Identification of Platelet-activating Factor in the Lymphocytes and Thrombocytes from Carp Cyprinus carpio

Toshiyasu Yamaguchi,* Shujiro Sakaki,* and Masaaki Takeuchi*
(Received April 13, 1990)

Platelet-activating factor (PAF, 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine) is a biologically active ether-linked phospholipid, which is implicated in anaphylactic and inflammatory reactions.1) This lipid mediator is produced by various types of cells from several mammalian species.2,3) Little is known of the existence of PAF in fish. We demonstrate here the isolation and identification of PAF from carp lymphocytes and thrombocytes.

Carp blood was collected from the caudal artery with a heparinized syringe after anesthetizing with MS-222 (100ppm). The thrombocyte-rich plasma (TRP) and lymphocytes were prepared from 28ml of carp blood by centrifugation (400×g, 30min, at room temperature) with lymphocyte separating solution (Nakarai, Japan): physiological saline (95:5). The TRP and lymphocyte suspension were incubated at 37°C for 15min with 2mM phenylmethylsulfonyl fluoride (PMSF) dissolved in dimethylsulfoxide (DMSO). To remove the PMSF, the samples were centrifuged for 15 min at 1,000×g, and subsequently, the lymphocytes and thrombocytes were resuspended in the saline with 4.8µM calcium ionophore A23187 dissolved in DMSO, and incubated at 25°C for 30 min.4)

Lipids were extracted from each of the lymphocyte and thromocyte suspensions using CHEM ELUTE CE1010 (Analytichem International, USA) with ethyl acetate. Phospholipids containing PAF were separated from the total lipids by Sep-Pak silica Cartridge (Waters, USA) with tert-butylmethylether.5) PAF was separated from other phospholipids by TLC (Merck, W. Germany) with chloroform: methanol: water (65:35:3).6)

1-O-hexadecyl-2-acetyl (perdeuterated)-sn-glycero-3-phosphocholine (C16:0 d3PAF) was synthesized by mixing deuterated acetic anhydride (Aldrich, USA) and 1-O-hexadecyl-2-lyso-sn-glycero-3-phosphocholine (Novabiochem, Switzerland) in the presence of a catalytic amount of perchloric acid using chloroform as the solvent.7) The C16:0 d3PAF (above synthesized) and the each resultant 1-O-alkyl-2-acetyl-sn-glycerol was converted to its tert-butyldimethylsilyl (t-BDMS) derivative.8) The t-BDMS derivatives of the authentic C16:0 PAF and the C16:0 dPAF were analysed in a mass spectrometer (JEOL DX300) coupled with a gas-liquid chromatograph with a Quadrex fused silica capillary column (25 m×0.25 mm i.d.), connected with a solvent cut injector. The column oven temperature was programmed from 200 to 320°C at a rate of 8°C/min.

Electron impact mass spectra of the t-BDMS derivatives of the authentic C16:0 PAF and the C16:0 dPAF showed their individual dominant peaks at m/z 117 and 120 which were produced by the rearrangement of the acetyl and dimethylsilanol moieties, and also the spectra did their [M-57]+ ions at m/z 415 and 418, respectively. The calibration curve for quantification of PAF was performed by measuring the peak areas of the ions at m/z 415 (C16:0 PAF) and 418 (C16:0 dPAF).9)

The each PAF from carp lymphocytes and thrombocytes was converted to its t-BDMS derivative in the same manner. Selected ion monitoring traces for the t-BDMS derivatives of PAF from carp lymphocytes and thrombocytes are shown in Fig. 1. When monitored by [M-57]+ ions at m/z 415 and 418, the retention time (7min 15s) of PAF derivative extracted from the carp completely coincided with that of the authentic standard. The C16:0 PAF contents of lymphocytes and thrombocytes were 3.8ng/108 cells and 4.0ng/106 cells, respectively.

The authors wish to thank Mr. Nishikawa, Taiyo Research Laboratory, for the GC/MS analysis.

References

Fig. 1. SIM trace of t-BDMS derivatives of PAF from ionophore A23187 stimulated carp lymphocytes.

* Department of Fishery Science, Faculty of Agriculture, Tohoku University, Tsutumi-dori Amamiya, Sendai, Miyagi 981, Japan (山口敏朗，東北大学農学部)