changes.[19] 6-Propionyl-2-(dimethylamino)naphthalene is sensitive to the polarity and degree of hydration of its environment.[20] Finally, a compound titled NK-529 has recently been introduced that apparently monitors the lateral phase separation of fatty acids in the bilayer.[9]

The fact that activation of phospholipase A_2 can be monitored during the time course of hydrolysis of DPPC LUV makes this system an excellent choice for studying the mechanisms of activation and possible effects of various activators and inhibitors. The experimental system described here provides a way to determine whether such regulators exert their effects through alterations of the properties of the membrane and/or the enzyme. Importantly, this system allows one to seek temporal correlations of the various events in the process.

Acknowledgments

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[19] J. R. Lakowicz, D. R. Bevan, B. P. Maliwal, H. Cherek, and A. Balter, *Biochemistry* **22,** 5714 (1983).
[20] G. Weber and F. J. Farris, *Biochemistry* **18,** 3075 (1979).

[23] Phospholipase Stereospecificity at Phosphorus

By KAROL S. BRUZIK and MING-DAW TSAI

Introduction[1]

Two types of stereochemical information can be obtained from studies of enzymatic reactions utilizing substrates which are stereogenically[2] (chirally) labeled at phosphorus.[3–10] (1) The steric course of the nucleophilic

[1] This is paper 21 in the series "Phospholipids Chiral at Phosphorus." For Paper 20, see Ref. 16.
[2] K. Mislow and J. Siegel, *J. Am. Chem.Soc.* **106,** 3319 (1984).
[3] F. Eckstein, *Angew. Chem. Int. Ed. Engl.* **22,** 423 (1983); F. Eckstein, P. J. Romaniuk, and B. A. Connolly, this series, Vol. 87, p. 197.
[4] P. A. Frey, J. P. Richard, H.-T. Ho, R. S. Brody, R. D. Sammons, and K.-F. Sheu, this series, Vol. 87, p. 213; P. A. Frey, *Tetrahedron* **33,** 1541 (1984).
[5] J. A. Gerlt, J. A. Coderre, and S. Mehdi, *Adv. Enzymol. Relat. Areas Mol. Biol.* **55,** 291 (1983).

displacement at phosphorus may be elucidated through synthesis and configurational analysis of chirally labeled substrates, coupled with configurational analysis of the product. The results can provide information on the possible involvement of the intermediary covalent phosphoenzyme complex along the reaction pathway, thus enabling insight into details of the elementary steps of enzymatic catalysis.[3,6,7] Two types of chirally modified substrates can be used for such studies: those labeled with oxygen isotopes (^{17}O and/or ^{18}O) or phosphorothioates. (2) Analysis of the stereospecificity of the enzyme toward isomers of different configuration at phosphorus can provide information on the stereochemical constraint of substrate binding and the three-dimensional architecture of the active site.[3,9] This type of study uses phosphorothioates only.

This chapter describes application of such stereochemical approaches to study phospholipases and other phospholipid-metabolizing enzymes using "phospholipids chiral at phosphorus." Figure 1 shows the structures of these compounds (**1–9**) and explains their nomenclature. The enzymes mentioned and their abbreviations are as follows: phospholipase A_2 (PLA_2), phospholipase C (PLC), phospholipase D (PLD), phosphatidylinositide-specific PLC (PI-PLC), lecithin–cholesterol acyltransferase (LCAT), and phosphatidylserine synthase (PS synthase).

Synthetic Procedures

The synthetic steps for chiral thiophospholipids **1–6** are described below. The synthesis of [^{17}O,^{18}O]phospholipids **7–9** is omitted here since the procedures are quite lengthy and the potential application of these compounds to other enzymes is more limited.

