Interaction of plant lipids with 14 kDa phospholipase A₂ enzymes

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Several structurally related plant lipids were isolated and their effect was assessed on the enzyme activity of group I (pancreatic and Naja mocambique venom) and group II (Crotalus atrox venom) phospholipase A₂ (PLA₂) enzymes, with labelled Escherichia coli as an enzyme substrate. The neutral monogalactosyldiacylglycerol (MGDG) and negatively charged diacylglycerol (DGGA) did not influence the enzyme activity of either group. Digalactosyldiacylglycerol (DGDG), another uncharged glycolipid, inhibited PLA₂ activity in a dose-dependent manner to 60–70% of the control. Sulphoquinovosyldiacylglycerol (SQDG), which is also anionic, activated both groups of PLA₂ enzyme. A similar activation was observed with the zwitterionic diacylglycerol-O-{N,N,N-trimethylhomoserine} (DGTS) and diacylglycerol-O-(hydroxymethyl)N,N,N-trimethyl)-β-alanine (DGTA). DGDG, SQDG and DGTS are dispersed homogeneously with low critical micelle concentrations (CMCs). The hydrodynamic radius of neutral DGDG is an order of magnitude larger than the charged lipids SQDG and DGTS. The inhibition of pig pancreatic PLA₂ by DGDG was dependent on substrate concentration. The intrinsic fluorescence spectra of the enzyme was not changed in the presence of native or hydrogenated DGDG. Thus the inhibition is most probably due to a non-specific interaction of plant lipids with the substrate. Different lengths and saturations of the fatty acyl chains of DGDG did not alter the inhibition of PLA₂, whereas deacylation abrogated the inhibitory effect. Both SQDG and DGTS activated pig pancreatic PLA₂ in a dose-dependent manner. Saturation of the double bonds of these lipids decreased the activating effect. The fluorescence of pig pancreatic PLA₂ incubated with SQDG and DGTS was enhanced by 2-fold and 3-fold respectively, suggesting the formation of a complex between enzyme and lipids. In conclusion, the effect of different plant lipids on PLA₂ activity depends on different structural elements of the polar head group and their charge as well as the degree of unsaturation of the fatty acyl chains.

INTRODUCTION

Phospholipase A₂ (PLA₂) enzymes play a key role in liberating free arachidonic acid from the sn-2 position of many phospholipids, thereby initiating the production of proinflammatory eicosanoids [1–4]. So far many PLA₂ enzymes have been characterized with respect to their primary and secondary structures. These enzymes are classified into high-molecular-mass PLA₂ (85–110 kDa) and low-molecular-mass PLA₂ (14 kDa) [1,2]. The low-molecular-mass PLA₂ is further classified into groups I, II and III. All the 14 kDa PLA₂ enzymes are secretory proteins. Group I comprises PLA₂ enzymes from pancreas and from the venom of snakes belonging to the families Elapidae and Hydrophiidae. Group II comprises PLA₂ enzymes from inflammatory exudates (synovial or ascites fluid), from cell membranes (platelets, spleen, lung, etc.) and from venom produced by snakes belonging to the families Crotalidae and Viperidae [2,5]. Group I and II PLA₂ enzymes exhibit more than 70% homology and the catalytic regions of these two groups of enzyme are highly conserved [3,6,7]. Group III comprises PLA₂ enzymes from bee venom and its primary structure is distinctly different from those of group I and II enzymes.

In many inflammatory diseases, high levels of 14 kDa (presumably group II) PLA₂ enzymes are detected: they are believed to be responsible for part of the inflammatory reactions. Injection of purified PLA₂ from synovial fluid and from snake venom into animal joints confirmed the development of an acute inflammatory response with oedema, swelling of synovial cells and hyperplasia [8,9]. Inhibition of such PLA₂ enzymes by xenobiotics is of potential therapeutic relevance. Several endogenous and exogenous agents such as lipocortins, cis-unsaturated fatty acids, gangliosides, manoalide, retinoids, flavonoids, aristolochic acid and synthetic lipids have been shown to inhibit PLA₂ enzymes [8,10–20].

In the present study we have used several polar lipids of plant origin that are not phospholipids, and hence are not substrates of PLA₂ enzymes, to study their interaction with 14 kDa PLA₂ enzymes. To the best of our knowledge these lipids, differing with respect to their functional groups, are not produced by animals or humans: the analysis of their interaction with PLA₂ might be of help in designing effective inhibitors of PLA₂ enzymes in the future.

