Catalytic Properties of Caseinolytic Protease Subunit of *Plasmodium knowlesi* and Its Inhibition by a Member of δ-Lactone, Hyptolide

ABSTRAK

The caseinolytic protease (Clp) system plays an essential role in the protein homeostasis of the malaria parasite, particularly at the stage of apicoplast development. The inhibition of this protein is known to have a lethal effect on the parasite and is therefore considered an interesting avenue for antimalaria drugs discovery. The catalytic activity of the Clp system is modulated by its proteolytic subunit (ClpP), which belongs to the serine protease family member and is therefore extensively studied for further inhibitors development. Among many inhibitors, the group of β-lactone is known to be a specific inhibitor for ClpP. Nevertheless, other groups of lactones have never been studied. This study aims to characterize the catalytic properties of ClpP of Plasmodium knowlesi (Pk-ClpP) and the inhibition properties of a δ-lactone hyptolide against this protein. Accordingly, a codon-optimized synthetic gene encoding Pk-ClpP was expressed in Escherichia coli BL21(DE3) and purified under a single step of Ni^{2+}-affinity chromatography, yielding a 2.20 mg from 1 L culture. Meanwhile, size-exclusion chromatography indicated that Pk-ClpP migrated primarily as homoheptameric with a size of 205 kDa. The specific activity of pure Pk-ClpP was 0.73 U μg^{-1}, with a catalytic efficiency k_{cat}/K_M of 0.05 μM^{-1} s^{-1}, with optimum temperature and pH of 50 °C and 7.0–7.5, respectively. Interestingly, hyptolide, a member of δ-lactone, was shown to inhibit Pk-ClpP with an IC_{50} value of 17.36 ± 1.44 nM. Structural homology modelling, secondary structure prediction, and far-UV CD spectra revealed that helical structures dominate this protein. In addition, the structural homology modeling showed that this protein forms a barrel-shaped homoheptamer. Docking simulation revealed that the inhibition was found to be a competitive inhibition in which hyptolide was able to dock into the catalytic site and block the substrate. The competitiveness of hyptolide is due to the higher binding affinity of this molecule than the substrate.