DPPsE, DPPsC, DPPsI, and DPPsS (**1–4**).[11–18] The procedure is out-

[6] J. R. Knowles, *Annu. Rev. Biochem.* **49,** 877 (1980); S. L. Buchwald, D. E. Hansen, A. Hassett, and J. R. Knowles, this series Vol. 87, p. 279.
[7] G. Lowe, *Philos. Trans. R. Soc. London, Ser. B* **293,** 75 (1981); G. Lowe, *Acc. Chem. Res.* **16,** 244 (1983).
[8] M.-D. Tsai, this series, Vol. 87, 235.
[9] M. Cohn, *Acc. Chem. Res.* **15,** 326 (1982).
[10] W. J. Stec, *Acc. Chem. Res.* **16,** 411 (1983).
[11] K. Bruzik, R.-T. Jiang, and M.-D. Tsai, *Biochemistry* **22,** 2478 (1983).
[12] R.-T. Jiang, Y.-J. Shyy, and M.-D. Tsai, *Biochemistry* **23,** 1661 (1984).
[13] M. D. Mateucci and M. H. Caruthers, *J. Am. Chem. Soc.* **103,** 3185 (1981).
[14] K. S. Bruzik, G. Salamonczyk, and W. J. Stec, *J. Org. Chem.* **51,** 2368 (1986).
[15] G. Lin and M.-D. Tsai, *J. Am. Chem. Soc.* **111,** 3099 (1989).
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[18] W. Loffredo and M.-D. Tsai, *Bioorg. Chem.* **18,** 78 (1990).

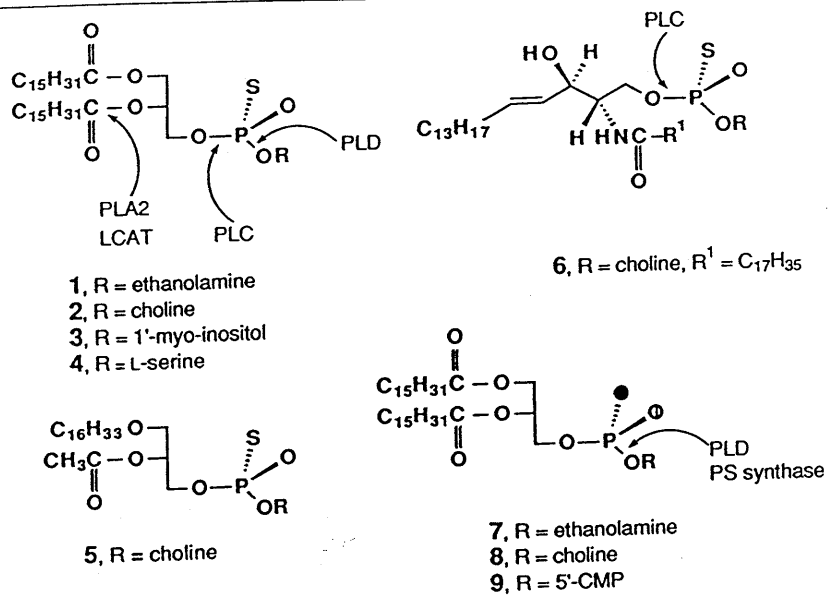

FIG. 1. Structures of chiral thiophospholipids (**1–6**) and [^{17}O,^{18}O]phospholipids (**7–9**). **1**, (R_p)-DPPsE; **2**, (R_p)-DPPsC; **3**, (S_p)-DPPsI; **4**, (R_p)-DPPsS; **5**, (R_p)-AGEPsC; **6**, (R_p)-SPsM; **7**, (R_p)-[^{17}O,^{18}O]DPPE; **8**, (R_p)-[^{17}O,^{18}O]DPPC; **9**, (S_p)-[^{17}O,^{18}O]CPD-DPG. It is a convention to use ⊙ and ● to denote ^{17}O and ^{18}O, respectively. In assigning the *R* and *S* configurations, it should be noted that the atomic number priority should be exhausted before considering atomic mass (i.e., OX has higher priority than ^{18}O) [R. S. Cahn, C. K. Ingold, and V. Prelog, *Angew. Chem., Int. Ed. Engl.* **5**, 385 (1966)] and that, even though all thiophospholipids shown have the same *relative* configuration, they have different *absolute* configurations due to different head group structures. For abbreviations of glycerophospholipids, the first two characters describe the structure of the glyceride moiety (e.g., DP, 1,2-dipalmitoyl; MP, 1-monopalmitoyl; DO, 1,2-dioleoyl). The third character, P/Ps, denotes phospho/thiophospho function. The fourth character denotes the head group structure (e.g., A, free acid; C, choline; E, ethanolamine; I, *myo*-inositol; S, L-serine). AGEPsC, 1-*O*-Hexadecyl-2-acetyl-3-thiophosphocholine; PAF, platelet-activating factor; CDP-DPG, cytidine 5′-diphospho-1,2-dipalmitoyl-*sn*-glycerol; SPsM, D-*erythro*-2,*N*-stearoylsphingosyl-1-thiophosphocholine.

