Chemistry and Physics of Lipids 165 (2012) 787–793

Contents lists available at SciVerse ScienceDirect

Chemistry and Physics of Lipids

journal homepage: www.elsevier.com/locate/chemphyslip

New cardiolipin analogs synthesized by phospholipase D-catalyzed transphosphatidylation

Anna O. Müller[a], Carmen Mrestani-Klaus[a], Jürgen Schmidt[b], Renate Ulbrich-Hofmann[a], Martin Dippe[a,*]

[a] Martin-Luther University Halle-Wittenberg, Institute of Biochemistry and Biotechnology, Kurt-Mothes-Str. 3, D-06120 Halle, Germany
[b] Leibniz Institute of Plant Biochemistry, Weinberg 3, D-06120 Halle, Germany

ARTICLE INFO

Article history:
Received 31 July 2012
Received in revised form
18 September 2012
Accepted 21 September 2012
Available online 8 October 2012

Keywords:
Cardiolipin analogs
Diphosphatidyl lipids
Ethanolamine derivatives
Head group
Phospholipase D
Transphosphatidylation

ABSTRACT

Cardiolipin (CL) and related diphosphatidyl lipids are hardly accessible because of the complexity of their chemical synthesis. In the present paper, the transphosphatidylation reaction catalyzed by phospholipase D (PLD) from *Streptomyces* sp. has been proven as an alternative enzyme-assisted strategy for the synthesis of new CL analogs. The formation of this type of compounds from phosphatidylcholine was compared for a series of N- and C2-substituted ethanolamine derivatives as well as non-charged alcohols such as glycerol and ethylene glycol. The rapid exchange of the choline head group by ethanolamine derivatives having a low molecular volume (diethanolamine and serinol) gave rise to an efficient production of the corresponding CL analogs. In contrast, the yields were comparably low in the reaction with bulky nitrogenous acceptor alcohols (triethanolamine, tris(hydroxymethyl)aminomethane, tetrakis(hydroxyethyl)ammonium) or the non-charged alcohols. Therefore, a strong dependence of the conversion of the monophosphatidyl to the diphosphatidyl compound on steric parameters and the head group charge was concluded. The enzyme-assisted strategy was used for the preparation of purified diphosphatidyldiethanolamine and diphosphatidylserinol.

© 2012 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Unlike the common membrane phospholipids, cardiolipin (CL) contains two phosphatidyl residues linked to the external hydroxyl groups of glycerol. This dimeric phospholipid specifically occurs in the membranes of bacteria and mitochondria harboring the respiratory complexes. Therefore, the role of CL in membrane organization and cellular energy metabolism has attracted much attention (Schlame, 2008). For example, this unique phospholipid is involved in the maintenance of the characteristic shape of the inner membrane (Khalifat et al., 2008). CL is crucial for the stabilization of the complexes of the respiratory chain (Arias-Cartin et al., 2012) and of mitochondrial carriers (Klingenberg, 2009). Besides its structural function, CL serves as a reservoir for the electrochemical gradient due to protonation of its head group at low pH (Haines and Dencher, 2002) and thus contributes to proton conduction (Lange et al., 2001). Additionally, it is involved in cell signaling related to oxidative stress (Gohil and Greenberg, 2009) and apoptosis (Wright et al., 2004; Joshi et al., 2009).

Because of its special structural features, the physicochemical properties of CL significantly differ from the corresponding monophosphatidyl compounds (Lewis and McElhaney, 2009). Since those properties are beneficial for the stabilization of lipid vesicles, CLs and structurally similar anionic compounds are of great interest for the preparation of liposomes used for DNA packing (Resina et al., 2009) and HIV prophylaxis (Malavia et al., 2011). Positively charged CL analogs, which mediate cellular nucleic acid uptake are attractive as components of liposome-based gene-delivery systems (Zhang et al., 2006).

However, the chemical synthesis of CL or structurally related compounds is complicated and involves various reaction steps (Paltauf and Hermetter, 1994). Phospholipase D (PLD)-catalyzed transesterification of the better accessible glycerophospholipids seems to be a useful alternative strategy to produce CL compounds. PLD is a well-known biocatalyst due to its capability to

Abbreviations: br, broad; dd, doubled doublet; dm, doubled multiplet; CL, cardiolipin; HPLC, high performance liquid chromatography; COSY, homonuclear correlation spectroscopy; DSS, 4,4-dimethyl-4-silapentane sodium sulfonate; FT-ICR, Fourier transform ion cyclotron resonance; HPTLC, high performance thin layer chromatography; m, multiplet; PA, phosphatidic acid; PC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; PE, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine; PG, phosphatidylglycerol; PLD, phospholipase D; q, quartet; t, triplet; THA, tetrakis(2-hydroxyethyl)ammonium bromide; TOCSY, total correlation spectroscopy; Tris, tris(hydroxymethyl)aminomethane.

* Corresponding author. Present address: Leibniz Institute of Plant Biochemistry, Weinberg 3, 06120 Halle, Germany. Tel.: +49 34555 821412; fax: +49 34555 27303.
E-mail address: Martin.Dippe@ipb-halle.de (M. Dippe).