MATERIALS AND METHODS

Materials

Fatty acid-free BSA and PLA₂ enzymes from pig pancreas, Naja mocambique and Crotalus atrox were obtained from Sigma Chemie (Buchs, Switzerland). Ochronomas danica (Chrysophyceae) and Chlamydomonas reinhardtii (Chlorophyceae) were grown in laboratory cultures as described previously and field thalli of Fucus serratus L. were used as a source of lipids [21–23]. Silica gel (40 μm) and Dynagel scintillation cocktail were obtained from J. T. Baker (B. V. Deventer, Holland). Silica gel TLC plates were from Merck (Basel, Switzerland). Palladium black was obtained from Fluka (Buchs, Switzerland). Escherichia

Abbreviations used: PLA₂, phospholipase A₂; CMC, critical micelle concentration; DGDG, digalactosyldiacylglycerol; DGGA, diacylglycerol α-glucuronide; DGTA, diacylglycerol-O-(hydroxymethyl)N,N,N-trimethyl)-β-alanine; DGTS, diacylglycerol-O-(N,N,N-trimethylhomoserine); MGDG, monogalactosyldiacylglycerol; SQDG, sulphoquinovosyldiacylglycerol.‡ To whom correspondence should be addressed.
coli cells labelled with [3H]oleic acid (specific radioactivity 10 Ci/mmol; Amersham, Bucks., U.K.) were prepared by the procedure of Patriarca et al. [24]. l-Tryptophan was obtained from Gibco (Basel, Switzerland) and pyrene was from Fluka. All reagents and organic solvents used were of analytical grade.

**Lipid extraction and purification**

Lipids were extracted, purified and identified by the methods described previously [21–23]. Briefly, material was extracted with 10 vol of methanol. After evaporation of solvent, the total lipids were dissolved in diethyl ether. Lipid extracts were fractionated by preparative flash chromatography on silica gel and mixtures of chloroform, acetone, methanol and formic acid as eluents [21–23]. For further purification of single lipids, the fractions were subjected to chromatography on silica gel plates in chloroform/methanol/water (65:25:4, by vol). The separated lipid spots were then subjected to chromatography on silica gel plates in chloroform/methanol/isopropanol/conc. NH$_3$ (65:35:0.5:5, by vol). In addition, sulphoquinovosyldiacylglycerol (SQDG) and diacylglycerol-$\alpha$-glycerol (DGGA) were separated in chloroform/acetone/methanol/formic acid/water (50:20:10:10:4, by vol). Lipid spots were detected with UV illumination after treatment with dichlorofluorescein, and eluted with methanol.

**Hydrogenation**

Palladium black (5 mg) was added to a solution of 5 mg of lipid dissolved in 1 ml of acetone and the mixture was flushed with hydrogen, tightly sealed and shaken overnight. After centrifugation the solvent was evaporated under nitrogen.

**Deacylation**

Digalactosyldiacylglycerol (DGDG; 40 mg) dissolved in 0.5 ml of KOH/water/methanol (1:2:20, w/v/v) was kept at 70 °C for 30 min. Water (0.5 ml) was added and the mixture extracted three times with 1 ml of diethyl ether/hexane (1:1, v/v). After the addition of 1 mol of 6 M HCl the free fatty acids were extracted with hexane. The aqueous phase was neutralized with 0.5 M KOH in methanol and dried in vacuum. The digalactosyldiglycerol (deacylated DGDG) obtained was eluted from the residue with methanol.

**Fatty acid analysis**

The fatty acid composition of different lipids was analysed by transesterification and gas chromatography as described previously [23].

**Assay of PLA$_2$ activity**

PLA$_2$ activity was assayed as described previously [25,26]. Briefly, PLA$_2$ activity was assayed with [3H]oleate-labelled autoclaved E. coli as the substrate. The reaction mixture of 350 µl contained 100 mM Tris/HCl, pH 8.0, 5 mM Ca$^{2+}$ and 2.85 × 10$^{8}$ autoclaved E. coli cells (corresponding to 10000 c.p.m. and 5.5 nmol of lipid phosphorus). The amount of protein was chosen such that a 6–15% hydrolysis of substrate was obtained when incubated at 37 °C for 120 min. The reaction components were mixed in the following order: buffer, calcium, water and different plant lipids or appropriate solvent. The reaction was started by adding the E. coli substrate. The reaction was terminated by adding 100 µl of 2 M hydrochloric acid and 100 µl of fatty acid-free BSA (100 mg/ml). The tubes were vortex-mixed and centrifuged at 20000 g for 5 min. An aliquot (140 µl) of the supernatant containing released [3H]oleic acid was mixed with scintillation cocktail Dynagel and counted in a Kontron Analytical BETAmatic I liquid-scintillation counter.

