Iron is universally required by all living cells. However, in aerobic environments, iron is found as highly insoluble ferric hydroxide complexes, which are forms that severely limit the bioavailability of iron. To acquire iron, microorganisms synthesize and secrete siderophores, compounds that chelate ferric iron and thereby form soluble iron complexes. In Gram-negative bacteria, all essential ions and nutrients are transported across the cell envelope in discrete steps. Transport across the cytoplasmic membrane is an energy-dependent high-affinity process, whereas transport across the outer membrane is primarily mediated by passive diffusion through nonspecific or substrate-specific porins. Because siderophore-iron complexes are found at exceedingly low concentrations in the external media, their rate of passive diffusion across the outer membrane is insufficient for supporting the requirements of cellular growth. Therefore, a class of high-affinity siderophore receptors exists within the outer membrane. They bind specific siderophore-iron complexes and promote their active transport into the periplasm, exploiting the electrochemical potential of the cytoplasmic membrane that is transduced to the outer membrane by the TonB-ExbB-ExbD complex. All TonB-dependent receptors possess a short sequence of residues at the NH₂-terminal, which is termed the TonB box (1, 3). It has been proposed that this region functions as a mediator of the physical interaction between TonB and TonB-dependent receptors. TonB spans the periplasmic space and physically interacts with siderophore receptors, resulting in energy transduction by a mechanism that is common to all TonB-dependent receptors (3).

FhuA in the outer membrane of *Escherichia coli* (4) is the receptor for ferrichrome-iron. In addition to binding ferrichrome-iron, FhuA also functions as the primary receptor for the structurally related antibiotic albomycin, for several bacteriophages (T1, T5, UC-1, and ϕ80), for the peptide antibiotic microcin 25, and for the bacterial toxin colicin M. Because the deletion of a surface-located linear sequence converted FhuA from an energy-dependent receptor into a general diffusion channel, it was concluded that FhuA (5) and other TonB-dependent receptors (6) act as ligand-specific gated porins. When wild-type FhuA was incorporated into an artificial lipid bilayer, it did not form channels. However, the addition of bacteriophage T5 resulted in the formation of stable, high-conductance ion channels, which were electrically similar but not identical to those observed with the channel-forming mutants of FhuA (7).

**General description.** The x-ray structure (Table 1) of FhuA is composed of a COOH-terminal β-barrel domain (residues 161 to 723) and an NH₂-terminal cork domain (residues 1 to 160), which fills the barrel interior (Fig. 1, A and B). According to a search through a database of protein structures (8), the fold of the cork domain has not been observed. In contrast to the typical trimeric arrangement found in porins, FhuA is monomeric. The barrel is formed by 22 antiparallel transmembrane β strands (β1 through β22). Loops connect adjacent strands; there are short periplasmic turns (T1 through T10) and longer surface-located loops (L1 through L11) (Fig. 1A). The FhuA barrel is larger than any barrel formed by the porins—it is 69 Å in height and has an elliptical cross section of 46 by 39 Å (Fig. 1, A and B). In common with other membrane proteins, two girdles of aromatic residues mark the boundary of an apolar cylindrical zone on the barrel surface (Fig. 1A). They are positioned to extend into the lipid bilayer and delineate the border between the lipid hydrocarbon chains and the polar head groups. The distance (34 Å) from the upper aromatic girdle to the apex of L4 and the distance to the apices of other surface-located loops of FhuA are substantially larger in comparison with the equivalent distance in the known crystallographic structures of porins (Fig. 1A). This feature may facilitate the use of these loops for the attachment of FhuA-specific bacteriophages. Porins, which have much shorter surface-located loops, also function as receptors for bacteriophages (2).

The single lipopolysaccharide (LPS) molecule that is noncovalently associated with the membrane-embedded outer surface of FhuA exhibits the expected chemical structure for *E. coli* K-12 LPS (9). Specifically, lipid A is composed of two linked phosphorylated glucosamines and six fatty acid chains, the inner core possesses two octose and two heptose residues, and the outer core contains three hexose residues. The LPS molecule is positioned so that the glucosamine moieties are placed slightly above the upper aromatic girdle. Five of the six alky1 chains are closely apposed with the barrel surface and are parallel to the barrel axis, as expected for the chains of the external LPS monolayer (Fig. 1A) (10).

