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Phosphorylation-dependent conformational changes in OmpR, an osmoregulatory DNA-binding protein of *Escherichia coli*

(signal transduction/response regulators/transcriptional activation/ion spray mass spectrometry/two-component regulatory system)

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ABSTRACT **Osmoregulated porin gene expression in *Escherichia coli* is controlled by the two-component regulatory system EnvZ and OmpR. EnvZ, the osmosensor, is an inner membrane protein and a histidine kinase. EnvZ phosphorylates OmpR, a cytoplasmic DNA-binding protein, on an aspartyl residue. Phospho-OmpR binds to the promoters of the porin genes to regulate the expression of *ompF* and *ompC*. We describe the use of limited proteolysis by trypsin and ion spray mass spectrometry to characterize phospho-OmpR and the conformational changes that occur upon phosphorylation. Our results are consistent with a two-domain structure for OmpR, an N-terminal phosphorylation domain joined to a C-terminal DNA-binding domain by a flexible linker region. In the presence of acetyl phosphate, OmpR is phosphorylated at only one site. Phosphorylation induces a conformational change that is transmitted to the C-terminal domain via the central linker. Previous genetic analysis identified a region in the C-terminal domain that is required for transcriptional activation. Our results indicate that this region is within a surface-exposed loop. We propose that this loop contacts the α subunit of RNA polymerase to activate transcription. Mass spectrometry also reveals an unusual dephosphorylated form of OmpR, the potential significance of which is discussed.**

In bacteria, adaptive responses are largely controlled by families of paired sensor–regulator proteins termed two-component regulatory systems (for reviews, see refs. 1 and 2). The first component, the sensor, is a histidine kinase that senses the environment and is autophosphorylated by ATP. The kinase phosphorylates the response regulator, altering its output. The specific response depends on the particular two-component system. For example, it may involve the regulation of transcription or the direction of rotation of the flagellar motor. In some systems, the histidine kinase is also a phosphatase that dephosphorylates the response regulator; in other systems, an additional protein provides this function.

In the case of the porin regulon, the histidine kinase EnvZ, in the inner membrane, senses a change in the osmolarity of the medium and is phosphorylated by ATP on a histidine residue (3, 4). The phosphoryl group is then transferred from EnvZ to an aspartate residue in OmpR, a cytoplasmic DNA-binding protein (4–8). Our current hypothesis, based on genetic analysis, is that at low osmolarity, low concentrations of phospho-OmpR (OmpR-P) interact with high-affinity sites at *ompF* to stimulate its expression. At high osmolarity, the concentration of OmpR-P increases and binds to low-affinity sites at *ompF* and *ompC*, repressing expression of *ompF* and stimulating expression of *ompC* (9, 10). The net result is that at low osmolarity, *ompF* is primarily expressed; at high osmolarity, OmpC is the major porin in the outer membrane (11). The porins differ from one another in their pore diameters, exclusion limit, and flow rate. OmpC, expressed at high osmolarity, has a smaller pore and a slower flow rate (12).

OmpR and its subfamily of response regulators are thought to consist of two domains. The N-terminal domain contains the phosphorylation site, and the C-terminal domain contains the DNA-binding site(s) (13, 14). The N-terminal domain is homologous to CheY, the chemotaxis regulator whose structure has been solved (15, 16). To date, no structural information exists regarding the C-terminal DNA-binding domain of OmpR, although crystallization has been reported (17).

OmpR-P controls the activity of RNA polymerase by direct interaction with the α subunit (18, 19). Genetic analysis has identified five amino acid residues in OmpR that are required for transcriptional activation: Arg-42 in the N-terminal domain and Pro-179, Glu-193, Ala-196, and Glu-198 in the C-terminal domain (20). It is thought that these residues define a region(s) required for interaction with the α subunit.

In other two-component systems, it has been shown that acetyl phosphate phosphorylates the response regulators CheY, NtrC, AlgR, NarQ, and NarX in the absence of the histidine kinase and ATP (21–24). We have used acetyl phosphate to phosphorylate OmpR and have examined the effects of limited proteolysis by trypsin to detect conformational changes in OmpR in response to phosphorylation. Our results suggest that OmpR undergoes a specific conformational change when phosphorylated and demonstrate that residues Arg-192 to Arg-199 are surface exposed, consistent with the hypothesis that this region contacts the α subunit of RNA polymerase.

