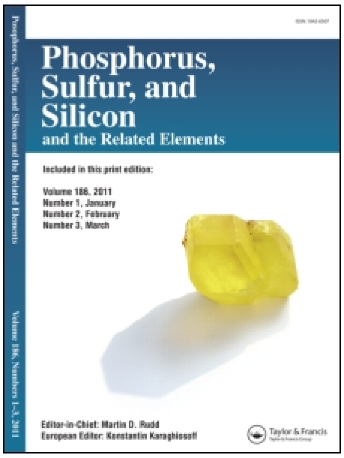

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Phospholipids Chiral at Phosphorus. 13. Stereochemical Comparison of Phospholipase A_2, Lecithin-Cholesterol Acyl Transferase, and Platelet-Activating Factor

Theresa Rosario-jansen[a]; Henry J. Pownall[b]; Joseph P. Noel[a]; Ming-Daw Tsai[a]
[a] Department of Chemistry, The Ohio State University, Columbus, Ohio, U.S.A. [b] The Methodist Hospital, Baylor College of Medicine, Houston, Texas, U.S.A.

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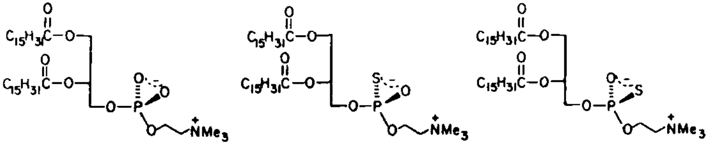
PHOSPHOLIPIDS CHIRAL AT PHOSPHORUS. 13. STEREOCHEMICAL COMPARISON OF PHOSPHOLIPASE A_2, LECITHIN-CHOLESTEROL ACYL TRANSFERASE, AND PLATELET-ACTIVATING FACTOR.

THERESA ROSARIO-JANSEN, HENRY J. POWNALL[a], JOSEPH P. NOEL, AND MING-DAW TSAI
Department of Chemistry, The Ohio State University,
Columbus, Ohio 43210, U.S.A. and [a]The Methodist Hospital,
Baylor College of Medicine, Houston, Texas 77030, U.S.A.

Abstract Thiophospholipids and thiophosphate analogs of platelet activating factor were synthesized and used to compare the stereochemical properties in three different biological systems. Phospholipase A_2 shows high stereospecificity, and this has been substantiated by computer graphics. Lecithin-cholesterol acyl transferase shows no stereospecificity. Platelet aggregation shows, preliminarily, a small degree of stereospecificity.

INTRODUCTION

Recently, chiral thiophospholipids[1] (Fig. 1) have been used to study the stereospecificity of phospholipase D(PL D)[2], phospholipase C(PL C)[3], and phospholipase A_2(PL A_2)[4] (Table I). It is intriguing that PL A_2 shows stereospecificity since the catalytic bond breaking is five bonds remote from the phosphorus. This result led us to investigate other phospholipid systems for their chiral discrimination, and to further examine the mechanism of PL A_2.



(1) DPPC (2) (R_p)-DPPsC (3) (S_p)-DPPsC

FIGURE 1. Structures of 1,2-dipalmitoyl-*sn*-phosphocholine (DPPC) and the R_p and S_p isomers of 1,2-dipalmitoyl-*sn*-glycero-3-thiophophocholine (DPPsC).

INTERACTIONS BETWEEN PHOSPHOLIPIDS AND PL A_2

The kinetic data listed in Table I strongly suggest the coordination of Ca^{2+} with the pro-S oxygen of DPPC.[4] We used computer graphics to model possible enzyme-substrate interactions. The fit (Fig. 2) is based on the assumption that the two water ligands of Ca^{2+} in the crystal structure of bovine pancreatic PL A_2[5] are substituted by the pro-S oxygen and the sn-2 carbonyl oxygen. The fit supports the mechanism whereby the N-1 nitrogen of His-48, acting as a general base, abstracts a proton from a nucleophilic water molecule, which, in turn, attacks the sn-2 carbonyl carbon.[6] The fit suggests two other interactions: the relatively invariant Phe-22 (in some cases Tyr) and Phe-106[7] create a "hydrophobic sandwich" for the sn-2 acyl chain. The second interaction involves the OH-group of Tyr-69, which may H-bond with the phosphate group. (Three variant sequences use Lys which could also act as a H-bond donor.)

Information from the above modeling studies should be treated with caution since both the enzyme and the substrate may assume a different conformation from that of the crystal structure. The point we emphasize is coordination of Ca^{2+} to the pro-R instead of the pro-S oxygen resulted in a very poor fit.

