Crystallizability of an engineered monomeric mutant of FK506-binding protein from *Shewanella* sp. SIB1: preliminary diffraction data analysis

Abstract

Background and Objective: A 22 kDa FK506-binding protein from a psychrophilic bacterium *Shewanella* sp. SIB1 (SIB1 FKBP22) is a member of peptidyl prolyl cis-trans isomerase (PPIase). This protein is homodimer with a V-shaped form, consisting of N and C-domains, that are connected through a long α-helix, responsible for dimerization and PPIase activity, respectively. Understanding on structural mechanisms behind the function of SIB1 FKBP22 is limited by unsuccessful attempts on crystallization of the full length SIB1 FKBP22 homodimer due to its flexibility and low stability. Despite the isolated N-domain, with the absence of α-helix and C-domain was successfully crystallized and structurally solved, the comprehensive structural arrangement of SIB1 FKBP22 remains missing. The objective of this study is to construct a crystallizable SIB1 FKBP22 derivatives consisting of N and C-domains with its α-helix that reflect full length of SIB1 FKBP22 and a platform for comprehensive structural analysis. **Materials and Methods:** A monomeric mutant of SIB1 FKBP22 was constructed by combining two gene fragments encoding Met 8-Ile 205 and Met 1-Ala 60 of the first and second monomer of SIB1 FKBP22, respectively, with three glycine residues. This design yielded the mutant has tandemly repeated N-domain connected to C-domain through a long α-helix hence designated as NNC-FKBP22. **Results:** The NNC-FKBP22 was monomeric in solution implying that it did not form a V-shaped dimeric structure. The crystallization of NNC-FKBP22 was attempted under sitting-drop vapor diffusion. The crystals were obtained under 1.0 M sodium citrate with 10 mM CHES/sodium hydroxide at pH 9.5. The crystal was in the space group of P212121 with unit cell dimension a = 94.635, b = 92.479 and c = 337.327 Å. A complete native data was collected from a rotating anode source to a resolution of 3.5 Å at 100 K with an R_{merge}value of 25.50. **Conclusion:** The NNC-FKBP22 was successfully crystallized implying that stabilization of SIB1 FKBP22 under protein engineering platform is promising approach to decipher its atomic structure. Protein engineering technique used in this study would also be applicable for other cold-adapted proteins with high structural flexibility and low stability.