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IN RUBBER LATEX. THESIS ADVISORS: DHIRAYOS WITITSUWANNAKUL
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Protease inhibitors (PI) were detected in the latex of rubber trees (*Hevea brasiliensis*). The PI was located mainly in the C-serum by screening assays with pronase while only little activity could be detected in the B-serum. Characterization of the C-serum protease inhibitor indicated that it was a thermostable protein with a very strong heat stable property. The PI in C-serum (CS-PI) was precipitated out from other proteins at a high concentration of acetone (80-95% v/v) and designated as *Hevea* protease inhibitor (HPI). It was shown as a single protein band with a calibrated subunit MW of 5.5 kD upon SDS-PAGE analyses. Heating of this HPI in boiling water for 15 and 30 min resulted in almost no PI activity loss, indicating it is a very thermostable protein with low subunit MW nature. Screening experiments on different protease classes of HPI showed that HPI was the most effective inhibitor for pronase (57.59 % enzyme activity inhibition), followed by chymotrypsin, trypsin (18.05 % and 14.47 % enzyme activity inhibition, respectively). A weaker inhibitor on papain (7.39 % enzyme activity inhibition) was observed. On the contrary, very mild PI activity was found for thermolysin inhibition (4.61% enzyme activity inhibition) and no PI activity against pepsin and protease from *Aspergillus saitoi* was observed in this comparative study. The HPI was further purified by passing through the Sephadex G-75 column. Two protein peaks with PI activity were obtained and designated as HPI-1 and HPI-2, respectively. Calibration for subunit MW determination showed both HPI-1 and HPI-2 possess the same MW of 5.5 kD upon SDS-PAGE. By gel filtration chromatography, the native MW of HPI-1 and HPI-2 were 20.8 kD and 11.7 kD, respectively. The results thus suggested that native HPI-1 existed as tetrahomomeric form and HPI-2 existed as dihomomeric form. The PI activity for both HPI-1 and HPI-2 after heating in boiling water for 30 min was decreased to 90% and 88% of the control inhibition, respectively. Both HPI-1 and HPI-2 have quite a broad range of pH stability. No effect or loss of the PI activity was observed between pH 3-11. However, their activities were rapidly decreased to 38% inhibition (HPI-1) and 50% inhibition (HPI-2) of the control level at pH 12. The pI values were determined to be 4.24 for HPI-1 and 4.17 for HPI-2, respectively.