Purification and Characterization of L-Kininogen from Pig Plasma

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Introduction Proteinaceous kininogens showed an inhibitory activity against many cysteine proteinases (Ohkubo et al., 1984; Sueyoshi et al., 1985). There are three types of kininogens, designated as high molecular weight kininogen (H-kininogen) with MW of 120 000, low molecular weight kininogen (L-kininogen) with MW of 68 000 and T-kininogen (also called thiostatin) with MW of 68 000, in mammalian blood plasma (Muller-Estert, 1982). Among these kininogens, T-kininogen is only detected in rat plasma. Kininogen consists of a heavy chain and a light chain. Both L- and H-kininogens are strong inhibitors of cathepsins B and L (Higashiama et al., 1986). To explain the inhibitory domains of heavy chain on kininogen molecule, L-kininogen was subjected to limited proteolysis with trypsin and divided into three functional domains (Salvesen et al., 1986; Vogel et al., 1988). Among these fragments, only domain 1 has no inhibitory activity on cysteine proteinases; domain 2 appears high inhibitory activity against m-calpain, papain and cathepsin L, whereas domain 3 can not inhibit m-calpain, but inhibits papain and cathepsin L strongly (Turk and Bode, 1991).

Fish muscle endogenous cysteine proteinases, such as lysosomal cathepsins and cytoplasmic calpains, were still active during postmortem storage or thermal process in surimi-based products. Cathepsins B, L (Jiang et al., 1996) as well as μ- and m-calpains (Jiang et al., 1991) showed myosin-proteolytic activity and resulted in postmortem autolysis of fish muscle. Moreover, cathepsins B and L could induce the thermal gel degradation of minced fish (Jiang et al., 1997). Although the structural and functional properties of kininogens in human blood have long been studied, the application of pig plasma kininogens to the surimi processing has not been studied. According to the previous studies, pig plasma kininogen could inhibit cathepsins and calpains which consequently decreases the postmortem autolysis and increases the gel forming ability of fish surimi. This study aims to purify and characterize the kininogens from pig plasma and subsequently provide an information for developing modori-inhibitors or freshness-keeping reagents.

Methodology: Fresh pig blood in 100 ppm EDTA solution was centrifuged to remove blood cells at 1000 x g for 10 min. The kininogen in plasma was then purified by DEAE-Sepharose FF, CM-Sepharose FF, and Sephacryl S-200 HR gel filtration. Cathepsin B inhibitory activity of kininogen was measured using Z-Phe-Arg-MCA as substrate (Jiang et al., 1996). One inhibitory unit of kininogen was defined as the amount of kininogen which can depress cathepsin B activity by 1 unit. The purity of the kininogen was assayed by SDS-PAGE, while protein concentration was measured using the dye-binding method (Bradford, 1976). The purified kininogen was characterized by measuring optimal pH and temperature for activity, pH and thermal stability, molecular weight, interaction of kininogen with proteinases, Ki value against papain (μM), cathepsins B (μM) and L (μM).

Results and Discussion: Pig blood plasma was assayed for the inhibitory activity against mackerel cathepsins B and L. The inhibitory activity was observed on washed fractions from DEAE-Sepharose FF chromatography. It was further fractionated by CM-Sepharose and Sephacryl S-200 chromatographies and purified to electrophoretic homogeneity (Fig. 1).

Properties: This inhibitor was considered to be a proteinaceous inhibitor. The MW was 55,000 estimated by SDS-PAGE and 60,000 by Sephadex G-75. According to Muller-Estert et al. (1982), the purified inhibitor was identified as L-kininogen. The purified inhibitor showed three inhibitory peaks on Superose 12 chromatography in the absence of 0.1 M NaCl. This phenomenon further supports it to be L-kininogen since L-kininogen tends to form larger aggregates from dimer (185,000) to decamer (780,000) in the absence of dissociating agents (Muller-Estert et al., 1982). The inhibitory activity of kininogen was stable at neutral and alkaline pH, but labile below pH 4.0, while there was still 90% activity left even after 30 min incubation at 80°C and about 80% activity left after 3 weeks storage at 4° or –20°C. The purified L-kininogen had inhibitory activity against mackerel cathepsins B, L, L-like and X, beef μ- and m-calpains, and papain (Table 1). However, it had no inhibitory effect on cathepsin D, trypsin and chymotrypsin. According to the inhibitory specificity, the purified inhibitor was a specific cysteine proteinase inhibitor. The concentration for inactivating 50% (ID_{50}) of mackerel cathepsin B, cathepsin L, cathepsin L-like, calpain I and calpain II activity were 0.50, 0.12, 0.12, 1.70 and 1.61 μg, respectively (Table 1). According to the calculation from Dixon plot, the inhibition constant (K_i) of L-kininogen against cathepsin L-like, m-calpain and μ-calpain were 0.19 nM, 111.02 nM and 35.36 nM, respectively. Among these proteinases, kininogen had a higher affinity to cathepsin L-like compared with calpains. The inhibition of the purified kininogen against these cysteine proteinases might be a competitive type.

