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Human Plasma Platelet-activating Factor Acetylhydrolase

OXIDATIVELY FRAGMENTED PHOSPHOLIPIDS AS SUBSTRATES*

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Human plasma platelet-activating factor (PAF) acetylhydrolase hydrolyzes the *sn*-2 acetyl residue of PAF, but not phospholipids with long chain *sn*-2 residues. It is associated with low density lipoprotein (LDL) particles, and is the LDL-associated phospholipase A_2 activity that specifically degrades oxidatively damaged phospholipids (Stremler, K. E., Stafforini, D. M., Prescott, S. M., Zimmerman, G. A., and McIntyre, T. M. (1989) *J. Biol. Chem.* 264, 5331–5334). To identify potential substrates, we synthesized phosphatidylcholines with *sn*-2 residues from two to nine carbon atoms long, and found the *V/k* ratio decreased as the *sn*-2 residue was lengthened: the C_5 homolog was 50%, the C_6 20%, while the C_9 homolog was only 2% as efficient as PAF. However, the presence of an ω-oxo function radically affected hydrolysis: the half-life of the *sn*-2 9-aldehydic homolog was identical to that of PAF.

We oxidized [2-arachidonoyl]phosphatidylcholine and isolated a number of more polar phosphatidylcholines. We treated these with phospholipase C, derivatized the resulting diglycerides for gas chromatographic/mass spectroscopic analysis, and found a number of diglycerides where the *m/z* ratio was consistent with a series of short to medium length *sn*-2 residues. We treated the polar phosphatidylcholines with acetylhydrolase and derivatized the products for analysis by gas chromatography/mass spectroscopy. The liberated residues were more polar than straight chain standards and had *m/z* ratios from 129 to 296, consistent with short to medium chain residues. Therefore, oxidation fragments the *sn*-2 residue of phospholipids, and the acetylhydrolase specifically degrades such oxidatively fragmented phospholipids.

Platelet-activating factor (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine, PAF[1]) is a potent phospholipid autacoid whose actions are mediated by receptors on the surface of target cells that specifically recognize PAF. At concentrations as low as 10^{-10} M it activates platelets, neutrophils, and other leukocytes, and it induces hypotension, increased vascular permeability, and shock in animals (reviewed in Refs. 1 and 2). The potency and nature of its effects suggest that the presence of PAF should be strictly controlled and, in fact, the enzymatic activities responsible for PAF biosynthesis are tightly regulated (1–3). Additionally, accumulation of PAF in some cells is controlled by its rate of degradation (4, 5). Degradation also plays a major role in the potential for PAF to circulate as a hormone or function as a locally acting autacoid due to the presence of plasma PAF acetylhydrolase. This enzyme inactivates PAF by hydrolyzing the *sn*-2 acetyl residue, and is exclusively responsible for the degradation of PAF in whole blood (6).

The majority (60–70%) of PAF acetylhydrolase in human plasma is associated with low density lipoprotein; the rest is associated with a subpopulation of high density lipoprotein that contains apolipoprotein E (HDL-with apoE) (6). The enzyme associated with LDL is immunologically equivalent to that in HDL and is transferred between the two lipoprotein particles in a pH-dependent fashion (6). There is a sharp deviation from Michaelis-Menten kinetics at low substrate concentrations for LDL-associated activity; hydrolysis of 1 nM PAF proceeds at only 1.7% of the predicted rate (7). HDL-associated activity is even less efficient than LDL-associated activity at low substrate concentrations, so that in whole plasma only LDL-associated activity appears to catalyze PAF hydrolysis (7). Lipoprotein-associated acetylhydrolase activity also rapidly degrades endothelial cell-associated PAF (8), indicating that stimulated endothelial cells express this PAF on their cell surface. It also shows that LDL can modulate

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[1] The abbreviations used are: PAF, platelet-activating factor (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine); GPC, *sn*-glycero-3-phosphocholine; HDL, high density lipoprotein; LDL, low density lipoprotein; *t*-BDMS, *tert*-butyldimethylsilyl; CV3988, *rac*-3-(*N*-*n*-octadecylcarbamoyloxy)-2-methoxypropyl-2-thiazolioethyl phosphate; Kadsurenone, 5-(prop-2-ene)-2(3,4-dimethoxyphenyl)-3a,α-methoxy-3-methyl-2,3,3a,6-tetrahydro-6-oxobenzofuran; L652,731, *trans*-2,5-bis(3,4,5-trimethoxyphenyl)tetrahydrofuran; WEB2086, 3-[4-(chlorophenyl)-9-methyl-11-thieno[3,2-*f*][1,2,4]triazolo-[4,3-*a*][1,4]diazepin-2-yl](4-morpholinyl)-1-propanone; L659,989, (±)-*trans*-2-(3-methoxy-5-methylsulfonyl-4-propoxyphenyl)-5-(3,4,5-trimethoxyphenyl)tetrahydrofuran; U66985, (1-*O*-octadecyl-2-acetyl-*sn*-glycero-3-phosphoric acid-6'-trimethylammoniumhexyl ester); WEB2170, 6-(2-chlorophenyl-8,9-dihydro-1-methyl-8-(4-morpholinylcarbonyl)-4H,7H-cyclopenta[4,5]thieno[3,2-*f*][1,2,4]triazo[4,3-*a*][1,4]diazepine; SRI63441, *cis*-(±)-1-[2-[hydroxy([tetrahydro-5-[(octadecylaminocarbonoyl)oxy]methyl]furan-2-yl)methoxyphosphinyloxy]ethyl]quinolinium hydroxide (inner salt); HPLC, high performance liquid chromatography; PC, phosphatidylcholine; TLC, thin layer chromatography; GLC, gas liquid chromatography.