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Proteolytic degradation of myofibrillar components by endogenous proteases in red sea bream muscle

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Abstract

Degradation of myofibrillar proteins is one of the causes of post-mortem muscle softening of fish. In order to clarify the endogenous proteases responsible for fish muscle softening, we injected specific protease inhibitors into the duct of Cuvier of live red sea bream to suppress the endogenous protease activities under similar physiological conditions. After sacrificing the fish, we confirmed the effects of protease inhibitors on degradation of myofibrillar components by western blot analysis during storage at 25°C. Degradation of myosin heavy chain (MHC) and β-connectin were significantly suppressed by leupeptin, diisopropyl fluorophosphate (DFP) (serine protease inhibitors), and *o*-phenanthroline (a metalloproteinase inhibitor). Hydrolysis of α-actinin was inhibited by E-64 (a cysteine protease inhibitor). Degradation of troponin I was suppressed by leupeptin, DFP, *o*-phenanthroline, and E-64. The limited degradation of tropomyosin was inhibited by DFP and *o*-phenanthroline. Our results suggested that endogenous serine proteases and metalloproteinases were involved in degradation of most of the myofibrillar components (MHC, β-connectin, troponin I, and tropomyosin) while α-actinin was hydrolyzed only by cysteine proteases in red sea bream muscle.

Keywords : post-mortem muscle softening, protease inhibitor, red sea bream, endogenous protease

I Introduction

Red sea bream (*Pagrus major*) is a popular aquacultured fish in Japan and its fillets are commonly served as fresh slices of raw fish "sashimi". For evaluation of freshness of raw fish fillets, its firmness is one of the important factors[1, 2]. However, post-mortem muscle softening occurs much faster in fish than livestock leading to reduction in commercial value[3, 4]. Therefore, clarification of the mechanism of post-mortem muscle tenderization in fish is a critical issue in the fisheries industry.

In general, it is considered that a major cause of the post-mortem muscle softening in fish is proteolysis of muscle structural proteins such as myofibrillar proteins and extracellular matrix (ECM) components. Some researchers have investigated the relationship between disintegration of myofibrillar proteins and post-mortem muscle tenderization[5, 6]. By immunohistological studies, it was revealed that the deterioration of Z-disk was related to post-mortem tenderization of red sea bream muscle during storage in ice[7]. Papa *et al.*[8] reported that α-actinin was released from the Z-disk and subsequently degraded during the early phase of post-mortem muscle softening. Also, the deterioration of collagen (a major component of ECM) during post-mortem fish muscle softening was studied[9-11]. Yamashita and Konagaya[9] reported that the non-helical region of type I collagen was hydrolyzed by cathepsin L of chum salmon muscle *in vitro*. In trout[10] and sardine[11] muscles, type V collagen was solubilized during chilled storage.

Endogenous proteases have been extensively studied as the

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