The Linker Region Plays an Important Role in the Interdomain Communication of the Response Regulator OmpR*

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Kirsten Mattison‡, Ricardo Oropeza‡, and Linda J. Kenney¶

From the Department of Molecular Microbiology and Immunology, Oregon Health and Science University, Portland, Oregon 97239

OmpR is the response regulator of a two-component regulatory system that controls the expression of the porin genes ompF and ompC in Escherichia coli. This regulator consists of two domains joined by a flexible linker region. The amino-terminal domain is phosphorylated by the sensor kinase EnvZ, and the carboxy-terminal domain binds DNA via a winged helix-turn-helix motif. In vitro studies have shown that amino-terminal phosphorylation enhances the DNA binding affinity of OmpR and, conversely, that DNA binding by the carboxy-terminal increases OmpR phosphorylation. In the present work, we demonstrate that the linker region contributes to this communication between the two domains of OmpR. Changing the specific amino acid composition of the linker alters OmpR function, as does increasing or decreasing its length. Three linker mutants give rise to an OmpF+ OmpC− phenotype, but the defects are not due to a shared molecular mechanism. Currently, functional homology between response regulators is predicted based on similarities in the amino and carboxy-terminal domains. The results presented here indicate that linker length and composition should also be considered. Furthermore, classification of response regulators in the same subfamily does not necessarily imply that they share a common response mechanism.

Two-component signaling systems are the predominant signal transduction pathways in prokaryotes, and the components are highly conserved (1). In Escherichia coli, a two-component system that consists of the sensor kinase EnvZ and the response regulator OmpR regulates the expression of the outer membrane porins OmpF and OmpC in response to environmental osmolarity. At low osmolarity, OmpF predominates in the outer membrane; OmpC is expressed at high osmolarity (2). In response to an unidentified environmental signal, the EnvZ kinase autophosphorylates at histidine 243 and transfers the phosphoryl group to aspartate 55 of OmpR (3–5). At low osmolarity OmpR-P1 activates transcription of ompF, whereas at high osmolarity OmpR-P represses ompF transcription and activates transcription of ompC (5, 6).

OmpR consists of an amino-terminal phosphorylation domain and a carboxyl-terminal DNA-binding domain, which are joined by a linker region (7, 8). The two domains of OmpR influence each other. Phosphorylation of OmpR at the amino-terminal aspartate 55 increases the DNA binding affinity of the carboxyl terminus (9–12). Conversely, the presence of specific DNA binding sites increases the steady-state amount of OmpR-P formed in vitro (13, 14). Our laboratory is interested in determining the mechanism responsible for this interdomain communication in OmpR.

The linker regions of response regulators are not highly homologous; however, they are relatively rich in glutamine, arginine, glutamate, serine, and proline residues (15, 16). These linker regions have no predicted secondary structure. Wootton and Drummond (15) termed these sequences Q-linkers because of the preponderance of glutamine residues. These authors suggested that the Q-linkers serve the simple role of tethering the two domains to allow functional interactions to occur. However, point mutants have been isolated in the linker region of both OmpR and the homologous regulator DmsR that disrupt protein function (17–19). It was therefore of interest to determine whether specific amino acids were required for interdomain communication of OmpR. In the present work, we have analyzed the effect of replacing the linker region with different amino acid sequences and found that some, but not all, linker substitutions allow OmpR-mediated transcriptional activation of the ompF and ompC genes.

OmpR orthologues are very highly conserved among enteric bacteria such as Salmonella typhi, Salmonella typhimurium, Yersinia enterocolitica, Yersinia pestis, Enterobacter cloacae, and Shigella flexneri. As such, although the linker sequences are conserved, it is impossible to determine whether this has functional significance, as the entire protein varies by only 1–8 residues. Among more divergent species such as Xenorhabdus nematophilus, Vibrio cholerae, and Pseudomonas aeruginosa, it appears that a central PGAP sequence (residues 128–131 in OmpR) is more highly conserved than the rest of the linker; this is where the previously isolated point mutants are located (17–19).