0009-3084/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.chemphyslip.2012.09.005

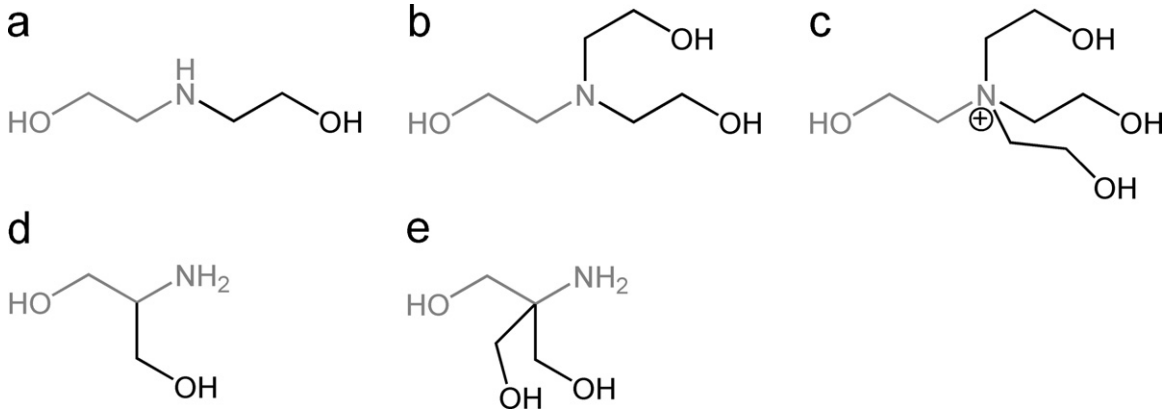

Fig. 1. Ethanolamine derivatives assayed for the enzymatic production of CL analogs. The ethanolamine moiety of the N-substituted derivatives diethanolamine (a), triethanolamine (b) and THA (c) and the C2-substituted derivatives serinol (d) and Tris (e) are drawn in gray.

exert a head group exchange reaction called transphosphatidylation. In phospholipid synthesis, especially bacterial PLDs such as the enzyme from *Streptomyces* sp. are used because they strongly favor transphosphatidylation of the acceptor alcohol over the undesired hydrolytic cleavage of the phospholipid substrate (Ulbrich-Hofmann et al., 2005). In contrast to other PLDs, this enzyme also mediates the conversion of phosphatidylglycerol (PG) to CL (D'Arrigo et al., 1996; Piazza and Marmer, 2007).

Interestingly, a similar reaction was observed in the enzymatic synthesis of phosphatidylethanolamine derivatives with multiple hydroxylated head groups (Dippe et al., 2008) leading to the formation of side products which were identified as dimeric phospholipids with a positively charged hydrophilic linker. In the present paper, we evaluate the applicability of this reaction for the synthesis of dimeric phospholipids from phosphatidylcholine and a series of N- and C2-substituted derivatives of ethanolamine or non-nitrogenous acceptors (glycerol and ethylene glycol). The reactions were compared with respect to chemical structure and charge of the used acceptor alcohols. Two of the CL analogs (diphosphatidylserinol and diphosphatidyldiethanolamine) are shown to be accessible in preparative scale.

2. Materials and methods

2.1. Materials

1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (PC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (PE), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-*rac*-glycerol, 1,2-dioleoyl-*sn*-glycero-3-phospho-*rac*-glycerol, CL from bovine heart, PLD from *Streptomyces* sp. (Type VII), and 2-bromoethanol were products from Sigma (USA). The phosphatidic acids (PAs) 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphate and 1,2-dioleoyl-*sn*-glycero-3-phosphate were from Avanti Polar (USA), and phosphatidyl-4-nitrophenol was synthesized from egg yolk phosphatidylcholine according to D'Arrigo et al. (1995). 1,2-Dioleoyl-*sn*-glycero-3-phosphoethylene glycol was produced by transphosphatidylation of the corresponding phosphatidylcholine by recombinant PLD from white cabbage according to the procedure described by Dippe et al. (2008) Its molecular mass was checked as described in Section 2.2.5. Ethylene glycol, water-free glycerol, tris(hydroxymethyl)aminomethane (Tris) and serinol were from Merck, Germany. All other reagents and solvents were purchased from Roth, Germany.

2.2. Methods

2.2.1. Determination of PLD activity

PLD activity was assayed in adaption to D'Arrigo et al. (1995) toward a micellar solution of the substrate phosphatidyl-4-nitrophenol (0.42 mM) in 0.21 mM sodium dodecyl sulfate, 3.3 mM Triton X-100, 120 mM $CaCl_2$ and 300 mM acetate buffer, pH 5.6. One unit (U) of enzyme activity represents the amount of enzyme releasing 1 μmol of 4-nitrophenol per minute.

2.2.2. Synthesis of tetrakis(2-hydroxyethyl)ammonium bromide (THA)

48 mmol of triethanolamine and 2-bromoethanol, respectively, were dissolved in 15 ml of nitromethane and heated under reflux at 95 °C for 10 h. Upon cooling, white crystals of THA were obtained, which were collected by filtration and washed with acetone. The crude product was re-crystallized from methanol/acetone/water (15:10:1, v/v/v) and dried in vacuum in the presence of P_4O_{10}, yielding 1.20 g (4.4 mmol) of pure THA. ^{1}H NMR (according to Section 2.2.6, in D_2O-DSS-external): δ 3.41 ppm (t, 8H, —CH_2—NR_3^+); δ 3.89 ppm (t, 8H, —CH_2—OH).