Inhibition of thermal gel degradation

After heating at 55°C for 90 min, degradation of myosin heavy chain (MHC) occurred in the presence of 0.12 units cathepsin L-like (Fig 2, Lane D). However, the degradation of MHC was inhibited by the addition of 0.06 (Fig 2, Lane E), 0.12 (Fig 2, Lane F) and 0.24 units (Fig 2, Lane G) of the purified kininogen. In mackerel surimi, cathepsins B and L induced modori phenomenon during heating process (Jiang et al., 1997). Mackerel muscle cathepsins B, L and L-like could proteolyze MHC of surimi and resulted in modori. However, their activities were substantially inhibited by the purified kininogen. Consequently, pig plasma kininogen could be a good additive for preventing modori during manufacturing the surimi-based products. Bovine plasma protein has been used to control the autolysis of Atlantic menhaden surimi due to the existence of proteinase inhibitory activity (Hamann et al., 1990). In summary, this study established the purification procedures of L-kininogen from pig plasma which is using DEAE-Sepharose, CM-Sepharose and Sephacryl S-200, and further investigated its role in preventing the thermal degradation of surimi gels.

References:

Jiang, S. T., Wang, J. H., and Chen, C. S. 1991. ***J. Agric. Food Chem.*** **39**, 237-241.

Jiang, S. T., Lee, B. L., Tsao, C. Y., and Lee, J. J. 1997. ***J. Food Sci.*** 62: 310-315.

Ohkubo, I., Kurachi, K., Takasawa, T., Shiokawa, H., and Sasaki, M. 1984. ***Biochemistry.*** **23**, 5691-5697.

Sueyoshi, T., Enjyoji, K., Shimada, T., Kato, H., Iwanaga, S., Bando, Y., Kominami, E., and Katunuma, N. 1985. ***FEBS Lett.*** **182**, 193-195.

Muller-Estert, W., Vohle-Timmermann, M., Boos, B., and Dittman, M. 1982. ***Biochim. Biophys. Acta.*** **706**, 145-152.

Higashiama, S., Ohkubo, I., Ishiguro, H., Kunimatsu, M., Sawaki, K., and Sasaki, M. 1986. ***Biochemistry.*** **25**, 1669-1675.

Turk, V. and Bode, W. 1991. ***FEBS Lett.*** **285**, 213-219.

Table 1. Inhibitory activity of the purified L-kininogen

Proteinase	Substrate	ID_{50}(μg)
Cysteine proteinase		
Papain (37.5 *p*mole)	Z-Phe-Arg-MCA	0.58
Cathepsin B (58.8 *p*mole)	Z-Phe-Arg-MCA	0.71
Cathepsin L (0.064 units)	Z-Phe-Arg-MCA	0.12
Cathepsin L-like (24 *p*mole)	Z-Phe-Arg-MCA	0.12
Metallo-proteinase		
μ-Calpain (46.6 *p*mole)	Casein	1.70
m-Calpain (29.8 *p*mole)	Casein	1.61
Serine proteinase		
Trypsin (1.3μg)	α-Benzoyl-L-Arginyl ethyl ester	>3.00
β-chymotrypsin (1.26μg)	α-Benzoyl-L-tyrosinyl ethyl ester	>1.50
Aspartic proteinase		
Cathepsin D (0.2μg)	Hemoglobin	>1.50

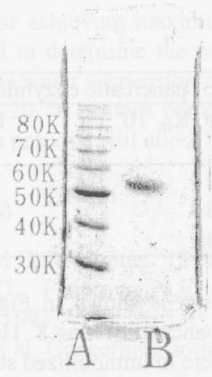

Figure 1. SDS-PAGE of kininogen from pig plasma (A: protein markers; B: purified kininogen)

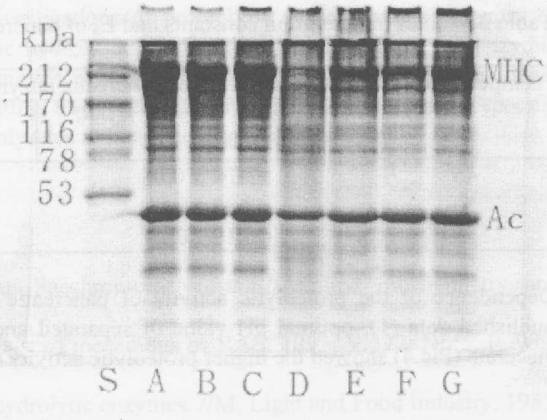

Figure 2. Electrophoretic profiles of actomyosin degradation by cathepsin L-like and inhibition by pig plasma L-kininogen. [samples on lanes C~G were incubated at 55°C for 90 min. Lane S: protein markers; Lane A & B: actomyosin without heating; Lane C: without proteinase and inhibitor; Lane D: without inhibitor; Lane E: with 0.06 units of L-kininogen; Lane F: with 0.12 units of L-kininogen; Lane G: with 0.24 uints of L-kininogen.]