lined in Fig. 2 (**10–15**). To the chloroform solution of 1,2-dipalmitoyl-*sn*-glycerol (**10**, 1 mmol, 5 ml) is added triethylamine (2 mmol) and *N,N*-diisopropylmethylphosphonamidic chloride (**11**, 1.2 mmol) at room temperature. After the completion of the reaction (5 min) the solvent and excess triethylamine are evaporated, and solid tetrazole (4 mmol) and the corresponding alcohol [1.1–3 mmol, *N*-tritylethanolamine or choline tosylate or (−)-2,3,4,5,6-pentabenzyl-*myo*-inositol or *N*-trityl-L-serine methoxymethyl ester] are added; all reactants are solubilized by adding acetonitrile–tetrahydrofuran (THF) (1 : 1, v/v). After 30 min solvents are

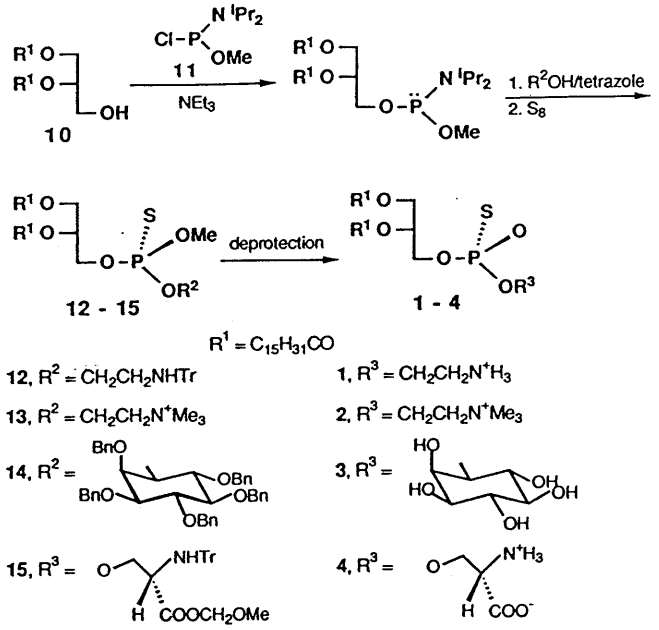

FIG. 2. Synthesis of phosphorothioate analogs of phospholipids **1–4** (via **10–15**). The products thus obtained should be mixtures of both diastereomers, but only one isomer is shown.

removed by evaporation and replaced with toluene. Elemental sulfur (10 mmol) is then added, and the reaction mixture is stirred for 12 hr at room temperature. The suspension is washed with triethylammonium bicarbonate buffer (1.5 *M*, pH 7.0), and the organic phase is concentrated and dried thoroughly under vacuum. Deprotection of the phosphate function in **12–14** is achieved by treatment of the triester with trimethylamine/toluene (1 : 1, v/v, 5 ml) at 50° for 20 hr. The deprotection of *N*-trityl-DPPsE is carried out with Zn^{2+}/acetic acid (200 mg/10 ml) at 45° during 9 hr. The pentabenzyl derivative of DPPsI is deprotected using ethanethiol/BF_3–etherate. In the case of DPPsS the deprotection of **15** is achieved by concentrated HCl in dry acetone followed by trimethylamine in toluene.

AGEPsC (**5**) *and SPsM* (**6**). Compounds **5** and **6** are obtained analogously as described above, except that the starting diacylglycerol **10** is replaced by 1-palmityl-2-palmitoyl-*sn*-glycerol for the synthesis of AGEPsC[19] (the 2-palmitoyl group is changed to a 2-acetyl group after the

[19] T. Rosario-Jansen, R.-T. Jiang, M.-D. Tsai, and D. J.Hanahan, *Biochemistry* **27**, 4619 (1988).

3-thiophosphocholine group has been introduced) and by D-*erythro*-3,*O*-*tert*-butyldiphenyl-2,*N*-stearoylsphingosine for the synthesis of SPsM.[20]

