Crystal Structure of PotD, the Primary Receptor of the Polyamine Transport System in Escherichia coli*

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PotD protein is a periplasmic binding protein and the primary receptor of the polyamine transport system, which regulates the polyamine content in Escherichia coli. The crystal structure of PotD in complex with spermidine has been solved at 2.5-Å resolution. The PotD protein consists of two domains with an alternating βαβ topology. The polyamine binding site is in a central cleft lying in the interface between the domains. In the cleft, four acidic residues recognize the three positively charged nitrogen atoms of spermidine, while five aromatic side chains anchor the methylene backbone by van der Waals interactions. The overall fold of PotD is similar to that of other periplasmic binding proteins, and in particular to the maltodextrin-binding protein from E. coli, despite the fact that sequence identity is as low as 20%. The comparison of the PotD structure with the two maltodextrin-binding protein structures, determined in the presence and absence of the substrate, suggests that spermidine binding rearranges the relative orientation of the PotD domains to create a more compact structure.

Polyamines, such as putrescine, spermidine, and spermine, are ubiquitous in all living organisms. They are involved in a wide variety of biological reactions, including nucleic acid and protein synthesis (1, 2). These compounds exist as linear polymers or as branched polyamines with variable lengths, and hence they are assumed to form a dimer. The protein in the asymmetric unit, connected by an almost precise translational symmetry with 1⁄2 of the unit cell parameters, confirms that there are two dimers in the asymmetric unit. The procedure for data collection was already reported (24).

MATERIALS AND METHODS

Structure Determination—Crystals, which belong to the monoclinic space group P21, with unit cell parameters a = 145.3 Å, b = 69.1 Å, c = 72.5 Å, and β = 107.6°, were grown according to the procedure described previously (24). The crystals contain four molecules in the asymmetric unit. The procedure for data collection was already reported (24).

The major heavy atom sites of K2PtCl4 and Pb(NO3)4 derivatives prepared by soaking were determined from their difference Patterson maps. The initial analysis of the x-ray data showed that the structure factors for the reflections with the odd h indices were much smaller than those with the even h indices (−F(h+1, k, l) > 0.5σ−F(h, k, l)) in a 2.5 Å resolution shell. This fact, along with the analysis of the heavy atom sites in the derivatives, confirmed that there are two dimers of the protein in the asymmetric unit, connected by an almost precise translational symmetry with 1⁄2 of the a-axis of the crystal (24). The heavy atom parameters were refined with the programs PROTEIN (25) and MLPHARE (26) against the 3.0 Å resolution data, including anomalous data from all derivatives. The latter program provided the mean figure of merit of 0.63. Solvent flattening (27) and noncrystallographic

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Crystal Structure of PotD

Table I

Structure determination statistics of PotD

| Data set | Instrument | Unique reflections | Number of water molecules | Residuals (all data) | Figures of merit |
|----------|------------|--------------------|----------------------------|----------------------|------------------|
| Native   | PF (2.5 Å) | 38,903             | 10,240                     | 0.85                 | 0.63             |
| Native   | DIP320 (2.7 Å) | 28,546         | 9,853                      | 0.80                 | 0.63             |
| Combined | (2.5 Å)     | 40,242             | 10,980                     | 0.82                 | 0.63             |
| PTCL1    | DIP320 (3.0 Å) | 9698             | 9,640                      | 0.78                 | 0.59             |
| PTCL2    | DIP320 (3.0 Å) | 9853             | 9,768                      | 0.75                 | 0.59             |
| PBNO     | DIP320 (3.0 Å) | 10,284           | 9,820                      | 0.77                 | 0.59             |
| UONO     | DIP320 (3.0 Å) | 9410             | 9,560                      | 0.77                 | 0.59             |
| KUOF     | DIP320 (3.0 Å) | 8649             | 9,040                      | 0.76                 | 0.59             |

Table II

Refinement statistics

|                   | Number of protein atoms (non-hydrogen) | Number of ligand atoms | Number of water molecules | R-factor | R-free | R.m.s. deviation in bond lengths | R.m.s. deviation in bond angles |
|-------------------|----------------------------------------|------------------------|--------------------------|----------|--------|-------------------------------|-------------------------------|
| Combined           | 10,240                                  | 40                     | 236                      | 0.199    | 0.280  | 0.016                         | 1.98°                         |
|                   | r.m.s. deviation in improper torsions   |                        |                          | 1.80°    |         |                               |                               |

RESULTS

Overall Structure of PotD—The crystal contains two dimeric molecules of the PotD protein in the asymmetric unit. The final structure refined at 2.5 Å resolution includes four identical protein molecules, each of which contains 325 amino acids, and one ordered spermidine molecule, in addition to 236 ordered water molecules in the asymmetric unit. The first two residues (aspartate residues 24 and 25) at the N terminus (since the signal sequence is eliminated in the crystallized PotD protein, Asp24 is defined as the N-terminal residue) are not well defined in the electron density map, and their conformations appear to be disordered in the crystal. The primary sequence with the secondary structure elements and the ribbon representation are shown in Fig. 1.

