Involvement of Endogenous Proteases in Abalone Muscle Softening

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The extracellular matrix (ECM) is greatly degraded by endogenous proteases in aquatic animals after death, thus leading to muscle softening. We revealed collagenases to participate in postmortem tissue self-degradation of *Haliotis discus hannai* by degrading type I collagen. Hence, recombinant matrix metalloproteinase 1 and 14 (rMMP1 and rMMP14) with high purity and enzymatic activities were expressed using a prokaryotic expression system. rMMP1 and rMMP14 effectively degraded type I collagen into small fragments and peptides. Tissue inhibitor of metalloproteinase (TIMP), an endogenous inhibitor of MMP, was also expressed using HEK 293F cells. Recombinant TIMP (rTIMP) showed great inhibitory activity toward rMMP1, but not rMMP14. Hence, it can significantly inhibit rMMP1's degradation activity toward collagen. Inhibition kinetics analyses revealed rTIMP to be a competitive inhibitor of rMMP1. Biolayer interferometry revealed rTIMP can effectively bind with rMMP1, with an equilibrium dissociation constant value of 263 nM. Furthermore, we cloned the full-length cDNA sequence of prolyl endopeptidase (PEP) from abalone. Recombinant PEP (rPEP) was expressed and characterized in detail. We for the first time determined the 1.5 Å crystal structure of rPEP. Using collagen peptides as substrates, HPLC-ESI-MS analysis confirmed that rPEP specifically cleaved at the carboxyl side of proline residues, suggesting its role in the degradation of collagen peptides. These results elucidate the possible mechanism of abalone muscle softening in the aspect of endogenous proteases.

Keywords: Abalone, Muscle softening, Matrix metalloproteinase 1, Prolyl endopeptidase, Collagen degradation