The cork domain, consisting mainly of a mixed four-stranded β sheet (βA through βD), extends from the periplasm to the ferrichrome-iron binding site (Fig. 1A). The electron density permits tracing of the cork domain beginning at Glu19. The cork domain is inclined by ~45° to the membrane normal, so that it sterically occludes most of the cross

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*Siderophore-Mediated Iron Transport: Crystal Structure of FhuA with Bound Lipopolysaccharide*

Andrew D. Ferguson, Eckhard Hofmann, James W. Coulton, Kay Diederichs, Wolfram Welte*
The presence of the cork domain suggests that the direct passage of ferrichrome-iron and small molecules through FhuA is not possible. This agrees with the finding that FhuA that has been reconstituted into planar lipid bilayers shows no channel conductance (5, 7). The cork domain is connected to the barrel wall by extensive hydrogen bonding. The number of hydrogen bonds observed in the FhuA–ferrichrome-iron complex is slightly reduced in comparison with FhuA in the absence of ligand. Given the large buried surface area (5000 Å²) between the inner barrel wall and the cork domain, we consider it unlikely that the entire cork domain detaches for ferrichrome-iron transport, for channel formation, or as a result of an interaction with TonB.

The cork domain delineates a pair of pockets within FhuA. The larger external pocket is open to the external environment and is restricted by barrel strands, surface-located loops, and cork domain apices A, B, and C (Fig. 1, A and B). The boundaries of the smaller periplasmic pocket are the barrel, cork domain loops, and the four-stranded β sheet (Fig. 1A).

The ferrichrome-iron binding site and the external pocket. Located in the external pocket of the FhuA–ferrichrome-iron complex is a single ferrichrome-iron molecule. The binding site for ferrichrome-iron is situated slightly above the external outer membrane interface (Fig. 1, A and B). Residues from apices A, B, and C of the cork domain and the barrel domain make direct hydrogen bonds or are in van der Waals contact with ferrichrome-iron atoms (Fig. 2). These residues are strongly conserved as assigned by the sequence alignment of ferrichrome-iron receptors from E. coli, Pantoea agglomerans, Salmonella paratyphi strain B, and Salmonella typhimurium (11, 12). Moreover, two water molecules have been identified in the binding site for ferrichrome-iron and may mediate the formation of additional hydrogen bonds between the ligand and FhuA (Fig. 3). Thus, the ferrichrome-iron binding site is coated with a tailored and complementary pattern of residues that tightly bind the ligand (dissociation constant $K_{Dp}$, 0.2 μM) (1, 13). The deletion of residues 236 to 248 from L3 resulted in the loss of ferrichrome-iron uptake (12), a result that is in accord with the composition of the binding site. Although none of the residues within L4 (residues 318 to 339) contribute directly to the high-affinity binding of ferrichrome-iron, the conformation of this loop is critical for the targeting of the ligand to its binding site (Fig. 1, A and B) (14). The inner walls of the external pocket, surface-located loops, and barrel strands from the ferrichrome-iron binding site to the external opening are lined by numerous aromatic residues (15). Ferrichrome-iron interacts favorably with aromatic residues, because it can be extracted from fungal extracts with benzoyl alcohol (16). Hydroxamate-type siderophores such as ferrichrome are uncharged at physiological pH and are not inherently hydrophobic. We propose that the interaction of aromatic residues with ferrichrome-iron involves electrostatic interactions between the quadrupole moment of the π electron system (17) and the dipoles of surface-located peptide bonds. Accordingly, the aromatic residues lining the inner walls of the external pocket function to extract ferrichrome-iron from the external medium; those found in the ferrichrome-iron binding site contribute to the high-affinity binding of ferrichrome-iron.

