Novel Proliferative Effect of Phospholipase A<sub>2</sub> in Swiss 3T3 Cells via Specific Binding Site*

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Phospholipase A<sub>2</sub> (PLA<sub>2</sub>) EC 3.1.1.4, which catalyzes the release of free fatty acids from the sn-2 position of glycerophospholipids, has been extensively studied from the viewpoint of eicosanoid production (Arita, H., Nakano, T., and Hanasaki, K. (1989) Prog. Lipid Res. 28, 273-301). Several lines of evidence suggest that PLA<sub>2</sub> is pathophysiological related to some disorders, including inflammation, and hyper-sensitivity. Despite this, little is known of the precise mechanism of the pathological processes as well as their intrinsic correlation with dysfunction. Here, we report a novel PLA<sub>2</sub> action on the proliferation of Swiss 3T3 fibroblasts via specific binding sites of approximately 200,000. Pancreatic type PLA<sub>2</sub> in the active form specifically recognized the sites and stimulated thymidine incorporation in DNA. Its inactive zymogen and other PLA<sub>2</sub>s from platelets, snake, and bee venoms showed much lesser activities. Although the physiological significance remains to be identified, our finding is the first to offer a new viewpoint on the effect of mammalian extracellular PLA<sub>2</sub> on cellular function.

In several inflammatory regions, levels of extracellular PLA<sub>2</sub> activity are described to be elevated, which has been thought to play an important role in mediating some inflammatory processes (1, 2). Mammalian extracellular 14-kDa PLA<sub>2</sub>s described thus far can be classified into two types, group I (PLA<sub>2</sub>-I) and group II (PLA<sub>2</sub>-II), based on their primary structures (3). Several studies have implicated the correlation of PLA<sub>2</sub>-II in the pathogenesis of inflammation (4, 5). We have recently found that some inflammatory factors dramatically increased PLA<sub>2</sub>-I secretion from several tissues and stimulated thymidine incorporation in DNA. Its inactive zymogen and other PLA<sub>2</sub>s from platelets, snake, and bee venoms showed much lesser activities. Although the physiological significance remains to be identified, our finding is the first to offer a new viewpoint on the effect of mammalian extracellular PLA<sub>2</sub> on cellular function.

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The abbreviations used are: PLA<sub>2</sub>, phospholipase A<sub>2</sub>; PLA<sub>2</sub>-I, group I phospholipase A<sub>2</sub>; PLA<sub>2</sub>-II, group II phospholipase A<sub>2</sub>; DSS, dextran sulfate; SDS, sodium dodecyl sulfate.
RESULTS AND DISCUSSION

When $^{125}$I-PLA$_2$-I (porcine) was incubated at 4 °C with Swiss 3T3 fibroblasts, it could bind specifically in a saturable manner. The specific binding of $^{125}$I-PLA$_2$-I reached equilibrium after 2 h and was stable for up to 2 h at 4 °C. Although about a two times higher level of binding was detected at 37 °C than at 4 °C, further binding experiments were performed at 4 °C to avoid internalization of the binding site as well as binding to the newly synthesized site.

Fig. 1A shows typical binding results of $^{125}$I-PLA$_2$-I to Swiss 3T3 cells. A Scatchard plot of the data yielded a straight line, suggesting the existence of a single class of binding site for PLA$_2$-I, with an equilibrium binding constant ($K_d$) value of 1.58 nM and a maximum binding capacity ($B_{max}$) of 8.34 fmol/10$^6$ cells (Fig. 1B). The relative inhibitory effects of various phospholipases on $^{125}$I-PLA$_2$-I equilibrium binding were examined, and the concentrations which inhibit half of the $^{125}$I-PLA$_2$-I specific binding (IC$_{50}$) were summarized in Table I. The IC$_{50}$ value of unlabeled porcine PLA$_2$-I corresponded well to the $K_d$ value. Rat and human PLA$_2$-I showed almost the same IC$_{50}$ values with porcine PLA$_2$-I, whereas the proenzymes of human and rat PLA$_2$-I, PLA$_{8S}$-II purified from rat and rabbit platelets, and toxic PLA$_{8S}$ from snake or bee venoms could not suppress the ligand binding at concentrations greater than 100 nM, demonstrating the specificity of PLA$_2$-I binding to the mature site for PLA$_2$-I derived from mammalian pancreas.

