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NOVEL TRYPSIN INHIBITOR FROM *Alismatis Rhizoma*

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Introduction

After Kunitz purified trypsin inhibitor from bovine pancreas, several research of trypsin inhibitor was performed. The serine protease inhibitor can be classified into at least 10 family, according to various schemes. It is very important to mammalian. For example, pancreatic secretory trypsin inhibitor in mammals is co-secreted with zymogens into the pancreatic juice and prevents premature trypsin activation of zymogens in the pancreatic duct. And trypsin inhibitors in plants inactivate insect's protease that digest their tissue. A trypsin inhibitor was isolated and purified from *Alismatis Rhizoma* which has been used as a galenic for diuretic and antiphlogistic.

Method

Purification was carried out by 0-80% saturated ammonium sulfate salting out, DEAE ion exchange chromatography, trypsin affinity column, and butyl toyopearl FPLC.

Result

The molecular weight of ARTI was estimated to be about 22 KDa by 12% SDS-PAGE and amino acid composition analysis. The chemically determined N-terminal and partial amino acid sequence(84 residue) of the ARTI did not

coincide with those of previously reported any type of trypsin inhibitor. In addition, it is assumed that this inhibitor did not have cysteine and tryptophane in their amino acid composition. This inhibitor forms tight, but reversible complexes with trypsin. Trypsin was specifically inhibited by this inhibitor, but other serine protease such as chymotrypsin, elastase were not inhibited. When N-benzoyl-L-arginine-4-methyl- coumarine-7-amide was used as a substrate of trypsin, IC_{50} of ARTI was observed at $0.087 \mu M$.