Structural characterization of a magnesium transporter of the SLC11/NRAMP family

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ABSTRACT

Divalent metal transporters (DMTs) of the SLC11/NRAMP family are proton coupled divalent transition metal symerptors and are evolutionary highly conserved. Our previous structure of the transporter of Staphylococcus capitis (ScaDMT) revealed conserved residues that coordinate the transition metal ion. Extensive sequence alignments show that evolutionary distant DMT homologues comprise divergent residues at this site. Those transporters have been proposed to function as NRAMP related Mg2+ transporters (NRAMTs) in bacteria. We selected EleNRMT as a promising candidate for biochemical characterization and proved that EleNRMT is a non proton coupled Mg2+ transporter. However, structural informations to explain the basis of this substrate selectivity were lacking.

Despite its small size (47 kDa), EleNRMT never crystallized alone. By combining thermostabilisation of EleNRMT by consensus mutagenesis and generation of two nanobodies binding EleNRMT simultaneously, we obtained cryoEM structures in presence and absence of Mg2+. At respective resolutions of 3.5Å and 4.1Å. The presence of the two nanobodies allowed the growth of crystals diffraction at 4.1Å. We used the high resolution cryoEM structure as a search model for molecular replacement. The crystals were soaked in a crystallization solution supplemented with Mg2+ and its anomalous diffraction properties were used allowing us to identify the substrate binding site. Overall, the structures of EleNRMT revealed a generally similar protein architecture compared to classical NRAMPs, with a restructured ion binding site whose increased volume provides suitable interactions with ions that likely have retained much of their hydration shell. The lack of proton coupling is explained by the absence in EleNRMT of signature proton acceptor residues in standard NRAMPs.

I- Generation of a thermostabilized mutant of EleNRMT

A Thermostabilisation of EleNRMT by consensus mutagenesis
B Biochemical stability of the EleNRMT® mutant

II- Structure determination of EleNRMT® in complex with two nanobodies by CryoEM

A Sample preparation of EleNRMT® in complex with Nb1 & Nb2
B CryoEM map of EleNRMT® without Mg2+

III- Combination of cryoEM and Xray crystallography

A Crystal phasing and soaks in Mg2+
B Identification of the ion binding site

IV- Visuialisation of residual density for Mg2+ using cryoEM

A CryoEM map of EleNRMT® with Mg2+

V- Structures explain absence of proton coupling in EleNRMT

A Region in vicinity of the ion binding site of ScaDMT on α9 and δ9 implicated in proton transport in NRAMPs and (B) corresponding region in EleNRMT. Residues involved in proton coupling in ScaDMT include a conserved histidine on α-helix 6b located close to the binding site and an intracellular H+ release aqueous pathway made of acidic and basic residues on α-helices 3 and 9. No proton acceptor is present at equivalent positions in EleNRMT.

2. Shi et al. (2014) Bio-Generic