Cysteine Protease-Cystatin System As a Modulator for Physical Properties of Proteins: A Study from the Aspect of Enzyme Chemistry and Food Processing

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Summary

Among a variety of food legumes, wheat is one of these which are most abundantly produced and most popularly consumed in the world. A great deal of research endeavors have been made so far to improve the quality of gluten, a mixture of gliadin and glutenin, which forms during the processing of wheat-based foods. Despite that, no extensive studies have been conducted, at least from a molecular aspect, on wheat proteases and their inhibitors that may serve as key factors involved in the processing. With this as a background, we noticed wheat cysteine proteases and their inhibitors, cystatins, and started a food-technological as well as enzyme-chemical study on the enzyme-inhibitor system as a factor modulating physical properties of food proteins.

We first isolated four cysteine proteases from wheat seeds and found that one of these, named gliadain, can almost specifically hydrolyze gliadin. To dissect biochemical properties of gliadain, we obtained its recombinant product by overexpression in *Escherichie coli*. For this in detail, the cording region of the cDNA encoding progliadain was inserted into an expression plasmid to produce it as a glutathione S-transferase (GST) fusion protein. The plasmid was then introduced into *E.coli* and forced to express the fusion protein by induction with isopropyl-1-thio-β-D-galactopyranoside. This resulted in producing it as an insoluble fraction which, however, was solubilized by treatment with a surfactant. We then purified the product by glutathion-affinity chromatography to obtain a single protein whose enzymatic activity was clearly observed. This is apparently the world first that succeeded in obtaining a recombinant cysteine protease of plant origin in the active form.

An activity assay based on the hydrolysis of a fluorescent substrate, Z-Phe-Arg-MCA, demonstrated that the gliadain preparation thus obtained is most active at pH 4.5 and that it most efficiently hydrolyzes gliadin, a prolamin-classed component of major storage proteins in wheat seeds. The assay suggests that this enzyme may be largely involved in the proteolysis during seed germination.

Salt effect on the enzymatic activity of gliadain were evaluated. It resulted that the enzyme preparation was active enough in the presence of NaCl, retaining 56% and 30% of its original activity at 1M and 3M NaCl, respectively. The result thus indicates that gliadain should be a salt-tolerant cysteine protease. Since the salt concentration in commercial bread items is usually around 0.2M, gliadain is almost fully active and may thus be successfully used for food processings in general as well as for baking in particular.

We then investigated the inhibitory effect of wheat cystatin on the gliadain preparation obtained as a GST fusion protein and found that the inhibition actually takes place efficiently. This investigation strongly suggests that the use of cystatin can optimize the cysteine protease-catalyzed reaction to proceed during baking and also that gliadain possibly modulate the proteolysis of seed storage proteins *in vivo*.