The PotD molecule has an ellipsoidal shape with dimensions of 30 × 40 × 55 Å. It consists of two distinct domains divided by a deep cleft. Each domain is formed by two noncontiguous polypeptide segments. Nevertheless, the two domains are very similar in the arrangements of their secondary structure elements. The first domain (N domain; residues 26–131 and 257–302) consists of five β-strands and six α-helices. The other domain (C domain; residues 132–256 and 303–348), with a larger size, contains five β-strands and seven α-helices. The β-sheet within each domain is flanked by several α-helices on both sides. The polypeptide chain crosses over three times between the two domains, which noncovalently interact with each other by an extensive interface. The three crossing segments and the interface form a deep cleft with approximate dimensions of 20 Å long, 5 Å wide, and 14 Å deep (Fig. 1B). The PotD protein, with its many β-α-β repeats, is classified as an α/β type. Of the amino acids, 40 and 18% are located in the α-helices and the β-sheets, respectively. The remaining amino acids (42%) belong to loops and coils. There is no substantial difference among the backbone structures of the four independent molecules in the asymmetric unit. Their root mean square deviations (r.m.s.) deviations for the superimposed Ca atoms are as low as 0.40 Å. However, when the Ca atoms of the N and C domains are superimposed separately among the corresponding domains in the asymmetric unit, the C domain shows a larger r.m.s. deviation value (0.42 Å) than the N domain (0.33 Å).

Subunit Contacts—The crystal contains two dimeric molecules in the asymmetric unit. The dimensions of the dimer are approximately 70 × 70 × 55 Å. Each monomer in a dimer is related by a noncrystallographic 2-fold axis (Fig. 2). The r.m.s. deviation values for the Ca atoms between these two related dimers was calculated to be 0.52 Å. The dimerization mainly involves the interactions between the N domain (βα, α1, and α2) and the C domain (αα, βα, and ββ).
b) and the C domain (α6, α7, and β1). Consequently, the two clefts in the dimer face each other and cross over the solvent region that is centered around the 2-fold axis. The dimer is stabilized by a network of many hydrogen bonds and van der Waals interactions. In fact, 14 residues participate in direct hydrogen bonds (Tyr30 to Asp141, Gly41 and Glu44 to Gln238, Tyr56 to Gly240 and Thr241, Thr58 to Met219, Glu60 to Glu220, and Asp212 to Asn216 and Glu220). These extensive interactions contribute to the maintenance of the dimer.

Spermidine Binding—Crystals of PotD have been grown in the presence of spermidine. Indeed, the omit map at 2.5-Å resolution shows an elongated electron density (Fig. 3), which is assigned to a spermidine molecule bound to the central cleft between the two domains. These densities have been found within all four molecules in the asymmetric unit, indicating that the bound spermidine molecules adopt the identical conformation. Interestingly, the spermidine molecule is bent within the PotD molecule, whereas all kinds of the crystal structures of spermidine in the Cambridge structural data base exhibit a linear shape (33, 34).

Another important feature in the binding site is that four acidic residues, Glu36, Asp168, Glu171, and Asp257, recognize the charged nitrogen atoms of spermidine through numerous ionic interactions (Fig. 4B). The conformations of these aromatic and acidic residues are conserved well among the four molecules in an asymmetric unit. One terminal amine group of propyl amine moiety in the spermidine forms the salt bridges with the carboxyl side chains of Asp168 and Glu171, and the hydrogen bonds with the side chain of Gln327 and Tyr85. The secondary amino group in the middle is recognized through the side chain of Asp257, and the other terminal amino group forms a salt bridge.
Fig. 3. Electron density showing the bound spermidine. Stereo view of the electron density for spermidine in the binding pocket and its environment. The electron density map was calculated with the coefficients $Fo - |Fc|$ and the phases from the refined protein and the solvent atoms with the spermidine molecule are omitted. The contour levels are at 3.5$\sigma$. The residues of the N domain are green, the C domain is blue, and the substrate is red. The conformation of the bound spermidine and its environment is shown in Fig. 4.