2.2.3. Kinetic measurements

PLD-catalyzed reactions were performed in an aqueous-organic two-phase system according to Hirche and Ulbrich-Hofmann (2000). 620 μl of substrate solution (1.24 μmol PC, 1,2-dioleoyl-*sn*-glycero-3-phosphoglycerol or 1,2-dioleoyl-*sn*-glycero-3-phosphoethylene glycol in diethyl ether) were mixed with 80 μl of enzyme solution (61.6 mU PLD in 300 mM sodium acetate buffer, 120 mM $CaCl_2$, pH 5.6). In the reactions containing PC, 96 μmol of acceptor alcohol was added to the aqueous phase. Before usage, stock solutions of amino alcohols were adjusted to pH 5.6 by addition of HCl. Reaction mixtures were shaken (400 rpm) in HPTLC screw flasks (Roth, Germany) at 30 °C. After a distinct reaction time (0–17 h), samples (25 μl) were taken from the organic phase which exclusively contained the phospholipids. The solvent was removed by air-drying and the residuals were re-dissolved in 25 μl of toluene, applied to silica 60 plates (Merck, Germany) and analyzed by HPTLC according to Dippe et al. (2008). After staining of the phospholipids with $CuSO_4/H_3PO_4$ (Touchstone et al., 1983), the resulting bands were evaluated by densitometry (densitometer CD60, Desaga, Germany). The concentrations of the individual phospholipids, which were identified according to their R_f values (Dippe et al., 2008), were calculated using standards of the corresponding PC, PA, PG, PE (for phospholipids having

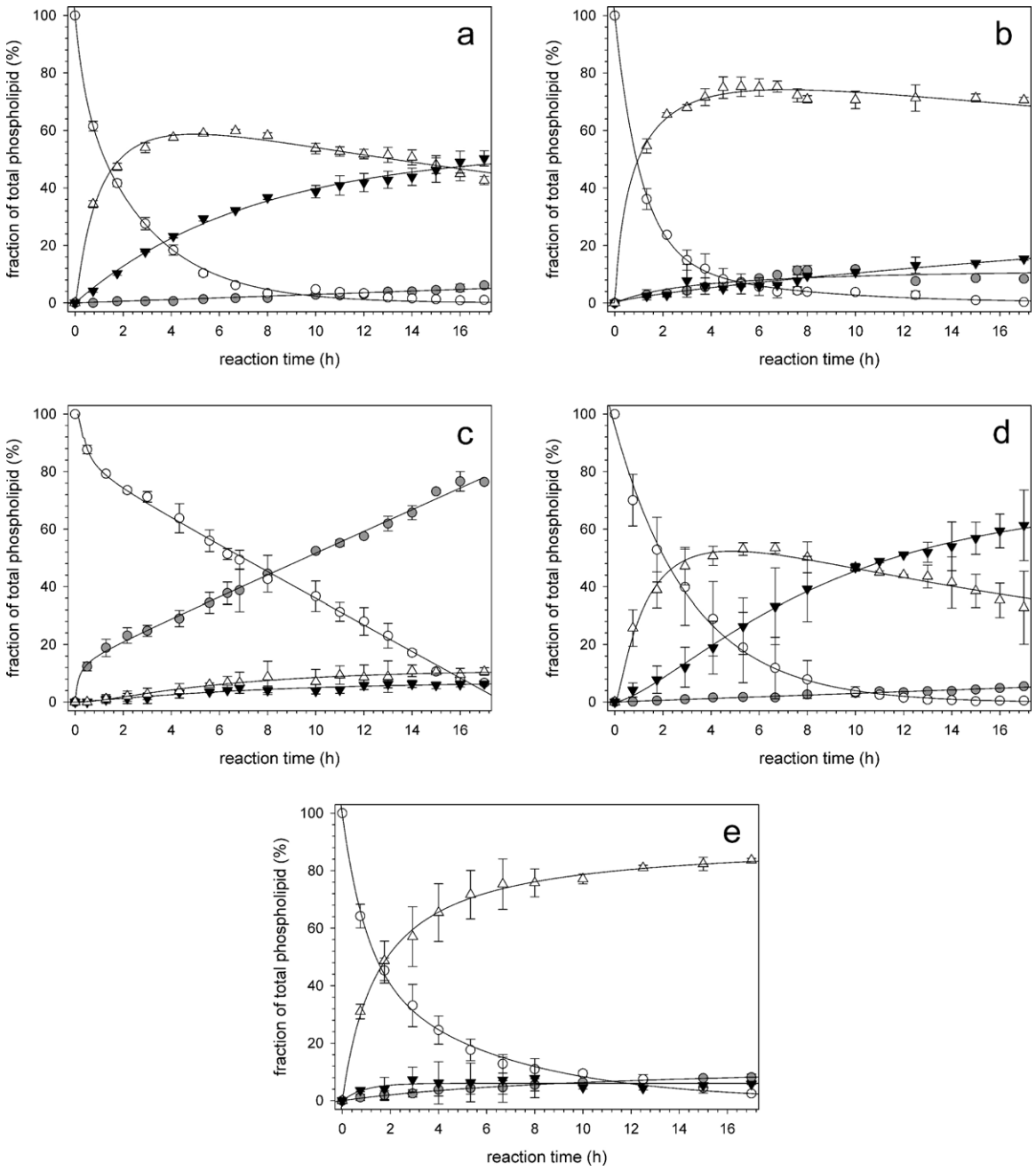

Fig. 2. Conversion of PC with ethanolamine derivatives by PLD-mediated transphosphatidylation. The reactions were performed in a two-phase system consisting of diethylether and acetate buffer, pH 5.6, in the presence of (a) diethanolamine, (b) triethanolamine, (c) THA, (d) serinol, or (e) Tris as described in Section 2.2.3. Transphosphatidylation of PC (○) to mono- (△) and diphosphatidyl products (▼) competes with the hydrolysis to PA (●).

ethanolamine-derived head groups), and CL. The presented data are the means resulting from two independent experiments.