The Q-linkers of response regulators vary greatly in length as well as in sequence composition. For example, two highly homologous regulators from the same subfamily, OmpR and PhoB, have linker regions of 15 and 6 amino acid residues, respectively (20, 21, 34). An understanding of the molecular mechanism for transmitting information between the two domains will depend upon understanding the interface that con-

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‡ Predoctoral fellow of the American Heart Association-Northwest Affiliate.
§ Recipient of a Consejo Nacional de Ciencias y Tecnicas (CONACyT) fellowship from the Mexican government. Current address: Instituto de Biotecnologia, Universidad Nacional Autonoma de Mexico, Ave. Universidad 2001, Cuernavaca, Morelos, 62210 Mexico.
¶ To whom correspondence should be addressed: Dept. of Molecular Microbiology and Immunology, L-220, Oregon Health and Science University, 3181 S. W. Sam Jackson Park Rd., Portland, OR 97239. Tel.: 503-494-1363; Fax: 503-494-6862; E-mail: KenneyL@ohsu.edu.

1 The abbreviation used is: P, phosphorylated (e.g. OmpR-P).
nected them. As the putative interdomain interface might vary with linker length, it is useful to understand the required length of the linker region for each regulator. Here we express OmpR constructs encoding linkers of various lengths and find that alterations in linker length impair OmpR function.

In our analysis of several linker mutants, it was striking that three different substitutions resulted in an OmpR’ OmpC phenotype. An examination of these OmpR mutants revealed that their porin gene expression profiles are similar, and yet they differ in their phosphorylation and DNA binding properties. Thus, there are different molecular mechanisms by which an OmpR linker mutant may present an OmpF OmpC phenotype in vivo.

EXPERIMENTAL PROCEDURES

Construction of Mutants—OmpR was subcloned into the Bluescript KS vector using HindIII and XbaI sites. To create some of the mutations, two complementary oligonucleotides containing the desired mutation were used in a PCR reaction as described (22, 23). These oligonucleotides are listed in Table I as group A. The linker is defined in this case based on structural determination by x-ray crystallography (20, 21). The linker is defined by the lack of electron density, and its edges are the first residue after the A 5 helix in the receiver (amino terminus) domain and the last residue before the β strand of the carboxyl-terminal DNA-binding domain. To create the linker substitutions, the endogenous SalI and DraI sites were removed from the pBluescript vector, and silent SalI and DraI sites were created in ompR flanking the linker region. This mutagenesis was by the same PCR reaction (22, 23). The oligonucleotides used for construction of this vector are listed as group B in Table I. Oligonucleotides corresponding to new linker sequences are listed as group C. The linker is defined by the lack of electron density, and its edges are the first residue after the A 5 helix in the receiver (amino terminus) domain and the last residue before the β strand of the carboxyl-terminal DNA-binding domain. To create the linker substitutions, the endogenous SalI and DraI sites were removed from the pBluescript vector, and silent SalI and DraI sites were created in ompR flanking the linker region. This mutagenesis was by the same PCR reaction (22, 23). The oligonucleotides used for construction of this vector are listed as group C in Table I. All of the linker mutants in the strain MH225.101 except Q9 were found to be expressed in soluble form by Western blot analysis.

Protein Purification—OmpR, G129D, P131S and GGK were expressed in soluble form by Western blot analysis.

RESULTS

Changes in the Sequence of the Linker Region of ompR Result in Altered Porin Expression—Previous results indicate that the linker is important for interdomain communication in OmpR signaling (17, 18, 28). To test this hypothesis more directly, we constructed ompR mutants containing non-native amino acids in place of the endogenous linker sequence. These sequences replaced amino acids 123–137 of the ompR sequence (QANELPGAPSQEEAV) with one of the following: QQGQGQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQ
are reported. Wild type OmpR, as well as Q15 and GST, activates expression of ompC. All three constructs demonstrate normal osmoregulatory profiles in the expression of this locus. Neither GGK nor ELVIS is capable of activating transcription from ompC.

Longer and Shorter Linker Sequences Abrogate ompR Function—Because ompRQ15 expressed both ompF and ompC, it enabled us to examine the effect of changing the length of the linker sequence without concern for the specific amino acid residues deleted. We constructed Q15 variants that added or removed amino acids from the linker region of ompRQ15.

The linker substitutions were examined in liquid β-galactosidase assays. The results are presented in Fig. 2. When Q15 is substituted with a longer linker region of 20 amino acid residues (Q20), activation of ompF is greatly diminished, although the construct still expresses ompF constitutively (Fig. 2A).

When OmpR contains a linker of 15 or 13 amino acids, it can activate expression of ompF to normal levels but fails to mediate repression of this locus at high osmolarity. When the linker region is shortened to 11 amino acids, some loss of activation is observed, and this construct still fails to repress ompF. With a linker length of 10 amino acids, OmpR is barely capable of ompF transcriptional activation. Further reduction of linker length completely abolishes transcriptional activation.