Fig. 4. Interactions of spermidine with protein atoms. A, a stereo picture showing hydrogen bonds, salt bridges, and van der Waals interactions. Hydrogen bonds and salt bridges are indicated by dotted lines. The spermidine molecule is shown by the shadowed ball-and-stick model. B, schematic diagram of hydrogen bonding and van der Waals interactions with spermidine.
with the side chain of Glu\textsuperscript{36} and a hydrogen bond with the side chain of Thr\textsuperscript{35}. These aromatic and acidic side chain atoms embed the spermidine molecule in the cleft so as to prevent no solvent access.

**DISCUSSION**

**Comparison with Other Periplasmic Binding Proteins**—Among the periplasmic binding proteins, the PotD backbone is most similar to that of MBP from *E. coli*, although their two sequences exhibit an identity less than 20%. In particular, the similarity between the two N domains is remarkable. When the two domains of PotD are optimally superimposed on the corresponding domains of MBP, the r.m.s. deviation values are evaluated to be 1.64 Å for the 100 C\textalpha atoms between the N domains and 2.63 Å for the 100 C\textalpha positions between the C domains. Furthermore, the active residues of the PotD and MBP proteins can be observed in similar regions of the two topologies (Fig. 5).

Another notable similarity between the two structures is that a conserved sequence motif is found in both PotD and MBP (35). This conserved sequence motif spans residues 46–54 (FT-KETGIK) of PotD, which corresponds to the loop between \(\alpha_1\) and \(\beta B\), and residues 53–61 (FEKDTGKIV) of MBP (Fig. 1). These sequences exhibit a remarkably similar conformation between the two molecules, as proved by the very small r.m.s. deviation value of 0.40 Å for the nine C\textalpha positions.

Open and Closed Forms—The crystal structures of MBP have been reported in both states of the open and closed forms (Brookhaven Protein Databank entry 1MBP, closed liganded form; 1OMP, open unliganded form), which correspond to the substrate-free form and the complex with the substrate, respectively. Substrate binding induces few conformational changes within each domain. However, it generates a substantial alteration in the relative orientation of the two domains. Substrate binding to MBP yields a hinge bending angle of 35° about an axis through the central hinge residues (residues 111 and 261) (36). The interdomain orientation of PotD is much closer to the closed form of MBP, indicating that both the PotD and MBP molecules adopt a similar domain arrangement upon binding the substrates (Fig. 6). These findings suggest that the spermidine-bound PotD molecule assumes the closed form, which presumably was converted from the open, ligand-free form.

Dimer Formation—The PotD molecule forms a dimer in this crystal structure, although PotD exists as monomeric form in the presence of the substrate (data not shown). The ionic interactions between the two subunits are so extensive that they are unlikely to have accidentally taken place during crystallization. All of the crystal structures of the periplasmic binding proteins exhibit monomeric molecules except for a MBP mutant crystal produced in the presence of maltose. This mutant crystal structure revealed a dimeric molecule (36). Furthermore, the MBP protein is purified as a dimer from an *E. coli* strain that is constitutive for the expression of the maltose system and that has been grown in the absence of maltose (37).

Therefore, we assume that the switch from the dimer to the monomer of the PotD protein may have physiological significance in polyamine transport.

Spermidine Recognition—The residues that participate in the recognition of spermidine spread over the two domains. The N domain comes into more extensive contact with the spermidine through the walls of the hydrophobic box, while the C domain provides the lid of the box. The ligand serves as a pin that links the two domains, and it is completely embedded in the protein atoms that lie between them (Fig. 4B).

The PotD protein can bind putrescine as well as spermidine, although the affinity of putrescine is much lower than that of spermidine. The dissociation constants \(K_d\) for spermidine and putrescine are 3.2 \(\mu\text{M}\) and 100 \(\mu\text{M}\), respectively (23). These \(K_d\) values reflect the spermidine-preferential recognition for the primary receptor of the polyamine transport system. The shorter putrescine molecule could possibly make ionic interac-

![Topological diagrams of PotD and MBP](http://www.jbc.org/)

**Fig. 5.** Topological diagrams of PotD and MBP. The \(\alpha\)-helices are represented as lettered cylinders, and the \(\beta\)-strands are indicated by arrows with numbers. Common secondary structure elements between PotD and MBP are shadowed. The active residues of the two binding proteins are shown as solid lines.
...ions with the acidic residues of Glu26 and Asp257, and form van der Waals interactions with the aromatic residues Trp24, Tyr27, Trp229, Trp255, and Tyr293. These interactions may stabilize the closed conformation. However, the smaller number of interactions, as compared with those with spermidine, would decrease the stability of the closed form.

