Supplementary Materials

Susceptibility of phosphonolipids to lipases and phospholipases

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1. Conditions of HPLC analysis

Analysis of synthesized (*R*)-diethyl 2-hydroxy-3-palmitoyloxypropylphosphonate (**2**) and (*R*)-diethyl 2,3-dipalmitoyloxypropylphosphonate [(*R*)-**1**)]was carried out using RP-HPLC system on ODS2 Spherisorb column (Waters, 150 mm x 4,6 mm x 3 μm, constant flow 1.0 mL/min, 30°C). Gradient timetable (%A:%B:%C, v/v/v): at 0 min, 8:82:10 for 1 min, at 5 min 8:72:20, at 6.5 min 3:40:57, at 14.5 min 3:40:57, at 15.5 min 8:82:10 until 22 min, where A = 1% HCOOH in water, B = acetonitrile and C = propan-2-ol.

Products of enzymatic hydrolysis of diethyl 2,3-dipalmitoyloxypropylphosphonate (**1**) were analyzed on chiral column Chiralpak® AD-H (250 mm x 4.6 mm ID x 5 μm, constant flow of 1.0 mL/min, 30°C). Gradient timetable (%A:%B, v/v): at 0 min 100:0, at 35 min 96:4, at 38 min 90:10, at 40 min until 47 min 100:0, where solvent A = hexane, solvent B = propan-2-ol.

The progress of hydrolysis or ethanolysis of DPPC, DPPnC (**4**) and soybean PC was monitored using a Betasil DIOL column (Thermo, 150 mm x 2.1 mm x 5 μm, constant flow 1.5 mL/min, injection volume 20 μL, 30°C). Gradient timetable (%A:%B:%C, v/v/v): at 0 min 0:43:57 for 1 min, at 5 min 3:40:57, at 8 min 10:40:50, at 13 min 10:40:50, at 13.1 min until 22 min 0:43:57, where A = 1% HCOOH containing 0.1% triethylamine, B = hexane and C = propan-2-ol. Retention times were 11.155 min for DPPC, 11.910 min for both 1-LPC and 2-LPC and 10.085 min for DPPA. In the conditions applied, retention times for soybean PC and DPPnC and products of their hydrolysis corresponded to the synthetic standards of DPPC, LPC and DPPA. The calibration curve for DPPC (M_w=735 g/mol) were performed based on six independent dilutions were injected three times in concentrations DPPC from 0.03 μg to 1.28 μg. Response of the detection method was fitted to the linear model and correlation coefficient value was R^2=0.991. Calibration curve developed for DPPC was used to confirm the known concentrations of soybean PC and DPPnC and, subsequently, for determination of these substrates concentration in the samples taken from reaction mixtures.

2. Conditions of GC analysis

(*R*)-diethyl 2,3-dihydroxypropylphosphonate **(3)** was analyzed on HP-5 capillary column (30 m × 0.32mm × 0.25 μm) using temperature program: 100 °C (hold 1 min), 100–200 °C (17 °C/min), 200–280 °C (30 °C/min), 280 °C (hold 1 min), injector 250 °C, detector 280 °C. The total time analysis was 10.55 min.

3. Synthesis of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC)

GPC (7.71 g, 30 mmol) was dissolved in methanol (50 mL) and added slowly to a solution of cadmium chloride hemi(pentahydride) $CdCl_2 \times 2½H_2O$ (6.85 g, 30 mmol) in water (20 mL). The suspension was mixed in 0 °C for 4 h. The precipitate was filtered off and washed with methanol (20 mL). The precipitate of GPC×$CdCl_2$ was lyophilized from distilled water to give a white powder, which was dried for 24 h in a drying pistol under vacuum over P_2O_5 at the boiling temperature of acetone.

Palmitic acid was dried by repeated co-evaporation with mixture of anhydrous CH_2Cl_2:benzene (1:1, v/v). The complex of GPC×$CdCl_2$ was suspended in the solution of palmitic acid (1.03 g, 4 mmol) in dry CH_2Cl_2 (30 mL) containing 4-(dimethyl-amino)pyridine (DMAP) (244

mg, 2 mmol) and finally, N,N-dicyclohexylcarbodiimide (DCC) (865 mg, 4.2 mmol) in a solution of CH_2Cl_2 (10 mL) was added. The suspension was stirred at room temperature under a nitrogen atmosphere. The progress of the reaction was monitored by HPLC and by TLC ($CHCl_3$:MeOH:H_2O, 65:25:4, v/v/v). Lipid spots were detected under a UV lamp after spraying a 0.05% primuline solution (acetone:water, 8:2, v/v). After 16 h, the precipitate was filtered off and DOWEX®50WX8 (H^+ form) was added to remove the cadmium chloride and 4-(dimethyl-amino)pyridine. The solution was stirred for 30 min, the ion-exchange resin was filtered off and the solvent was evaporated in vacuo. The crude product was purified on a silica-gel column (eluent: $CHCl_3$:MeOH:H_2O, 65:25:4, v/v/v). The corresponding fractions (checked on TLC with the primuline test) were collected and evaporated to give appropriate 1,2-diacyl-*sn*-glycero-3-phosphocholines (610 mg, yield 83%).