Interestingly, the results obtained with the ompC-lacZ fusion clearly show that a decrease in transcriptional activation occurs with increasing and decreasing linker length (Fig. 2B). Linkers of 20, 13, and 11 residues exhibit normal patterns of osmoregulated expression of ompC. However, deviation from the wild type length of 15 decreases the level of transcription observed. When only 10 residues separate the amino- and carboxyl-terminal domains of OmpR, no transcription is de-
tected in logarithmically growing cells. Linkers of even shorter length similarly have no function. Thus, changing the length of the linker that separates the two domains of OmpR severely curtails its function in vivo. This finding has serious implications for function (signal output) and may limit the validity of comparisons between OmpR and closely related response regulators. For example, the recently crystallized OmpR homologue DrrD from *Thermotoga maritima* has a linker length of only 5 residues, and the limited interdomain interface observed may not be relevant to the structure of OmpR (30–32) (see "Discussion").

Sequence Changes in the Linker Affect OmpR Phosphorylation and Dephosphorylation by the Kinase EnvZ—Since two previously identified point mutants (G129D and P131S) of the OmpR linker region were OmpF− OmpC−, it was of interest that the GGK linker substitution also resulted in this phenotype (17, 18). To characterize the OmpF− OmpC− phenotype observed in OmpR mutants with a defective linker, we expressed and purified OmpRG129D, OmpRP131S, and OmpRGGK for in vitro analysis. We hypothesized that the OmpC− phenotype associated with substitutions in the linker resulted from a defect in interdomain communication, and thus it was of interest to compare the effects of DNA binding on phosphorylation and vice versa.

We performed kinase assays to determine whether phosphotransfer from the EnvZ kinase to the linker mutants was similar to wild type OmpR. Fig. 3 shows the results of this experiment. In Fig. 3a, it is apparent that wild type OmpR is phosphorylated upon incubation with [γ-32P]ATP and EnvZ (lanes 1–3). The phosphorylated EnvZ is turned over as OmpR is phosphorylated and OmpR-P is dephosphorylated. Upon the addition of a 2-fold molar excess of the high affinity OmpR...
binding site from the ompF promoter (Fig. 1), slightly more OmpR-P is visible (lanes 4–6). In Fig. 3b, P131S is phosphorylated (lanes 1–3), similar to the wild type OmpR (Fig. 3a). The addition of F1 DNA in this case only slightly increases the amount of P131S-P. G129D is also phosphorylated by EnvZ-P (Fig. 3c, lanes 1–3), and in this case, the addition of DNA does not affect the phosphoprotein levels (lanes 4–6). GGK is phosphorylated by EnvZ-P (Fig. 3d, lanes 1–3), but the addition of DNA has a remarkable effect on the level of GGK-P (lanes 4–6), as GGK-P increases over time in the phosphorylation reaction when DNA is present. In summary, all three linker mutants are phosphorylated by EnvZ-P, but the effect of DNA on the GGK linker substitution is dramatic, causing an increase in turnover of GGK-P (24).

In vitro, the EnvZ kinase capable of phosphorylating both OmpR and dephosphorylating OmpR-P. When EnvZ is the phosphodonor, the observed increase in OmpR-P formation upon the addition of DNA is due to a decrease in the phosphatase activity of EnvZ (14, 24). We can quantify the effect of DNA on the phosphatase activity of EnvZ, using an assay that detects the release of P<sub>i</sub>, (33). Upon incubation of OmpR, EnvZ, and ATP there is a large increase in P<sub>i</sub> production over time.