2.2.4. Preparative phospholipid transformation

Phospholipid conversions were performed similar to the kinetic experiments (Section 2.2.3) but in a larger scale. The reaction mixtures contained 28.3 mg (37.2 μmol) of PC in 18.6 ml of diethyl ether and 2.4 ml of enzyme solution (148 mU PLD in 300 mM sodium acetate buffer, pH 5.6 containing 120 mM $CaCl_2$ and 2.88 mmol acceptor alcohol). After shaking (400 rpm) for 17 h at 30 °C, the organic phase was separated and washed with 5 ml of 1 mM ethylendiaminetetraacetic acid (adjusted with NaOH to pH 8.0) and water, respectively. The solvent was removed by rotary evaporation and the residual was re-dissolved in 5 ml of $CHCl_3$. For separation of phospholipids, 1 ml of the chloroform solution was applied to a silica HPLC column (Lura Si 100, 100 μm, 4.3 × 250 mm) (Phenomenex, USA). The stepwise elution of serinol-containing transphosphatidylation products was achieved by a linear gradient of $CHCl_3/CH_3OH/H_2O$ (7:4:1, v/v/v) at a flow rate of 1 ml min^{-1}, whereas for phospholipids having diethanolamine head groups $CHCl_3/CH_3OH$/25% NH_3 (7:4:0.05, v/v/v) was used. Phospholipid-containing fractions were identified by a light scattering detector Sedex 45 (Sedere, France).

2.2.5. Mass spectrometry

The positive ion high resolution ESI mass spectra were obtained from a Bruker Apex III Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer (Bruker Daltonics, Billerica, USA)

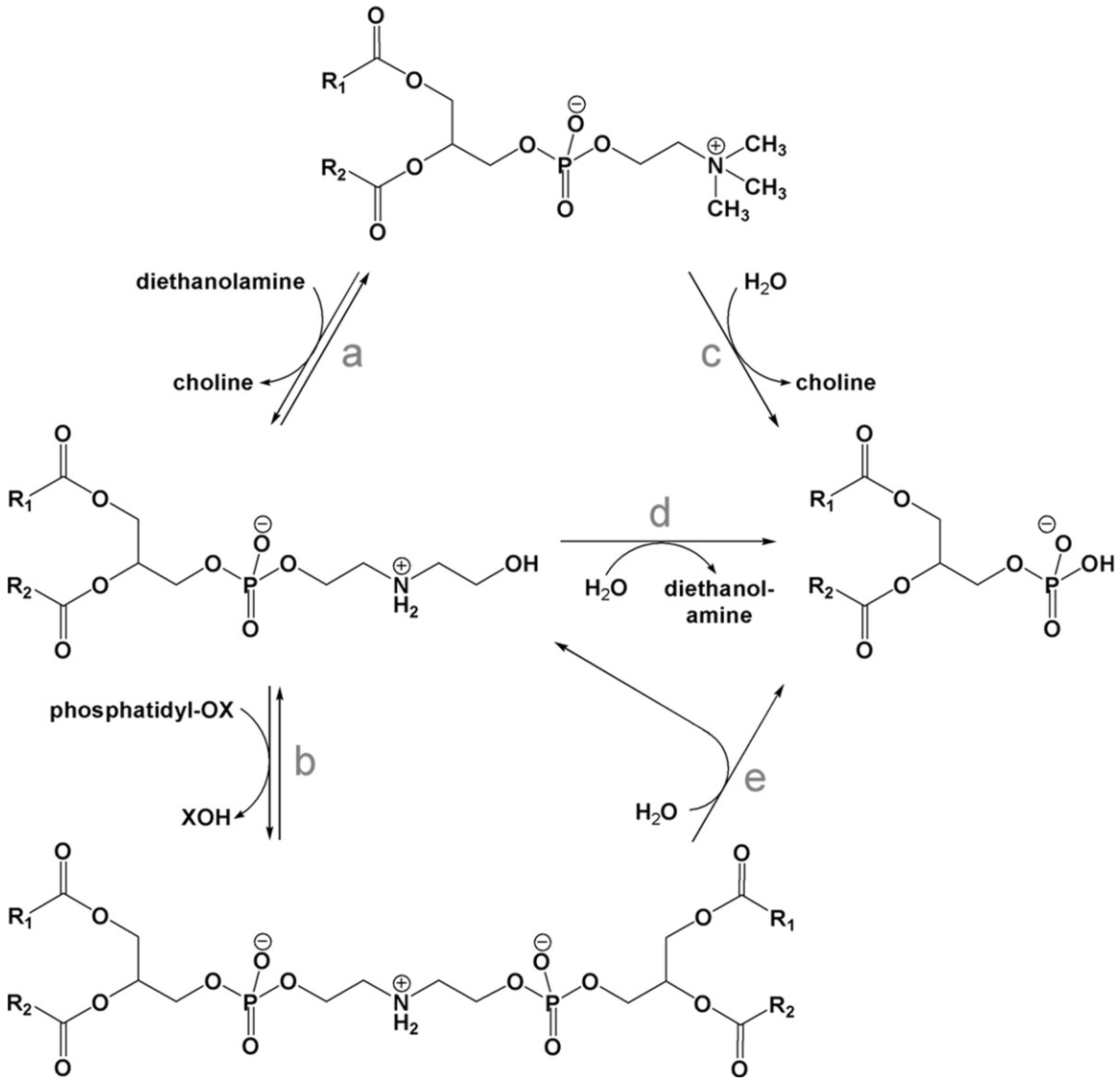

Fig. 3. Scheme of the PLD-catalyzed reaction of PC with diethanolamine. In a first transphosphatidylation step (a), PC is converted to phosphatidyldiethanolamine, which is subsequently transformed to its CL analog by reaction with a phosphatidyl donor (X = choline or diethanolamine moiety) (b). Hydrolytic degradation of PC (c) and both transphosphatidylation products (d and e) yields PA.