4. Synthesis of 1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (1-palmitoyl LPC) and 1-hydroxy-2-palmitoyl-*sn*-glycero-3-phosphocholine (2-palmitoyl LPC)

1-palmitoyl LPC:

The enzymatic hydrolysis of DPPC was catalyzed by phospholipase A_2 (PLA_2) from porcine pancreas in reversed micelles was carried out in a thermostated (40 °C) batch with magnetic stirring. A reversed micellar solution containing PLA_2 was prepared by injecting, with strong magnetic stirring, 17.6 μL of Tris–HCl buffer (pH 8.5, 0.1 M) with $CaCl_2$ (0.75 M), and 26.3 μL (263 U) aqueous solution of the enzyme to preincubated flasks (40 °C for 0.5 h) containing 14 mg of dioctyl sulfosuccinate sodium salt (AOT) dissolved in 1.1 mL of isooctane. The reaction was started by the addition of a preincubated (40 °C for 0.5 h) solution of 41 mg DPPC (0.05 mmol) dissolved in 1.1 mL of isooctane. The progress of enzymatic reactions was monitored by TLC ($CHCl_3$:MeOH:H_2O, 65:25:4, v/v/v) and HPLC. The reaction was completed after about 10 min. 1-Palmitoyl LPC was purified on the silica gel column chromatography ($CHCl_3$:MeOH:H_2O, 65:25:4, v/v/v). The fractions containing 1-palmitoyl LPC were collected and evaporated to dryness at 45 °C *in vacuo* to give 24.5 mg of 1-palmitoyl LPC (purity 96.8% by HPLC).

2-palmitoyl LPC:

Reaction was started by the addition of Lipozyme® (40 U) to DPPC (50 mg, 0.07 mmol) dissolved in 2.5 mL of 96% ethanol. The mixture was shaken vigorously at 25 °C. The progress of reaction was monitored by TLC ($CHCl_3$:MeOH:H_2O, 65:25:4, v/v/v) and HPLC. When the reaction was completed (12 h), the enzyme was filtered off and washed with 10 mL of methanol. Solvents were removed at 45 °C on a rotary evaporator *in vacuo*. The residue was diluted in 0.3 mL of $CHCl_3$, placed in an ice-bath and 5 mL of chilled acetone (−20 °C) was added to precipitate 2-palmitoyl LPC. The precipitate was washed with cold acetone (6 x 10 mL) and the solvent was removed by decantation each time. Finally, the acetone was evaporated at 45 °C *in vacuo* to afford 32 mg of 2-palmitoyl LPC product (purity 91% by HPLC).

Table S1. Concentration of substrates during hydrolysis or ethanolysis of soybean PC, DPPC and (*R*)-DPPnC in *sn*-1 position catalyzed by lipases and Lecitase® Ultra

t[h]	Lecitase® Ultra			Novozym 435			Lipozyme®		
	Soybean PC [%]	**DPPC [%]**	**DPPnC [%]**	**PC [%]**	**DPPC [%]**	**DPPnC [%]**	**PC [%]**	**DPPC [%]**	**DPPnC [%]**
0	100±0	100±0	100±0	100±0	100±0	100±0	100±0	100±0	100±0
0.5	95.4±7.0	90.0±3.0	96.0±3.8	84.5±11.7	86.2±6.0	68.6±10.8	97.9±2.0	89.4±11.9	86.0±13.5
1	92.4±5.5	87.9±6.9	80.2±6.7	69.3±7.4	79.4±6.1	68.2±12.6	51.3±7.1	69.6±1.8	48.5±11.4
2	84.7±9.5	82.8±7.1	74.1±7.6	37.1±6.1	66.9±5.9	50.0±9.3	23.9±11.4	66.3±13.7	33.5±12.4
4	81.2±14.0	76.3±8.2	56.4±12.4	9.4±8.1	51.4±7.9	39.9±4.0	8.0±4.2	23.0±4.2	16.1±9.2
6	77.1±6.7	54.9±9.9	51.2±9.6	4.5±5.4	42.5±7.0	16.0±7.7	0.8±0.3	18.4±12.7	6.2±2.5
8	68.0±4.7	47.8±3.2	48.3±5.8	3.6±1.5	21.7±2.0	8.9±12.2	1.0±0.4	14.6±12.9	7.1±6.1
24	18.2±12.9	45.6±6.7	41.2±11.9	1.4±2.5	0.7±0.2	3.0±7.3	0.9±0.5	1.0±0.2	1.5±0.8
48	13.6±10.9	45.6±10.2	40.7±3.5	0.7±1.2	0.6±2.4	0.8±0.7	0.6±0.5	0.9±2.0	0.5±0.5