Consensus Motif—The highly conserved sequence motif observed in MBP and PotD suggests a common functional role in the transport system, although mutations introduced in this region of MBP do not clearly cause a transport defect (21, 38). When the PotD structure is optimally superimposed onto the MBP structure, the motif is located in the surface loop of the N domain (Fig. 1), which is distant from the substrate binding site. On the other hand, it is directly connected to the loop α4A to α5, which participates in the substrate binding and is opposite the hinge between the domains. This motif, which also lies on the molecular surface, does not participate in the dimerization. It may be possible that the physiological role of the consensus sequence is to interact with the membrane components, PotB and/or PotC, which could be an initial switch to release the substrate from the protein.

Interactions with the Membrane-bound Components—In the course of periplasmic receptor-dependent transport, the substrate is initially recognized by a specific binding protein. The subsequent translocation across the cytoplasmic membrane requires a set of membrane protein components. The membrane-bound components in the polyamine transport machinery are three nonidentical proteins (PotA, -B, and -C). Polyamine uptake appears to be initiated by the formation of a complex between the two membrane-bound components (PotB and PotC) and the periplasmic receptor (PotD). The substrate-free PotD protein slightly inhibits spermidine uptake to the cytoplasm (23). This result implies that the closed form of PotD is preferentially recognized by the membrane components.

In spite of many relevant reports (38–43), mutational analyses have not clearly identified the interface of periplasmic binding proteins with their membrane protein components yet. However, it should be noted that the consensus sequence lies in the N domain, which shows a higher similarity of PotD and MBP in terms of the three-dimensional structure. Furthermore, in most of the periplasmic binding proteins including PotD and MBP (Fig. 5), the folding topology of the N domain is more conserved than the C domain (22). Therefore, it is likely that the interface of PotD with the membrane components is located in the N domain rather than the C domain.

