Structural Basis of Gating by the Outer Membrane Transporter FecA

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Introduction

In response to iron deficiency, most microbes secrete organic chelators called siderophores, which are designed to sequester ferric iron. Regulatory mechanisms, responsive to both the internal and external iron concentration, control the transcription of genes involved in iron uptake. The ferric citrate uptake (*fec*) genes are responsible for the transport of ferric citrate from the external medium into the cytoplasm. Proteins required for each phase of these energy-dependent transport processes have defined functions and are localized to specific cell envelope compartments. Embedded within the outer membrane is FecA, which performs two mutually independent functions: It binds and transports ferric citrate, and it is required to initiate transcription of the *fecABCDE* transport operon but not the regulatory *fecIR* genes.

Methods and Materials

Full-length FecA was expressed by using *E. coli* in the presence of seleno-L-methionine and was purified in the presence of 5 mM dithiothreitol. Well-ordered crystals of FecA were grown by using the hanging-drop vapor diffusion technique to a final size of 300 × 100 × 10 µm. These crystals were mounted in cryoloops and flash-frozen by direct immersion into liquid propane. All diffraction data were collected at 100K by using a cryostream apparatus with synchrotron radiation from Structural Biology Center beamlines ID-19 and BM-19 at the APS. All x-ray diffraction data were reduced with HKL2000. These crystals have the symmetry of space group $P2_12_12$, with one molecule per asymmetric unit and a Matthews coefficient of 3.06 $Å^3$ per dalton (corresponding to a solvent content of 59%). Phase information was derived from multiwavelength anomalous diffraction (MAD) data collected from a single crystal measured at three wavelengths corresponding to the peak, inflection point, and high-energy remote of the selenium x-ray fluorescence spectrum. Of 14 selenium sites, 13 were located with Shake-and-Bake and were refined with SHARP. Initial phases were improved by density modification and phase extension to 2.5-Å resolution by using CNS. A structural model for unliganded FecA was built into the experimental electron density map with O and refined with CNS. Dinuclear ferric citrate was soaked overnight into apo-FecA crystals. The liganded FecA structure was solved by molecular replacement by using the unliganded structure as the search model [1].

Results

The crystal structure of full-length FecA (741 residues) is composed of three domains (Fig. 1). A monomeric 22-stranded β-barrel is formed by residues 222 to 741. *In vivo*, these antiparallel strands traverse the outer membrane. Adjacent strands are connected by solvent-accessible extracellular loops containing two α-helices and three β-strands. Turns are oriented toward the periplasm. When viewed along its axis, the barrel has ellipsoidal dimensions (35 × 47 Å) and is 65 Å in height, extending 30 Å above the external interface of the upper leaflet of the outer membrane. Two girdles of aromatic residues inscribe the membrane-embedded hydrophobic surface of the receptor.

The plug domain (residues 80 to 221) is located inside the barrel, made up of five helices, two β-strands, and a mixed four-stranded β-sheet that is tilted by about 45° with respect to the membrane plane (Fig. 1). The plug domain prevents the direct passage of ferric citrate across the outer membrane and separates the external and periplasmic pockets, which are located above and below the four-stranded β-sheet. The extracellular pocket of FecA is lined with positively charged residues (ferric citrate is negatively charged). The equivalent region of FhuA and FepA are lined predominantly with aromatic and/or hydrophilic residues. The electrostatic properties of these pockets confer specificity on the basis of the chemical attributes of the siderophore. The flexible 79-residue domain of FecA, termed the NH_2-terminal extension (residues 1 to 79), which resides entirely within the periplasm and transmits the liganded status of the receptor to FecR, was not visible in the electron density maps and could not be modeled.

Discussion

Noncovalently bound within the external pocket of FecA is a single dinuclear ferric citrate molecule. Three loops of the plug domain — apices A (Thr^{138}), B (Arg^{155}), and C (Ser^{180}) — extend above the plane of the upper leaflet of the outer membrane. In the liganded structure, apices A, B, and C, together with residues located on

