外国語要旨

The biological functions of cyclic phosphatidic acid and its derivatives

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Cyclic phosphatidic acid (cPA) is a phospholipid mediator composed of a cyclic phosphate ring at *sn*-2 and *sn*-3 positions of the glycerol backbone, which was originally isolated from the myxoamoebae of a true slime mold, *Physarum polycephalum*. From previous studies, it has been found that cPA has potent biological functions, such as inhibition of cancer cell invasion and metastasis *via* suppression of autotaxin (ATX) activity, inhibition of nociceptive response by primary afferent C-fiber, and attenuation of ischemia-induced delayed neuronal cell death. Therefore, we have been investigating for the development of an effective therapeutic agent for some diseases, including cancer and neurodegeneration.

It has been reported that the unique structure of cPA is entirely required for biological activities. In order to protect the cyclic phosphate ester moiety from hydrolysis, we synthesized a derivative of cPA, racemic-2-*O*-carba-cPA (2ccPA), in which one on the phosphate oxygen atoms at *sn*-2 position is replaced with a methylene group. This derivative has shown more potent inhibitory activities than that of natural cPA. As it is well known that each enantiomer shows same/different biological activities in many cases, the determination of biological activities of enantiopure 2ccPA is strongly required for further 2ccPA studies.

We synthesized sulfur analogs of cPA, 3-*O*-thia-cPAs (3ScPAs), replacing a phosphate oxygen with a sulfur atom. As it has been reported that the variation in the acyl chain of 2ccPA shows different effects on some biological functions, we examine the effects of 3ScPAs with different fatty acid moieties (16:0, 16:1, 18:0 or 18:1). And as same as 2ccPA, the determination of biological activities of enantiopure 3ScPA is strongly required for further studies.

Recently, it has been reported that cPA is generated intracellularly by phospholipase D2 (PLD2) in mammalian cells. However, its biological function is not yet clarified.

From these previous results, in this study, we aimed to examine the effects of derivatives of cPA and its enantiomers on biological functions and to study the role of

PLD2 on cPA formation and cell motility.

In chapter 1, we compared the effects of (*R*)-2ccPA and (*S*)-2ccPA on ATX activity, cancer cell invasion, and nociceptive reflex. It was shown that enantiopure 2ccPA had inhibitory effects as well as racemic-2ccPA. Then their effects were not significantly different from each other.

In chapter 2, we studied racemic-3ScPAs action toward ATX, cancer cell invasion, nociceptive reflex, and ischemia-induced delayed neuronal cell death. Different species of the acyl chain of racemic-3ScPAs did not significantly affect biological functions. Then, we examined (*R*)-3ScPA and (*S*)-3ScPA effects on ATX activity and found that the chirality of 3ScPA was not involved in ATX inhibition.

In chapter 3, our experimental results were also consistent with quantum mechanics and molecular mechanics calculations. The predicted binding energy for 2ccPA was smaller than that of cPA or 3ScPA. From highest occupied molecular orbital - lowest unoccupied molecular orbital gap value, the order of binding strength, was determined as follows; 2ccPA>3ScPA>cPA. The predicted binding energies for enantiopure cPA and its derivatives did not shown significant difference.

In chapter 4, the extract from PLD2-transfected cells had higher potential for cPA formation in comparison with that of empty vector-transfected cells. It was shown that the migration of cancer cells was significantly suppressed by transfection with PLD2. Then PLD2 inhibitor recovered the suppression of migration of PLD2-trasfected cells.

From these results, it was shown that 2ccPA and 3ScPA have higher biological activities than that of natural cPA. As the chirality of 2ccPA and 3ScPA may not be critical for these biological functions, racemic-2ccPA and -3ScPA are considered to be utilized as effective compounds for therapeutic application. Moreover, it is suggested that cPA intracellularly generated by PLD2 inhibits the motility of cancer cells.