second coincident TonB could not use the Ton-box in binding, so its affinity would be much lower (as has been observed). Binding of the substrate to the transporter likely induces an order-to-disorder transition in the Ton-box (8), and this disordered Ton-box is the recognition element for TonB. Because the TonB-transporter interaction is transient (i.e., TonB may interact serially with multiple outer membrane transporters to perform multiple transport cycles), the presence of disorder (high entropy) of the Ton-box is a useful strategy for obtaining high specificity without high affinity (30). The disordered Ton-box may be useful for sweeping out a larger volume within the periplasm, thus increasing the probability of encountering TonB. The conserved salt bridge between the Ton-box and TonB may be critical for longer range electrostatic attraction and nucleation of β strand formation of the Ton-box. Lastly, the structure of this complex, particularly the Ton-box:TonB region, may be useful in structure-based lead compound discovery for novel antibacterials. The addition of Ton-box peptides to media inhibits bacterial growth (31), and the expression of plasmidic domains of TonB inhibits transporter function (32). Both of these results indicate that disruption of the physiological TonB:Ton-box interface may provide a drug-discovery paradigm.

References and Notes
12. Materials and methods are available as supporting materials on Science Online.
19. Single-letter abbreviations for the amino acid residues are as follows: K, lys; L, Leu; P, Pro; Q, Gln; T, Thr; and Y, Tyr.
34. This work supported by NIH grants DK59999 to D.D.S., M.D.P., C.N.B., and M.C.W. and GM02055 to D.D.S.). Data were collected at Southeastern Regional Collaborative Access Team (SER-CAT) 2-ID beamline at the Advanced Photon Source, Argonne National Laboratory. Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under contract no. W-31-109-Eng-38. Coordinates and structure factors (accession number 2GSK) will be submitted to the Protein Data Bank. We thank P. Loli, R. Nakamoto, and E. Perozo for critical reading of the manuscript. This paper is dedicated to the memory of Professor Robert J. Kadner.

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Materials and Methods
SOM Text
Figs. S1 to S6
Table S1
References
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Structure of TonB in Complex with FhuA, E. coli Outer Membrane Receptor

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The cytoplasmic membrane protein TonB spans the periplasm of the Gram-negative bacterial cell envelope, contacts cognate outer membrane receptors, and facilitates siderophore transport. The outer membrane receptor FhuA from Escherichia coli mediates TonB-dependent import of ferrichrome. We report the 3.3 Ångstrom resolution crystal structure of the TonB carboxyl-terminal domain in complex with FhuA. TonB contacts stabilize FhuA’s amino-terminal residues, including those of the consensus Ton box sequence that form an interpretone β sheet with TonB through strand exchange. The highly conserved TonB residue arginine-166 is oriented to form multiple contacts with the FhuA cory, the globular domain enclosed by the β barrel.

Iron is an essential element in bacteria for a number of redox processes (1). It is highly insoluble in its ferric (Fe3+) form at physiological pH. To overcome the low bioavailability of Fe3+, Gram-negative bacteria have evolved strategies for high-affinity Fe3+ uptake. One well-characterized strategy involves transduction of energy from the cytoplasmic membrane (CM) to the outer membrane (OM), resulting in active transport of Fe3+ chelated to molecules known as siderophores. Central to this process, the CM-anchored energy-transducing protein TonB spans the periplasm and contacts OM-embedded receptors that bind metal-chelated siderophores. TonB, through uncharacterized conformational changes, transduces energy from the CM’s proton motive force to the receptor and facilitates siderophore transport into the periplasm. Existing structural data that describe key protein components of the siderophore transport cycle highlight outstanding questions regarding the overall molecular mechanism (2).

The CM protein TonB is composed of three domains. An N-terminal domain (residues 1 to 33) is anchored to the CM and makes contacts with proteins ExbB and ExbD to form an energy-transducing complex. A C-terminal domain (residues 155 to 239) directly contacts receptors in the OM. An intermediate domain (residues 34 to 154) contains a region of alternating Pro-Glu and Pro-Lys repeats located between residues 66 and 100. There is currently no known structure for the entire TonB protein. However, three structures have been reported for the TonB C-terminal domain. Its oligomeric states are variable and correlate with lengths of the recombinant constructs used to obtain the structures: a tightly interwoven dimer, residues 155 to 239 (3); a loose dimer, residues 148 to 239 (4); and a monomer, residues 103 to 239 (5). The monomeric C-terminal domain consists of two α helices positioned on the same face of a four-stranded antiparallel β sheet, strand β4 being located at the C terminus of TonB.

