Appl Biochem Biotechnol (2011) 165:1494–1506
DOI 10.1007/s12010-011-9370-4

Large-Scale Production of Phospholipase D from *Streptomyces racemochromogenes* and Its Application to Soybean Lecithin Modification

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Received: 22 April 2011 / Accepted: 2 September 2011 /
Published online: 21 September 2011

Abstract Phospholipase D (PLD) catalyzes transphosphatidylation, causing interconversion of the polar head group of phospholipids and phospholipid hydrolysis. Previously, we cloned PLD103, a PLD with high transphosphatidylation activity, from *Streptomyces racemochromogenes* strain 10-3. Here, we report the construction of an expression system for the PLD103 gene using *Streptomyces lividans* as the host bacterium to achieve large-scale production. The phosphatidylcholine (PC) hydrolysis activity of *S. lividans* transformed with the expression plasmid containing the PLD103 gene was approximately 90-fold higher than that of the original strain. The recombinant PLD103 (rPLD103) found in the supernatant of the transformant culture medium was close to homogeneous. The rPLD103 was indistinguishable from the native enzyme in molecular mass and enzymatic properties. Additionally, rPLD103 had high transphosphatidylation activity on PC as a substrate in a simple aqueous one-phase reaction system and was able to modify the phospholipid content of soybean lecithin. Consequently, the expression system produces a stable supply of PLD, which can then be used in the production of phosphatidyl derivatives from lecithin.

Keywords Phospholipase D · Lecithin · Phospholipid · Soybean · Transphosphatidylation · Phospholipid modification · Oil manufacture · Phosphatidylcholine · Phosphatidylserine · Phosphatidylglycerol

Electronic supplementary material The online version of this article (doi:10.1007/s12010-011-9370-4) contains supplementary material, which is available to authorized users.

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Introduction

Lecithin, a by-product of the process of obtaining edible oils and fats from vegetable oils and animal fats, is defined as a mixture of phospholipids (PLs) with additional minor components. The PL content of lecithin is predominantly phosphatidylcholine (PC), with smaller amounts of phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylserine (PS), and phosphatidylinositol. Owing to their interfacial activity, emulsification properties, and ability to form liposomes, lecithin-derived PLs are included in food products, cosmetics, and pharmaceuticals as an emulsifier, stabilizer, and antioxidant [1–5]. Of the minor PL constituents of lecithin, PG provides emulsification stability to oil-in-water emulsions over a wide range of temperatures [6]. Additionally, it has been reported that PG protects human retinal pigment epithelial cells against *N*-retinyl-*N*-retinylidene ethanolamine-induced apoptosis, which is suspected to cause age-related macular degeneration [7]. PS, which is found in animal brains, has been suggested as a food supplement to decrease and/or prevent senile dementia [8]. A series of studies indicated that PS may be a valuable food supplement for athletes; PS has been demonstrated to hasten recovery, prevent muscle soreness, and decrease circulating cortisol concentrations during and after exercise, including cycling, weight training, and endurance running [9–11].

Phospholipase D (PLD; E.C.3.1.4.4), which is widely distributed in bacteria, fungi, plants, and animals, catalyzes the hydrolysis of PLs, releasing phosphatidic acid (PA) and the alcohol moiety. In addition to its hydrolytic activity, PLD catalyzes transphosphatidylation. In this reaction, polar head modified phosphatidyl alcohols are synthesized from the phosphatidyl moiety donor PL in the presence of alcohol [12] (Fig. 1). Thus, PLD is of interest to applied enzymological approaches utilizing the synthesis of phosphatidyl derivatives for economic benefit, as well as in the basic research. The reactions of PLD occur with different degrees of selectivity between hydrolysis and transphosphatidylation activity, depending on the source of the enzyme. In addition, PLD can competitively react with newly synthesized phosphatidic derivatives as they hydrolyze the de novo products, preventing the synthesis of the transphosphatidylation products. To reduce hydrolysis by PLD in the transphosphatidylation reaction, the reactions are often carried out in a biphasic system consisting of an aqueous solution of PLD and an organic solution of the PL substrate to avoid the exposure of the de novo PLs to PLD [13]. However, the biphasic