Table S2. Concentration of substrates during hydrolysis of soybean PC, DPPC and (*R*)-DPPnC in *sn*-2 position by phospholipases A_2

t[h]	PLA_2 from porcine pancreas			PLA_2 from bovine pancreas			PLA_2 from bee venom		
	Soybean PC [%]	**DPPC [%]**	**DPPnC [%]**	**PC [%]**	**DPPC [%]**	**DPPnC [%]**	**PC [%]**	**DPPC [%]**	**DPPnC [%]**
0	100±0	100±0	100±0	100±0	100±0	100±0	100±0	100±0	100±0
0.5	71.1±3.5	97.7±1.3	64.6±4.2	97.9±2.0	86.6±11.9	88.2±10.8	98.8±1.0	96.8±1.9	97.8±1.6
1	65.1±6.8	87.4±3.6	56.2±2.4	51.4±7.1	70.9±2.2	56.2±6.6	96.6±3.3	96.6±3.3	94.6±6.2
2	62.5±6.4	84.7±5.9	47.8±8.2	28.7±8.6	67.5±5.7	49.8±8.1	92.1±6.8	94.2±3.1	92.6±5.8
4	51.2±14.0	72.6±9.1	30.0±6.2	8.2±4.0	36.0±8.6	27.0±6.0	80.1±8.0	91.5±5.4	88.4±3.8
6	19.8±8.1	55.5±4.5	26.6±2.6	1.0±0.1	16.6±6.4	15.6±7.5	71.0±9.5	84.4±6.2	82.3±6.0
8	1.5±1.1	25.6±7.7	16.8±11.7	1.0±0.2	12.9±10.9	12.3±5.0	57.7±11.7	81.02±12.1	75.5±6.7
24	1.1±1.1	4.1±6.1	1.2±1.1	0.9±0.0	1.2±0.2	1.7±0.5	53.5±5.2	69.7±8.3	71.1±10.3
48	0.8±0.8	1.5±1.7	1.0±0.9	0.7±0.7	1.1±2.0	0.7±0.6	53.4±10.8	68.1±8.0	65.4±7.3

Table S3. Concentration of substrates during hydrolysis of of soybean PC, DPPC and (*R*)-DPPnC by phospholipases D

t[h]	PLD from *Streptococcus* sp.			PLD from *Streptomyces chromofuscus*			PLD from white cabbage		
	Soybean PC [%]	**DPPC [%]**	**DPPnC [%]**	**PC [%]**	**DPPC [%]**	**DPPnC [%]**	**PC [%]**	**DPPC [%]**	**DPPnC [%]**
0	100±0	100±0	100±0	100±0	100±0	100±0	100±0	100±0	100±0
0.5	52.9±6.8	76.3±9.9	88.1±7.6	89.3±11.5	91.8±2.1	97.7±0.2	95.5±3.0	95.5±3.0	96.9±1.5
1	39.4±4.6	55.1±4.9	87.5±5.6	86.9±10.6	71.6±9.6	96.5±0.3	90.1±10.0	90.4±9.4	94.1±5.4
2	36.1±5.6	44.4±6.3	84.2±4.7	86.2±11.9	49.0±9.1	90.9±5.0	87.1±9.4	87.7±10.9	93.5±6.3
4	35.9±8.0	39.4±5.7	84.0±6.8	62.7±4.2	46.4±8.2	82.2±10.9	83.6±7.4	82.7±8.8	88.2±8.4
6	34.7±10.5	39.3±5.9	83.2±6.6	57.2±10.2	41.0±7.0	81.4±7.3	80.0±8.2	77.8±9.6	85.6±10.4
8	34.0±6.0	39.1±8.4	82.6±5.3	45.2±3.5	39.7±3.3	79.5±9.8	71.7±6.0	72.1±6.5	80.6±8.8
24	34.5±8.6	38.2±7.1	81.3±10.1	32.6±11.1	36.7±9.8	77.5±6.6	67.0±7.8	69.4±5.8	80.5±9.2
48	33.8±7.7	37.2±7.8	80.8±5.3	31.7±6.5	32.2±6.9	77.1±5.4	62.9±10.0	62.7±7.5	79.8±11.3