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Structural and Functional Comparison of Untagged and Tagged EmrE Protein

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Abstract : EmrE, a member of the small multidrug resistance protein family in bacteria is considered to be the archetypical member of its family. It confers host resistance to a wide variety of quaternary cation compounds (QCCs) driven by proton motive force. Generally, purification yield is a challenge in all membrane proteins because of the difficulties in their expression, isolation and solubilization. EmrE is extremely hydrophobic which make the purification yield challenging. We have purified EmrE protein using two different approaches: organic solvent membrane extraction and hexahistidine (his6) tagged Ni-affinity chromatographic methods. We have characterized changes present between ligand affinity of untagged and his6-tagged EmrE proteins in similar membrane mimetic environments using biophysical experimental techniques. Purified proteins were solubilized in a buffer containing n-dodecyl-β-D-maltopyranoside (DDM) and the conformations in the proteins were explored in the presence of four QCCs, methyl viologen (MV), ethidium bromide (EB), cetylpyridinium chloride (CTP) and tetraphenyl phosphonium (TPP). SDS-Tricine PAGE and dynamic light scattering (DLS) analysis revealed that the addition of QCCs did not induce higher multimeric forms of either proteins at all QCC:EmrE molar ratios examined under the solubilization conditions applied. QCC binding curves obtained from the Trp fluorescence quenching spectra, gave the values of dissociation constant (Kd) and maximum specific one-site binding (Bmax). Lower Bmax values to QCCs for his6-tagged EmrE shows that the binding sites remained unoccupied. This lower saturation suggests that the his6-tagged versions provide a conformation that prevents saturated binding. Our data demonstrate that tagging an integral membrane protein can significantly influence the protein.

Keywords : small multidrug resistance (SMR) protein, EmrE, integral membrane protein folding, quaternary ammonium compounds (QAC), quaternary cation compounds (QCC), nickel affinity chromatography, hexahistidine (His6) tag
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