Separation of Diastereomers.[11] Separation of R_p and S_p isomers is achieved by stereospecific hydrolysis of the R_p isomer catalyzed by PLA_2 (see Results). (R_p + S_p)-DPPsC (1 g) is dissolved in chloroform/ether (60 ml, 1/5) and the solution treated with PLA_2 (bee venom, 1000 units) in buffer (3.5 ml, 10 m*M* Tris-Na, pH 7.2, 2 m*M* $CaCl_2$, 0.2 m*M* EDTA). The resulting emulsion is stirred at room temperature, and the reaction is monitored by ^{31}P nuclear magnetic resonance (NMR) (see below). When approximately 80% of (R_p)-DPPsC is hydrolyzed, the mixture is concentrated and the unreacted DPPsC separated from (R_p)-MPPsC (lyso-DPPsC) by chromatography on silica gel using chloroform/methanol/water (70 : 30 : 4, v/v). The fraction containing unreacted DPPsC is again subjected to hydrolysis by PLA_2 as described above, except the reaction is allowed to proceed until the formation of small quantities of (S_p)-MPPsC can be detected by ^{31}P NMR. Column chromatography on silica gel afforded pure (S_p)-DPPsC. The fraction containing pure (R_p)-MPPsC from the first column is subjected to reacylation using palmitoyl anhydride in the presence of 4-dimethylaminopyridine to give (R_p)-DPPsC. Alternatively, (R_p)-DPPsC can be obtained by the hydrolysis of (R_p + S_p)-DPPsC catalyzed by PLC from *Bacillus cereus,* which hydrolyzes (S_p)-DPPsC specifically.[11]

(R_p + S_p)-DPPsE is also separated into (R_p)- and (S_p)-DPPsE as described above. (R_p + S_p)-DPPsI is separated into (R_p)-DPPsI and (S_p)-MPPsI,[15,16] and (R_p + S_p)-DPPsS is separated into (R_p)-MPPsS and (S_p)-DPPsS by PLA_2.[18] Reacylation of (S_p)-MPPsI and (R_p)-MPPsS is not performed due to presence of other acylation sites in the molecules. Separation of (R_p + S_p)-DPPsI into (R_p)- and (S_p)-DPPsI is also achieved by column chromatography on silica gel at the stage of protected derivatives **14** using carbon tetrachloride/acetone (100 : 1, v/v) as the eluting solvent.[17]

Configurational Analysis

[^{17}O,^{18}O]DPPE.[21] Analysis of [^{17}O,^{18}O]DPPE is presented in Fig. 3. Diastereomers of [^{17}O,^{18}O]DPPE are first converted to [^{16}O,^{17}O,^{18}O]DPPA (**16**) and then to 1-[^{16}O,^{17}O,^{18}O]phosphopropane-1,2-diol (**17**). The configuration of **17** is determined by converting **17** to **18** followed by ^{31}P NMR analysis as described by Buchwald and Knowles.[22] The basis of the ^{31}P

[20] K. S. Bruzik, *J. Chem. Soc. Perkin Trans. 1,* p. 423 (1988).
[21] K. S. Bruzik and M.-D. Tsai, *J. Am. Soc. Chem.* **106,** 747 (1984).
[22] S. L. Buchwald and J. R. Knowles, *J. Am. Chem. Soc.* **102,** 6601 (1980).

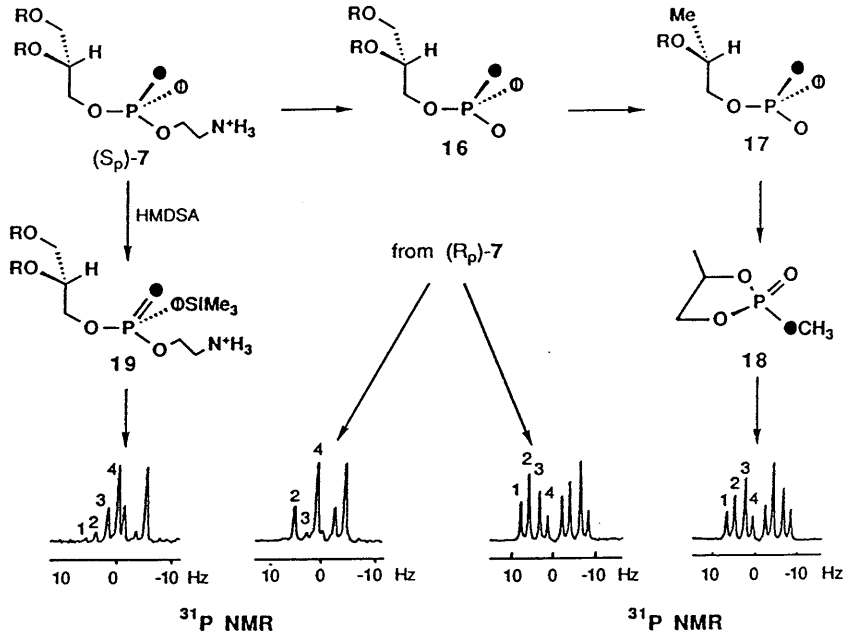

FIG. 3. Stereochemical analysis of P-chiral oxygen-labeled phospholipids. Although only single structures are shown for **18** and **19**, each consists of a mixture of two diastereomers arising from methylation in **18** and silylation in **19**. Each ^{31}P NMR spectrum consists of eight lines (four lines for each diastereomer of the triester derivative). Only one set is labeled (peaks 1–4) in each spectrum, which arise from different ^{18}O-labeled species: 1, unlabeled; 2, P—^{18}O; 3, P=^{18}O; 4, ^{18}O—P=^{18}O. The ratio of intensity of lines 2 and 3 determines the diastereomeric purity and configuration.