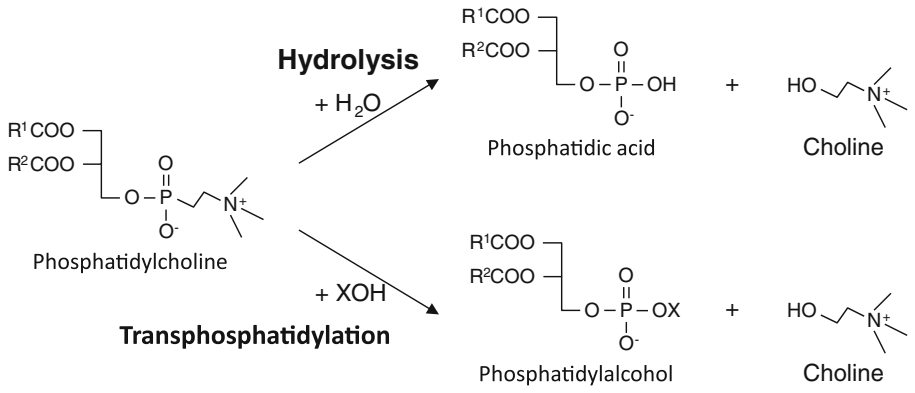

Fig. 1 Reaction scheme for the hydrolysis and transphosphatidylation of phosphatidylcholine by phospholipase D

system requires enormous quantities of organic solvents, often including toxic compounds unavailable in the food-industrial, cosmetic, and medical fields.

Recently, we isolated and cloned the PLD (PLD103) native to *Streptomyces racemochromogenes* strain 10-3 [14–16]. This PLD has PG-, PS-, and PE-producing transphosphatidylation activity with a high conversion rate of 82–97% in a simple one-phase system, where the PC was dispersed in an aqueous solution in the presence of a small amount of detergent. The synthesis of transphosphatidylation products by PLD103 in the one-phase system was comparable to that of other *Streptomyces* PLDs in the biphasic system. Besides the advantage that the reaction system using PLD103 can avoid the usage of organic solvent, *Streptomyces* strains have been used as a source of natural antibiotics for many years, and *Streptomyces* enzymes, such as transglutaminase and glucose isomerase, are already utilized in food-industrial field [17, 18]. Therefore, the PLD103 would be useful for food, cosmetic, and pharmaceutical fields. A strain of *Streptomyces* species *Streptomyces lividans* is generally recognized as safe and has been widely used for the eukaryotic and prokaryotic protein productions [19–21]. In the current study, we constructed an overexpression system of PLD103 in *S. lividans* to achieve large-scale production of the enzyme.

Materials and Methods

Materials

The *Streptomyces* high-copy number replicative cloning vector pIJ702 [22] and its host strain *S. lividans* TK23 [23] were kindly provided by Dr. Tomohisa Kuzuyama of the Biotechnology Research Center, the University of Tokyo, Tokyo, Japan. Egg yolk lecithin (PC-98N, PC content more than 98%) was a gift from Kewpie Fine Chemicals (Tokyo, Japan). Fractionated soybean lecithin (SLP-PC70; PC content more than 70%) was purchased from the NOF Corporation (Tokyo, Japan). Other PLs were purchased from Avanti Polar Lipids (Alabaster, AL, USA).

Construction of Expression Plasmids in *S. lividans*

To amplify the full-length *pld103* gene, a primer set of *Bam*HI/10-3/preF (3′-ATGCGGATCCGCGGAGGTGTGGTCGTACGAGGACA-5′) and *Bam*HI/10-3/postR (3′-ATGCGGATCCGCAGTCTCTCGATCAGAGTTCCACACC-5′) was used. These primers were designed using the nucleotide sequences of the 5′- and the 3′-flanking regions of the open reading frame of the *pld103* gene and contain *Bam*HI-digested sites (indicated by underlined nucleotides) at their 5′-ends. The 2.6-kbp product was digested with the *Bam*HI restriction enzyme and ligated into the *Bam*HI-digested site of pUC118 (Takara Bio, Ohtsu, Japan) to generate pld103/pUC118; the nucleotide sequence of the insert was then determined. The *Escherichia coli* and *S. lividans* shuttle vector (pES) was constructed by ligation of the pUC and pIJ cassettes as summarized in Fig. 2. The pUC cassette carrying a replication site (*ColE1*) and the ampicillin-resistance gene *bla* was amplified from the pUC19 vector (Takara Bio) by PCR using the *Bgl*II/pUC/bla/Fw (3′-ATGCAGATCTGACGAAAGGGCCTCGTGATACGC5′) and *Kpn*I/pUC/ori/Rv primers (3′-ATGCGGTACCGGCGCTCTTCCGCTTCCTCG-5′), which contain a *Bgl*II or *Kpn*I restriction site (indicated by underlined nucleotides), respectively, at the 5′-end. The pIJ cassette carrying the replication sites *rep* and *ori* and the thiostrepton-resistance gene (*tsr*) was amplified