Ferrichrome-iron–induced conformational changes and transmembrane signaling. Comparing the structure of FhuA to its complex with ferrichrome-iron reveals two distinct conformations: the ligand-free and ligand-loaded conformations. In the barrel domain, the coordinates of the backbone atoms of FhuA and its complex with ferrichrome-iron are very similar (root mean square deviation, 0.42 Å), except for minor differences in the periplasmic turns T8 and T9 (Fig. 4). Key differences between the structures are localized in the cork domain. In the ferrichrome-iron binding site, an induced fit mechanism is observed. Apex B (residues 98 to 100) is translated 1.7 Å upward toward ferrichrome-iron, resulting in the formation of multiple hydrogen bonds with the ligand. All loops of the cork domain between apex A and the periplasmic pocket follow this trans-
The four-stranded β sheet and the loops of the cork domain that are situated below apex C and the periplasmic pocket remain stationary (Fig. 4).

As a dramatic exception to the otherwise overall conservation of the secondary structure of FhuA upon ferrichrome-iron binding, a helix [termed the switch helix (residues 24 to 29)] that is located in the periplasmic pocket in the ligand-free conformation is completely unwound in the FhuA–ferrichrome-iron complex (Figs. 4 and 5B). The switch helix contains a number of inherently hydrophobic residues, and in the ligand-free conformation, it fits into a complementary hydrophobic pocket that is formed by select residues from T8, T9, and βA. Upon ferrichrome-iron binding, the upward translation of selected loops of the cork domain disrupts the interaction of this pocket with the hydrophobic face of the switch helix, thus promoting its destabilization. The stabilization of helices in short peptides due to interactions with hydrophobic side chains has been observed and theoretically discussed (18). All residues from Arg31 to the NH2-terminus (Glu19) assume an opposite direction of the former helix axis. Glu19 is placed near Arg31 from βD, in the center of the periplasmic pocket, 17.3 Å away from its former α-carbon position (Figs. 4 and 5B) (19). All residues from Glu19 to the NH2-terminus, including the TonB box (residues 7 to 11), are disordered in both the FhuA and the FhuA–ferrichrome-iron structures. As a result of this helix-coil transition, Trp22 occludes the periplasmic end of the putative channel-forming region, and the location of the TonB box in the ligand-bound conformation is changed (Fig. 5B) (20).

These observed allosteric transitions are in agreement with previous antibody recognition studies. All monoclonal antibodies that were bound to sequences between residues 21 to 59 discriminated between ligand-free and ligand-occupied FhuA (21). The incubation of purified FhuA with ferrichrome-iron, colicin M, and (to a lesser extent) dh80 increased the relative amount of the FhuA-TonB complex that was cross-linked as compared to the amount of the FhuA-TonB complex that was cross-linked in the absence of ligand (22). The ability of these TonB-dependent FhuA-specific ligands to promote the physical interaction between FhuA and TonB suggests that allosteric transitions observed in the cork domain of the FhuA–ferrichrome-iron complex may be similar (notably, the unwinding of the switch helix). The unwinding of the switch helix is a clear periplasmically disordered conformational change, which signals the ligand-loaded status of the receptor and therefore the need for TonB-dependent energy transduction. Considering that siderophore receptors must compete for a limited amount of TonB (23), efficient signal transduction across the cell envelope to indicate the occupancy of the receptor is essential for the physiology of the bacterial cell (24).

A mechanism of ferrichrome-iron transport. A model for the transport of siderophores by TonB-dependent receptors is necessarily subject to constraints imposed by structural data, studies of ligand binding, phenotypes of genetic mutants, and residue conservation among different FhuAs. We propose the following basic model. After the initial physical interaction between ferrichrome-iron and the surface-located loops of FhuA, the ligand is partitioned from the external medium into the external pocket by its affinity for aromatic residues. It is then bound with high affinity by an induced fit mechanism, resulting in an allosteric transition. Subsequent transport of ferrichrome-iron to the periplasm is dependent on the disruption of the binding site. We propose that the formation of the FhuA-TonB complex and the subsequent energy transduction induce a further allosteric transition to reduce the stability of the ferrichrome-iron binding site. A disruption of the induced fit binding mechanism may be effected by a small shift of apices A, B, and C toward the periplasm as a consequence of energy being transduced by TonB.

