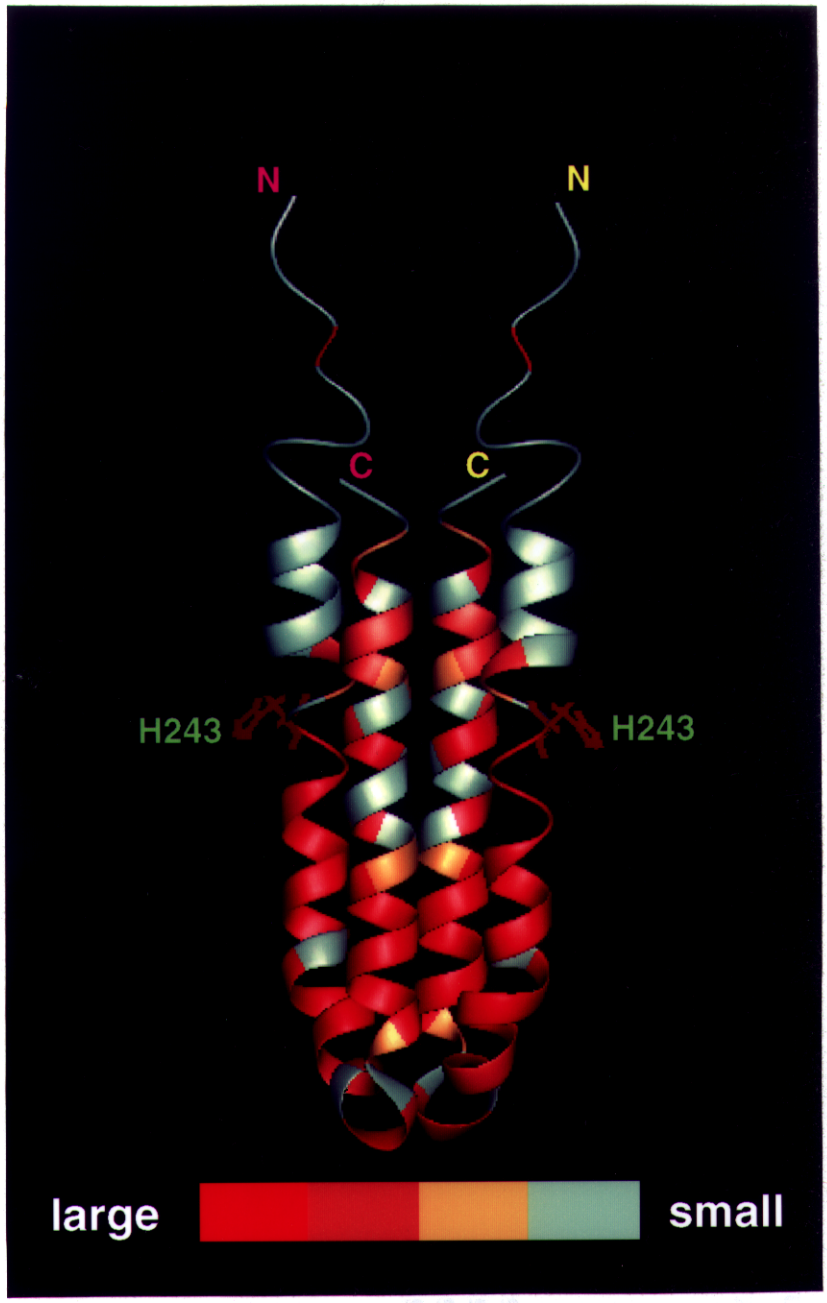

Figure IV-1. Summary of OmpR titration effects on domain A.

The OmpR-induced changes in intensity of the backbone amide peaks of domain A are classified into four groups and represented on the ribbon diagram of domain A. His-243 as well as the N- and C- termini are marked. This figure was generated by QUANTA.

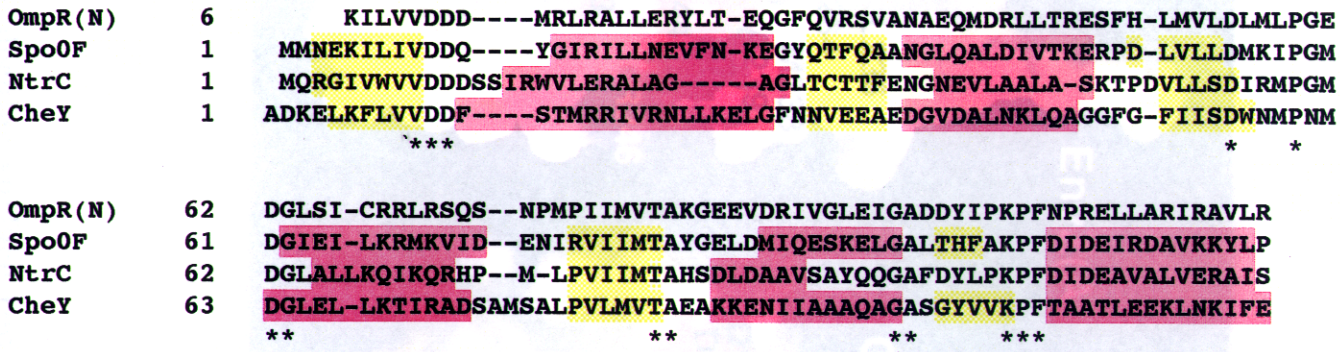

Figure IV-2. Sequence alignment of OmpR (residues 6-121) with the corresponding region of other response regulator proteins.