MATERIALS AND METHODS

Materials. Trypsin (L-1-tosylamido-2-phenylethyl chloromethy ketone-treated), soybean trypsin inhibitor, and acetyl phosphate were from Sigma. $H_3{}^{32}PO_4$ was from New England Nuclear/DuPont; Rainbow molecular size standards were from Amersham. Poly(vinylidene difluoride) membrane was from Millipore.

Buffers. Buffers used were TGED [10 mM Tris·HCl, pH 7.6/5% (vol/vol) glycerol/0.1 mM EDTA/0.1 mM dithiothreitol] and sample buffer (0.5 M Tris·HCl, pH 8.8/32% glycerol/0.04% bromophenol blue/6% SDS).

Purification of OmpR Protein. OmpR was purified according to Jo *et al.* (25) and stored at −20°C in TGED containing 50% glycerol. The protein was >95% pure based on Coomassie blue staining of SDS gels and was a single peak on a C_4 column. The concentration of OmpR was determined by the BCA assay from Pierce and was compared to bovine serum albumin standards.

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Abbreviations: OmpR-P, phospho-OmpR; AMP-PNP, adenylyl imidodiphosphate.

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Synthesis of Acetyl [^{32}P]Phosphate. Dilithium acetyl [^{32}P]phosphate was prepared according to Stadtman (26). Purity was determined by TLC; the concentration was determined as described (27).

OmpR Phosphorylation. Ten micrograms of protein was incubated with 50 mM Tris·HCl, pH 7.6/50 mM KCl/20 mM $MgCl_2$ in 15 μl. Phosphorylation was initiated by acetyl [^{32}P]phosphate addition and stopped by addition of sample buffer. The reaction mixture was spotted onto nitrocellulose filters and washed extensively with 0.1% SDS/1 mM EDTA. The filters were dried and then assayed in a Beckman LS 1801 scintillation counter.

OmpR Dephosphorylation. OmpR was phosphorylated as before and applied to a NAP-5 column (Pharmacia) equilibrated with phosphorylation buffer lacking acetyl phosphate. Appropriate fractions were pooled and sampled for phosphorylation level by filter binding. Twenty-five microliters was added to tubes containing 25 μl of 0.2 M sodium citrate (pH 2.4), 2 M NaOH (pH 13), or 100 μg EnvZ115 plus 2 mM adenylyl imidodiphosphate (AMP-PNP). Samples from each were measured by filter binding after 15 and 30 min.

Limited Proteolysis with Trypsin. OmpR (usually 12 μg) was phosphorylated with acetyl phosphate for 30 min and then added to a tube containing 140 mM imidazole (pH 7.6) and 1 mM EDTA. Unphosphorylated OmpR was present in a duplicate tube and trypsin was added (protein/trypsin = 60:1, wt/wt). At intervals, 2.5 μg of protein was removed into 5 μl of cold soybean trypsin inhibitor in a 5-fold excess to trypsin and quick-frozen. Sample buffer was added and thawed samples were loaded onto an SDS/14% polyacrylamide gel.

Protein Sequencing of the Peptides. To prepare peptides for sequencing, samples were digested as before but were electrophoresed in a Tricine gel system (28). Fragments were transferred to a poly(vinylidene difluoride) membrane in 10 mM Caps, pH 11/10% (vol/vol) methanol, stained for 1 min with Coomassie blue, and destained in 50% methanol. The relevant bands were excised and sequenced in an Applied Biosystems model 473A sequencer by automated Edman degradation.

Microbore HPLC–Ion Spray Mass Spectrometry. A Waters 600-MS system controller was used at a flow rate of 1 ml/min in the gradient mode. The mobile phase solvents were 0.02% trifluoroacetic acid in H_2O (solvent A) and 0.02% trifluoroacetic acid in CH_3CN (solvent B). Gradient conditions were 1–85% solvent B over 40 min. A preinjector split ratio of 19:1 was established by splitting one side to a Waters Delta-Pak C_{18} 300-Å 3.9 × 150 mm "dummy" column and the other side to a 1 × 150 mm C_4 microbore reversed-phase column (Brownlee; Aquapore OD-300, 7 μM) with an in-line loop injector (Rheodyne 7125). The microbore column effluent led to the mass spectrometer via a 100-μm-i.d. fused silica capillary tube (final flow rate = 50 μl/min). Ion spray mass spectrometry was carried out on a Sciex API-III LC/MS/MS triple quadrupole instrument (PE Sciex, Thornhill, ON, Canada) calibrated over a range of 50–2400 mass units using polypropylene glycols. Samples were analyzed using a 1.0-msec dwell time and a 0.2-atomic mass unit step size over a m/z range of 300–2400. Mass measurement accuracy was >0.02%.