When viewed along the barrel axis, the external pocket is connected to the periplasmic pocket in one segment of the barrel cross section by a narrow water-filled channel. We designated this segment as the putative channel-forming region (Fig. 5A). Located directly below apex B is a short coil containing the strongly conserved residues Leu106, Asn107, and Gly108 (21). Subtle conformational changes of this and other loops of the cork domain between apex B and the periplasmic pocket of FhuA would suffice to allow the permeation of ferrichrome-iron through the putative channel-forming region (Figs. 4 and 5A). We therefore propose that, after the formation of the FhuA-TonB complex, a channel opens in this region by the rearrangement of loops of the cork domain.

Among TonB-dependent receptors, there are few regions of strict sequence conservation. However, sequence alignments of FhuA proteins identify a series of strongly conserved residues (25) that are positioned on the inner barrel wall of the putative channel-forming region. These residues coat an extended inner barrel surface from the ferrichrome-iron binding site to the periplasmic pocket of FhuA (Fig. 5A). The arrangement of these residues may function as a series of low-affinity binding sites for the surface diffusion of ferrichrome-iron through FhuA. The weak adsorption of ferrichrome-iron to the inner barrel wall could mediate both the rapid diffusion (26) of the ligand inside the putative channel-forming region and, by its binding, confinement to this region.

This proposal is supported by previous studies involving channel-forming deletion.

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**Table 1. Crystallographic data.** FhuA and SeMet-FhuA were purified by immobilized ligand affinity chromatography with 0.05% dimethyldodecylamine-N-oxide and exchanged into 0.8% dimethyldodecylamine-N-oxide (35). For SeMet-FhuA, 2.5 mM of reduced glutathione was added to all buffers. Crystals of FhuA and SeMet-FhuA were grown with the hanging drop vapor diffusion technique by mixing 5 μl of protein at 6.5 mg/ml with an equal volume of reservoir solution (0.1 M sodium cacodylate, pH 6.4, 1% polyethylene glycol (PEG), 2000 mononethyl ether, 20% glycerol, 3% PEG 200, 0.8% dimethyldodecylamine-N-oxide, and 1% cis-inositol) (36). FhuA and SeMet-FhuA in complex with ferrichrome-iron (Fc) were crystallized under similar conditions. All crystals grew within 7 days to a final size of 350 by 350 μm and 0.25 μm in thickness at 18°C. They belonged to the primitive hexagonal space group P61 (a = b = 171.4 Å; c = 85.7 Å), with one molecule per asymmetric unit, a Matthews coefficient of 4.82 Å3/dalton, and a solvent content of 74.3%. Native data were collected at 100 K from flash-frozen crystals of FhuA and SeMet-FhuA complexed with Fc to resolutions of 2.5 and 2.7 Å, respectively. MAD data were collected from a SeMet-FhuA-Fc complex crystal to a resolution of 3.05 Å, allowing the structure to be solved. For details of structure solution and refinement, see (37). Parentheses denote the highest shell. Phasing power is the mean value of heavy atom structure factor amplitude divided by lack of closure. Rsym = Σh,S|Fo.h| − |Fc.h|/Σh,S|Fo.h| where |Fc.h| is the average of symmetry-related Fc.h.