The average rate of P<sub>i</sub> release in the presence of OmpR is 7.1 nmol/ml/min (Fig. 4A, filled triangles). The P131S mutant possesses a net ATPase activity (P<sub>i</sub> release) of 6.6 nmol/ml/min, close to the level observed with wild type OmpR (Fig. 4A, filled circles). When G129D is incubated with EnvZ and ATP, there is a low rate of P<sub>i</sub> production, 1.0 nmol/ml/min (Fig. 4A, open squares). In the presence of GGK, P<sub>i</sub> production is barely above background (0.3 nmol/ml/min, Fig. 4A, open diamonds). When specific DNA such as the region upstream of the ompC promoter (C1-C2-C3) is included in the ATPase reaction, the level of OmpR-stimulated ATPase activity decreases dramatically, falling to 0.3 nmol/ml/min (Fig. 4B, filled triangles). None of the linker mutants follows this pattern. The ATPase activity in the presence of P131S does not decrease substantially in the presence of C1-C2-C3 (4.1 nmol/ml/min, only a 2-fold decrease) (Fig. 4B, filled circles). The P<sub>i</sub> release stimulated by G129D is also not affected by the presence of DNA, decreasing 1.5-fold to 0.7 nmol/ml/min (Fig. 4B, open squares). These differences are small when compared with the 20-fold reduction in phosphate turnover seen with wild type OmpR (Compare filled triangles in Fig. 4, A and B). Both P131S and G129D are known to bind DNA (see below), and yet DNA binding does not increase the stability of the mutant phosphoproteins as it does for wild type OmpR-P (Compare circles or triangles in Fig. 4, A and B).

We observed that all three of the mutants are capable of protecting the ompF promoter to the same extent as wild type OmpR, as expected by their OmpF<sup>+</sup> phenotypes. Therefore, only the ompC binding data are shown in Fig. 5.

Fig. 5, A–C, reveals that the three linker mutants have different DNA binding properties at the ompC promoter region. Both OmpR-P and P131S-P are capable of protecting the ompC promoter sequences from −104 to −40 as shown in Fig. 5A. With the G129D point mutant, a different pattern of protection is observed (Fig. 5B). Whereas OmpR-P is capable of protecting the entire ompC upstream region from −104 to −40, G129D-P binds only at the high affinity C1 site, from −104 to −75. Even at protein concentrations up to 2 μM G129D-P, no binding is evident at the C2 or C3 regions. As observed with the point mutant G129D-P, the substituted linker mutant GGK-P binds only to the high affinity C1 region (Fig. 5C). It seems likely that the OmpC<sup>−</sup> phenotypes of two of the linker mutants (G129D and GGK) are due to a DNA binding defect at the −35 proximal regions of the promoter (sites C2 and C3). The P131S point mutant may be incapable of interacting productively with RNA polymerase to initiate transcription because it can bind to the entire ompC promoter region but does not activate transcription from this locus. Thus, the underlying defects in G129D and GGK are distinct from that of P131S, and yet they all give rise to an OmpF<sup>+</sup> OmpC<sup>−</sup> phenotype (see “Discussion”).

**DISCUSSION**

**Changing the Length of the OmpR Linker Inhibits Transcriptional Activation**—We chose to use Q15 as a basis for our studies of OmpR linker length to eliminate concerns that specific amino acid residues were being added or deleted. We discovered that a linker of 13–15 amino acid residues is optimal for OmpR function, with shorter linkers gradually decreasing the ability of the protein to activate transcription of the porin genes (Fig. 2). This finding has important implications for the likelihood of a similarity in the activation mechanisms of closely related response regulators. One OmpR family member, PhoB, which shares a high degree of sequence homology with OmpR, has a linker of only 6 amino acid residues (34). Chimeric protein studies with CheY/PhoB have shown that α-helix 5 in the amino terminus of PhoB inhibits transcriptional activation by the carboxyl terminus; inhibition is relieved when the amino terminus is phosphorylated (35). This is unlike the signal propagation mechanism that controls OmpR signaling. Both the amino and carboxyl termini of OmpR are necessary for its function, and neither domain is inhibitory (36). Similarly, although the FixJ family members UhpA and NarL share high sequence similarity, the carboxyl terminus of NarL is inhibited by its amino terminus, whereas that of UhpA is not (37, 38).

NarL has a short interdomain linker of 6 amino acid residues, and the linker of UhpA is 16 residues long (38). Thus, even within the fixJ family of response regulators to which UhpA and NarL belong, there is a correlation between linker length and functional differences in the activation mechanism. In
any attempt to predict functional homology based on sequence analysis, it may be useful to consider the length of the interdomain linker as well as the respective domain architectures.

An additional point worth noting is that longer linker sequence also reduced transcriptional activation by OmpR. Thus, a functional OmpR linker must be 13–15 amino acid residues in length. Because a linker of 20 amino acid residues should provide a similar increase in local concentration of the amino and carboxyl termini as a linker of 15 amino acid residues, it seems likely that the linker of OmpR plays some active role in directing the appropriate interaction between the two domains (13, 39, 40). Evidence supporting this view comes from our studies of limited proteolysis with trypsin, in which we found that cleavage sites in the linker were sensitive to both phosphorylation and DNA binding in the amino- and carboxyl-terminal domains, respectively (13, 41).