Identification of the binding proteins responsible for $^{125}$I-PLA$_2$-I binding was achieved by cross-linking experiments using a bifunctional cross-linker, DSS. $^{125}$I-PLA$_2$-I was bound to Swiss 3T3 cells at 4 °C, treated with 0.15 mM DSS, and then analyzed by polyacrylamide gel electrophoresis in the presence of SDS. As shown in Fig. 2, a single band at an apparent $M_r$ of 210,000 was detected only in the treatment with DSS, while formation of the cross-linked complex was completely blocked by the presence of excess unlabeled PLA$_2$-I during the $^{125}$I-PLA$_2$-I binding. Under nonreducing conditions, the same $M_r$ position was specifically labeled (data not shown). Assuming that the $^{125}$I-labeled complex contains a single molecule of both receptor and PLA$_2$, subtracting the mass of the PLA$_2$-I (14 kDa) suggests that the binding site for PLA$_2$-I has a mass of approximately 200 kDa.

Lambeau et al. (25) have recently reported the specific binding protein in rat synaptic membranes which was recognized by neurotoxic snake venom PLA$_{8S}$s, while mammalian PLA$_2$ does not recognize this binding site. Thus, the PLA$_2$-I binding site we characterized in this study might differ significantly from the neurotoxic PLA$_2$ binding site.

As one of the physiological functions of the PLA$_2$-I binding site in Swiss 3T3 cells, we found the effect of PLA$_2$-I on $[^3H]$thymidine incorporation into acid-insoluble DNA. When quiescent cells were incubated with PLA$_2$-I alone for 24 h, DNA synthesis of 3T3 cells was stimulated in a dose-dependent manner as shown in Fig. 3. This effect of PLA$_2$-I was synergistically enhanced in the presence of 100 nM insulin, which by itself showed weak mitogenic activity. Similar synergistic effects of PLA$_2$-I were observed in the presence of platelet-derived growth factor (2 ng/ml) or endothelin (10 nM). The mitogenic effects of PLA$_2$-I could not be affected by the treatment with indomethacin, demonstrating a direct effect of PLA$_2$-I without any involvement of the growth-promoting prostanoids in Swiss 3T3 cells (36). Similar proliferative effects were observed in the treatment with rat and

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**Table I**

Inhibition of $^{125}$I-PLA$_2$-I specific binding to Swiss 3T3 cells

| PLA$_2$          | IC$_{50}$ (nM) |
|------------------|---------------|
| PLA$_2$-I (porcine) | 1.0           |
| PLA$_2$-I (rat)   | 1.0           |
| PLA$_2$-I (human) | 1.4           |
| rPLA$_2$-I (human)$^a$ | 1.3           |
| proPLA$_2$-I (rat) | >300          |
| proPLA$_2$-I (human) | >300          |
| rproPLA$_2$-I (human)$^a$ | >300          |
| PLA$_2$-II (rat)  | >100          |
| Bee venom         | >1,000        |
| N. naja venom     | >1,000        |
| C. adamanteus venom | >1,000        |

$^a$ r, recombinant.
human PLA$_2$-I, whereas pro-PLA$_2$-I (human and rat), PLA$_2$-II (rat and rabbit), and toxic PLA$_2$s (snake and bee venoms) had no measurable effects on DNA synthesis. Treatment of Swiss 3T3 cells with PLA$_2$-I (10 nM) for 2 h at 37°C resulted in stimulation of the 3-O-methyl-d-[1-$^3$H]glucose transport which is known to link the cell proliferation to increase in cell number (insulin only, 5.4 ± 0.38 × 10$^4$ cells; PLA$_2$-I treatment, 7.33 ± 0.76 × 10$^4$ cells; n = 3). Thus, these findings suggest the occurrence of mitogenic effects of PLA$_2$-I via specific receptors on Swiss 3T3 cells. In order to rule out the possibility that the effect of PLA$_2$-I occurs via liberation of free fatty acids from membrane phospholipids, we examined liberation of fatty acids by porcine PLA$_2$-I or rat PLA$_2$-II from Swiss 3T3 cells labeled with [3H]oleic acid or [3H]arachidonic acid. Neither liberation of free fatty acids nor release of lactate dehydrogenase was detected up to 1 μM concentration of both enzymes treated for 24 h, although 5 μM A23187 liberated a large amount of labeled fatty acids (data not shown). These data demonstrate that binding as well as proliferative effects of PLA$_2$-I can be clearly distinguished from its phospholipid hydrolyzing activity, and the absence of-binding affinity of PLA$_2$-II cannot be attributed to its direct effect on the membrane phospholipids. Other evidence from the binding profile of PLA$_2$-I, showing it not to be affected in the presence of EDTA (10 mM), further supports this specificity, because phospholipid hydrolysis by PLA$_2$-I completely depends on submillimolar amounts of calcium (28). Recently, we found the same PLA$_2$-I binding sites in some tissues of several animal species, especially in vascular function. These findings suggest some role of PLA$_2$-I in vascular function, which is now under further investigation. In conclusion, our finding provides a new aspect of phospholipase A$_2$ in the modulation of cellular functions.

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