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mutants of FhuA. The excision of some surface-located linear sequences from TonB-dependent siderophore receptors resulted in the formation of nonspecific open diffusion channels. Specifically, the deletion of residues 322 to 355 (residues from β7, L4, and β8) and 335 to 355 (residues from L4 and β8) from FhuA abolished TonB-dependent ferrichrome-iron transport while permitting the nonspecific diffusion of ferrichrome-iron, sodium dodecyl sulfate, and maltodextrins across the outer membrane (5, 27, 28). Small fluctuations in the conductance patterns of channel-forming FhuAs were observed; they were different from those of porins and possibly resulted from changes in the cross-sectional diameter of the channel. The binding of bacteriophage T5 to FhuA induced similar channels (7). Considering the structure, the removal of residues 340 to 355 from β8 would disrupt critical connections between the barrel wall and apex C of the cork domain, resulting in a higher degree of conformational flexibility of the coil segments around apices B and C and of more remote regions of the cork domain. The result may be the transient opening and closing of an aqueous channel (Fig. 5A). The location and structure of the channel may be similar to that formed in vivo by the FhuA-TonB complex. This suggestion is supported by planar lipid bilayer experiments involving the FhuA–bacteriophage T5 complex. The addition of ferrichrome-iron to either chamber of the bilayer apparatus resulted in a reduction in channel conductance; ferrichrome-iron may have bound to a distorted binding site (7).

We postulate that ferrichrome-iron is liberated from its high-affinity binding site and diffuses to the periplasm through a channel similar in structure and size to that induced by the binding of bacteriophage T5. This surface diffusion model resembles the model that was postulated for the permeation of...
sugars through the glycoporins (29). When ferrichrome-iron reaches the periplasmic pocket of FhuA, it is bound by the high-affinity periplasmic binding protein FhuD ($K_D$, 0.1 μM) (1, 30), thereby ensuring unidirectional transport across the cell envelope (31). We further postulate that other TonB-dependent siderophore receptors undergo similar ligand-induced allosteric transitions, transport their cognate siderophore through channels by surface diffusion, and therefore utilize a common siderophore-mediated iron transport mechanism.  

This proposed mechanism suggests an explanation for the evolution of high-affinity receptors for different siderophore-iron complexes by Gram-negative bacteria. Only the external aromatic pocket and the high-affinity binding site must be tailored to different ligands. Ligand-induced allosteric transitions and transport are common mechanistic features and are essentially receptor independent. The inherent flexibility of this design is advantageous in adapting siderophore receptors such as FhuA for the fungal siderophore ferrichrome (which is an obvious advantage, given variations in iron supply for bacteria). Moreover, the correlation between bacterial virulence in vivo and the expression of high-affinity TonB-dependent iron acquisition systems, including receptors for transferrin, lactoferrin, and heme and ferric siderophores (1, 32), indicates adaptations that allow bacteria to survive in the interstitial spaces and the bloodstream of host organisms. The high affinity and specificity of TonB-dependent siderophore receptors make them ideal targets for the design of novel antibacterial agents such as siderophore-antibiotic conjugates (33). The principle relies on the specific recognition of the outer membrane receptor by the siderophore moiety, thereby ensuring transport of the conjugate through the receptor and into the periplasm of the bacterial cell.

Fig. 5. The putative channel-forming region with bound ferrichrome-iron complexes. (A) Stereoview of the putative channel-forming region in ribbon representation as viewed from the external environment along the barrel axis. This is an enlargement of the upper left portion of Fig. 1B. The barrel is colored blue, and the cork domain is shown in yellow. The putative channel-forming region is indicated by a circle (diameter, 10 Å). Strictly conserved side-chain residues (Arg297 and Asn299 from β7, Asp348, Gln349, and Asp270 from β9; and Asn346, Gln348, and Gln444 from β10) that are thought to be involved in the surface diffusion of ferrichrome-iron complexes through the putative channel-forming region are shown in green. (B) Stereoview illustrating the unwinding of the switch helix as a result of an allosteric transition that was induced for ferrichrome-iron binding. The barrel strands (shown in blue) are represented as thin lines for clarity. Cork domain strands (βA through βD, Glu19, Trp22, and Arg128 are labeled (34). The switch helix in the ligand-free conformations and the coil in the ligand-loaded conformations are shown in purple and yellow, respectively.