RESULTS

The major problem studying the properties of the OmpR phosphoprotein has been the difficulty in isolating the phosphorylated form in sufficient quantity for analysis. Because of the competing EnvZ kinase and phosphatase activities, generating OmpR-P required achieving a favorable balance between these two opposing reactions. This often involved preparing OmpR-P by phosphorylation from a mutant form of EnvZ that had a reduced level of phosphatase activity (7). In general, it has not been possible to phosphorylate >5–10% of the EnvZ protein, making it even more difficult to generate reasonable levels of OmpR-P. To circumvent this problem, we tried acetyl phosphate as a phosphorylating agent for OmpR. Previous results have demonstrated the utility of this agent for phosphorylating other response regulators (21–24).

OmpR Phosphorylation by Acetyl Phosphate. A time course of phosphorylation is shown in Fig. 1*A*. Ten micrograms of OmpR was incubated with acetyl [^{32}P]phosphate for the times indicated. Phosphorylation increases linearly for 30 min and reaches a steady state that is constant for another 30 min.

Various amounts (0–20 μg) of purified OmpR were phosphorylated by acetyl [^{32}P]phosphate for 30 min, the peak of the time course in Fig. 1*A*. The data show that OmpR phosphorylation is linear with increasing concentrations of OmpR (Fig. 1*B*). This enabled us to calculate the amount of protein phosphorylated, which varied from about 60% to >90%, depending on the time and reactant concentrations.

If acetyl phosphate phosphorylates OmpR appropriately, then OmpR-P should exhibit the properties of an acyl phosphate and should be sensitive to dephosphorylation by the phosphatase activity of EnvZ (29). OmpR-P was prepared as usual, and the unincorporated acetyl [^{32}P]phosphate was removed by gel filtration. OmpR-P was added to acidic or basic solutions or to a solution containing EnvZ115. Phosphorylation was assayed after 15 and 30 min. It is apparent that the phosphoprotein intermediate is relatively acid-stable and ex-

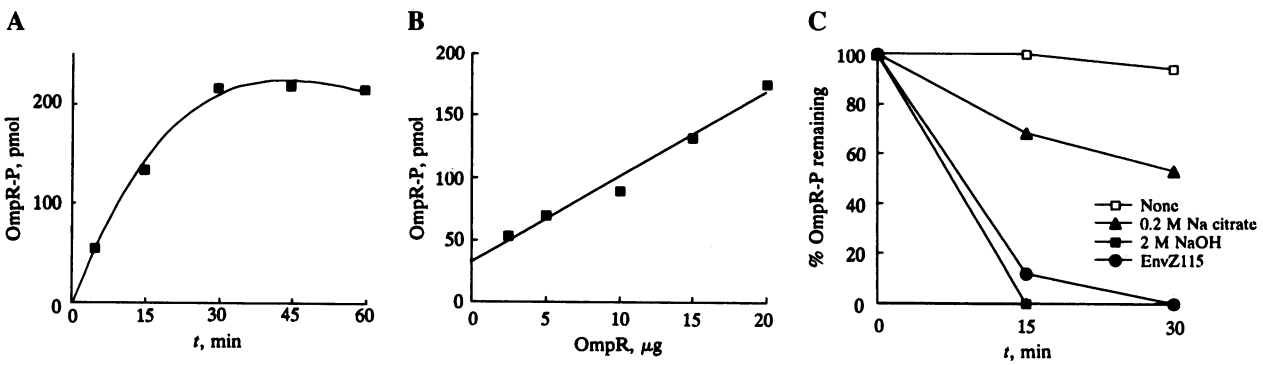

FIG. 1. Phosphorylation of OmpR by acetyl [^{32}P]phosphate. (*A*) Time course. Phosphorylation was initiated by addition of radiolabeled acetyl phosphate and stopped by the addition of sample buffer at the times indicated. (*B*) Dependence on protein concentration. The amount of protein varied as shown; the reaction time was 30 min. (*C*) OmpR-P dephosphorylation. One hundred micrograms of OmpR was phosphorylated for 30 min; acetyl [^{32}P]phosphate was removed by gel filtration. Twenty-five-microliter aliquots were added to duplicate tubes containing 25 μl of phosphorylation buffer without acetyl phosphate (□), 0.2 M sodium citrate at pH 2.4 (▲), 2 M NaOH (■), or 100 μg of EnvZ115 plus 2 mM AMP-PNP (●). At 15 and 30 min, 20 μl was removed into sample buffer and spotted onto nitrocellulose filters. Maximal OmpR-P was determined immediately after gel filtration.