equipped with an infinity cell, a 7.0 T superconducting magnet (Bruker), an RF-only hexapole ion guide and an external electron spray ion source (Agilent). Nitrogen was used as drying gas at 150 °C. The sample solutions (in CH_3OH) were introduced continuously via a syringe pump with a flow rate of 120 μl h^{-1}. The data were evaluated by the Bruker XMASS 7.0.8 software.

The following molecular masses were found (the theoretical values are given in parentheses):

- di(1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho)serinol: *m*/*z* 1405.0277 $[M+H]^+$, (1405.0268, calculated for $[C_{77}H_{148}NO_{16}P_2]^+$);
- di(1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho)diethanolamine: *m*/*z* 1419.0429 $[M+H]^+$, (1419.0424, calculated for $[C_{78}H_{150}NO_{16}P_2]^+$); and
- 1,2-dioleoyl-*sn*-glycero-3-phosphoethylene glycol *m*/*z* 743.5226 $[M-H]^-$, (743.5232, calculated for $[C_{41}H_{76}O_9P]^-$).

2.2.6. ^1H NMR spectroscopy

Spectra of the phospholipid solutions (2 mM in CD_3Cl/CD_3OD (7:4, v/v)) were recorded at 300 K on a Bruker Avance 400 spectrometer (Rheinstetten, Germany). Chemical shifts were referred to internal tetramethylsilane at 0 ppm. Standard methods were used to perform 1D and 2D experiments, pulse programs being taken from the Bruker Software Library. Resonance assignments were made by combined analysis of H,H-COSY and TOCSY spectra.

2.2.6.1. Di(1-palmitoyl-2-oleoyl-sn-glycero-3-phospho)serinol. δ 0.89 ppm (t, ^3J = 6.7 Hz, 12H, CH_3—); δ 1.28 ppm (br m, 80H, —$(CH_2)_n$—); δ 1.61 ppm (br m, 8 H, —CH_2—acyl C3); δ 2.03 ppm (br m, 8H, —CH_2CH=$CHCH_2$—); δ 1.33 ppm (br, 8H, —CH_2CH_2CH=$CHCH_2CH_2$—); δ 2.33 ppm (q, ^3J = 7.2 Hz, 8H, —CH_2—acyl C2); δ 3.99 ppm (br, 4H, —CH_2OR—CHOR—CH_2—O—PO_2—); δ 4.18 ppm (*AB*X, m, 2H,–CH_AH_BOR–CHOR–CH_2–O–PO_2–); δ 4.42 ppm (*AB*X, dd, ^3J = 12.1 Hz, ^2J = 2.5 Hz, 2H, —CH_AH_BOR—CHOR—CH_2—O—PO_2—); δ 5.16 ppm (br, 2H, —CH_2OR—*CH*OR—CH_2—O—PO_2—); δ 5.35 ppm (m, 4H, —*CH*=*CH*—); δ 3.99 ppm (br, 4H, —PO_2—O—CH_2—); δ 3.35 ppm (buried by CD_3OD, 1H, —PO_2—O—CH_2—*CH*(NH_3)—).

2.2.6.2. Di(1-palmitoyl-2-oleoyl-sn-glycero-3-phospho) diethanolamine. δ 0.86 ppm (br m, 12H, CH_3—); δ 1.27 ppm (br m, 80H, —$(CH_2)_n$—); δ 1.61 ppm (br m, 8 H, —CH_2—acyl C3); δ 2.03 ppm (br m, 8H, —CH_2CH=$CHCH_2$—); δ 1.32 ppm (br, 8H,

$-CH_2CH_2CH{=}CHCH_2CH_2-$); δ 2.32 ppm (m, 8H, $-CH_2-$acyl C2); δ 4.01 ppm (t, ^3J = 6.3 Hz, 4H, $-CH_2OR-CHOR-CH_2-O-PO_2-$); δ 4.20 ppm (m, 2H, $-CH_AH_BOR-CHOR-CH_2-O-PO_2-$); δ 4.39 ppm (buried by H_2O, 2H, $-CH_AH_BOR-CHOR-CH_2-O-PO_2-$); δ 5.24 ppm (br m, 2H, $-CH_2OR-CHOR-CH_2-O-PO_2-$); δ 5.35 ppm (m, 4H, $-CH{=}CH-$); δ 4.20 and 4.15 ppm (dm, 2H, $-PO_2-O-CH_2-$); δ 3.23 ppm (br m, 2H, $-PO_2-O-CH_2-CH_2-NH_2-$); δ 3.81 ppm (m, 2H, $-NH_2-CH_2-CH_2-O-PO_2-$); δ 3.13 and 3.01 ppm (dm, 2H, $-NH_2-CH_2-CH_2-O-PO_2-$).