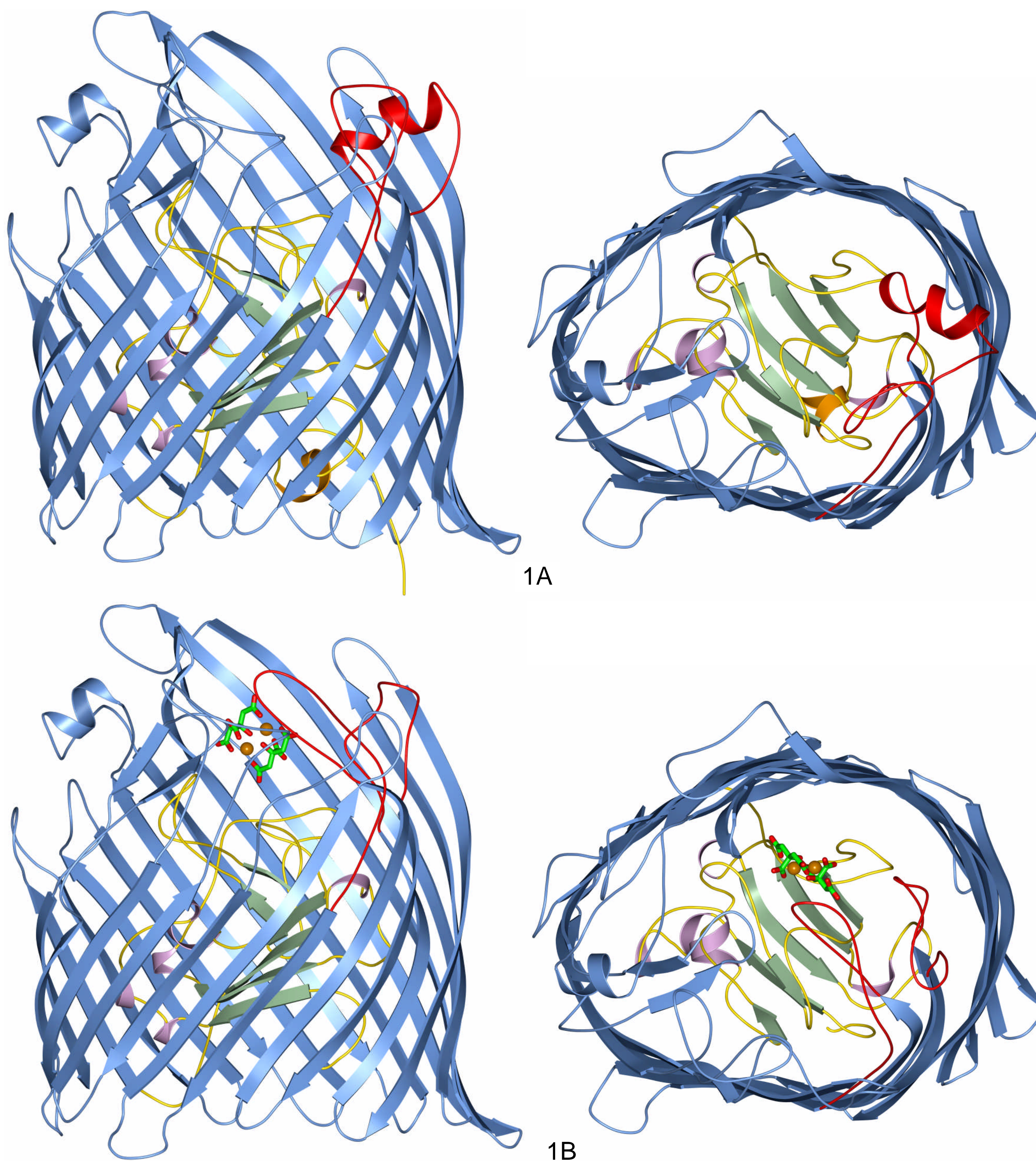

FIG. 1: Crystallographic structure of FecA in the (A) unliganded and (B) liganded conformation. The 22-stranded barrel is blue. The mixed four-stranded beta-sheet of the plug is green; loops are yellow; and helices are violet. The seventh and eighth extracellular loops of the barrel are red. The switch helix, located in the periplasmic pocket of FecA, is orange and is observed only in the unliganded conformation. The ferric citrate molecule is shown in ball-and-stick representation with the oxygen atoms in red, carbon atoms in green, and ferric iron atoms shown as orange spheres.

extracellular loops 4, 5, 8, and 11, form hydrogen bonds and electrostatic interactions with dinuclear ferric citrate. Additional van der Waals contacts are provided by Leu^{156} from the plug domain and Phe^{333} from the barrel.

Formation of the liganded complex substantially affects the conformation of the barrel and the plug domain of FecA. Binding of ferric citrate causes both minor and major changes in the spatial arrangement and conformation of five extracellular loops (Fig. 1). Minor changes (<0.5 Å) are observed in the fourth, fifth, and ninth extracellular loops. Strikingly, the seventh extracellular loop (residues 516 to 535) is translated by up to 11 Å, and it changes conformation such that helix 2 (residues 522 to 529) is unwound. Residues 562 to 581 from the eighth extracellular loop are also translated by up to 15 Å.

Allosteric transitions are propagated across the outer membrane by the plug domain via shifts (0.5 to 0.7 Å) toward the ferric citrate molecule of apices A and C, via shifts of apex B away from the siderophore, and via the concerted downward movement (0.7 to 2.0 Å) toward the periplasm of βE and βF. In the liganded structure, an NH_2-terminal segment located within the periplasmic pocket of FecA, termed the switch helix, unwinds to assume a flexible extended conformation (as judged by the absence of interpretable electron density before residue Asn^{95}). Similar changes have been observed in the 3-D structures of multiple liganded complexes of FhuA. The unwinding of the switch helix signals the occupancy of the receptor in the periplasm such that energized TonB molecules can effectively discriminate between unliganded and liganded receptors.

Acknowledgments

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References

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