Possible Involvement of the Interconversion between Dimer and Monomer in the Regulation of The Reactivity of *Tc*cys4, a Cystatin from Cacao

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Phytocystatins are cysteine-proteinase inhibitors from plants implicated in the endogenous regulation of protein turnover, programmed cell death, and defense mechanisms against pathogens. We identified four cystatins ORFs named *Tc*CYS1, *Tc*CYS2, *Tc*CYS3 and *Tc*CYS4 using the data from two cDNA libraries corresponding to resistant and susceptible interactions between *T. cacao* and *Moniliophthora perniciosa*. These ORFs were sub cloned, and His-Tag fused proteins expressed in *E.coli* using pET28a vector. Recombinant proteins were obtained by affinity chromatography in a single step of purification. Recombinant proteins showed papain inhibition by colorimetric method with BApNA substrate with Ki 203.2, 220.7, 152.4, 158.9 for *Tc*CYS1, *Tc*CYS2, *Tc*CYS3 and *Tc*CYS4, respectively. We examined the biochemical and structural properties of cacaocytatins, under heat-stress conditions. The *Tc*CYS1 protein show thermo unstable. The *Tc*CYS3 and *Tc*CYS4 were thermo stable, but the enzyme inhibitory reactivity of *Tc*CYS4 was reduced by heating at 65 °C for 10 min. In analysis by non-denaturing PAGE, *Tc*CYS4 demonstrated a band shift at 65 °C, corresponding with the decline of inhibitory reactivity. The protein band showing slower mobility at 65°C most likely corresponds to the dimeric form of *Tc*CYS4. This was confirmed by size exclusion chromatography analysis. The dimeric form of *Tc*CYS4 was stable during storage at 4 °C, suggesting that dimerization is an intrinsic property of *Tc*CYS4. The endogenous *Tc*CYS4 was purified from cacao tissues and its oligomerization properties tested. The considerable difference of the affinity for enzyme between the dimeric and the monomeric forms may indicate some involvement of this conversion in the regulation of *Tc*CYS4 in vivo.
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