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REFERENCES
1. Tabor, C. W., and Tabor, H. (1984) Annu. Rev. Biochem. 53, 749–790
2. Pegg, A. E. (1988) Cancer Res. 48, 759–774
3. Kashiwagi, K., Hosokawa, N., Furuchi, T., Kobayashi, H., Sasaki, C., Yoshikawa, M., and Igarashi, K. (1990) J. Biol. Chem. 265, 20893–20897
4. Kashiwagi, K., Suzuki, T., Suzuki, F., Furuchi, T., Kobayashi, H., and Igarashi, K. (1991) J. Biol. Chem. 266, 20922–20927
5. Kashiwagi, K., Miyamoto, S., Suzuki, F., Kobayashi, H., and Igarashi, K. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 4529–4533
6. Furuchi, T., Kashiwagi, K., Kobayashi, H., and Igarashi, K. (1991) J. Biol. Chem. 266, 20928–20933
7. Pistocho, R., Kashiwagi, K., Miyamoto, S., Nukui, E., Sadakata, Y., Kobayashi, H., and Igarashi, K. (1993) J. Biol. Chem. 268, 146–152
8. Ames, G. F.-L. (1986) Annu. Rev. Biochem. 55, 397–426
9. Furlong, C. E. (1987) in Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology (Neidhardt, F. C., Ingraham, J. L., Low, K. B., Magasanik, B., Schaechter, M., and Umbarger, H. E., eds) pp. 768–796, American Society for Microbiology, Washington, D.C.
10. Kashiwagi, K., Endo, H., Kobayashi, H., Takio, K., and Igarashi, K. (1995) J. Biol. Chem. 270, 25377–25382
11. Kang, C. H., Shin, W. C., Yamagata, Y., Gokcen, S., Ames, G. F.-L., and Kim, S. H. (1991) J. Biol. Chem. 266, 23893–23899
12. Oh, B. H., Pandit, J., Kang, C. H., Nikaido, K., Gokcen, S., Ames, G. F.-L., and Kim, S. H. (1993) J. Biol. Chem. 268, 11348–11355
13. Sack, J. S., Saper, M. A., and Quiocho, F. A. (1989) J. Mol. Biol. 206, 171–191
14. Sack, J. S., Trakhanov, S. D., Tsiganik, I. H., and Quiocho, F. A. (1989) J. Mol. Biol. 206, 193–207
15. Tame, J. R. H., Murshudov, G. N., Dodson, E. J., Neil, T. K., Dodson, G. G., Higgins, C. F., and Wilkinson, J. A. (1994) Science 264, 1578–1581
16. Pfleger, J. W., and Quiocho, F. A. (1988) J. Mol. Biol. 200, 163–180
17. Luecke, H., and Quiocho, F. A. (1990) Nature 347, 402–406
18. Quiocho, F. A., and Vyas, N. K. (1984) Nature 310, 381–386
19. Vyas, N. K., Vyas, M. N., and Quiocho, F. A. (1988) Science 242, 1290–1295
20. Mowbray, S. L., and Cole, L. B. (1992) J. Biol. Chem. 267, 155–175
21. Sharff, A. J., Rodseth, L. E., Spurlino, J. C., and Quiocho, F. A. (1992) Biochemistry 31, 10657–10663
22. Spurlino, J. C., Lu, G.-Y., and Quiocho, F. A. (1991) J. Biol. Chem. 266, 5202–5219
23. Kashiwagi, K., Miyamoto, S., Nukui, E., Kobayashi, H., and Igarashi, K. (1993) J. Biol. Chem. 268, 19358–19363
24. Sugiyama, S., Matsushima, M., Saisho, T., Kashiyagi, K., Igarashi, K., and Morikawa, K. (1996) Acta Crystallogr. D, in press
25. Steigemann, W. (1974) Die Entwicklung und Anwendung von Rechenverfahren und Rechenprogrammen zur Strukturanalyse von Proteinen am Beispiel des Trypsin-Trypsininhibitor Komplexes, des Frelen Inhibitors und der L-Asparaginase. Ph. D. thesis, Technische Universität München
26. Leslie, A. G. W. (1988) in Proceedings of the Daresbury Study Weekend, 5–6 February 1988 (Bailey, S., Dodson, E., and Phillips, S., eds) pp. 25–31, SERC Daresbury Laboratory, Warrington, United Kingdom
27. Wang, B. C. (1985) Methods Enzymol. 115, 90–112
28. Brünger, A. T. (1992) Acta Crystallogr. 48, 832–847
29. Jones, T. A., Zou, J. Y., Cowan, S. W., and Kjeldgaard, M. (1991) Acta Crystallogr. 47, 110–119
30. Brünger, A. T., Kuriyan, J., and Karplus, M. (1987) Science 235, 458–460
31. Jones, T. A. (1978) J. Appl. Crystallogr. 11, 268–272
32. Laskowski, R. A., MacArthur, M. W., Moss, D. S., and Thornton, J. M. (1993) J. Appl. Crystallogr. 26, 283–290
33. Giglio, E., Liquori, A. M., Puliti, R., and Ripamonti, A. (1966) Acta Crystallogr. 20, 683–688
34. Huse, Y. and Itaka, Y. (1969) Acta Crystallogr. B 25, 498–509
35. Matsu, Y., and Nishikawa, K. (1994) FEBS Lett. 345, 23–26
36. Sharff, A. J., Rodseth, L. E., Szmelcman, S., Hofnung, M., and Quiocho, F. A. (1995) J. Mol. Biol. 246, 8–13
37. Richarme, G. (1982) Biochem. Biophys. Res. Commun. 105, 476–481
38. Zhang, Y., Conway, C., Rosato, M., Suh, Y., and Manson, M. (1992) J. Biol. Chem. 267, 22813–22820
39. Duplay, P., Szmelcman, S., Bedouelle, H., and Hofnung, M. (1987) J. Mol. Biol. 194, 663–673
40. Duplay, P., and Szmelcman, S. (1987) J. Mol. Biol. 194, 675–678
41. Vermersch, P. S., Tesner, J. J. B., Lemon, D. D., and Quiocho, F. A. (1990) J. Biol. Chem. 265, 16592–16603
42. Mowbray, S. L. (1992) J. Mol. Biol. 217, 418–440
43. Binnie, R. A., Zhang, H., Mowbray, S., and Hermodson, M. A. (1992) Protein Sci. 1, 1642–1651
44. Kraulis, P. J. (1991) J. Appl. Crystallogr. 24, 946–950
45. Brünger, A. T. (1992) Nature 355, 472–474
46. Engh, R. A., and Huber, R. (1991) Acta Crystallogr. 47, 392–400
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