Secondary structure elements of each protein are shown in colored boxes; helices in pink and strands in yellow. Residues conserved among all five proteins are marked with an asterisk. Alignment was performed using HSSP (Dodge et al., 1998) and Ψ-BLAST (Zhang et al., 1998) programs.

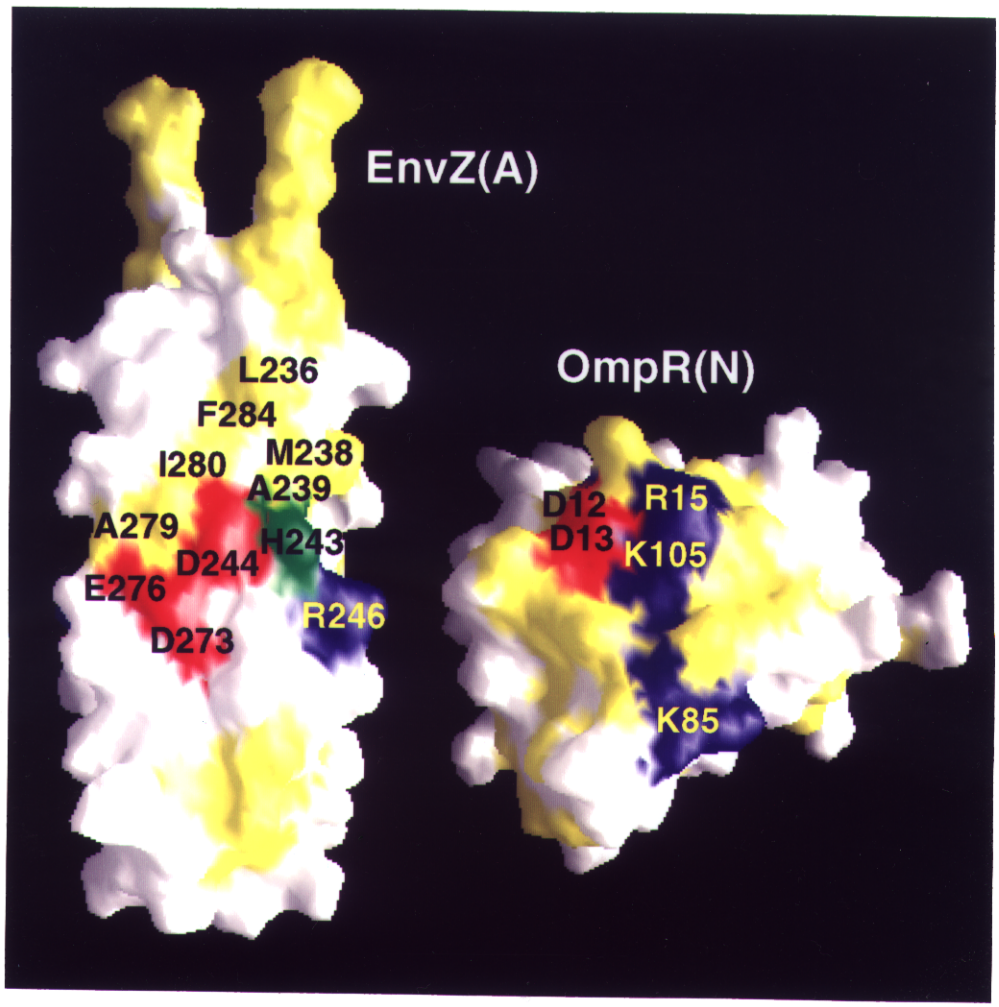

Figure IV-3. The molecular surface representation of EnvZ domain A structure [EnvZ(A)] determined by NMR and OmpR structure [OmpR(N)] predicted by the homology modeling.

Residues located in close proximity to His-243 of EnvZ and to Asp-55 of OmpR are labeled: hydrophobic residues in yellow, acidic residues in red, and basic residues in blue. This figure is generated using GRASP.