3. Results and discussion

3.1. PLD-mediated formation of CL analogs from ethanolamine derivatives

To examine the production of CL-related phospholipids from PC, transphosphatidylation reactions were performed with PLD from *Streptomyces* sp. in the biphasic reaction system acetate buffer–diethyl ether. As acceptor alcohols, compounds deriving from ethanolamine by substitution of one, two or three amine hydrogen atoms by hydroxyethyl groups (Fig. 1a–c) or of one or two hydrogen atoms at the C2 position by hydroxymethyl groups (Fig. 1d and e) were used.

The time course of the reactions is shown in Fig. 2. In the reaction with diethanolamine (Fig. 2a), a fast accumulation of phosphatidyldiethanolamine was observed in the initial period of the reaction. As depicted in the reaction scheme (Fig. 3), this phospholipid is produced by transphosphatidylation of PC. Due to a free hydroxyl function in its head group, phosphatidyldiethanolamine is able to participate in a subsequent step of transphosphatidylation yielding the corresponding symmetric diphosphatidyl compound. Thereby, PC or a second phosphatidyldiethanolamine molecule was used as donor for the transferred phosphatidyl moiety. Thus, the formation of the CL analog is limited by the concentration of the monophosphatidyl compound, which is in accordance with its slow increase (Fig. 2a). After 17 h reaction time, 50% diphosphatidyldiethanolamine were formed. In contrast to this strong preference for transphosphatidylation, the hydrolytic production of PA from PC or the transphoshatidylation products (Fig. 3) is marginal (Fig. 2a).

Similar to diethanolamine, the more bulky triethanolamine is rapidly transformed into its monophosphatidyl product (Fig. 2b). However, the generation of the CL analog was comparably slow. Since only 15% diphosphatidyl product was reached after 17 h, a prolonged reaction time seems to be required to reach a maximum product yield. The differences in the reaction rates may be related to the different molecular volume of both head group alcohols introduced into the phospholipid structure. This assumption is supported by a similar trend observed for the C2-substituted ethanolamines (Fig. 2d and e), where the small-sized serinol is preferred compared to the highly branched homologue Tris. In the reaction with serinol, the yield of the diphosphatidyl product was remarkably high, whereas the concentration of the corresponding CL analog of Tris reached only 6% in the same reaction time. That means that PLD from *Streptomyces* sp. efficiently catalyzes the transfer of a single phosphatidyl moiety to all of the acceptor alcohols mentioned above (Fig. 2; Dippe et al., 2008), but the modification of the introduced head group by a second phosphatidate seems to be strongly size-controlled. Obviously, this difference results from increased steric requirements of the phospholipid acceptor limiting the access to the active site.

In contrast to the acceptor alcohols of the reactions mentioned above (Fig. 2a, b, d, and e), THA (Fig. 1c) does not allow any significant transphosphatidylation (Fig. 2c). The high molecular volume of THA (195.3 Å^3, http://www.molinspiration.com) even impairs the initial generation of phosphatidyl-THA. As indicated by the strongly enhanced formation of PA (Fig. 2c), PC is mainly converted by hydrolysis.

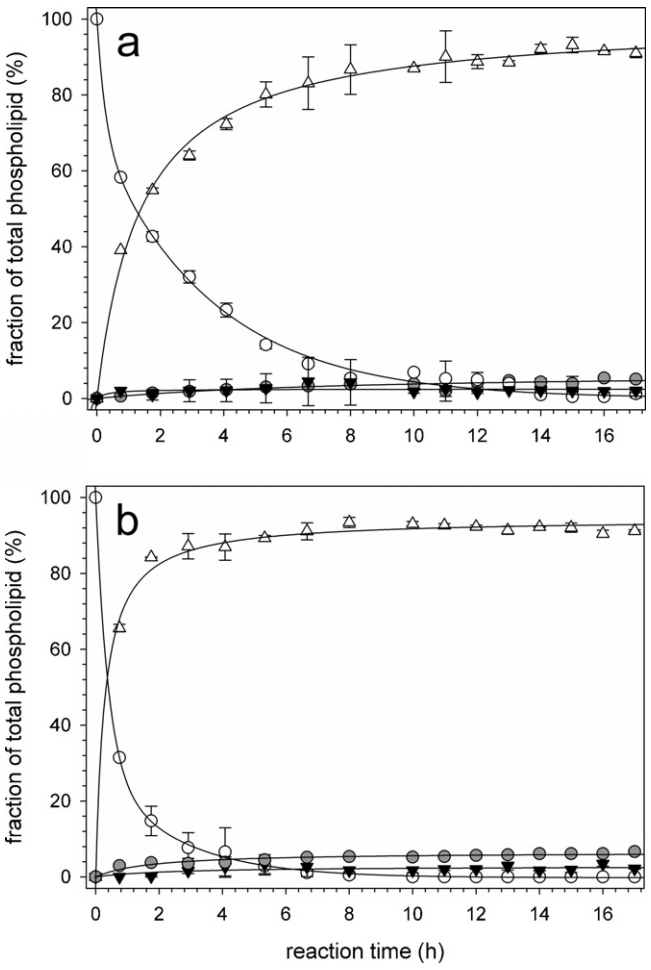

Fig. 4. Conversion of PC with glycerol (a) and ethylene glycol (b) by PLD-mediated transphosphatidylation. The reactions were performed in a two-phase system consisting of diethylether and acetate buffer, pH 5.6, as described in Section 2.2.3. Transphosphatidylation of PC (○) to mono- (△) and diphosphatidyl products (▼) competes with the hydrolysis to PA (●).