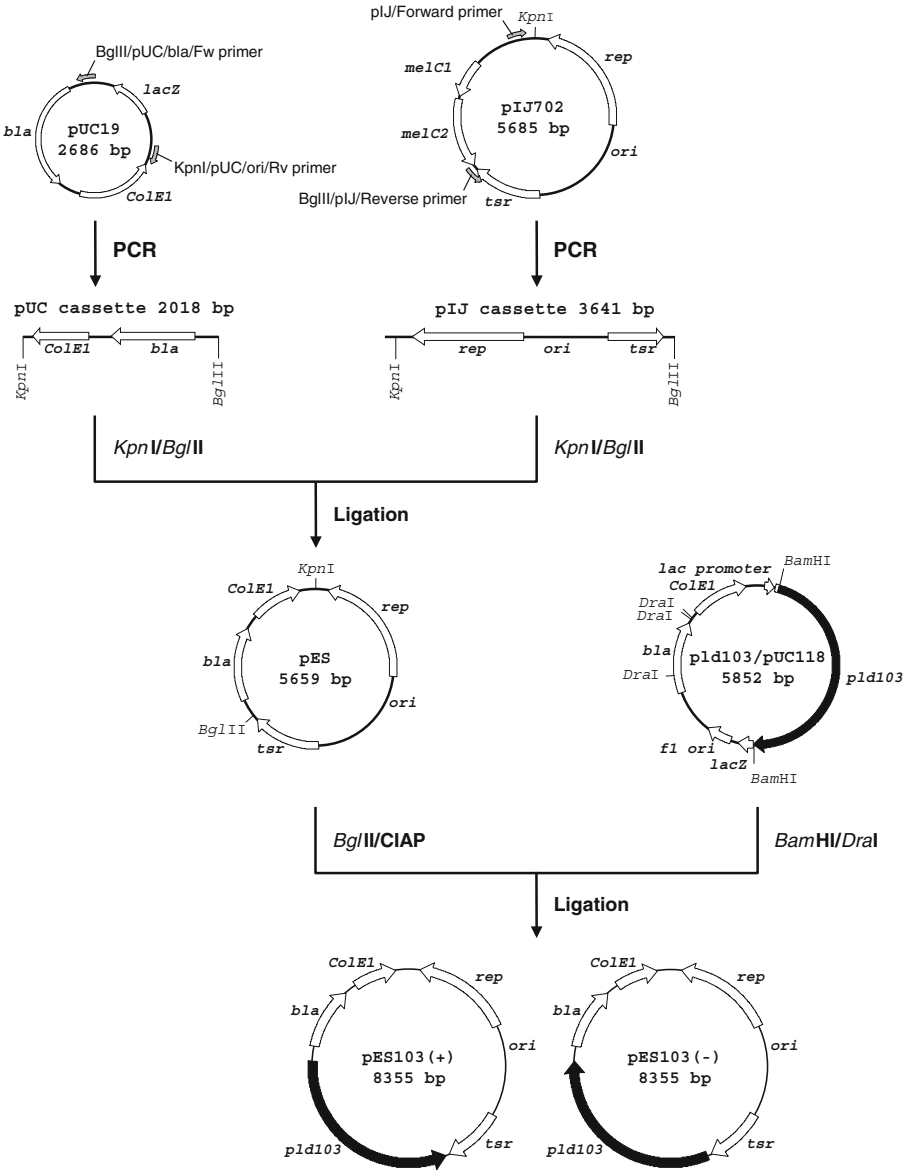

Fig. 2 Construction of expression plasmids pES103(+) and pES103(−). Strategies of plasmid construction are described in the "Materials and Methods" section

from pIJ702 by PCR using pIJ/forward (3′-AATGGAAGGCTGCTGCATTTCGT CACGTGA-5′) and *Bgl*II/pIJ/reverse primers (TGAGGAGATCTTCACTGACGAATC GAGGTC). The *Bgl*II/pIJ/reverse primer contains a *Bgl*II-digested site at its 5′-end (indicated by underlined nucleotides). Each amplified DNA fragment was treated with *Bgl*II and *Kpn*I restriction enzymes, and the products were gel-purified. The 2.0-kbp fragment of the pUC cassette and the 3.7-kbp fragment of the pIJ cassette were ligated with T4 DNA ligase and transformed into *E. coli* DH5α (Nippon Gene, Tokyo, Japan). The bacterial cells

carrying pES were selected on LB plates containing 100 μg/ml ampicillin. The pld103/pUC118 plasmid was digested with *Bam*HI and *Dra*I, and the resulting 2.6-kbp *pld103* gene was ligated into the *Bgl*II site in the pES vector to yield pES103. The direction of the *pld103* gene in the pES103 vector was confirmed by colony PCR and subsequent cycle sequencing with the primers pUC/bla/R (3′-GCGCACATTTCCCCGAAAAGTGCC-5′), pIJ/tsr/Fw (3′-GAGGATCGACAGGAATCTCGCGG-5′), and 10-3/AS3 (3′-AGACCTGGCGCAGGGTCTGCTC-5′). The transformation of the host cell *S. lividans* TK23 with the pES103 vector was carried out as described by Kieser et al. [24]. *S. lividans* transformants were cultured at 28 °C in tryptic soy broth (TSB) medium (BD Difco, Franklin Lakes, NJ, USA) supplemented with 10 μg/ml thiostrepton (Sigma-Aldrich, St. Louis, MO, USA) on a reciprocal/orbital shaker.