**PLA$_2$ enzyme activity with plant lipids**

Briefly, the reaction mixture of 350 µl contained 100 mM Tris/HCl, pH 8.0, 5 mM Ca$^{2+}$, 2.0 mM plant lipids (liposomes) and 5 µg of pig pancreatic PLA$_2$ enzyme. Incubation was performed at 37 °C for 120 min. The reaction was terminated by adding 3.5 ml of methanol. After extraction, the solvent was evaporated under nitrogen. The lipids were dissolved in 200 µl of chloroform/methanol (1:1, v/v). The volume was reduced to 20 µl and the lipids were spotted on a thin-layer silica gel plate and subjected to chromatography with chloroform/methanol/water (65:25:4, by vol). The reaction products were detected by UV illumination after treatment with dichlorofluorescein.

**Determination of the critical micelle concentration (CMC)**

The CMC was measured by using pyrene as a polarity probe [27]. Pyrene emission spectra were obtained with a Perkin–Elmer spectrometer at room temperature (23 °C). The excitation wavelength was chosen as 335 nm with a slit width of 5 nm, and the emission scan was done from 360 to 440 nm with a slit width of 2.5 nm. The fluorescence intensities of the highest vibrational band, $I_{37}$ (373 nm) and $I_{33}$ (385 nm), in the emission spectrum were measured as peak heights [27].

**Light-scattering measurements**

Buffer, calcium chloride and lipid solutions were filtered under pressure through Nalgene filters (0.2 mm) to free them from dust particles. The measurements were performed with a standard DLS goniometer (ALV, Langen, Germany) equipped, for maximum performance, with single-mode fibre receiving optics [28]. The measurements were done at three scattering angles ($\theta$) of 45, 90 and 135 with a $\lambda = 514$ nm Ar laser line. Measurements were performed at 25 °C with a 200 mM concentration of lipids. The dynamic light-scattering signal was processed with an ALV 5000 digital correlator (ALV). This device calculates on-line the correlation function $g(\tau)$, i.e. the average $g(\tau) = [I(\tau)A(\tau + \tau)]/[I]^{2}$.

The information about the observed system is contained in the function $g(\tau)$. In the simplest case of a very dilute suspension of monodisperse scattering particles, $g(\tau)$ is a simple exponential:

$$g(\tau) = e^{-\tau/\tau_R}$$

(1)

From the relaxation time $\tau_R$ one can extract the diffusion coefficient of the scattering particles by the relation $D = 1/q^2\tau_R$ [$q$ is the magnitude of the scattering vector, $q \approx \sin(\theta)/2\lambda$] and this in turn allows the determination of the size of dissolved macromolecules in a range from few µm down to 1 nm [29,30]. However, this simple case is seldomly encountered in practice. In general $g(\tau)$ has to be expressed in terms of a spectrum $A(\tau R)$ of relaxation times as:

$$g(\tau) = \int_{0}^{\infty} A(\tau R) e^{-\tau/\tau_R} d\tau_R$$

(2)

**Intrinsic fluorescence measurements**

The relative intrinsic fluorescence intensity of pig pancreatic PLA$_2$ with and without plant lipids was monitored with a Perkin–Elmer Luminescence Spectrophotometer LS50. A reaction mix-
ture of 2.0 ml in 1 cm path length quartz cuvettes contained 100 mM Tris/HCl buffer, pH 8.0, 5 mM Ca\(^{2+}\), PLA\(_2\) [75 µg (2.7 µM)] and various plant lipids (85 µM). Fluorescence spectra were measured between 300 and 450 nm after excitation at 280 nm. Correction for non-specific interaction of PLA\(_2\) owing to internal absorption by plant lipids was performed with a tryptophan standard. No effect of plant lipids on the fluorescence of the tryptophan standard was observed.