tremely base-labile, as expected for an acyl phosphate (Fig. 1*C*). The addition of EnvZ115 in the presence of 2 mM AMP-PNP caused complete dephosphorylation as expected (29). We conclude that acetyl phosphate is phosphorylating OmpR at the normal site.

Limited Trypsin Proteolysis. The lack of structural information for OmpR prompted us to use limited proteolysis by trypsin to investigate its structure. There are 34 putative sites for trypsin cleavage in the OmpR protein. An initial experiment indicated that only a few sites were available to trypsin, since predominantly large fragments were obtained (protein/trypsin = 60:1; data not shown). An extended time course of digestion of OmpR is shown in Fig. 2. The fragments were separated on a Tricine gel after digestion (28). Lanes 2–6 represent OmpR digested for 0–120 min. After 30 min, there was a major fragment observed of ≈25 kDa (fragment I). Two additional fragments were also observed in the 21- to 15-kDa range (fragments II and III). After 60 min of digestion, the higher molecular mass band (fragment II) was entirely converted to the smaller fragment (fragment III), which was then resistant to further digestion.

We reasoned that it might be possible to observe changes in the protein structure upon phosphorylation if a conformational change altered the availability of any of these sites to trypsin. Lanes 7–10 show the result of digestion of OmpR-P by trypsin. Digestion was much slower compared to unphosphorylated OmpR, as evidenced by the presence of intact protein remaining even after 2 hr (lane 10). The appearance of fragment I is much slower. However, the most striking difference is that when OmpR is phosphorylated, the site that converts fragment II to fragment III is protected from digestion. This suggests that a conformational change results from phosphorylation of OmpR and that this change alters the sensitivity of this specific tryptic site.

N-Terminal Sequencing of the OmpR Trypsin Fragments. To identify the OmpR fragments, we scaled up the digestion. Fifty micrograms of OmpR was digested with trypsin for 30 min, and the fragments were isolated on a Tricine gel (Fig. 3). The gel was transferred to poly(vinylidene difluoride), and the stained fragments were excised and sequenced. The largest three fragments (I–III) have the N terminus of OmpR. The two smallest fragments (IV and V) have N termini that correspond to amino acids 193 and 200 of OmpR, respectively.

Similar results were obtained for OmpR-P. Fragments I and II also have the N terminus of OmpR. Because trypsin digestion of OmpR-P proceeds more slowly, we did not obtain sufficient amounts of the smaller fragments for amino acid sequence analysis. It seems likely that they correspond to fragments IV and V of OmpR, and data presented below support this view.

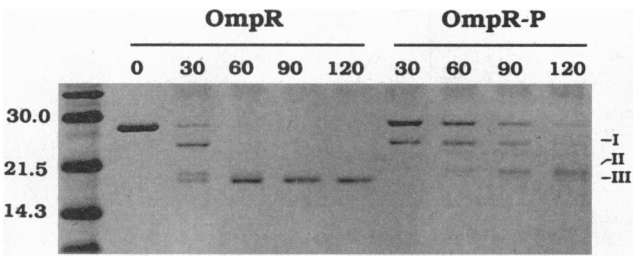

FIG. 2. Time course of OmpR digestion by trypsin. Lane 1, Rainbow molecular size markers. Lane 2, 2.5 μg of undigested OmpR. Lanes 3–6, 12.5 μg of OmpR was digested with 0.21 μg of trypsin and at 30, 60, 90, and 120 min (indicated above the gel lanes), 2.5 μg was removed into trypsin inhibitor, quick-frozen, and then separated on a Tricine gel. Lanes 7–10, results of digestion when OmpR was phosphorylated with 20 mM acetyl phosphate for 60 min prior to trypsin digestion.

