Transmembrane gate movements in the type II ABC importer BtuCD-F during nucleotide cycle

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ATP-binding cassette (ABC) transporters are ubiquitous integral membrane proteins that translocate substrates across cell membranes. The alternating access of their transmembrane domains towards the inner and outer leaflet of the membrane powered by the closure and reopening of the nucleotide binding domains is proposed to drive the translocation events. Despite clear structural similarities, evidences for considerable mechanistic diversity start to accumulate within the importers subfamily. We present here a detailed study of the gating mechanism of a type II ABC importer - the BtuCD-F vitamin B₁₂ importer from E. coli. With site-directed spin labeling (SDSL), spin labels were introduced at key positions in the translocation channel. Continuous wave and pulsed electron paramagnetic resonance (EPR) spectroscopy was used to reveal conformational dynamics during transport cycle in detergent solubilized and liposome reconstituted BtuCD-F complex. The translocation gates of the BtuCD-F complex undergo conformational changes in line with a ‘two-state’ alternating-access model. However, binding of ATP drives the gates to an inward-facing conformation, in contrast to the better characterized type I importers specific for maltose, molybdate or methionine. In the presence of ATP, an excess of vitamin B₁₂ promotes the dissociation of BtuF and restore the gates conformation similar to that in BtuCD. The EPR data allow a productive translocation cycle of the vitamin B₁₂ transporter to be modeled.