NMR method is explained in detail elsewhere.[8,23] Such a procedure has allowed elucidation of the absolute configuration of [^{17}O,^{18}O]DPPE. Now that the absolute configuration has been established, it is no longer necessary to go through the above steps. Instead, the distinction between diastereomers of [^{17}O,^{18}O]DPPE is made possible by silylation with hexamethyldisilazane (HMDSA) followed by ^{31}P NMR analysis of the resulting mixture of diastereomeric triesters (**19**), each having a distribution of various isotopomers (Fig. 3).[21,24] Diastereomers of singly labeled [^{18}O]DPPE can be analyzed by exactly the same method (see the spectrum in Fig. 4).

The configuration of other [^{17}O,^{18}O]phospholipids can be established

[23] M.-D. Tsai and K. S. Bruzik, *Biol. Magn. Reson.* **5**, 129 (1984).
[24] K. S. Bruzik and M.-D. Tsai, *J. Am. Chem. Soc.* **104**, 863 (1982).

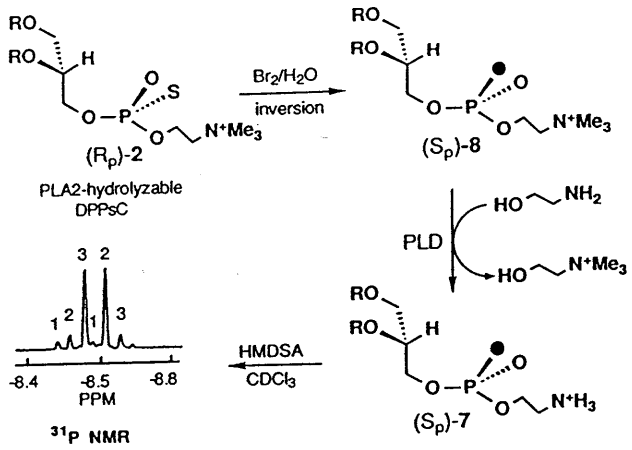

FIG. 4. Chemical correlation of the configuration of DPPsC to that of [^{16}O,^{18}O]DPPE. The method of configurational analysis of [^{16}O,^{18}O]DPPE is the same as that for [^{17}O,^{18}O]DPPE described in Fig. 3. The only difference is that peak 4 due to ^{18}O—P═^{18}O is missing, but the diastereomeric purity and configuration are determined only by the ratio peak 2/peak 3.

by chemical correlation with [^{17}O,^{18}O]DPPE based on the known steric course of PLD-catalyzed transphosphatidylation.[25]

Chiral Thiophospholipids. The configurations of chiral thiophospholipids are determined by chemical correlation with [^{18}O]DPPE as shown in Fig. 4. The PLA_2-hydrolyzable isomer of DPPsC (see Results) is desulfurized with bromine/$H_2{}^{18}O$, and the resulting [^{16}O,^{18}O]DPPC is subjected to the transphosphatidylation reaction in the presence of ethanolamine to give the corresponding [^{16}O,^{18}O]DPPE.[12] The configuration of this sample is determined as S_p by the silylation/^{31}P NMR method. Since the steric course of the desulfurization is inversion[26–28] and that of the transphosphatidylation reaction is retention[24] (see Results), the R_p configuration can be assigned to the PLA_2-hydrolyzable isomer of DPPsC. The configurations of the diastereomers of DPPsE are determined analogously.[12] The configurations of DPPsI,[15,16] DPPsS,[18] and AGEPsC[19] are determined on the basis of the stereospecific hydrolysis by PLA_2, whereas that of SPsM[20] is determined on the basis of the stereospecific hydrolysis by PLC. Now that the absolute configurations have been established, the diastereomers of chiral

[25] K. S. Bruzik and M.-D. Tsai, *Biochemistry* **23**, 1656 (1984).
[26] G. Lowe, G. Tansley, and P. M. Cullis, *J. Chem. Soc., Chem. Commun.*, p. 595 (1982).
[27] D. Sammons and P. A. Frey, *J. Biol. Chem.* **257**, 1138 (1982).
[28] B. A. Connolly, F. Eckstein, and H. H. Füldner, *J. Biol. Chem.* **257**, 3382 (1982).