The OM receptor FhuA is the Escherichia coli transporter of the hydroxamate siderophore
ferrichrome. Structurally homologous TonB-dependent OM receptors include the E. coli transporters of cobalamin (BtuB) (6), ferric citrate (FecA) (7), and ferric enterobactin (FepA) (8). The crystal structure of FhuA demonstrates (9, 10) a porin-like protein, with a 22-stranded C-terminal β barrel domain that encloses a globular N-terminal cork domain. Ferrichrome binds to the receptor via extracellular loops and apical regions of the cork domain. The cork domain occludes the lumen of the barrel, preventing passive diffusion of ferrichrome. Characteristic of TonB-dependent OM receptors, FhuA possesses an N-terminal consensus sequence termed the Ton box (TTITVTA) that was not visible in the reported three-dimensional structures of FhuA.

We crystallized a purified complex of TonB (residues 33 to 239) and FhuA (residues 1 to 725) and collected data on a single TonB-FhuA crystal (11). The TonB-FhuA crystal structure consists of one protomer of TonB in complex with one protomer of FhuA (Fig. 1). The model was refined at a resolution of 3.3 Å to $R_{	ext{cryst}} = 28.4\%$ and $R_{	ext{free}} = 32.9\%$ (table S1). The overall folds of the FhuA and TonB protomers are in agreement with previously reported structural data. The N-terminal globular cork domain (residues 19 to 160) of FhuA is enclosed by the C-terminal β-barrel domain (residues 161 to 725). The cork domain possesses a four-stranded β sheet between residues 47 and 154. The barrel domain is composed of 22 antiparallel β strands. The β strands terminate in 11 long loops on one face of the barrel and 10 short turns on the other face (Fig. 1A). Given the membrane topology of FhuA, the loops and turns are exposed to the extracellular environment and periplasm, respectively. A molecule of ferriocin was observed bound to FhuA in a position not much different from that observed for the ligand-loaded FhuA structure 1QFF.

TonB residues 158 to 235 were observed in the TonB-FhuA structure (see supporting online text). The fold of the TonB protomer consists of a three-stranded β sheet ($\beta_1$-$\beta_2$ between residues 174 and 197, $\beta_3$ between residues 223 and 231), a short helix $\alpha_1$ (residues 165 to 170), and a longer helix $\alpha_2$ (residues 203 to 210) (Fig. 1B). TonB interacts with the periplasm-exposed face of FhuA such that TonB helices $\alpha_1$ and $\alpha_2$ are oriented toward FhuA, with the TonB β sheet (composed of $\beta_1$ to $\beta_3$) distal to the FhuA barrel (Fig. 1A). The TonB protomer occupies approximately one-half of the periplasm-exposed surface area of FhuA and occludes the barrel lumen from the edge containing periplasmic turns 8 to 10 to a boundary delineated by a straight line between periplasmic turns 1 and 7 (Fig. 1B). Inspection of the TonB-FhuA structure relative to FhuA 1QFF revealed minor structural rearrangements [root mean square deviation (RMSD) = 0.23 Å] upon TonB binding. Structural changes proximal to the bound TonB are localized to the periplasmic turns of the FhuA barrel as well as the periplasm-exposed loop at the C terminus of the cork domain (fig. S1). Larger structural changes were observed in TonB upon complexing.
The overall Ca-Ca RMSD between FhuA-complexed TonB and TonB 1U07 is 0.93 Å. The largest main-chain deviations are localized mainly to the C terminus from residues Ile232 to Thr235. The Ca-Ca deviation relative to 1U07 at TonB Ile232 is 2.0 Å, whereas at Thr235 the Ca-Ca deviation is 3.5 Å.

We observed extensive and continuous electron density N-terminal to FhuA Glu19 and extending toward TonB. This unambiguous electron density allowed us to build FhuA residues 9 to 18 into the model, encompassing five of the six residues in the conserved FhuA Ton box consensus (Fig. 1C). The FhuA Ton box forms a parallel β interaction with β3 of the TonB C-terminal domain, extending the β sheet. FhuA residues Ile9, Thr10, Val11, and Ala13 interact with TonB residues Val225, Val226, Leu229, and Lys231, respectively (table S2). These interactions result in the formation of an interprotein β sheet with TonB β1 to β3 are labeled. (C) Superposition of the TonB-FhuA crystal structure (TonB, yellow coil; FhuA, blue coil) and monomeric C-terminal TonB (PDB code 1XX3; pink coil). The Ton box and N terminus of FhuA, and the C terminus of TonB, from the crystal structure are labeled. FhuA periplasmic turns 7, 8, 9, and 10 (T8, T9, T10) are also marked for reference. Yellow arrow indicates direction toward the C terminus of FhuA-complexed TonB. Pink arrow indicates direction toward the C terminus of monomeric TonB.

The FhuA cork and barrel domains interact with TonB Arg166. Cut-away view showing FhuA and TonB protomers in cartoon representation; β strands are shown as flat arrows, helices as flat coils. The FhuA cork domain (residues 19 to 160) is colored green; the remaining FhuA residues are colored blue. TonB is colored yellow. TonB Arg166 and interacting FhuA residues (Ala26, Glu56, Ala593, and Asn154) are shown as sticks colored by atoms (as in Fig. 1C). Strands of the central β sheet of the FhuA cork domain (β1 to β4) are labeled.