References and Notes
10. Crystallization of FhuA is dependent on the presence of stoichiometric amounts of LPS. If LPS is completely removed from FhuA protein preparations or if an excess of LPS is present in such preparations, the growth of FhuA crystals is inhibited. We propose that LPS remained bound to FhuA throughout the process of purification and crystallization and that it did not adsorb to FhuA during isolation. Because it is known that LPS is localized to the outer leaflet of the outer membrane, the location of bound LPS marks its position relative to the upper aromatic girdle of FhuA and to the outer membrane.
11. Strongly conserved residues found in the ferrichrome-iron binding site are Arg151 from apex A, Gly206 and Gin310 from apex B, Phe115 and Tyr116 from apex C, Tyr244 and Trp246 from L3, Tyr213 and Tyr215 from β7, Phe231 from β9, and Phe217 from β8.
13. The binding site possesses a higher affinity for ferrichrome than for apoferrichrome [P. Boulanger et al., Biochemistry 35, 14216 (1996)]. In the ligand-loaded structure, Tyr244 comes in close contact with the iron atom of the ferrichrome-iron molecule. This observation may explain the decreased affinity for apoferrichrome.
15. Residues lining the external aromatic pocket are Tyr225 from L4, Tyr244 from β8, Tyr231 from β9, Phe236 and Phe337 from L8, and Tyr312 from L9, Tyr705 from L11, and Phe708 from β22.
19. The insertion of a dipeptide into the fhuA gene after residue Arg128 results in a complete loss of all FhuA function [G. Carmel et al., J. Bacteriol. 172, 1861 (1990)].
20. The tryptophan emission spectra were measured for FhuA and the FhuA–ferrichrome-iron complex [K. Locher and J. Rosenbusch, Eur. J. Biochem. 247, 779 (1997)]. The tryptophan fluorescence was shown to decrease after the addition of the ferrichrome-iron, which suggests a change in accessibility.
Structure of the MscL Homolog from Mycobacterium tuberculosis: A Gated Mechanosensitive Ion Channel

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Mechanosensitive ion channels play a critical role in transducing physical stresses at the cell membrane into an electrochemical response. The MscL family of large-conductance mechanosensitive channels is widely distributed among prokaryotes and may participate in the regulation of osmotic pressure changes within the cell. In an effort to better understand the structural basis for the function of these channels, the structure of the MscL homolog from Mycobacterium tuberculosis was determined by x-ray crystallography to 3.5 angstroms resolution. This channel is organized as a homopentamer, with each subunit containing two transmembrane α helices and a third cytoplasmic α helix. From the extracellular side, a water-filled opening approximately 18 angstroms in diameter leads into a pore lined with hydrophilic residues which narrows at the cytoplasmic side to an occluded hydrophobic apex that may act as the channel gate. This structure may serve as a model for other mechanosensitive channels, as well as the broader class of pentameric ligand-gated ion channels exemplified by the nicotinic acetylcholine receptor.

The sensing of physical forces within a cell’s environment is primarily mediated by a specialized class of membrane proteins known as mechanosensitive (MS) ion channels. MS channels have evolved the ability to transduce mechanical strain into an electrochemical response (1), enabling cells to respond to stimuli such as sound, touch, gravity, and pressure. Although several putative MS channels have been cloned, the large-conductance mechanosensitive channels (MscL) of prokaryotes have been most extensively characterized, primarily through the efforts of Kung and co-workers (2–4). MscL, first isolated by...

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R E S E A R C H A R T I C L E S