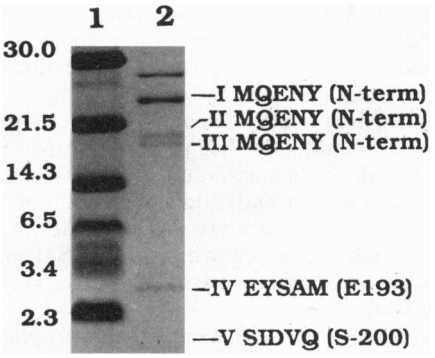

FIG. 3. Trypsin digestion of OmpR. Lane 1, Rainbow molecular size markers. Lane 2, 60 μg of OmpR protein digested with 1 μg of trypsin for 30 min. The stained bands of a duplicate gel were excised and sequenced. The Roman numerals are OmpR fragments produced and their N-terminal amino acid sequence identified by Edman degradation. Band V is not visible on this gel since less protein was used; it was visible on the Immobilon blot sequenced.

Ion Spray Mass Spectrometry. To obtain an accurate measurement of molecular masses, the intact OmpR protein, OmpR-P, and the trypsin digests of each were subjected to microbore HPLC-ion spray mass spectrometry using a C_4 reversed-phase column. The masses resulting from this analysis are shown in Table 1.

OmpR has a measured mass of 27,355 Da (calculated, 27,354 Da). Mass spectrometry identified five major peptides produced from digestion of OmpR. These five peptides correspond to residues 1–192, 1–190, 1–142, 193–239, and 200–239 of OmpR, respectively. Comparison of these results with the data shown in Figs. 2 and 3 led us to the following conclusions. Fragment I is a mixture of two peptides (1–192 and 1–190). Fragment II corresponds to peptide 1–142. We were unable to identify fragment III. Fragments IV and V correspond to peptides 193–229 and 200–239, respectively. These conclusions are summarized in Fig. 4.

OmpR-P has a measured mass of 27,438 Da (calculated, 27,434 Da), an increase of about 83 Da compared to OmpR; thus, OmpR-P is phosphorylated at only one site. The peptides resulting from digestion of OmpR-P are the same as those from OmpR (Fig. 4). Fragments I and II contain the phosphoryl group (Table 1 legend), demonstrating that the phosphorylation site is in the N-terminal domain, probably at Asp-55.

As noted above, the site in OmpR that is cleaved by trypsin to produce fragment III and is protected by phosphorylation was not identified. We know it is an N-terminal fragment from sequence analysis and that it is only slightly smaller than fragment II (Figs. 2 and 3). There are several potential cleavage sites that are N-terminal to Lys-142, and likely candidates are Arg-122, Arg-121, Arg-117, and Arg-115. Any of these residues could correspond to the site that is protected by phosphorylation. The identified site at Lys-142 and the unidentified site N terminal to it closely bracket a region of OmpR that likely serves as the linker between the N- and C-terminal domains. This region has been called the Q-linker in other two-component systems (30). It is noteworthy that under the digestion conditions we employ, the small but intact C-terminal fragments (IV and V) can be isolated. Hence, these relatively small peptides must assume a stable structure.

We were surprised to find that the mass spectrum of phosphorylated OmpR actually contained three peaks. One of these corresponds to OmpR-P; another corresponds to OmpR. This was expected, since phosphorylation by acetyl phosphate is incomplete and the phosphoprotein is somewhat unstable. The third peak has a measured mass of 27,339 Da and this is about 16 Da *less* than OmpR itself (OmpR*; Table 1). One

Table 1. Ion spray mass spectrometry

Sample	Mass, Da		% error	Proposed modification
	Calculated	Measured		
OmpR	27,354	27,355	+0.004	None
OmpR-P	27,434	27,438	+0.014	+ H_2PO_3
OmpR*	27,336	27,339	+0.011	− H_3PO_4
OmpR fragment I	21,918	21,917	−0.004	Tryptic fragment 1–192
OmpR fragment I	21,704	21,702	−0.009	Tryptic fragment 1–190
OmpR fragment II	16,180	16,180	−0.000	Tryptic fragment 1–142
OmpR fragment IV	5,454	5,454	−0.000	Tryptic fragment 193–239
OmpR fragment V	4,587	4,587	−0.000	Tryptic fragment 200–239

Summary of mass spectrometry of OmpR, OmpR-P, and digests of each. Fragments I, II, IV, and V were identified in both OmpR and OmpR-P tryptic digests. Fragments I and II contained the phosphoryl group in the OmpR-P sample.

interpretation of this decrease in mass is that it represents a loss of H_3PO_4 instead of H_2PO_3, suggesting that the dephosphorylated form of OmpR may be different from the unphosphorylated protein. The presence of three species in the mass spectrum of the OmpR-P sample was highly reproducible. However, it is not possible to estimate the percentage of OmpR* in the sample, as the efficiencies with which the various forms ionize may be different.