TABLE I
^{31}P NMR CHEMICAL SHIFTS OF THIOPHOSPHOLIPIDS[a]

	DPPsC		DPPsE		DPPsS		DPPsI		AGEPsC		SPsM	
Conditions	R_p	S_p	R_p	S_p	R_p	S_p	R_p	S_p	R_p	S_p	R_p	S_p
$CDCl_3$	56.12	56.07	59.82	59.95	Unresolved		Unresolved		56.20	56.27	57.3	57.1
CH_3OD	60.88	60.80	60.10	60.08	Unresolved							
D_2O[b]	57.13	57.20			58.73	58.84	57.05	57.45			56.4	56.7
$CDCl_3/Et_3N$					59.13	59.29						

[a] Data in ppm relative to 85% H_3PO_4. From Refs. 11, 12, 15, 18–20; also, DOPsC R_p 56.591, S_p 56.615 ppm ($CDCl_3$).
[b] Containing 5% Triton X-100, 0.1 *M* Tris, pH 8.0.

thiophospholipids can be differentiated directly by ^{31}P chemical shifts listed in Table I.

Results

Steric Course. The steric course of the reaction is illustrated by the transphosphatidylation catalyzed by PLD from cabbage.[24] As shown in Fig. 5, (R_p)-[^{16}O,^{18}O]DPPE was methylated, and the resulting (R_p)-[^{16}O,^{18}O]DPPC was used as a substrate for PLD (cabbage leaves) in the presence of ethanolamine. The resulting [^{16}O,^{18}O]DPPE was analyzed by ^{31}P NMR after silylation. Since the spectra of silyl derivatives obtained

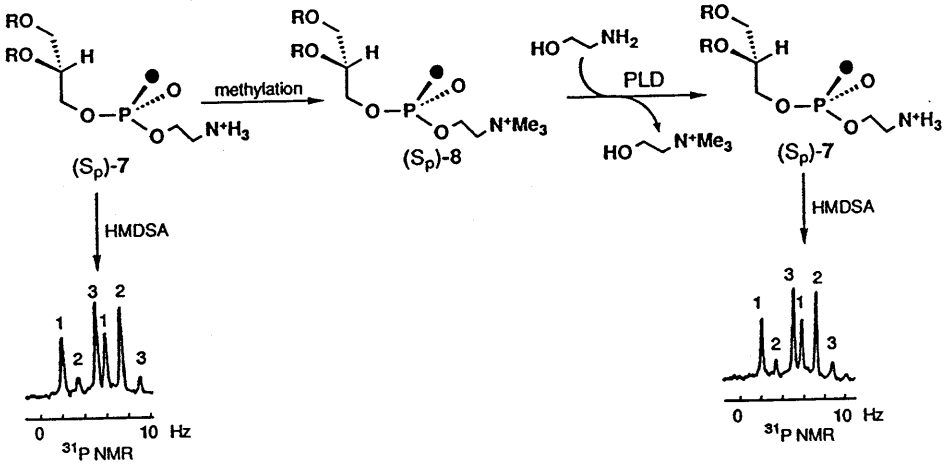

FIG. 5. Determination of the steric course of PLD-catalyzed transphosphatidylation.

TABLE II
STERIC COURSE OF PHOSPHOLIPID-METABOLIZING ENZYMES[a]

Enzyme	Source	Substrate	Product	Steric course	Ref.
PLD	Cabbage	[^{17}O,^{18}O]DPPE	[^{16}O,^{17}O,^{18}O]DPPA	Retention	25
PLD	Cabbage	[^{18}O]DPPC	[^{18}O]DPPE	Retention	24
PS synthase	*Escherichia coli*	[^{17}O,^{18}O]CDP-DPG	[^{17}O,^{18}O]DPPS	Retention	*b*
PS synthase	Yeast	[^{17}O,^{18}O]CDP-DPG	[^{17}O,^{18}O]DPPS	Inversion	*b*
PI-PLC	*Bacillus cereus*	(R_p)-DPPsI	*exo*-cIPs[c]	Inversion	15
PI-PLC-I	Guinea pig	(R_p)-DPPsI	*exo*-cIPs[c]	Inversion	16
PI-PLC-II	Guinea pig	(R_p)-DPPsI	*exo*-cIPs[c]	Inversion	16