Electrophoresis Analyses and N-Terminal Amino Acid Sequence Analysis

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed by the method of Laemmli [25] on a 12.5% polyacrylamide gel using a discontinuous buffer system. The molecular mass under denaturing conditions was determined using the LMW marker kit (GE Healthcare Bio-Science, Little Chalfont, Buckinghamshire, UK). The N-terminal amino acid sequence of the purified PLD protein was determined by the direct protein sequencing method described by Hirano and Watanabe [26] using a protein sequencer (model PPSQ-23A; Shimadzu, Kyoto, Japan) equipped with an online HPLC (model LC-10A; Shimadzu).

PLD Hydrolysis Assay

The PLD hydrolysis activity was assayed using PC-98N as the substrate by measuring the formation of choline with choline oxidase and peroxidase based on the method of [27], with some modifications [14]. One unit was defined as the amount of enzyme that liberates 1 μmol of choline per minute. To determine the pH optimum, PLD activity was assayed using reaction mixtures made with 20 mM of different buffers at different pH ranges (pH 4, 5, and 6=sodium acetate–acetic acid; pH 7, 7.5, and 8=Tris–HCl; pH 9 and 10=boric acid–NaOH). To determine the temperature optimum, the experiments were carried out at different temperatures. To determine the pH stability of PLD, the enzyme solution (5 U/ml) was incubated in 0.2 M buffer at different pH at 25 °C for 4 h, and the residual activity was determined. To determine the thermal stability of PLD, the PLD solution (2.5 U/ml) was incubated at different temperatures (20 °C, 30 °C, 37 °C, 40 °C, 50 °C, 60 °C, 70 °C, or 80 °C) for 10 min. After chilling on ice, residual activity was determined.

Transphosphatidylation Assay and Soybean Lecithin Modification

The transphosphatidylation activity of PLD was assayed as previously reported [14]. Six milliliters of a reaction mixture composed of 0.12 U of PLD enzyme, 0.1% (*w*/*v*) PC-98N, 4 mM sodium deoxycholate, 20 mM MOPS–NaOH buffer (pH 7.5) or 20 mM sodium acetate buffer (pH 5.5), 10 mM $CaCl_2$, and 20% (*w*/*v*) glycerol, L-serine, or 2-aminoethanol hydrochloride was incubated at 37 °C. One milliliter of the reaction mixture was transferred at intervals of 30–300 min into a test tube containing 0.2 ml of 0.1 M NaOH in order to terminate the enzyme reaction. For the soybean lecithin modification assay, 0.1% soybean lecithin was substituted for PC-98N. The PLs in the reaction mixture were extracted by the method of Brigh and Dyer [28]. The chloroform phase was

separated and evaporated, and the resultant lipid residue was dissolved in 100 μl of chloroform. Then, 10 μl of the PL solution was applied to a silica gel 60 thin-layer chromatography (TLC) plate (Merck, Darmstadt, Germany). The plate was developed with chloroform/methanol/acetic acid (40:15:6, *v*/*v*). Spots on the plate were visualized by iodine vapor. The intensities of the visualized spots were measured using Gel-Pro Analyzer software (version 3.1; Media Cybernetics, Silver Spring, MD, USA). The transphosphatidylation conversion rate (in percent) was defined as $[PX] \times 100/[PX] + [PC] + [PA]$, and the selectivity (in percent) was defined as $[PX] \times 100/[PX] + [PA]$, where PX is the transphosphatidylation product.