DISCUSSION

Acetyl phosphate can phosphorylate OmpR directly, yielding high levels of OmpR-P. The physiological significance of our results depends upon the same residue being phosphorylated *in vivo* as *in vitro*. However, the phosphoprotein from acetyl phosphate has all of the characteristics of the phosphoprotein produced by phosphorylation by EnvZ, the cognate histidine kinase. The phosphoprotein is acid-stable, is base-labile, requires Mg^{2+}, and is dephosphorylated by EnvZ115 plus AMP-PNP. Our results are in good agreement with other response regulators that can be phosphorylated by small molecular weight phosphodonors such as carbamyl phosphate, phosphoramidate, and acetyl phosphate (21–24).

The mass spectrometry data indicate that OmpR is phosphorylated on only one residue, with an accompanying increase in mass of about 83 Da. A phosphorylated fragment representing residues 1–142 was isolated, indicating that the phosphorylation site is in the N-terminal half of the protein and is probably Asp-55. In addition, the purified OmpR protein is not phosphorylated, since the theoretical and measured masses are virtually identical (Table 1). OmpR-P is rather unstable (5), and we think it unlikely that the phosphorylated species can be purified from whole cells as has been claimed (31).

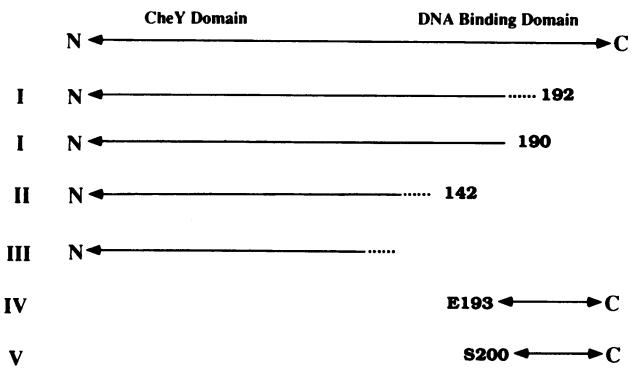

FIG. 4. Summary of tryptic digestion of OmpR and resulting fragments. N, N terminus; C, C terminus. Roman numerals correspond to fragments in Figs. 2 and 3; the numbers on the right correspond to the sequence at the cleavage site.

Previous studies on OmpR have suggested a two-domain model (13, 14). The N-terminal domain resembles CheY and contains the site for phosphorylation by EnvZ, Asp-55. The C-terminal domain binds DNA. These two domains are connected by a flexible linker, which in other response regulators has been termed the Q-linker (30). This model predicts protease-sensitive sites in the linker region, which we have confirmed. There are at least two trypsin-sensitive sites in the linker region of OmpR, Lys-142 and another site ≈20 amino acid residues toward the N terminus. Cleavage at Lys-142 produces fragment II; cleavage at the other site produces fragment III (Figs. 2 and 4).

The sensitivity of the linker region to trypsin cleavage is distinctly affected by phosphorylation. In the absence of phosphorylation, fragment II is converted to fragment III. When OmpR is phosphorylated, this cleavage site is protected (Fig. 2). We suggest that phosphorylation of the N-terminal domain induces a conformational change that is transmitted to the C-terminal domain via this linker region, resulting in an increased affinity of the latter domain for DNA. The altered protease sensitivity we observe reflects this conformational change. This interpretation is entirely consistent with studies on the cAMP receptor protein, which found that the hinge region between the N-terminal cAMP-binding domain and the C-terminal DNA-binding domain was an important determinant of the conformational change that occurred upon cAMP binding. The majority of mutations that permit cAMP receptor protein function in the absence of the allosteric effector cAMP alter residues in this region (32, 33). Our results are consistent with studies comparing the NMR ^{15}N-^{1}H spectra of the CheY·Mg^{2+} complex and phospho-CheY in the presence of Mg^{2+}. It was suggested that significant structural changes occur in the CheY protein upon phosphorylation (34). Earlier reports using ^{19}F NMR also showed that the environment of phenylalanine residues distant from the phosphorylation site changed when CheY was phosphorylated (35).