RESULTS

The structure and nomenclature of the different glycerolipids used and their major constituent fatty acids are given in Table 1. Monogalactosyldiacylglycerol (MGDG), DGDG and SQDG are regular lipid constituents of all photosynthetic plant tissues [31]. DGGA and the betaine lipids diacylglyceryl-\(O\)-(\(N,N,N\)-trimethylhomoserine) (DGTS) and diacylglyceryl-\(O\)-(hydroxymethyl)(\(N,N,N\)-trimethyl)-\(\beta\)-alanine (DGTA) are produced by the chrysophycean alga \(O.\ danica\) [21,32] and by other lower plants. DGGG and SQDG contain an acidic polar group, whereas MGDG and DGDG are neutral glycolipids. DGTS and DGTA contain a zwitterionic polar group of the betaine type. To study their interaction with PLA\(_2\) activity, the lipids were used in both native and hydrogenated (saturated) forms.

Figure 1 shows the interaction of different plant lipids with pig pancreatic PLA\(_2\) (group I). DGGA and MGDG did not influence the PLA\(_2\) activity, whereas the enzyme was inhibited by DGDG. The same effect was observed with the native and hydrogenated forms of the lipid used. In contrast, PLA\(_2\) enzyme was activated by the sulpholipid SQDG and the betaine lipids DGTS and DGTA in their native forms. The activation, however, was less pronounced when the hydrogenated instead of the native forms of these lipids were used. A similar change in the activity was observed with these lipids when PLA\(_2\) from \(N.\ mocambique\)

### Table 1 Structure of different plant lipids used for PLA2 inhibition experiments

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Structure</th>
<th>Abbreviations</th>
</tr>
</thead>
<tbody>
<tr>
<td>DGGA</td>
<td>Diacylglyceryl-(\alpha)-glucuronide</td>
<td>20:4/22:5</td>
</tr>
<tr>
<td>MGDG</td>
<td>Monogalactosyldiacylglycerol</td>
<td>18:3/18:3</td>
</tr>
<tr>
<td>DGDG</td>
<td>Digalactosyldiacylglycerol</td>
<td>18:3/18:2+18:4</td>
</tr>
<tr>
<td>SQDG</td>
<td>Sulphoquinovosyldiacylglycerol</td>
<td>18:3/18:2+18:4</td>
</tr>
<tr>
<td>DGTS</td>
<td>(Diacylglyceryl-(O)-((N,N,N)-trimethyl)-homoserine)</td>
<td>14:0/18:2</td>
</tr>
<tr>
<td>DGTA</td>
<td>Diacylglyceryl-(O)-(hydroxymethyl)((N,N,N)-trimethyl)-alanine</td>
<td>18:0 18:2/22:5</td>
</tr>
</tbody>
</table>
Figure 1  Effect of different plant lipids on pig pancreatic PLA2

Protein concentrations (1.3 ng) were adjusted to yield 6–15% hydrolysis. The reaction mixture (350 µl) containing 5.5 nmol of *E. coli* phospholipid, 100 mM Tris/HCl buffer, pH 8.0, 5 mM Ca²⁺ and 70 µM native (open bars) or hydrogenated (hatched bars) plant lipids was incubated at 37 °C for 2 h. Means ± S.D. values for three determinations are given.

Table 2  Physicochemical parameters of plant lipids at 25 °C and pH 8.0

The hydrodynamic radius, *r*ₜ, was obtained from dynamic light-scattering intensity decays at 45°, 90° and 135°.

<table>
<thead>
<tr>
<th>Plant lipid</th>
<th>CMC (µM)</th>
<th><em>r</em>ₜ (nm)</th>
</tr>
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<tbody>
<tr>
<td>DGDG</td>
<td>13.1</td>
<td>1740, 1680, 1520</td>
</tr>
<tr>
<td>SQDG</td>
<td>6.3</td>
<td>150, 80, 70</td>
</tr>
<tr>
<td>DGTS</td>
<td>5.1</td>
<td>170, 103, 98</td>
</tr>
</tbody>
</table>

Figure 2  Dose-dependent inhibition of group I [pig pancreatic (●) and *N. mocambique* (■)] and group II (*C. atrox* (▲)) PLA2 enzymes by DGDG

Protein concentrations of different enzymes (1.3 ng of pancreatic PLA₂, 1.0 ng of *N. mocambique* PLA₂, or 75 ng of *C. atrox* PLA₂) were adjusted to yield 6–15% hydrolysis. The reaction mixture (350 µl) containing 100 mM Tris/HCl buffer, pH 8.0, 5 mM Ca²⁺, 5.5 nmol *E. coli* phospholipid and the indicated concentrations of DGDG was incubated at 37 °C for 2 h. Inhibition is expressed as a percentage of the control. Values are the means of three determinations.