Results

Expression of rPLD103

For large-scale production of recombinant proteins, expression systems using *E. coli* as a host bacterium have been widely utilized and are available commercially. We attempted to use an *E. coli* system for recombinant PLD production. However, the recombinant PLD formed an inclusion body in the host cells, and we were unable to obtain an active form of the enzyme (Y. Nakazawa, unpublished data). For that reason, we chose to produce rPLD103 in *S. lividans* based on the recombinant *Streptoverticillium cinnamoneum* PLD production system developed by Ogino et al. [29], with some modifications. A 2,690-bp genomic DNA fragment of *S. racemochromogenes* strain 10-3 containing the *pld103* gene sequence and its putative promoter and terminator regions [16] were amplified by PCR using a gene-specific primer set. The PCR product was then ligated into the *E. coli* and *S. lividans* shuttle vector pES (Fig. 2). Two plasmids were generated, pES103(+) and pES103 (−), with the gene running in opposite directions. Each plasmid was transformed into the host bacteria *S. lividans* TK23. The presence of the plasmid in the host bacteria was confirmed by PCR using the shuttle vector-specific primer set. Production of the PLD103 enzyme was confirmed by assaying the PC-hydrolyzing activity of the bacterial culture supernatant. The time course of the secretion of PLD from the *S. lividans* transformants is shown in Fig. 3a, b. Both transformants harboring pES103(+) and pES103(−) had similar profiles, with the highest enzyme activity at 60 h post-initiation of the culture. At this point, both transformants produced approximately 30 U/ml of PLD, whereas wild-type *S. lividans* TK23 did not produce any PLD activity (Fig. 3c). Compared to the original PLD103 strain, *S. racemochromogenes* 10–3 (0.33 U/ml), the PLD activity of the transformants was approximately 90-fold higher.

As shown in Fig. 3d, the culture supernatant from the pES103(+) and pES103(−) transformants contained a single band with a molecular mass of 55 kDa observed by SDS–PAGE followed by Coomassie Brilliant Blue staining. This molecular mass is similar to that of the native PLD103 (nPLD103). The N-terminal amino acid sequence of both proteins was determined to be NH_2-ASPTPHLDSVEQTLRQVSPG, which exactly matches the nPLD103 sequence. Therefore, the proteins displayed as strong bands on the SDS–gel were identified as the recombinant PLD103 proteins. The PLD103 precursor possesses a potential signal cleavage site between Ala26-Ala27; the 26 N-terminal amino acid residues are removed post-translationally in the *S. racemochromogenes* strain 10-3 [16]. The N-terminal sequences of the two rPLD103 proteins indicate that post-translational cleavage of the precursor occurred in the same fashion as in the original *S. racemochromogenes* strain, thus producing active enzyme.