[a] When oxygen-labeled substrates were used, the experiments were usually performed for both R_p and S_p isomers, whereas when thiophospholipid was used, only one isomer could be studied due to stereospecificity of the enzyme.
[b] C. R. H. Raetz, G. M. Carman, W. Dowhan, R.-T. Jiang, W. Waszkuc, W. Loffredo, and M.-D. Tsai, *Biochemistry* **26**, 4022 (1987).
[c] cIPs, *myo*-inositol 1,2-cyclic phosphorothioate.

from the substrate and from the product were nearly identical, the retention of configuration at phosphorus in the transphosphatidylation reaction was inferred. This suggests that the reaction proceeds by a two-step mechanism involving a phosphatidyl-enzyme intermediate. Results of the analysis of the steric course of reactions catalyzed by phospholipases and related enzymes are listed in Table II.

Stereospecificity. The results on stereospecificity are summarized in Table III. The enzymes which catalyze a P–O bond substitution or cleav-

TABLE III
STEREOSPECIFICITY OF PHOSPHOLIPID-METABOLIZING ENZYMES

Enzyme	Source	Substrate	Preferred isomer	Ref.
PLA_2	Bee venom	DPPsC, DPPsE	R_p	11, 12
PLA_2	*Naja naja*	DPPsC	R_p	11, 12
PLA_2	*Crotalus adamanteus*	DPPsC	R_p	11, 12
PLA_2	Porcine pancreas	DPPsC	R_p	11, 12
PLD	Cabbage	DPPsC	S_p	12
PLC	*Clostridium perfringens*	DPPsC, SPsM[a]	S_p	11, 12, 20
PLC	*B. cereus*	DPPsC, DPPsE, SPsM[a]	S_p	11, 12, 20
PI-PLC	*B. cereus*	DPPsI	R_p	15
PI-PLC-I	Guinea pig uterus	DPPsI	R_p	16
PI-PLC-II	Guinea pig uterus	DPPsI	R_p	16
LCAT	Human plasma	DPPsC, DOPsC	No preference	30
PAF receptor	Rabbit platelets	AGEPsC	S_p	19

[a] The configuration of SPsM was not determined by an independent method. Instead, the stereospecificity of PLC was used to assign the configuration of SPsM.[20]

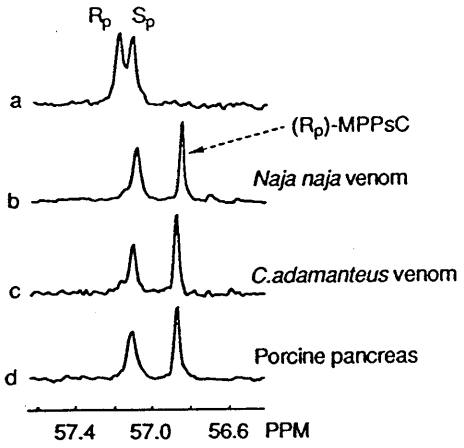

FIG. 6. ^{31}P NMR spectral analysis of the hydrolysis of (R_p + S_p)-DPPsC catalyzed by PLA_2 from various sources showing the stereospecific hydrolysis of (R_p)-DPPsC. (a) Starting (R_p + S_p)-DPPsC; (b–d) products of PLA_2 reactions. (From Bruzik *et al.*[11])

age (e.g., PLC, PLD, and PI-PLC) all exhibit a stereospecificity without surprise. The result of PLA_2 is more unexpected. In view of the rather broad specificity of this enzyme with respect to the structure of the phosphate head group, and the fact that the hydrolysis reaction occurs five bonds away from the negatively charged oxygen atoms of the phosphate group, the high stereospecificity of this enzyme with respect to configuration at phosphorus was not expected. However, ^{31}P NMR analysis in Fig. 6 indicates that PLA_2 is specific for the R_p isomer of DPPsC.

The result with PLA_2 has several impacts. (1) The stereospecificity of PLA_2 constitutes a basis for the enzymatic separation of various thiophospholipids as discussed above. (2) Since the stereospecificity is not expected to depend on the type of phospholipids for the same enzyme, PLA_2 and PLC can be used to determine the phosphorus configuration of other thiophospholipids. PLA_2 is particularly useful due to its broad substrate specificity, and it has been used to assign the configurations of DPPsI, DPPsS, and AGEPsC. (3) The result of PLA_2 was interpreted to suggest that the phosphate group functions as a "remote stereochemical control" in substrate binding via stereospecific coordination of Ca^{2+} to the *pro-S* oxygen of natural phospholipids.[29] Such an interpretation was further supported by detailed kinetic studies, particularly the observation that the ratio $V_{max}(R_p)/V_{max}(S_p)$ dramatically decreases on substitution of the hard metal Ca^{2+} by the soft metal Cd^{2+} (see data in Table IV). This result was

[29] T.-C. Tsai, J. Hart, R.-T. Jiang, K. Bruzik, and M.-D. Tsai, *Biochemistry* **24**, 3180 (1985).

