論文内容の要旨

Characterization of murine cytosolic phospholipase $A_2\delta$

マウス細胞質型ホスホリパーゼ $A_2\delta$の性質と機能解析

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Cytosolic phospholipases A_2 ($cPLA_2$s) are enzymes that hydrolyze ester bonds at the *sn*-2 position of glycerophospholipids (simply termed phospholipids, hereafter) in a Ca^{2+} dependent manner and liberate 1-acyl-lysophospholipids and unsaturated fatty acids. Human $cPLA_2\delta$ was first identified as a gene up-regulated in psoriatic skin. Murine $cPLA_2\delta$ ($mcPLA_2\delta$) was discovered in our research group by searching the mouse genome database by using relatively conserved regions within murine $cPLA_2\alpha$, β and γ as queries. $cPLA_2\delta$ has a primary structure similar to $cPLA_2\alpha$: a C2 domain on its N-terminus and a catalytic domain on its C-terminus. To explore the physiological and pathological roles of $cPLA_2\delta$, it is important to characterize biochemical properties including enzyme kinetics, substrate specificities, and effects of known PLA_2 inhibitors using purified enzyme preparation.

Real time quantitative PCR analysis revealed that mcPLA$_2\delta$ is exclusively expressed in placenta, testis and tongue epithelium layer. The anti-mcPLA$_2\delta$ rabbit polyclonal antibody was generated and the endogenous mcPLA$_2\delta$ protein expression was examined. The endogenous mcPLA$_2\delta$ protein was detected in placenta and tongue epithelium layer. The expression of mcPLA$_2\delta$ was quite limited, which contrasted with cPLA$_2\alpha$ that is expressed ubiquitously. Together with a reported increase of human cPLA$_2\delta$ expression in psoriatic skin, this tissue-specific expression pattern of mcPLA$_2\delta$ suggests the inducible nature of cPLA$_2\delta$ and its specific roles in respective tissues.

The recombinant mcPLA$_2\delta$ was prepared from Sf9 cells utilizing (His)$_6$-tag affinity chromatography and enzymatic properties of mcPLA$_2\delta$ were characcterized. Since mcPLA$_2\delta$ was originally identified as a cPLA$_2$ paralogue, first, PLA$_2$ activity of mcPLA$_2\delta$ were examined. As expected, mcPLA$_2\delta$ exhibited PLA$_2$ activity that requires mM of Ca^{2+} ion. As for substrate preferences, mcPLA$_2\delta$ displayed PLA$_2$ activity with a broad substrate specificity. To be more precise, mcPLA$_2\delta$ did not show preferences for phosphatidylcholine over phosphatidylethanolamine and linoleoyl- over arachidonoyl-phospholipids in PLA$_2$ activity.

Further analyses revealed the unexpected strong PLA$_1$ activity of mcPLA$_2\delta$, which is about 100-fold higher than the PLA$_2$ activity. By the action of PLA$_1$ activity, phospholipids are hydrolyzed its ester bonds at the *sn*-1 position and saturated fatty acids and 2-acyl-lysophospholipids are released. mcPLA$_2\delta$ showed a slight preference for phosphatidylethanolamine over phosphatidylcholine in PLA$_1$ activity. The liquid chromatography-mass spectrometry (LC=MS) based PLA$_1$ assay revealed that

mcPLA$_2\delta$ hydrolyzes all the phospholipids examined, such as phosphatidylserine, phosphatidylinositol, phosphatidylglycerol and phosphatidic acid, and produces 2-acyl-lysophospholipids. Several enzymes that exhibit PLA$_1$ activity have been clones. However, cPLA$_2\delta$ does not show any sequence similarity with those PLA$_1$ enzymes.

Recently, it is reported that 2-acyl-lysophospholipids are more potent than 1-acyl-lysophospholipids in the activation of some lysophospholipid G-protein-coupled receptors, including LPA$_6$ and GPR55. Saturated fatty acids are reported to be involved in developing metabolic disorders and inflammation. Therefore, in contrast to previously-identified PLA$_2$s, cPLA$_2\delta$ produces 2-acyl-lysophospholipids and saturated fatty acids, and may play novel roles *in vivo*.