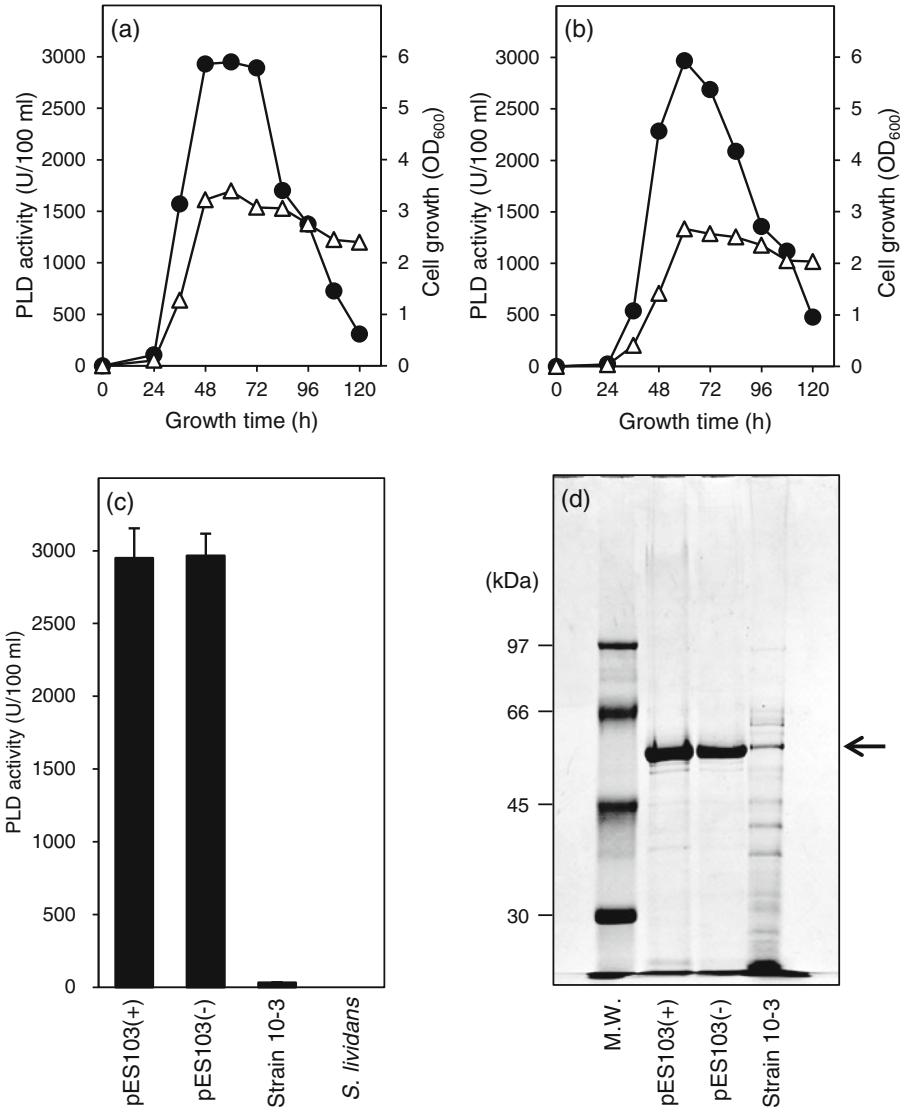

Fig. 3 Production of recombinant PLD103 in *S. lividans* TK23. Selective transformed *S. lividans* TK23 harboring pES103(+) or pES103(−) plasmid were cultured in 100 ml of TSB medium containing 10 μg/ml thiostrepton at 28 °C. **a**, **b** Time course of PLD production in *S. lividans* TK23 harboring the pES103(+) (**a**) and pES103(−) (**b**) plasmid. *Closed circles* indicate PLD activity, whereas the *open triangles* show cell growth (OD_{600}) **c** PLD activity of the culture supernatants of the *Streptomyces* strains. Phosphatidylcholine-hydrolytic activities in the culture supernatants of *S. lividans* TK23 harboring pES103(+) and pES103(−), *S. racemochromogenes* strain 10-3, and wild-type *S. lividans* TK23 were compared at 60 h. Experiments were repeated four times, and the *error bars* represent the standard deviation. **d** Electrophoretic analysis of the recombinant and the native PLD103. Culture supernatants of *S. lividans* TK23 harboring pES103(+) and pES103(−) and *S. racemochromogenes* strain 10–3 were analyzed by SDS–PAGE. The protein bands corresponding to the native or the recombinant PLD103 are indicated with an *arrowhead*. Each PLD was identified based on the N-terminal amino acid sequence

Enzymatic Properties of rPLD103 and Native PLD103

To compare the biochemical characterizations of the nPLD103 and the rPLD103, the rPLD103 protein was purified from the culture supernatant of the transformant harboring the plasmid pES103(−), as described in a previous study [16]. The optimum pH and temperature requirements for rPLD103 activity were determined to be 7.5 °C and 55 °C, respectively; these values are the same as for the native enzyme (Fig. 4). The PC hydrolysis activity of both the rPLD103 and the native enzyme is stable in the pH 4–9 range, and more than 75% of the original activity remained after a 4-h incubation. Both of the PLD enzymes remained active after a 10-min exposure to a 10–60 °C temperature range, displaying over 80% of the original activity. In a previous report [16], we found that the nPLD103