Missense mutations in the linker of OmpR have been described (G129D and P131S) (36). These mutations interfere with the ability of OmpR-P to repress transcription at *ompF*, perhaps by altering the conformational change that occurs upon phosphorylation. Alternatively, they may affect oligomerization. Such interactions may be required for repression, since it seems likely that DNA looping is involved (37, 38).

The most trypsin-sensitive sites in OmpR are not in the linker region. Rather, they lie in a C-terminal region between residues 193 and 200. This observation argues strongly that this region of the protein is surface-exposed and that this is true independent of the phosphorylation state of the protein. Missense mutations that alter residues in this region of OmpR have also been described (E193K, A196V, and E198K) (20). In particular, these mutations interfere with the ability of OmpR-P to activate transcription at both *ompF* and *ompC*. OmpR-P activates transcription by interaction with the C-terminal domain of the α subunit of RNA polymerase (18, 19),

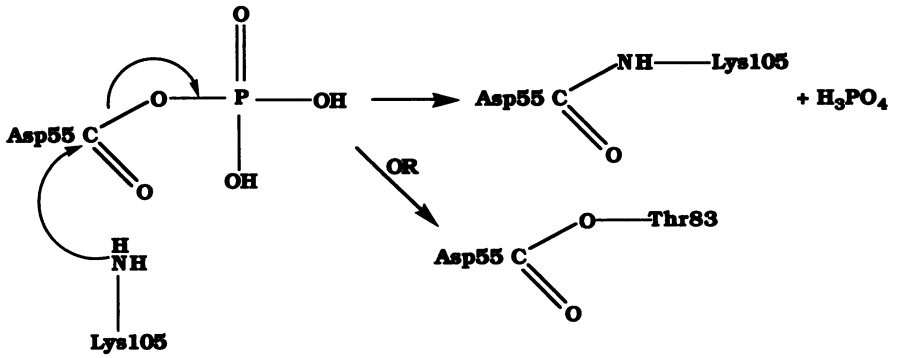

FIG. 5. A possible reaction intermediate of OmpR-P.

and it has been suggested that these mutations prevent transcriptional activation by altering the interaction of OmpR-P with the α subunit. Our results support this interpretation. Clearly, the region of OmpR-P that contacts α must be surface-exposed.

Mass spectrometry of trypsin-digested OmpR and OmpR-P identified C-terminal fragments consisting of residues 193–239 and 200–239. These fragments were isolated when most of the N terminus was degraded, suggesting that these relatively small fragments assume a stable structure. It is tempting to speculate that this region may represent at least part of the DNA-binding domain of OmpR. Results from genetic analysis support this view. The missense mutation that most clearly affects the ability of OmpR-P to bind DNA (V203M) is located in this region (36). Our results suggest that it is possible to purify this small domain in reasonable quantities and would provide a means to test these predictions directly. This is an especially important issue, since no standard DNA-binding motif can be recognized in OmpR or any member of this subfamily of response regulators (1, 39).

The third species observed in the mass spectrum of the OmpR-P sample (OmpR*) remains incompletely defined. Its mass differs from the phosphorylated protein by about 99 Da and from OmpR by about 16 Da. One explanation for this species is that it represents the loss of H_3PO_4 upon dephosphorylation instead of H_2PO_3. Perhaps Lys-105, located at the phosphorylation site in the corresponding CheY structure, attacks the carbonyl of Asp-55, displacing H_3PO_4, resulting in the formation of an isopeptide bond (Fig. 5). This mechanism explains the observed mass, but it requires hydrolysis of the newly formed isopeptide bond for regeneration of the unphosphorylated protein. Perhaps this is achieved by EnvZ. Alternatively, a proximal serine, threonine, or tyrosine might be involved, leading to the formation of an ester bond, which would be more readily hydrolyzed. One potential candidate is Thr-87, which is located near the active site in the CheY structure and is conserved in OmpR (Thr-83). It is possible, however, that OmpR* is aberrant and that it was detected simply because the level of OmpR-P produced by acetyl phosphate is very high.

The potential significance of OmpR* must await further analysis. However, many response regulators have autophosphatase activity; CheY-P, for example, has a half-life of only a few seconds. Mutations such as K109R, which corresponds to Lys-105 of OmpR, exhibit a slower rate of dephosphorylation (40). Dephosphorylation is not stimulated by CheZ, suggesting a role for this lysine subsequent to phosphorylation. Mass spectrometry should be useful for analyzing a similar mutation in OmpR.

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