J. Grad. Sch. Biosp. Sci.,
Hiroshima Univ. (2003), **42** : 75～76

Studies on phospholipases A_2 in the gills of the red sea bream

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Phospholipase A_2 (phosphatide 2-acyl hydrolase, EC 3.1.1.4) (PLA_2) hydrolyzes the fatty-acyl ester bond at the sn-2 position of glycerophospholipids. PLA_2 has now become a large superfamily of distinct enzymes that play a central role in diverse cellular processes including phospholipid digestion and metabolism, host defense and signal transduction. Secretory PLA_2s have been comprehensively investigated in land animals and are now found to consist of twelve molecular species. On the other hand, there is little of information about the enzymology of fish PLA_2. Although it is known that PLA_2 exists in the digestive tissues of the fish, only limited information exists on the physiological role of PLA_2 in non-digestive tissues of fish. In the present study, I purified PLA_2 from the non-digestive tissue, the gills, of the red sea bream. I further investigated the localization of protein and the gene expression of gill PLA_2 and an activating enzyme of pro-PLA2 in the red sea bream.

Purification and cDNA cloning of phospholipases A_2 from the gills of the red sea bream

PLA_2 activity was investigated in various tissues of the red sea bream. The specific activity of PLA_2 in the gills was significantly higher than that in other tissues, such as the adipose tissue, intestine and hepatopancreas. Therefore, I tried to purify PLA_2 from the gill filaments of red sea bream to near homogeneity by sequential chromatography. G-1, G-2 and G-3 PLA_2s were purified and all showed a single band. The exact molecular mass values of G-1, G-2 and G-3 PLA_2s were 14,040, 14,040 and 14,005 Da, respectively. G-1, G-2 and G-3 PLA_2s had a Cys 11 and were all identical in N-terminal amino acid sequences from Ala 1 to Asn 56. A full-length cDNA encoding G-3 PLA_2 was cloned by RT-PCR and RACE methods, and G-3 PLA_2 was found to be classified to group IB PLA_2 from the deduced amino acid sequence. G-1, G-2 and G-3 PLA_2s had a pH optimum in an alkaline region at around pH 9-10 and required Ca^{2+} essentially for enzyme activity, using a mixed-micellar phosphatidylcholine substrate with sodium cholate. These results demonstrate that three group I PLA_2s, G-1, G-2 and G-3 PLA_2s, are expressed in the gill filaments of red sea bream.

Localization of phospholipase A_2 in the gills of the red sea bream

It was reconfirmed that the level of PLA_2 activity is extremely high in the gills compared with other tissues, and gill PLA_2 was detected only in the gills by immunoblotting and inhibition test using anti-gill PLA_2 monoclonal antibody. The level of PLA_2 activity and protein expression in the gills are well correlated; Fish can be roughly divided into high and low groups based on the level of PLA_2 activity. Gill PLA_2 was detected in the gills of the high group, but not the low group by immunoblotting. In the gills of the high group, gill PLA_2 was detected in the mucous cells and pavement cells located on the surface of gill epithelia by immunohistochemistry. On the other hand, positive signals were observed only in the mucous cells by *in situ* hybridization. I also isolated inactive pro-PLA_2, having AR propeptide, preceding the mature enzyme from the gill

extract. These results suggest that gill PLA_2 is synthesized as an inactive pro-PLA_2 in the mucous cells and is secreted to the surface of gill epithelia.

Purification of an activating enzyme of gill PLA_2

I investigated the pro-PLA_2 activating enzyme (PAP) in the gills of the red sea bream by measuring the increase of PLA_2 activity, using the gill pro-PLA_2 as a substrate. As PAP activity was found to distribute in the membrane fraction of the gills, I tried to purify PAP from the fraction of the gills. Two membrane-bound trypsin-like serine proteases (approximately 200 kDa) were partially purified from the gills of the red sea bream, and these two enzymes were found to activat the gill pro-PLA_2.

Key words: phospholipase A_2, pro-PLA_2 activating enzyme, gills, red sea bream