Reassessment of the Catalytic activity and substrate specificity of FKBP35 from Plasmodium Knowlesi using Protease-Free Assay

ABSTRACT

FK506-binding protein35 of Plasmodium knowlesi (Pk-FKBP35) is a member of peptidyl prolyl cis-trans isomerase (PPIase) and is considered as a promising avenue of antimalarial drug target development. This protein is organized into the N-terminal domain responsible for PPIase catalytic activity followed and the tetratricopeptide repeat domain for its dimerization. The protease-coupling and protease-free assays are known to be the common methods for investigating the catalytic properties of PPIase. Earlier, the protease-coupling assay was used to confirm the catalytic activity of Pk-FKBP35 in accelerating cis-trans isomerization of the peptide substrate. This report is aimed to re-assess the catalytic and substrate specificity of Pk-FKBP35 using an alternative method of a protease-free assay. The result indicated that while Pk-FKBP35 theoretically contained many possible cleavage sites of chymotrypsin, experimentally, the catalytic domain was relatively stable from chymotrypsin. Furthermore, under protease-free assay, Pk-FKBP35 also demonstrated remarkable PPIase catalytic activity with kcat/KM of 4.5 + 0.13 × 105 M−1 s−1, while the kcat/KM of active site mutant of D55A is 0.81 + 0.05 × 105 M−1 s−1. These values were considered comparable to kcat/KM obtained from the protease-coupling assay. Interestingly, the substrate specificities of Pk-FKBP35 obtained from both methods are also similar, with the preference of Pk-FKBP35 towards Xaa at P1 position was Leu>Phe>Lys>Trp>Val>Ile>His>Asp>Ala>Gln>Glu. Altogether, we proposed that protease-free and protease-coupling assays arereliable for Pk-FKBP35.