PB-29

# Purification and characterization of a trypsin inhibitor from egg of skipjack tuna, *Katsuwonus pelamis*

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## Introduction

Protein inhibitors are proteins or peptides capable of inhibiting catalytic activities of proteolytic enzymes. They are grouped primarily as either serine, cysteine, aspartic or metallo-proteinase inhibitors. Protease inhibitors have been known since the end of the last century in nematodes and human blood serum, and their ubiquitous distribution in microorganisms, animals and plants has been widely documented. They are classified into many families based on homology in amino acid sequences and similarity in topology of the S-S bridges and reactive sites. The best studied are the member of the Kazal-type inhibitor family. Kazal-type trysin inhibitors(Pancreatic Secretory Trypsin Inhibitors-PSTIs), first described by Kazal, are mainly present in the pancreas and pancreatic juice of all animals and many of them have been purified and well characterized. PSTIs from pancreas of mammals consist of 52-57 amino acids and their amino acid sequences are very homologous. In contrast, trypsin inhibitors from bird's pancreas consist of 69-72 amino acids and data on them are relatively meagre(Zhao M. *et al.*, 1996). In this study, we report the purification and characterization of a trypsin inhibitor from egg of skipjack tuna, *Katsuwonus pelamis.*

## Materials and Methods

*Assay of trypsin inhibitory activity* Trypsin inhibitory activity was determined by measuring the remaining hydrolytic activity of trypsin towards the substrate 0.2 mM N-benzoyl-L-arginine p-nitroanilide (BAPNA) after pre-incubation with inhibitor. The following buffer was used for enzyme assay: 50 mM Tris-HCl, pH 8.0 with 20 mM $CaCl_2$ was terminated by addition of 0.5 ml of 30% acetic acid.

One unit of antiproteolytic activity was defined as the amount of an inhibitor which reduced by half the activity of 2 mg of enzyme.

*Polyacrylamide gel electrophoresis* Polyacrylamide gel electrophoresis was carried out in the presence of 0.1% SDS using a 12.5% slab gel. A SDS-trypsin inhibitor complex, incubated either with or without β-mercaptoethanol for 5 min at 95℃, was used for the samples(Laemmli U. 1970) The standard molecular materials were myosin (200 kDa), β-galactosidase (116.25 kDa), phosphorylase b (97.4 kDa), serum albumin (66.2 kDa), ovalbumin (45 kDa), carbonic anhydrase (31 kDa), trypsin inhibitor (21.5 kDa), lysozyme (14.4 kDa) and aprotinin(6.5 kDa).

## Results

A trypsin inhibitor was isolated from egg of skipjack tuna, *Katsuwonus pelamis.* The trypsin inhibitor was purified by ammonium sulfate precipitation, Sephadex G-100 gel chromatography, DEAE-Sephacel ion exchange chromatography and high performance liquid chromatography(HPLC) on C18 column. The molecular weight of the purified trypsin inhibitor as estimated by gel chromatography on Sephadex G-100 was approximately 78 kDa. The molecular weight of the reduced trypsin inhibitor on SDS-PAGE was 38 kDa. It is supposed to be a dimer composed of two identical subunits. The purified trypsin inhibitor was stable in the pH range of 4.0 to 10.0. The activity was stable up to 37℃, but rapidly become unstable at temperature higher than 55℃. The effect of metal ions including $K^+$, $Na^+$, $Mg^{2+}$, $Ca^{2+}$ on inhibitory activity was examined. The trypsin inhibitory activity was increased by these metal ions, and calcium ion was the most potent activator. The purified trypsin inhibitor contains 4 cysteines, and there are many highly charged amino acids such as Glx, Asp and Lys in the protein.

## Reference

Laemmli U. 1970. Cleavage of structural protein during assembly of the head obacteriophage T4. *Nature(London).* 227, 680-685.

Zhao M, Naude RJ, Muramoto K, Oelofsen W. 1996. Purification and characterization of ostrich pancreatic secretory trypsin inhibitor. *Int J Peptide Protein Res.* 48, 174-181.