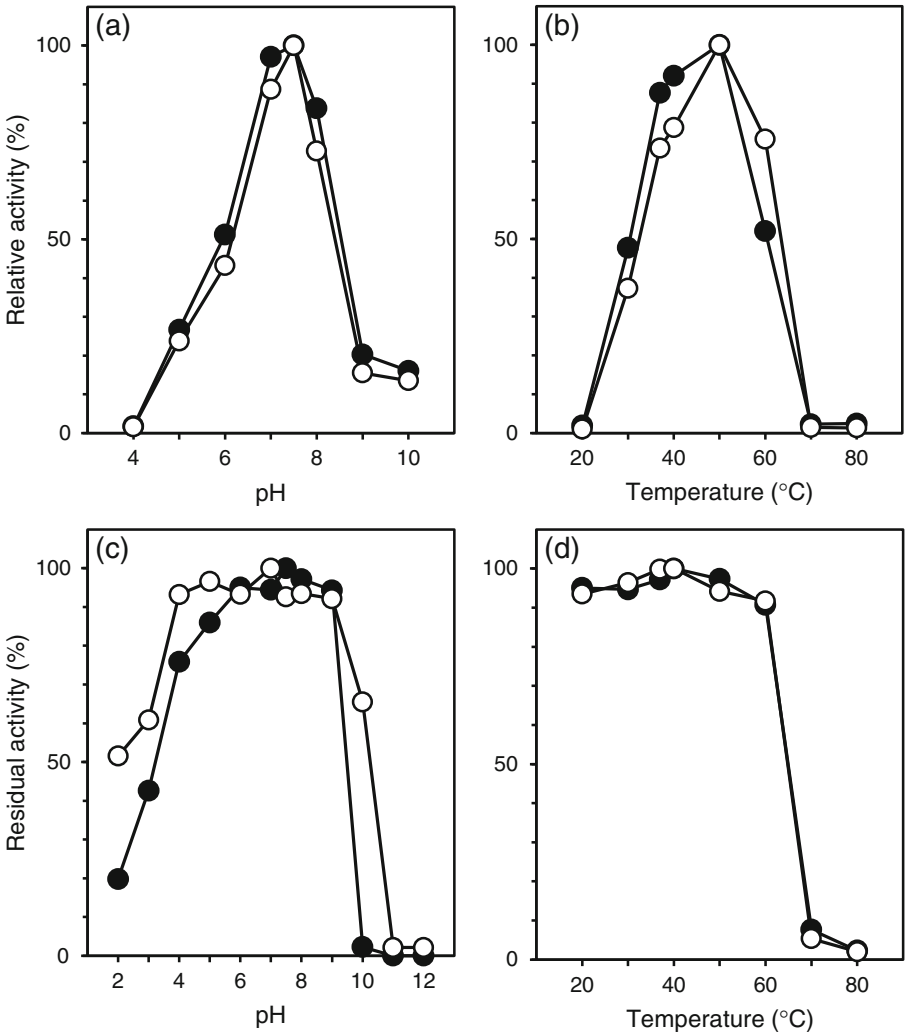

Fig. 4 Enzymatic properties of the recombinant and the native PLD103. **a** pH stability, **b** temperature stability, **c** optimum pH, and **d** optimum temperature of the recombinant (*open circles*) and native (*closed circles*) PLD103

possesses a characteristic pH optimum, with the greatest PC-hydrolyzing activity at slightly alkaline conditions, as opposed to the majority of the streptomycete PLDs, which have a slightly acidic optimum pH. This unique characteristic was observed in the rPLD103.

In the PG- and PS-producing transphosphatidylation reactions, the nPLD103 converts substrate PC to transphosphatidylated products with a high conversion rate and selectivity for transphosphatidylation instead of hydrolysis [16]. Therefore, the conversion rates and the selectivity of the PG- and PS-producing transphosphatidylation reactions by rPLD103 were compared to nPLD103. The transphosphatidylation activities of rPLD103 were examined in an aqueous one-phase reaction mixture in which the 0.1% (*w*/*v*) PC substrate was dispersed in the presence of 4 mM deoxycholate. In the PG-producing reaction with PC as substrate and glycerol as acceptor, 85.6% of the PC was converted into PG. Of the reaction products, 88.0% resulted from transphosphatidylation (Table 1). In the PS-producing reaction, 80.2% of the substrate was converted into PS with 86.9% of the reaction product a result of transphosphatidylation (Table 1). These values are comparable to those of nPLD103; thus, the overexpression system employed here successfully produced active enzyme with high transphosphatidylation activity in the simple aqueous one-phase reaction system.

Modification of PL Contents in Soybean Lecithin

Soybean lecithin modification was examined using the partially purified rPLD103. Culture supernatants from the transformant harboring pES103(−) were brought to ammonium sulfate precipitation (65% saturation); the resulting precipitate was resuspended and dialyzed against distilled water. The PL content of the original soybean lecithin was determined as 65.7% PC, 27.9% PE, and 6.4% PA (Table 2). The soybean lecithin was dispersed at a final concentration of 0.1% in a reaction mixture containing 0.02 U/ml of the rPLD103 and 20% choline chloride, glycerol, or L-serine. The pH of the reaction mixture was adjusted to 7.5 and 5.5, corresponding to the optimum pH for PC hydrolysis activity of PLD103 and the majority of the known PLDs from *Streptomyces*, respectively [30–33]. During the reaction, aliquots of the reaction mixture were collected at several time intervals and the PL contents of the aliquots determined by TLC (Fig. S1). The increase in transphosphatidylation products reached a plateau at different post-initiation time points of the reaction, ranging from 30 to 180 min, depending upon the reaction. In the slightly alkaline reaction, the amount of transphosphatidylation products decreased slightly after reaching the plateau, probably because of the optimum condition requirements for hydrolytic reactions by PLD103 [16]. Figure 4 displays the ratio of PL content in each reaction mixture when the transphosphatidylation reaction reached a plateau. In the

Table 1 Production of PG and PS by transphosphatidylation using nPLD103 and rPLD103

	PG production		PS production	
	Conversion (%)	Selectivity (%)	Conversion (%)	Selectivity (%)
nPLD103	87.8±1.8	89.7±1.3	79.2±1.5	89.5±0.4
rPLD103	85.6±2.7	88.0±2.4	80.2±3.8	86.9±3.1

Percentages represent transphosphatidylation conversion rate and selectivity. Methods for estimating these values are described in "Materials and Methods"