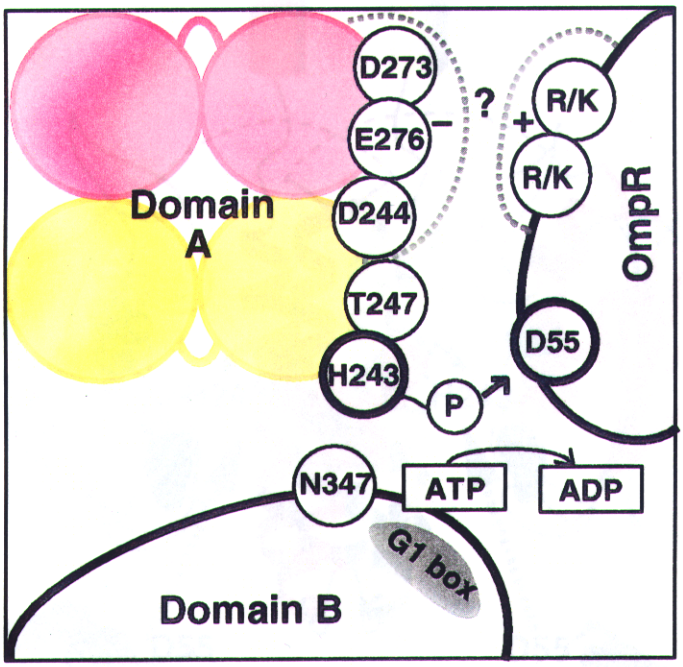

Figure IV–4. EnvZ–OmpR interaction model.

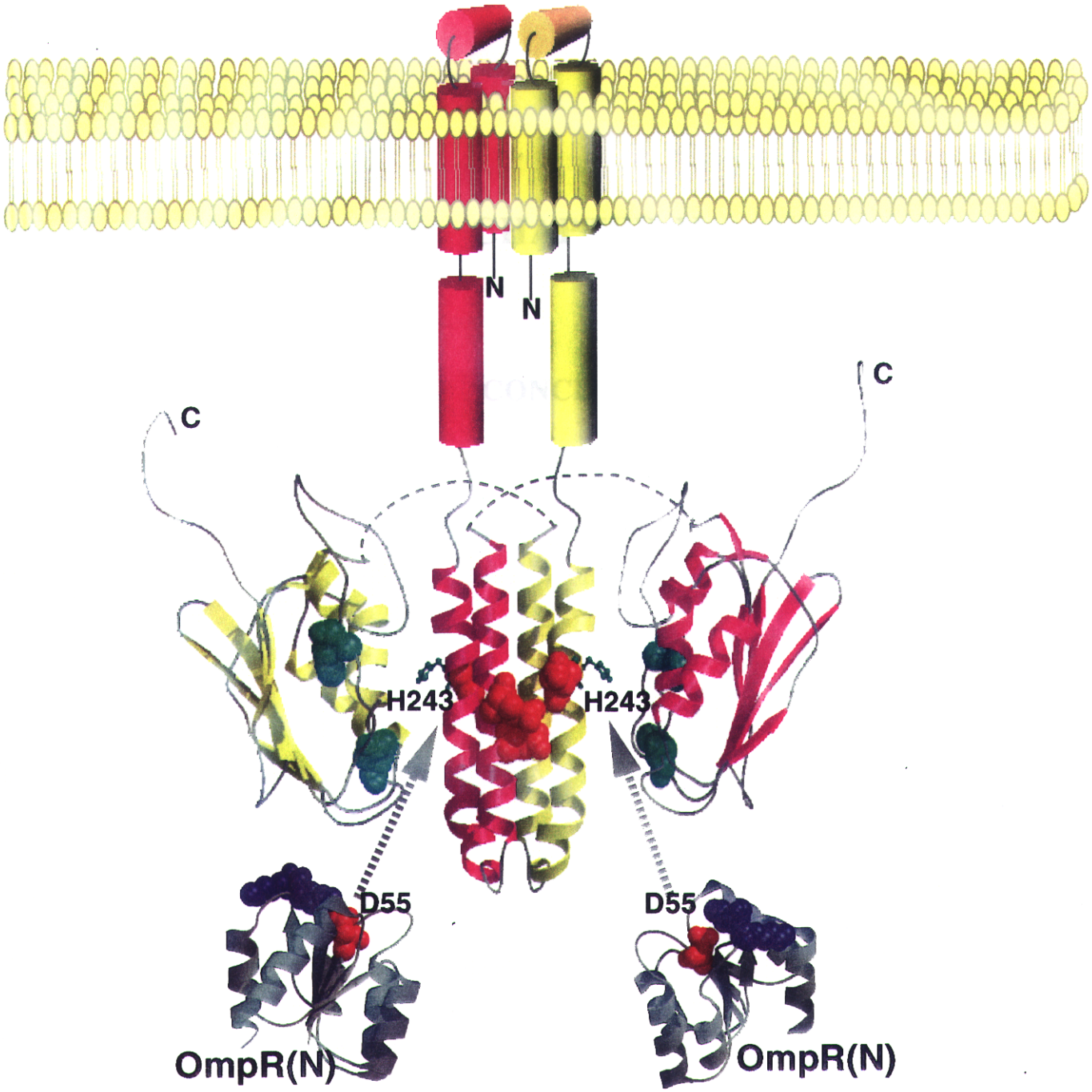

Figure IV–5. Overall molecular architecture of Osmosensor EnvZ.

The ribbon diagrams of domains A and B are based on their NMR–derived structures and each subunit is colored as in Figure III–4a. Asn–247 and Phe–287 are shown in space filling representation and colored in green. His–243 is shown as a ball and stick in green. The ribbon diagram of the predicted structure of OmpR is shown in gray. Acidic and basic residues are shown in space filling representation and colored in red and blue, respectively. This figure is generated using MOLSCRIPT and Raster3D.