Inhibition and Substrate Specificity Properties of FKBP22 from a Psychrotrophic Bacterium, Shewanella sp. SIB1

ABSTRACT

SIB1 FKBP22 is a peptidyl prolyl cis–trans isomerase (PPIase) member from a psychrotrophic bacterium, Shewanella sp. SIB1, consisting of N- and C-domains responsible for dimerization and catalytic PPIase activity, respectively. This protein was assumed to be involved in cold adaptation of SIB1 cells through its dual activity of PPIase activity and chaperone like function. Nevertheless, the catalytic inhibition by FK506 and its substrate specificity remain unknown. Besides, ability of SIB1 FKBP22 to inhibit phosphatase activity of calcinuerin is also interesting to be studied since it may reflect wider cellular functions of SIB1 FKBP22. In this study, we found that wild type (WT) SIB1 FKBP22 bound to FK506 with IC50 of 77.55 nM. This value is comparable to that of monomeric mutants (NNC-FKBP22, C-domain+ and V37R/L41R mutants), yet significantly higher than that of active site mutant (R142A). In addition, WT SIB1 FKBP22 and monomeric variants were found to prefer hydrophobic residues preceding proline. Meanwhile, R142A mutant has wider preferences on bulkier hydrophobic residues due to increasing hydrophobicity and binding pocket space. Surprisingly, in the absence of FK506, SIB1 FKBP22 and its variants inhibited, with the exception of N-domain, calcineurin phosphatase activity, albeit low. The inhibition of SIB1 FKBP22 by FK506 is dramatically increased in the presence of FK506. Altogether, we proposed that local structure at substrate binding pocket of C-domain plays crucial role for the binding of FK506 and peptide substrate preferences. In addition, C-domain is essential for inhibition, while dimerization state is important for optimum inhibition through efficient binding to calcineurin.