

BEAMLINES

X6A, X29

PUBLICATION

P.D. Pawelek, N. Croteau, C. Ng-Thow-Hing, C.M. Khursigara, N. Moiseeva, M. Allaire, and J.W. Coulton, "Structure of TonB in Complex with FhuA, E. coli Outer Membrane Receptor," *Science*, **312**, 1399-1402 (2006).

FUNDING

Canadian Institutes of Health Research

FOR MORE INFORMATION

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STRUCTURE OF TonB IN COMPLEX WITH FhuA, E. COLI OUTER MEMBRANE RECEPTOR

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Iron, an essential element for most living organisms, is highly insoluble at physiological pH, making it difficult for bacteria to acquire. In order to obtain sufficient amounts of iron, bacteria have developed high affinity uptake systems. For the first time, interactions between two proteins of the ferric hydroxymate uptake (Fhu) system are seen at atomic resolution. Crystals of a complex of outer membrane receptor FhuA and its energizing partner, TonB, give us insights into the mechanism by which E.coli acquires iron. Learning about this mechanism could help in the development of targeted antibiotics.

Bacteria, like most living organisms, need iron to survive. Because of the low bioavailability of iron, bacteria synthesize high-affinity, iron-chelating low molecular weight compounds called siderophores that form metal:siderophore conjugates.

The size of a metal:siderophore conjugate like ferrichrome prohibits entry into the bacteria via simple diffusion through porin-like channels. Alternatively, bacteria have developed uptake systems for these conjugates. We study the Ferric Hydroxymate Uptake system (Fhu) that works as follows (**Figure 1**): The outer membrane receptor FhuA, a 22-strand β -barrel protein with a cork domain, binds iron-bound ferrichrome. TonB that has been energized by the ExbB/D cytoplasmic membrane complex comes into contact with FhuA and allows ferrichrome to pass into the periplasmic space. Once in that space, ferrichrome binds to the protein FhuD that brings it to the FhuB/C



Authors (from left) Natalia Moiseeva, Marc Allaire, Nathalie Croteau, and Peter Pawelek

complex, an ABC transporter that finally transports it into the cytoplasm.

We first showed that upon binding of the ferrichrome-iron conjugate, a conformational change is transmitted to the periplasmic side of FhuA, resulting in the unwinding of a switch helix, thus signaling the ligand-loaded status of the receptor (**Figure 2A,B**). The next step involves the binding of TonB to the FhuA receptor; however the molecular mechanism whereby this occurs was unknown. By obtaining diffraction-quality crystals of a 1:1 FhuA:TonB complex and the use of synchrotron radiation, we have been able to shed light on how ferrichrome enters the periplasmic space.

The resulting crystal structure of this complex reveals that residues from the FhuA Ton box form a parallel β -interaction with the β 3-strand of the central β -sheet of the C-terminal domain of TonB (**Figure 2C**). This interaction positions the highly conserved TonB residue, Arg166, to contact FhuA residue Glu56, which is also located in a conserved motif. From this we are able to predict the functional importance of this FhuA-TonB ionic interaction.

What does the crystal structure of the FhuA-TonB complex tell us about interactions of TonB with a cognate OM receptor and the transport of metal-chelated siderophore? From our structural data combined with findings from previous studies, we propose that the interprotein β -sheet formed between the receptor Ton box, and the C-terminal domain of TonB is required to position the TonB helix close to the receptor cork domain. In the FhuA-TonB structure, this results in TonB Arg166

forming an electrostatic interaction with FhuA cork residue Glu56. It was recently proposed that hydration of the cork domain may render it prone to disruption by TonB through transmission of a relatively small force perpendicularly applied to it.

Given its position proximal to the FhuA cork domain, TonB Arg166 is positioned to mediate such a mechanical shearing or pulling force to the cork domain. This may result in cork disruption, allowing siderophore translocation into the periplasm.

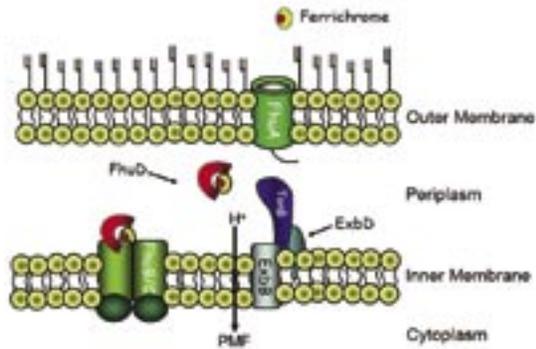


Figure 1. The Ferric Hydroxamate Uptake system (Fhu)

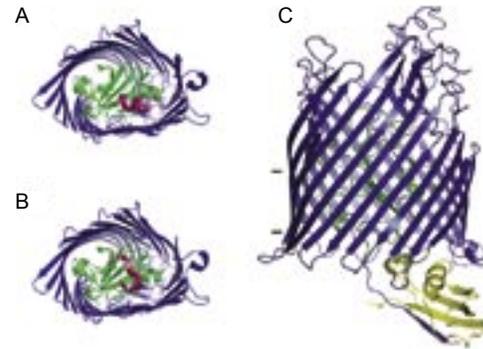


Figure 2. Changes occurring at the periplasmic side of FhuA. (A) Prior to iron-ferrichrome conjugate binding on the FhuA receptor. (B) Unwinding of the switch helix upon iron-ferrichrome conjugate binding. (C) Binding of the C-Terminal domain of TonB to the FhuA conserved Ton box.