Experiments were repeated four times. Values are the means±standard deviations

Table 2 Phospholipid content of the original and the PL-modified lecithin

Lipids	Original	Choline chloride (%)[a]		Glycerol (%)[a]		L-Serine (%)[a]	
		pH 5.5 180 min[b]	pH 7.5 120 min[b]	pH 5.5 180 min[b]	pH 7.5 120 min[b]	pH 5.5 180 min[b]	pH 7.5 30 min[b]
PC	65.7±4.4	78.5±1.1	74.1±0.8	5.3±0.7	19.5±3.4	6.2±2.4	4.6±0.4
PA	6.4±4.0	16.8±0.9	20.0±0.8	18.8±1.1	4.6±1.5	1.0±0.9	25.0±0.2
PE	27.9±1.4	4.7±0.2	5.9±0.4	3.9±0.7	8.1±0.7	14.8±2.2	6.9±0.6
PG	–	–	–	72.1±1.6	67.9±2.4	–	–
PS	–	–	–	–	–	78.0±0.1	63.5±0.6

[a] Percentages represent the ratios of each PL contents to total PLs. Lecithin modification was performed in the presence of 20% (*w*/*v*) phosphatidyl group acceptors—choline chloride, glycerol, and L-serine—and 0.02 U/ml of rPLD103. The PLs in original and modified lecithins were developed by thin-layer chromatography and visualized with iodine vapor

[b] The increase in the transphosphatidylation products reached a plateau at the time indicated (see Fig. S1). The PL contents of the PL-modified lecithin indicated here occurred at the time when the reaction reached a plateau. Experiments were repeated four times, and error bars represent standard deviations

presence of choline chloride, a slight increase in PC (78.5% at pH 5.5; 74.1% at pH 7.5) was observed after the reaction. In the original soybean lecithin, there is small amount of PE. In this case, PE would also provide a phosphatidyl group for transphosphatidylation. However, PLD103 exerted a high hydrolytic activity on PC and PE [14], which may result in hampering the PC-producing transphosphatidylation. In the presence of glycerol, PG increased to 72.1% at pH 5.5 and 67.9% at pH 7.5 after the reaction. When L-serine was added to the mixture, PS increased to 63.5% at pH 5.5 and 78.0% at pH 7.5 after the reaction. The production of PG and PS using soybean lecithin as a phosphatidyl group donor was relatively lower than when using purified PC, probably because of the low concentration of PC in the lecithin.

Discussion

In this study, we constructed an overexpression system for PLD103. The shuttle vector (pES) used in this study was prepared with reference to the pUC702 vector previously published by Ogino et al. [29]. To reduce the size of the plasmid, we removed some regions, e.g., *melC1*, *melC2*, and the *lac* promoter, which are unnecessary for the expression of the recombinant protein. Using pES103(+) or pES103(−), we achieved large-scale production of the rPLD103, in which PC hydrolytic activity was approximately 90-fold higher than the original PLD from *S. racemochromogenes* 10-1. Ogino et al. [29] improved the production of recombinant PLD in the *S. lividans* system approximately 50-fold compared to the original strain, *S. cinnamoneum*. Therefore, the elimination of unnecessary elements from the plasmid might explain the relatively high production of PLD in our system.

In the lecithin modification assay, rPLD103 displayed a pH-dependent weak hydrolytic reaction that degrades the transphosphatidylation products. Additionally, slightly acidic conditions appear to be preferred for PC and PG production, whereas slightly alkaline conditions appear to be preferred for PS production. Therefore, further studies using altered pH conditions are necessary to optimize the production of phosphatidyl derivatives. For example, switching the pH of the reaction mixture from alkaline to acidic and vice versa

after the reaction has been allowed to run for a finite period of time may be effective in improving production. The rPLD103 produced both PG and PS from soybean lecithin. The transphosphatidylation reaction of PLD may enable to synthesize various phosphatidyl derivatives of hydroxyl compounds, such as phenols, sugars, nucleotides, vitamins, and others, in addition to glycerol and L-serine [34–38]. Phosphatidyl derivatives may improve the functioning of ingredients found in food supplements due to their emulsification properties, antioxidant abilities, and liposome formation abilities. Thus, it would be worthwhile to examine the transphosphatidylation reaction using hydroxyl compounds as acceptors to obtain rare PL from lecithin. The large-scale production of rPLD103 established here ensures a stable supply of the PLD with high transphosphatidylation activity and allows us to attempt to synthesize the phosphatidyl derivatives of various compounds from abundant lecithin.

Conclusions

We successfully established an overexpression system of rPLD103 in *S. lividans* TK23. The rPLD103 conserved the enzymatic properties of native PLD103. In a simple aqueous one-phase system, the rPLD103 had high transphosphatidylation activity when using PC as a phosphatidyl group donor. The enzyme also effectively converted PC and PE in soybean lecithin into PG or PS in the presence of glycerol or L-serine, respectively. Therefore, rPLD103 could be used to obtain PG and PS from the lecithin that is abundantly available from edible oil production processing.

Acknowledgments We thank Kazuaki Hirabayashi, Teppei Kikuchi, and Eri Yamamoto for their technical assistance.

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