25. Strictly conserved side-chain residues that are thought to be involved in the surface diffusion of ferrichrome-iron through the putative channel-forming region may be Arg257 and Asn299 from β7; Asp318, Glu326, and Asp339 from β9; and Asn436, Glu438, and Met440 from β10.
28. Strand β7 is composed of residues 294 to 317, L4 is composed of residues 318 to 339, and strand β8 is composed of residues 340 to 366.
31. A more complicated mechanism could avoid the possible steric blockage of the surface-located pocket. However, the TonB-dependent binding and uptake of the Fhu-specific toxin colicin M through FhuA would require that the putative channel-forming region remain open from the external environment to the periplasm for an extended period of time and therefore contradict such a mechanism [C. J. Lazdunski et al., J. Bacteriol. 180, 4993 (1998); R. M. Stroud et al., Curr. Opin. Struct. Biol. 8, 525 (1998)].
34. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
35. Because FhuA is difficult to purify to homogeneity in large amounts, one prerequisite for crystallization and subsequent structural analysis by x-ray crystallography was the development of an overexpression system and efficient protocols for the rapid purification of FhuA [A. D. Ferguson et al., Protein Sci. 7, 1636 (1998)]. A recombinant FhuA was constructed by splicing a hexahistidine tag and three additional amino acids (SHHHHH-HGS) [34] at a previously identified surface location (amino acid 405) [C. S. Mocek et al., J. Bacteriol. 176, 4250 (1994)]. Functional assays confirmed (21) that the recombinant protein FhuA405His (native FhuA is fully active as a bacteriophage receptor and is also active for TonB-dependent ferrichrome-iron transport at levels comparable to those of the wild type) was secreted into the periplasm and associated with the outer membrane. To generate selenomethionyl-labeled FhuA (SMe-FhuA), plasmid pHK405 was transformed into the methylotrrophic E. coli strain DL41 and grown as recommended [S. Doublé, Methods Enzymol. 276, 523 (1997)].
37. Phase information was derived from multiple anomalous dispersion (MAD) [W. A. Hendrickson, Science 254, 51 (1991)] data that were measured at three wavelengths that corresponded to the point of inflection, the peak of the selenium absorption profile, and a remote point. All data were reduced and processed with the XDS software package [W. Kabash, J. Appl. Crystallogr. 21, 916 (1988)]. The 10 selenium sites were located with SOLVE [T. C. Terwilliger and J. Berendzen, Acta Crystallogr. D53, 571 (1997)], and MAD phases were obtained with SOLVE and SHARP [E. de La Fortelle and G. Bricogne, Methods Enzymol. 277, 367 (1996)]. Initial phases, which were calculated to a resolution of 3.05 Å, were improved by solvent flattening with the program DM [K. Cowtan, Acta Crystallogr. D50, 760 (1994)]. The resulting electron density was of sufficient quality to build a model with the program O [T. A. Jones et al., Acta Crystallogr. A47, 110 (1991)] and unambiguously place a ferrichrome-iron molecule and a LPS molecule. The model was refined with the programs X-PLOR [A. T. Brünger, X-PLOR 3.1 (Yale University, New Haven, CT, 1992)] and CNS [A. T. Brünger et al., Acta Crystallogr. D54, 905 (1998)] and was used to solve the structure of FhuA to a resolution of 2.5 Å by difference Fourier techniques. Standard protocols for simulated annealing and minimization as implemented in CNS were used for refinement. Individual restrained B-factor refinement was justified as judged by a substantial drop in Rwork (A. T. Brünger, Nature 355, 472 [1992]). The current FhuA model contains residues 19 to 723, 1 LPS, 1 ferrichrome-iron molecule, and 52 water molecules. The average B factors for main-chain and side-chain atoms, and the LPS, and ferrichrome-iron molecules are 63, 66, 75, and 49 Å², respectively. Refinement is not complete for both structures. For the FhuA model, the Rwork is 24.2% (48,359 reflections), and the Rfree is 28.3% (2309 reflections); for the FhuA–ferrichrome-iron complex, the Rwork is 26.5% (10,672 reflections), and the Rfree is 28.1% (1532 reflections). All residues lie in allowed regions of the Ramachandran plot, and all residues that are explicitly mentioned in the text reside in good electron density. Protein Data Bank accession codes are 2tpn and 1tpf for FhuA and the FhuA–ferrichrome-iron complex, respectively.
38. All figures were prepared with the programs MOLSCRIPT [P. Kraulis, J. Appl. Crystallogr. 24, 946 (1991)] and Raster-3D [E. A. Merritt and D. J. Bacon, Methods Enzymol. 277, 505 (1997)], except for Fig. 3, which was prepared with the program O.
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