Figure 3  Effect of substrate concentration on the inhibition of pig pancreatic PLA2 by DGDG

(A) Pig pancreatic PLA₂ (1.3 ng) in 100 mM Tris/HCl buffer, pH 8.0, containing 5 mM Ca²⁺ was incubated at 37 °C for 2 h with the indicated concentrations of *E. coli* phospholipid in absence (○) or presence (●) of 20 µM DGDG. (B) Inhibition of pig pancreatic PLA₂ activity by 20 µM DGDG. Inhibition is expressed as a percentage of the control. Values are the means of three determinations.

(group I) and *C. atrox* (group II) venom were used (results not shown). On the basis of these results, the focus of further studies was the inhibition of pig pancreatic PLA₂ enzyme by DGDG and the activation by SQDG and DGTS.

The physicochemical behaviours of DGDG, SQDG and DGTS are summarized in Table 2. These lipids have small CMCs with a fairly homogeneous dispersion of large micelles. All three samples exhibited remarkably simple relaxation spectra: a fairly narrow dominant peak contributing more than 80% to the correlation function was accompanied by few spurious peaks. The lipids most probably have a spherical structure. With SQDG and DGTS an angle dependency in the hydrodynamic radius was observed.

The dose-dependent inhibition of pig pancreatic PLA₂ by native DGDG was approx. 70 % with 240 µM of lipid suspension (Figure 2). A similar inhibition of PLA₂ from venoms of *N. mocambique* and *C. atrox* was obtained by using native DGDG (Figure 2, inset). The apparent IC₅₀ of DGDG calculated by a Lineweaver–Burk plot ranged from 10 to 27 µM of added lipid for all three PLA₂ enzymes investigated. The effect of DGDG on PLA₂ did not change as a function of lipid fatty acids (100 % C₁₈ or 50 % C₁₄ and 50 % C₁₆ fatty acids). This indicates that the chain length of the constituent fatty acids seems not to modulate the inhibitory effect of the lipid.

To study the mechanism of inhibition by DGDG, we examined its inhibition of pig pancreatic PLA₂ as a function of substrate concentration (Figure 3). PLA₂ activity in the presence and in the absence of 20 µM DGDG increased almost linearly with increasing substrate concentration (Figure 3A). The percentage inhibition by DGDG was approx. 50 % at lower substrate
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Figure 4 Activation of pig pancreatic PLA<sub>2</sub> by SQDG and DGTS

The reaction mixture (350 µl) containing PLA<sub>2</sub> enzyme (see the legend to Figure 1), 100 mM Tris/HCl buffer, pH 8.0, 5 mM Ca<sup>2+</sup>, 5.5 nmol of E. coli phospholipid and the indicated concentrations of native (●) or hydrogenated (■) SQDG or DGTS were incubated at 37 °C for 2 h. Activation is expressed as a percentage of the control. Values are the means of three determinations.

Figure 5 Intrinsic fluorescence spectra of pig pancreatic PLA<sub>2</sub> in the presence of different plant lipids

The reaction mixture (2.0 ml) contained 75 µg of PLA<sub>2</sub> (2.7 µM) in 100 mM Tris/HCl buffer, pH 8.0, 5 mM Ca<sup>2+</sup> and 85 µM of native or hydrogenated (indicated with a suffixed H) plant lipid. The control contained the native lipid only. Fluorescence spectra were recorded after excitation at 280 nm.

DISCUSSION

Understanding the molecular interactions between the enzyme and agents modulating the activity of PLA<sub>2</sub> is a prerequisite for designing new powerful inhibitors. Recently, detailed studies were performed with aminoacyl phospholipid analogues and lipids containing carbohydrate units for the inhibition of group I PLA<sub>2</sub> enzymes (pancreatic and cobra venom PLA<sub>2</sub>) [17–20]. In these studies the charge of the head group, various chain lengths and degrees of unsaturation of aminoacyl fatty acids were examined for their inhibitory potency on PLA<sub>2</sub> [17–20]. In the present study we examined the effect on pig pancreatic PLA<sub>2</sub> of several plant glycerolipids with neutral, negatively charged or zwitterionic polar head groups.