**Substitutions in the OmpR Linker Reveal New Classes of OmpR Mutants**—Four different linker sequences were used to examine whether the sequence of the linker region was important for OmpR function. Of these, only the ELVIS linker did not activate transcription (Fig. 1), suggesting that the linker region may require flexibility and hydrophilicity to allow appropriate interactions between the amino- and carboxyl-terminal domains of OmpR. In contrast to the other substitutions, GGK activates transcription from one locus (ompF) and not the other (ompC). GGK is also the only construct that mediates repression of ompF expression at high osmolarity. The GGK linker is expected to be flexible, which may improve its ability to activate transcription over that of the ELVIS construct. The
five positively charged residues concentrated in the linker region of GGK may allow OmpR to more readily adopt a conformation that represses ompF transcription at high osmolarity than one that allows expression of ompC (see Fig. 6). Q15 and GST both contain hydrophilic residues and are predicted to be flexible. Thus, hydrophilicity and flexibility, as well as the length requirement mentioned above, seem to be important requirements for OmpR linker function.

We have shown that OmpR can retain its basic function of transcriptional activation at the ompF and ompC promoters when the endogenous amino acid sequence of the linker is altered (Table I and Fig. 1). However, there are subtleties in porin gene regulation that are not maintained in the mutants with substituted linkers, as none of the constructs tested displayed wild type osmoregulatory profiles (Fig. 1, A and B). With both OmpRQ15 and OmpRGST, the ability to repress transcription of ompF at high osmolarity was lost (Fig. 1A). Note that this does not result from constitutive expression of both porin genes, as osmoregulation of ompC is normal in these assays (Fig. 1B).

We present a model in Fig. 6 that incorporates these findings. We propose that unphosphorylated OmpR does not play a role in the osmoregulation of porin genes, because it interacts with the promoter regions with low affinity (10). At low osmo-
larity, OmpR-P exists in a conformation that activates expression of ompF but binds only at the high affinity C1 site and as such fails to activate transcription from the ompC promoter (Fig. 6A, circles). At high osmolarity, wild type OmpR-P is capable of adopting a conformation that represses transcription of ompF (Fig. 6B, squares) or one that allows binding at the downstream sites of the ompC promoter and activation of ompC expression (Fig. 6B, triangles). The model predicts that two different conformations of OmpR-P co-exist in the cell at high osmolarity, one of which represses ompF and another that activates ompC. The DNA binding sites at the ompF and ompC promoter regions may induce these alternate conformational changes. We can explain the phenotypes of the linker mutants using this model (Fig. 6C). GGK-P is capable of activating and repressing ompF but is unable to undergo the conformational change that would allow binding at the C2 and C3 sites of the ompC promoter. Q15-P and GST-P are capable of activating transcription from both ompF and ompC but cannot access the conformation that would direct repression loop formation and repress transcription at ompF.

Mutations That Confer an OmpF+ OmpC− Phenotype Do So by Different Mechanisms—The three linker mutants described in the present work share an OmpF+ OmpC− porin profile but have different molecular defects. G129D and GGK fail to bind the promoter-proximal C2 and C3 sites of the ompC promoter region, and this explains their failure to activate transcription from ompC (Fig. 5). However, a closer examination of mutant phosphorylation properties indicates that although the mutants have similar DNA binding properties, the G129D and GGK substitutions have altered the protein in different ways. The G129D substitution appears to have only quantitatively altered the effect of DNA on the stability of the phosphoprotein (Figs. 3 and 4). GGK, however, has a dramatic phenotype (24). The results obtained with GGK in assays of phosphotransfer and P1 release are diabolically opposed to those seen with wild type OmpR.

P131S can protect the same sites at ompC to which OmpR binds, indicating that this residue change most likely interferes with the ability of the P131S protein to contact RNA polymerase, as suggested by previous studies (Figs. 4B and 5) (17).

We have thus examined the molecular phenotypes of three OmpR linker mutants with the same porin profiles and found that no two behave in the same way. This result, together with the possibilities for conformational changes in OmpR-P revealed by the linker substitutions and depicted schematically in Fig. 6, underscores the sophisticated mechanism by which OmpR and EnvZ control the osmoregulation of porin gene expression.

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