Verification of Modulator Effects of Cystatins in Food Processing Using Proteases at High Salt Concentrations

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Summary

It has long been practiced to hydrolyze food proteins with endogenous and/or exogenous proteases for the purpose of improving physical and sensory protein qualities. This enzymatic process has also been carried out often in the presence of added salt. When animal and fish materials undergoes the process, involved enzymes are primarily cysteine proteinases (CP). Thus, how to modulate CP reactions determines the quality of the resulting products. Meanwhile, our group has found proteinaceous CP inhibitors, *i. e.*, cystatins, in rice, wheat, corn and soybean as common foods and investigated them as factors for use in modulating the CP reactions. In this context, the present study was undertaken to evaluate the modulator effect of cystatins, with special reference to rice oryzacystatin (OC) as the first well-defined phytocystatins.

A raw squid was cut into slices, each being 5 cm in length and 1 cm in width. Slices were dipped in 1.5M NaCl and incubated at 4 °C in the presence of the internal organs having an endogenous CP activity. About 5 hr after the start of incubation, the squid muscle got tendered, with formation of a weakly viscous texture. Over-tenderization occurred 72 hr after the start, and the muscle structure was degraded, with an excessive viscosity increase. To stop the enzymatic reaction at the incubation time of 5 hr, we tried to add OC which had been prepared from rice. In detail, rice bran was treated with water and the resulting extract was heated at 80 °C for 30 min. The supernatant obtained by filtration was freeze-dried into a power to be used as an OC preparation. It was found that the addition of this preparation to the squid during incubation could control the muscle degradation; it was possible to modulate the quality of the incubation product by adding it at the time of 5 hr.

In the meantime, our group was engaged in analysis of a three-dimensional solution structure of purified OC in terms of the heteronuclear signal quantum-coherence and the nuclear Overhauser effect. As a result, OC was found to be constituted with an α-helix region and five anti-parallel β-sheet regions. It was also found that the CP-inhibitory activity of OC involved its main structure, 13E-D97, comprising the first (53Q-G57) and second (83P-W84) binding loops.

In the present study, we observed that the addition of final concentrations (0.5-1.5M) of NaCl to an OC solution did not affect the circular dichroism of OC. Though speculative at present, no critical conformational change may take place with OC, even when this is treated under a condition that simulates the practical "shiokara" production in which NaCl is used normally at 3M. We will evaluate the effectiveness of added OC as a CP modulator in the actual processes for salting fish.