TABLE IV
KINETIC PARAMETERS OF PLA_2 AND LCAT

Enzyme/metal ion	Substrate	K_m (mM)	V_{max} (mmol/min/mg)	Ref.
PLA_2/Ca^{2+}	DPPC	1.67	1850	29
	(R_p)-DPPsC	0.85	76	
	(S_p)-DPPsC	0.30	0.044	
PLA_2/Cd^{2+}	DPPC	6.4	17.6	29
	(R_p)-DPPsC	0.24	0.069	
	(S_p)-DPPsC	—	0.0044	
LCAT	DPPC	0.032	0.021	30
	(R_p)-DPPsC	0.064	0.023	
	(S_p)-DPPsC	0.07	0.02	
	DOPC	0.07	0.066	
	(R_p)-DOPsC	0.072	0.056	
	(S_p)-DOPsC	0.056	0.05	

also used to propose a substrate-binding model which is being tested by protein engineering techniques. (4) The same approach can be used to test whether the phosphate group of phospholipids is involved in a specific interaction in other systems. For example, in contrast to PLA_2, LCAT shows little variation in K_m and V_{max} on sulfur substitution and configuration change at phosphorus[30] (see Table IV). The results suggest that the interaction between the phospholipid substrate and LCAT does not involve stereospecific binding to the phosphate group. The approach has also been used in the interaction of AGEPC (PAF) with PAF receptors.[19]

An obvious question for the use of thiophospholipids is their reactivity relative to the natural substrate. As shown in Table IV, the active isomer of DPPsC has approximately 4% reactivity relative to DPPC. On the other hand, sulfur substitution has no effect in the case of LCAT. The effects in PLC and PLD have not been quantitatively determined, but it was estimated to be of the order of 1% for PLC and PI-PLC and much smaller for PLD.

Another question which has been raised occasionally is whether the observed stereospecificity could be due to differences in the physical properties of the two isomers, instead of differences in the binding to the active site. We have observed that some of the physical properties of the bilayers of chiral thiophospholipids, particularly the thermotropic property, are very sensitive to the configuration at phosphorus. This aspect is

[30] T. Rosario-Jansen, H. J. Pownall, J. P. Noel, and M.-D. Tsai, *Phosphorus Sulfur* **30**, 601 (1987); T. Rosario-Jansen, H. J. Pownall, R.-T. Jiang, and M.-D. Tsai, *Bioorg. Chem.* **18**, 179 (1990).

beyond the scope of this chapter. However, since all of the studies of phospholipases described in this chapter employed micelles or mixed micelles of substrates, the configurational effect on the physical properties should not be responsible for the observed stereospecificity.

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[24] Phospholipase A_2: Microinjection and Cell Localization Techniques

By DAFNA BAR-SAGI

Introduction

Phospholipase A_2 (PLA_2) is a calcium-requiring esterase that catalyzes the hydrolysis of glycerophospholipids specifically at the *sn*-2 position to produce a fatty acid and a lysophospholipid.[1,2] As mentioned elsewhere in this volume, the activity of PLA_2 has been postulated to play an important regulatory role in several metabolic pathways. For example, PLA_2 catalyzes the release of arachidonic acid, the first and rate-limiting precursor in the biosynthesis of prostaglandins. In addition, the activity of the enzyme is part of the phosphoglyceride deacylation–reacylation cycle and as such mediates the rapid metabolic turnover of membrane phospholipids. Furthermore, there is increasing evidence in support of the participation of PLA_2 in the generation of receptor-mediated transmembrane signals.

This chapter is specifically concerned with two approaches, microinjection and cell localization, to analyze the biological properties of cellular PLA_2. As both approaches rely primarily on the availability of anti-PLA_2 antibodies, methods for obtaining suitable anti-PLA_2 antibodies are also included.

[1] H. van den Bosch, *Biochim. Biophys. Acta* **604**, 191 (1980).
[2] H. M. Verheij, A. J. Slotboom, and G. H. de Haas, *Rev. Physiol. Biochem. Pharmacol.* **91**, 91 (1981).