Abstract

FrpC protein produced by *Neisseria meningitidis* in a human host belongs to the family of bacterial RTX toxins due to the presence of RTX domain. FrpC possesses a calcium-dependent auto-catalytic cleavage activity which is localized within its 177 amino-acids long segment Self-Processing Module (SPM). As the SPM is naturally intrinsically disordered protein without bound Ca^{2+}, the calcium binding is crucial for SPM folding which is followed by the auto-catalytic processing. The elucidation of the SPM structure may be the key step for understanding of enzymatic and biological function. The structure of folded SPM itself can be characterized only with difficulties due to the presence of flexible loop according to preliminary NMR data. The subject of this work is the description of SPM using fluorescence methods, characterization of ions binding to SPM and structural changes occurring during Ca^{2+} binding.

In this work, the ion binding properties of SPM segment and its ion-induced folding was characterized. It was found that the dissociation constant k_D of 17 μM coincided with the folding of SPM into the native calcium-bound state which occurs in the concentration range between 1 and 20 μM Ca^{2+}. In the attempt to characterize the structure of ion binding site, the fully active single tryptophan mutants W451F and W519F together with Tb^{3+}, a luminescent analog of Ca^{2+}, were used. Due to the FRET between tryptophan and Tb^{3+} as donor and acceptor, respectively, the determination of the 6 Å distance between bound Tb^{3+} and 451W tryptophan residue of SPM was possible. The other part of this work is also a methodical insight to the properties of Tb^{3+} ion usage, whose absorption properties vary while bound in a hydrophobic binding site or located in the solvent. Understanding the influence of electrostatic interactions between two tryptophan in the tertiary structure of proteins by geometric analysis of the possible Trp-Trp configurations from the protein structures from the PDB database enables to discover the most probable Trp-Trp configurations within the SPM protein.

Keywords: auto-catalytic cleavage, RTX protein, FrpC protein, calcium-binding sites, calcium, terbium, *Neisseria meningitidis*