The plant lipids selected for this study resemble the natural substrate for PLA<sub>2</sub> with respect to their diglyceride moiety. They differ, however, from the natural phospholipids by the polar head group, which is linked to the sn-3 position not by a phosphodiester bond but by an O-glycosidic bond in glycolipids and by an O-ether linkage in betaine lipids [21,31,32]. In the absence of a phosphodiester group from the polar head, these plant lipids are not accepted as substrates for the hydrolysis of the sn-2 fatty acyl ester. Because these lipids resemble substrates for PLA<sub>2</sub> in their geometry but are not hydrolysed by it, it was of interest to study their interaction with PLA<sub>2</sub> enzymes.

The glycolipids MGDG and DGGA, containing a neutral galactose and negatively charged glucuronic acid respectively, did not influence the activity of pig pancreatic PLA<sub>2</sub>. In contrast,
DGDG, another neutral glycolipid that differs from MGDG by an additional galactose moiety, clearly inhibited the PLA₂ enzyme, indicating that the additional galactose might be essential for inhibition. Different fatty acid chain lengths at the sn-1 and sn-2 positions (C₁₈:1/C₁₈:0) in their native and hydrogenated forms did not change the effect of DGDG. This is in accord with the fact that acylamino lecithins containing a 20-carbon chain with zero to five cis-double bonds had all the same inhibitory potency [19]. Dijkman et al. [18], in contrast, showed that acylamino lipid analogues containing linoleic acid or linolenic acid caused a 4–8-fold higher inhibition than the fully saturated octadecanoic acid-containing inhibitor. Deacylation of DGDG, however, abrogated the inhibitory potency towards these 14 kDa PLA₂ enzymes indicating that the intact lipid is necessary for an effective inhibition. Several investigators demonstrated that within the primary structure of the phospholipase polypeptide a cluster of positively charged amino acids at positions 53, 56 and 57 are responsible for the preference for a polar head group [17, 33–35]. In addition, studies with (R)-2-acylamino inhibitors revealed that anionic derivatives, because of their stronger binding, are more effective inhibitors than zwitterionic molecules [18]. Among the plant lipids examined, neutral glycolipid DGDG was the only compound exerting an inhibitory effect. These results reveal that apart from the charge effect in molecules that affect the activity of PLA₂ enzymes indicating that the additional galactose might be essential for diffusive motion in the absence of interactions because DGDG is a neutral lipid. However, the effective radius of SQDG and DGTS is increased at 45°; this might be due to interactions between these charged aggregates (Table 2). The CMC of DGDG is 13.1 mM and inhibits PLA2 activity with an IC₅₀ of 10–27 mM, suggesting that both monomeric and aggregated lipid forms inhibit the enzyme activity. The transition from the monomeric to the aggregated state did not significantly influence the inhibitory power of DGDG.

The intrinsic fluorescence reflects conformational changes in proteins due to substrate or ligand binding [8, 13, 14, 16]. Thus it was expected that fluorescence measurements would provide information on the binding of plant lipids to PLA₂. Surprisingly, DGDG, which acts as an inhibitor, did not change the intrinsic fluorescence of pancreatic PLA₂, suggesting that either the lipid did not interact with the enzyme or its binding to the enzyme did not alter the intrinsic fluorescence. From fluorescence measurements and substrate relief experiments it might rather be concluded that the inhibition by DGDG is more probably due to a non-specific interaction with the substrate than to a competition with the substrate for the active site.

In contrast, the fluorescence intensity of pig pancreatic PLA₂ was drastically enhanced by the sulpholipid SQDG. An increase in fluorescence was also observed with DGTS. Such an increase has been interpreted as representing the transition from the inactive to the active hypothetical states of the enzyme–bilayer complex [36–39]. Because both SQDG and DGTS in their native form activated the PLA₂ enzyme, the observed spectral and catalytic properties are in line with this interpretation.

The plant glycerolipids used in the present investigation structurally resemble several lipid type PLA₂ inhibitors such as (R)-1-alkylthio-2-acylamino phospholipids and (R)-2-acylamino phospholipids [17–19]. The results provide additional information on the importance of different structural elements of the lipid molecule in terms of designing new effective PLA₂ inhibitors of therapeutic value. Finally, the effect of DGDG as a PLA₂ inhibitor might be of some interest because this lipid is a major constituent of all photosynthetic plants, including vegetables consumed by humans and animals [31]. Further studies should focus on the kinetics and dynamics of PLA₂ inhibition/activation by these plant lipids as well as on their susceptibilities to the digestive lipases other than PLA₂ enzymes in the mammalian system.

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