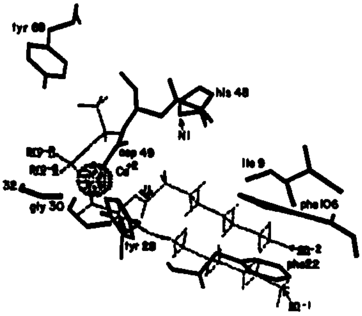


FIGURE 2. Computer modeling of active site residues of PL A_2 (solid lines) and L-dimyristoylphosphatidylethanolamine (dashed lines). The double arrow points to the sn-2 carbonyl carbon.

TABLE I Summary of Kinetic Data[a].

Substrate	PL $A_2(Ca^{2+})$ K_m (mM)	PL $A_2(Ca^{2+})$ V_{max} (μmol/min/mg)	PL $A_2(Cd^{2+})$ K_m (mM)	PL $A_2(Cd^{2+})$ V_{max} (μmol/min/mg)	LCAT K_m (mM)	LCAT V_{max} (μmol/hr/mg)
DPPC	1.67	1850	6.4	17.6	0.032	1.25
DPPsC (R_p)	0.85	76	0.24	0.069	0.064	1.36
DPPsC (S_p)	0.30	0.044		0.0044	0.07	1.19
DPPsC (R_p + S_p)	2.1	64			0.06	1.39

[a] Both PL C and PL D are specific to the S_p isomer of DPPsC[2,3], but quantitative kinetic data have not been obtained.

DPPsC AS A SUBSTRATE OF LCAT

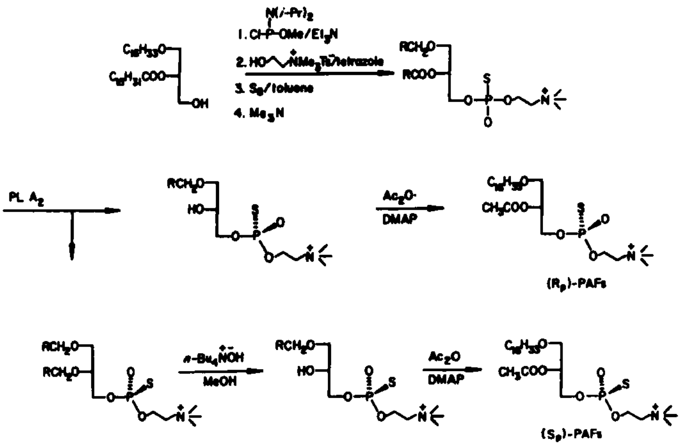
DPPsC was used as a test substrate to probe the stereospecificity of lecithin-cholesterol acyl transferase (LCAT) purified from human plasma. The substrates were model high density lipoproteins (HDL) composed of non-hydrolyzable 1-palmityl-2-oleyl-sn-glycero-3-phosphocholine (POPC ether)[8], the test DPPsC, free cholesterol, and apolipoprotein A-1 in a molar ratio of 90:10:4:1, respectively. We found the substrate saturation curves for the production of cholesterol esters by LCAT are similar to that of DPPC, irrespective of the chirality at phosphorus. This is reflected in both the V_{max} and K_m values given in Table I.

LCAT catalyzes the transfer of the sn-2 acyl chain of lecithins to cholesterol, forming cholesterol esters. Most common phospholipids are LCAT substrates if they are placed in a fluid environment such as HDL[9] or POPC[8] ether. Our present results show that the configuration of the phosphorus in unimportant, consistent with our view that the polar head group is a relatively weak determinant of acyl donor activity in LCAT, in contrast to the stereospecific interactions of the phosphate group of phospholipids with PL A_2.

THIOPHOSPHATE ANALOGS OF PLATELET-ACTIVATING FACTOR

1-O-Alkyl-2-acetyl-sn-glycero-3-phosphocholine (PAF) has a remarkable potency toward platelet aggregation, illiciting full aggregation at 10^{-10} to 10^{-9} molar in rabbit platelets.[10] (R_p)- and (S_p)-1-O-hexadecyl-2-acetyl-sn-glycero-3-thiophosphocholine (PAFs) were synthesized according to Scheme I and used to determine the stereospecificity of the aggregation response, using aggregometry on washed rabbit platelets.[11] PAF (Bachem) untreated with PL A_2, was used as a standard. Our preliminary results indicate that the (S_p)-isomer has about 38% activity

SCHEME 1



compared to the standard, whereas the ($\underline{R}_p$)-isomer has less than 10% activity. More quantitative testing is in progress in order to enhance our understanding of the structure-activity relationship of PAF.

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