Fig. 3. Residues from the FhuA cork and barrel domains interacting with TonB Arg166. Cut-away view showing FhuA and TonB protomers in cartoon representation; β strands are shown as flat arrows, helices as flat coils. The FhuA cork domain (residues 19 to 160) is colored green; the remaining FhuA residues are colored blue. TonB is colored yellow. TonB Arg166 and interacting FhuA residues (Ala26, Glu56, Ala593, and Asn154) are shown as sticks colored by atoms (as in Fig. 1C). Strands of the central β sheet of the FhuA cork domain (β1 to β4) are labeled.

Fig. 2. Interactions of the FhuA Ton box with the C-terminal domain of TonB. (A) The interprotein β sheet formed between the FhuA Ton box and the central β sheet of the TonB C-terminal domain. TonB and FhuA residues are shown as sticks. Coloring as in Fig. 1C. TonB strands β1 to β3 are labeled. FhuA periplasmic turns 8 and 9 (T8, T9) are also labeled for reference. (B) View along the plane of the interprotein β sheet showing potential hydrogen bonding between two rotamers of TonB Gln160 and the main-chain carbonyl oxygen atom of FhuA Thr12. FhuA residues are shown as sticks and are colored as in (A). TonB is shown as a yellow coil. Rotamers (“a” and “b”) of TonB Gln160 are shown as sticks colored by atom (carbon, green; nitrogen, blue; oxygen, red). TonB strands β1 to β3 are labeled. (C) Superposition of the TonB-FhuA crystal structure (TonB, yellow coil; FhuA, blue coil) and monomeric C-terminal TonB (PDB code 1XX3; pink coil). The Ton box and N terminus of FhuA, and the C terminus of TonB, from the crystal structure are labeled. FhuA periplasmic turns 7, 8, 9, and 10 (T8, T9, T10) are also marked for reference. Yellow arrow indicates direction toward the C terminus of FhuA-complexed TonB. Pink arrow indicates direction toward the C terminus of monomeric TonB.
the C-terminal TonB domain. We do not observe TonB β4 in our structure. Instead, the C terminus of TonB extends away from the complex, such that the FhuA Ton box forms a parallel β interaction with TonB β3 (Fig. 2C). This observation clearly demonstrates that the complexation of TonB to FhuA occurs via strand exchange.

Our in vitro surface plasmon resonance studies (12, 13) indicated that TonB-FhuA complexation involves a kinetically limiting conformational rearrangement. The present structure showing exchange of TonB β4 with the FhuA Ton box corroborates these biophysical observations and suggests that formation of the interprotein β sheet is the initial committed step of TonB-FhuA complexation. The complex that we observe in this structure is likely the 1:1 high-affinity complex identified by biophysical methods. Interactions between a FhuA Ton box peptide and residues from TonB β3 and β4 were recently reported (5). Ton box residues may therefore form low-affinity encounter interactions with TonB β3 and β4 before formation of the stable interprotein β sheet. TonB Glu160 likely participates in these encounter interactions, perhaps facilitating displacement of TonB β4. A cysteine cross-linking study (14) mapped a complex network of interactions between TonB residues 159 and 164 such that each TonB residue interacted with multiple residues in the BtuB Ton box. This is consistent with TonB residues at or near Glu160 being involved in multiple transient encounter interactions before high-affinity complex formation.

The TonB-FhuA interface has a mean interfacial accessible surface area (AASIA) (15) of 1299 Å². The calculated shape correlation statistic (S) for this interface is 0.60, indicating that it has a surface complementarity similar to that observed for cork-barrel interfaces of TonB-dependent OM receptors (16). The TonB-FhuA interface is composed of a network of 17-residue pairs bound by hydrophilic interactions (table S2). All pairs were observed to have well-defined electron density for both FhuA and TonB side chains. Within this interface, a single electrostatic interaction occurs between FhuA cork residue Glu56 and TonB Arg166, located on TonB α1 (Fig. 3). A hydrogen bond is also formed between TonB Arg166 and the main-chain carbonyl oxygen atom of FhuA Ala26, located in the switch helix region. Ferrichrome binding to FhuA was previously observed to result in a 17.3 Å translocation of the FhuA N terminus and unwinding of the switch helix (9). Therefore, it appears that unwinding of the FhuA switch helix upon ligand binding occurs in order to stabilize FhuA interactions with TonB Arg166. FhuA barrel residues Ala591 and Asn594, located in or near periplasmic turn 8, also form hydrogen bonds with TonB Arg166.

What does the crystal structure of the TonB-FhuA complex tell us about interactions of TonB with a cognate OM receptor and the transport of metal-chelated siderophore? Given our structural data, combined with findings from previous studies

References and Notes


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Materials and Methods

SOM Text

Figs. S1 and S2

Tables S1 and S2

References

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