3.2. PLD-mediated formation of CL and its analog from ethylene glycol

Beside the ethanolamine derivatives described in Section 3.1, glycerol and ethylene glycol were used for the transphosphatidylation of PC. Glycerol is the central part of the natural CL and both alcohols are – in contrast to the ethanolamine derivatives – uncharged. Surprisingly, in the reaction with glycerol only small amounts of CL were formed (Fig. 4a), although the molecular volume of glycerol (87.1 Å^3, http://www.molinspiration.com) is very similar to that of serinol (91.2 Å^3) which strongly promotes the production of its CL analog (Fig. 2d). Similar results were obtained for the even less voluminous ethylene glycol (Fig. 4b). Therefore, the lack of a positive charge in the head group of PG or phosphatidylethylene glycol seems to be restrictive for the subsequent transphosphatidylation step leading to CL. Obviously, beside its size, also the charge of the head group which serves as nucleophile is a significant factor in the transformation of phospholipids to CL analogs, whereas this charge is not essential in the formation of the monophosphatidyl derivative where 91% of products are reached (Fig. 4a and b).

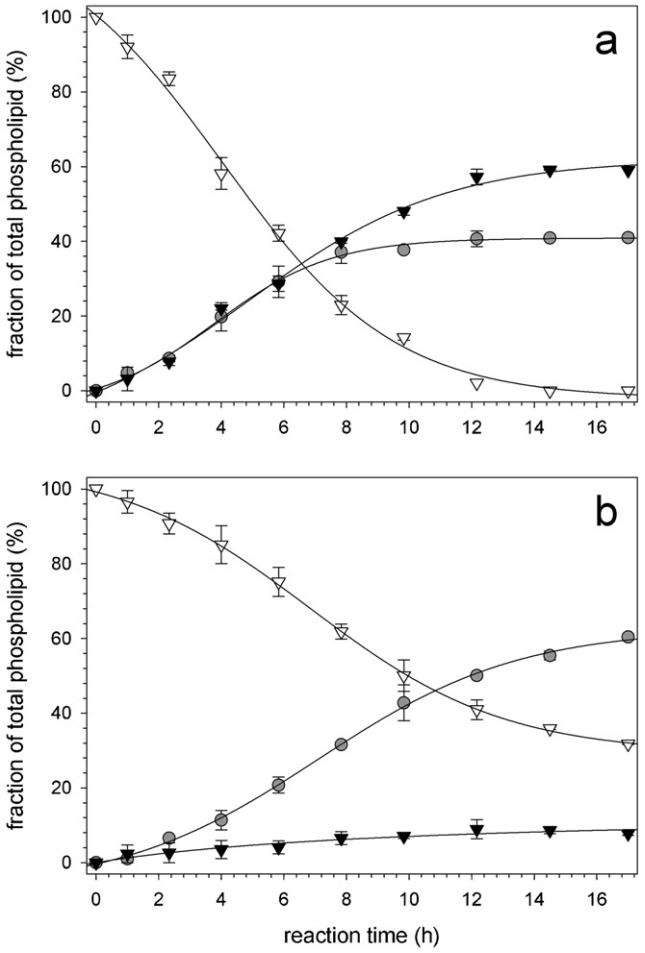

Fig. 5. PLD-catalyzed formation of CL and its analog of ethylene glycol from monophosphatidyl compounds. PG (a) and phosphatidylethylene glycol (b) were transformed in a two-phase system consisting of diethylether and acetate buffer, pH 5.6, in the absence of any acceptor alcohol as described in Section 2.2.3. The interconversion of the monophosphatidyl compound (▽) to CL or the corresponding CL analog (▼) competes with the hydrolytic formation of PA (●).

In contrast, the formation of CL is much more successful when starting from pure PG in the absence of PC and glycerol (Fig. 5a). Under these conditions PG was completely converted into CL (59%) and PA (41%) after 17 h. Surprisingly, however, the transformation of phosphatidylethylene glycol into its CL analog (Fig. 5b) is less favorable. This substrate is mainly converted by hydrolysis, probably because of the close vicinity of the free hydroxyl group to the phosphodiester bond.

3.3. Preparative synthesis of diphosphatidyldiethanolamine and -serinol

The two CL analogs diphosphatidyldiethanolamine and -serinol, which were shown to be produced with high yields (Section 3.1), were prepared from 28.3 mg (37.2 μmol) of PC and purified by HPLC on silica. Analogous to the kinetic experiments (Fig. 2a and d), 57% and 62% of the total PC were transformed into diphosphatidyldiethanolamine and -serinol after 17 h of reaction time. After product purification, 2.6 mg (1.8 μmol) of di(1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho)diethanolamine and 4.3 mg (3.1 μmol) of di(1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho)serinol could be recovered, corresponding to final yields of 10% and 17%. The substances were homogeneous in HPTLC performed as described in Section 2.2.3. The molecular masses determined by high-resolution mass spectrometry were in accordance with the theoretical masses. The experimental data of the ^{1}H NMR spectroscopy confirmed the identity of the compounds.

