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Spectroscopic analyses of Gram-negative bacterial iron transport reactions

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Gram-negative bacteria, including both commensal and pathogenic species, acquire iron with TonB-dependent uptake systems. Using fluorescence spectroscopic analyses of the *Escherichia coli* outer membrane (OM) protein FepA and its cell envelope partner TonB, it was described dynamic actions of both proteins during the uptake of ferric enterobactin (FeEnt). When FeEnt interacts with fluoresceinated FepA, the quenching of light emissions reflects its binding and transport process as a series of conformational motions in the receptor protein. This simple experimental system involves genetically engineering Cys sulfhydryls in any of 7 surface loops of FepA and fluorophore maleimides reacting with them. Spectroscopic or microscopic fluorescence intensity changes, ultimately mirrored cellular uptake that depleted FeEnt from solution. The TonB-ExbBD inner membrane complex transfers energy to OM iron transporters like FepA. (GFP)-TonB hybrid proteins were used to investigate its activity. Fluorescence microscopic characterization of the (GFP)-TonB hybrids revealed an unexpected, restricted localization of TonB in the central region of the cell envelope. Fluorescence polarization measurements demonstrated energy-dependent motion of TonB in living cells, which likely was rotation. The findings show that TonB undergoes energized motion in the bacterial cell envelope and that ExbBD couples this activity to the electrochemical gradient. The results portray TonB as an energized entity in a regular array underlying the OM bilayer, which promotes metal uptake through OM transporters by a rotational mechanism.

Biography

Phillip E Klebba received his doctorate in Biochemistry at the University of California, Berkeley, working with the discoverer of siderophores, Dr. Joe . Neilands. He performed post-doctoral study with Drs. Leon Rosenberg at Stanford University and Hiroshi Nikaido at UC Berkeley, and was a visiting professor with Drs Maurice Hofnung, Institut Pasteur, Alain Charbit, Institut Necker, and Ron Kaback, UCLA. He is Head of Biochemistry and Molecular Biophysics at Kansas State University. His research interests focus on biophysical approaches to problems in membrane transport, especially iron acquisition by bacteria.

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