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Screening and Isolation of Chymotrypsin Inhibitors

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Protease inhibitors play an important role in several pathological conditions associated with excessive proteolysis. Recently, from two species of Korean native leeches(*Hirudo nipponia and Whitmania edentula*), elastase inhibitors(guamerin I and guamerin II, respectively) were identified from total body extracts and their primary structures and kinetic properties were verified. Now we have purified several new chymotrypsin inhibitors from the leech *H. nipponia* by a sequential procedure of gel filtration chromatography, anion exchange chromatography and reverse phase HPLC. Multiple inhibitors were seperated into homogeneity from the final HPLC. These were commonly small-sized(less than 10kDa) inhibitors; molecular weights were confirmed by MALDI-mass spectrometry or 16.5% Tricine/SDS-PAGE. Amino acid sequences have been revealed completely or partially by automated Edman degradations after reduction/alkylation and/or enzymatic digestion. Interestingly, they were cysteine-rich polypeptides with similar structural(e.g. primary sequences and cysteine spacing) and physiochemical(e.g. low isoelectric point, hydrophobicity and thermal stability) characteristics like guamerin I or II. However, kinetic properties were profoundly different from guamerin-type inhibitors. Some of the new inhibitors could act specifically on chymotrypsin with moderately low Ki values but others inhibited elastase and trypsin.

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Screening of New Thrombin Inhibitor on Blood Sucking Animal

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Among blood-sucking animal, leech has not significant digestive enzymes in saliva, unlike most animals, and there are small peptides and proteins which are important for overcoming the host's haemostatic mechanisms. Hirudin is a representative peptide in leech saliva. It acts as a anticoagulant by inhibiting thrombin which is a key enzyme in blood clotting. It was identified that another *Hirudo* species, *Hirudo nipponia*, has some anti-thrombin activity in their saliva. From this species, thrombin inhibitor was purified by a simple method. The molecular weight of purified inhibitor was determined as 12 KDa by gel electophoresis. Mass spectrometry, however, showed the mass of this protein is 6307 Da. This inhibitor strongly inhibited thrombin to prevent blood coagulation, and also the amino acid composition of the inhibitor was similar to other hirudin variants.