4. Conclusions

In addition to its ability to exchange the head group alcohol, bacterial PLD is able to catalyze the conversion of phospholipids with polyhydric head groups into diphosphatidyl compounds. This enzymatic two-step reaction allows the synthesis of new CL analogs bearing head groups with positive charge and different volume which are scarcely accessible by chemical synthesis. However, product yields are strongly dependent on both the molecular volume and the charge of the used acceptor alcohol. Diphosphatidyldiethanolamine and -serinol are two newly described compounds that can be easily produced in significant yields. In contrast to the initial transphosphatidylation of PC yielding the monophosphatidyl compound, the subsequent transfer of a second phosphatidyl moiety is hindered if the first attached head group alcohol is sterically challenging or uncharged.

Acknowledgements

We thank the LIPOID GmbH (Ludwigshafen, Germany) for the gift of phospholipids and Christa Kuplens and Katja Fröhlich for their excellent technical assistance. The financial support by the State of Sachsen-Anhalt and by the Graduiertenkolleg 1026 (Deutsche Forschungsgemeinschaft, Bonn, Germany) is gratefully acknowledged.

References

Arias-Cartin, R., Grimaldi, S., Arnoux, P., Guigliarelli, B., Magalon, A., 2012. Cardiolipin binding in bacterial respiratory complexes: structural and functional implications. Biochim Biophys Acta 181, 1937–1949.
D'Arrigo, P., Piergianni, V., Scarcelli, D., Servi, S., 1995. A spectrophotometric assay for phospholipase D. Analytica Chimica Acta 304, 249–254.
D'Arrigo, P., de Ferra, L., Pedrocchi-Fantoni, G., Scarcelli, D., Servi, S., Strini, A., 1996. Enzyme-mediated synthesis of two diastereomeric forms of phosphatidylglycerol and diphosphatidylglycerol (cardiolipin). Journal of the Chemical Society, Perkin Transactions 1, 2657–2660.
Dippe, M., Mrestani-Klaus, C., Schierhorn, A., Ulbrich-Hofmann, R., 2008. Phospholipase D-catalyzed synthesis of new phospholipids with polar head groups. Chemistry and Physics of Lipids 152, 71–77.
Gohil, V.M., Greenberg, M.L., 2009. Mitochondrial membrane biogenesis: phospholipids and proteins go hand in hand. Journal of Cell Biology 184, 469–472.
Haines, T.H., Dencher, N.A., 2002. Cardiolipin: a proton trap for oxidative phosphorylation. FEBS Letters 528, 35–39.
Hirche, F., Ulbrich-Hofmann, R., 2000. The interdependence of solvent, acceptor alcohol and enzyme source in transphosphatidylation by phospholipase D. Biocatalysis and Biotransformation 18, 343–353.
Joshi, A.S., Zhou, J., Gohil, V.M., Chen, S., Greenberg, M.L., 2009. Cellular functions of cardiolipin in yeast. Biochimica et Biophysica Acta 1793, 212–218.
Khalifat, N., Puff, N., Bonneau, S., Fournier, J.B., Angelova, M.I., 2008. Membrane deformation under local pH gradient: mimicking mitochondrial cristae dynamics. Biophysical Journal 95, 4924–4933.
Klingenberg, M., 2009. Cardiolipin and mitochondrial carriers. Biochimica et Biophysica Acta 1788, 2048–2058.
Lange, C., Nett, J.H., Trumpower, B.L., Hunte, C., 2001. Specific roles of protein–phospholipid interactions in the yeast cytochrome bc_1 complex structure. EMBO Journal 20, 6591–6600.
Lewis, R.N., McElhaney, R.N., 2009. The physicochemical properties of cardiolipin bilayers and cardiolipin-containing lipid membranes. Biochimica et Biophysica Acta 1788, 2069–2079.
Malavia, N.K., Zurakowski, D., Schroeder, A., Princiotto, A.M., Laury, A.R., Barash, H.E., Sodroski, J., Langer, R., Madani, N., Kohane, D.S., 2011. Liposomes for HIV prophylaxis. Biomaterials 32, 8663–8668.
Paltauf, F., Hermetter, A., 1994. Strategies for the synthesis of glycerophospholipids. Progress in Lipid Research 33, 239–328.
Piazza, G.J., Marmer, W.N., 2007. Conversion of phosphatidylcholine to phosphatidylglycerol with phospholipase D and glycerol. Journal of the American Oil Chemists Society 84, 645–651.

Resina, S., Prevot, P., Thierry, A.R., 2009. Physico-chemical characteristics of lipoplexes influence cell uptake mechanisms and transfection efficacy. PLoS ONE 4, e6058.
Schlame, M., 2008. Cardiolipin synthesis for the assembly of bacterial and mitochondrial membranes. Journal of Lipid Research 49, 1607–1620.
Touchstone, J.C., Levin, S.S., Dobbins, M.F., Beers, P.C., 1983. Analysis of saturated and unsaturated phospholipids in biological fluids. Journal of Liquid Chromatography 6, 179–192.
Ulbrich-Hofmann, R., Lerchner, A., Oblozinsky, M., Bezakova, L., 2005. Phospholipase D and its application in biocatalysis. Biotechnology Letters 27, 535–544.
Wright, M.M., Howe, A.G., Zaremberg, V., 2004. Cell membranes and apoptosis: role of cardiolipin, phosphatidylcholine, and anticancer lipid analogues. Biochemistry and Cell Biology 82, 18–26.
Zhang, Z.Y., Ugwu, S., Zhang, A., Ahmad, M.U., Ahmad, I., Chiu, S., Lee, R.L., 2006. A novel cationic cardiolipin analogue for gene delivery. Pharmazie 61, 10–14.