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Published in:
European Journal of Biochemistry


Published: 01/01/1989

Document Version
Publisher's PDF, also known as Version of Record (includes final page, issue and volume numbers)

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Conformational transmission in condensed lipid model compounds
Chain packing modification by head-group-induced conformational changes in the glyceryl backbone

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(Received November 29, 1988/February 28, 1989) – EJB 88 1388

On changing the phosphorus coordination from four to five via a trigonal bipyramidal geometry in phospholipid model systems, a conformational change is initiated around the C2-C3 linkage caused by enhanced electrostatic repulsion between the atoms O3 and O2, situated in the axis of the trigonal bipyramid. This is supported by the absence of conformational change upon substitution of O2 by methylene. With solid-state cross-polarization and magic-angle spinning 13C-NMR it is shown that such a conformational transmission leads to an increased packing density of the lipid chains. It seems to us that changes in lipid packing caused by conformational transmission may also occur when pentacoordination is present transiently, as under biological conditions, thereby inducing an activation of the transport proteins that are embedded in the membrane.

Much research has been carried out to elucidate the mechanisms for activation of transport proteins in membranes. A detailed molecular concept has not emerged yet, although an important role has been attributed to protein-induced hexagonal H2 lipid structures in transport processes [1, 2]. The possibility that lipids themselves are able to modify the conformation of incorporated proteins has become subject for further research only very recently [3–5]. Since structural changes of the polar head group can easily be initiated in phospholipids [6–9], we expect their influence on transport processes to be significant.

Recently Meulendijks et al. [10] provided first experimental verification of a mechanism proposed by Merkelbach et al. [11] for alterations in the lipid chain packing, induced by the formation of transient pentacoordinated phosphorus in the head group with a trigonal bipyramidal geometry. The build-up of such a transient pentacoordinated phosphorus atom can be realized by a nucleophilic attack on the tetracoordinated phosphorus atom in the phosphate group. For an intramolecular addition (e.g. via the carboxylate anion in phosphatidylserine), the pentacoordinated phosphorus with a trigonal bipyramidal geometry is then actually promoted by shielding one of the negative charges of the phosphate diester with the ammonium group of the serine moiety (see Fig. 1).

The changes in lipid packing are supposed to create a domain with a fluidity different from other parts of the membrane. A protein that is incorporated in such a domain may then be activated [5, 12].

Since the pentacoordinated phosphorus formed in water is transient, conformational inductions have been studied with model lipids containing a stabilized pentacoordinated phosphorus atom on the head group. The experiments were performed in chloroform, thus presenting the monomeric phase. It has been shown for compound 2A that a conformational change in the glyceryl backbone is present with respect to lipids with a tetracoordinated phosphorus atom in their head groups as in compound 3A [10]. The molecular basis is found in the fact that the extra ligand on phosphorus changes the chemical bond properties, resulting in an enhanced electron density on the oxygen atom O3 in the axis of the trigonal bipyramid (see Fig. 2).

Therefore, an increased coulombic repulsion between the vicinal oxygen atoms O3 and O2 in the atomic sequence P-O-C-C-O is present in compound 2A, which causes a conformational change around the C2-C3 bond toward a trans orientation of O3 and O2 (g † conformation, see Fig. 3). This conformational change is absent in compound 2B, in which O2 is substituted for methylene.

The pentacoordinated phosphorus concept has proved to be a general mechanism for conformational changes in other

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Abbreviation. CP-MAS, cross-polarization and magic-angle spinning.

Fig. 1. Proposed trigonal bipyramidal geometry after intramolecular addition in phosphatidylserine
phosphorylated biomolecules containing the P-O-C-C-O atomic sequence in the axial location of the trigonal bipyramid. For these specific conformational changes we introduced the indication ‘conformational transmission’ [13 – 16].

In the present paper, we will extend the conformational transmission concept from an organic monomeric phase to a condensed phase. For that purpose we selected the corresponding lipid silatrane 1 A (see Fig. 2) with trigonal bipyramidal geometry [17], thereby introducing conformational transmission [18,19] and the possibility for solidification from the liquid state. For that purpose we used the deoxy form of the lipid 1 A, containing a pentacoordinated silicon atom with a trigonal bipyramidal geometry, with the corresponding tetracoordinated phosphorus system 3 A. However, to prevent an intrinsic chemical difference, which may influence the conformational transmission effect, we used the deoxy silatrane lipid 1 B as an additional reference compound. The two silatranes, 1 A and 1 B, are isosteric, but differ in their C2-C3 conformation, since in the deoxy form the vicinal coulombic repulsion is absent (vide supra). For completeness, the deoxy tetracoordinated phosphorus system, compound 3 B, was also included in our set of model compounds.

EXPERIMENTAL PROCEDURES

NMR spectroscopy

The solution 1H- and 13C-NMR spectra were obtained at 200.13 MHz and 50.32 MHz, respectively, with a Bruker AC 200 NMR spectrometer. In order to facilitate the elucidation of very complicated 1H patterns, some spectra were recorded on an AM 300 NMR spectrometer. Samples were routinely dissolved in C2HCl3. The solid-state CP-MAS 13C- and 29Si-NMR spectra were recorded at 75.48 MHz and 59.63 MHz, respectively, on a Bruker CXP 300 NMR spectrometer equipped with a double air-bearing CP-MAS probe. The samples were stored in Kel-F capsules [23] which were sealed and used in aluminium oxide MAS rotors. By using these capsules moisture was kept out and the sample amount could, in principle, be as low as a few milligrams, without spinning instability problems. The temperature was about 300 K. The chemical shifts were related to tetramethylsilane (or in the case of 13C, via adamantane). The cross-polarization measurements were carried out with flipback of the proton magnetization and spin temperature inversion. For the CP-MAS experiments we used a time domain of 1000 data points and spectral widths of 17.2 kHz for 13C and 20 kHz for 29Si. A pulse width of 6 µs and contact and acquisition times of 2.5 ms and 29 ms for 13C, and 6 ms and 10 ms for 29Si were used. The pulse delay was 2 s for the silatranes and 3 s for the phosphates. The samples were rotated with 3 kHz. The reported chemical shifts were averaged values of at least two measurements with a deviation of less than ± 0.05 ppm.

Synthesis

The enantiomerically pure precursor 1,2-sn-dihexadecyglycerol was synthesized according to the method of Yamauchi et al. [24] starting from D-mannitol. The racemic modification of the alcohol was obtained starting from racemic 2,2-dimethyl-1,3-dioxolane-4-methanol (Janssen Chimica).

2-Hydroxymethylnonadecanol

To a stirred suspension of 1.8 g (75 mmol) NaH in 30 ml dry N,N-dimethylformamide was added 7.2 g (45 mmol) diethyl malonate. After keeping the solution at 85°C for 1 h, 7.2 g (23 mmol) heptadecyl bromide was added dropwise at room temperature. The mixture was allowed to stand for 72 h, after which 200 ml H2O was added. Then the solution was brought to pH 5 with 6 M hydrochloric acid. Extraction with diethyl ether, subsequent treatment with H2O and thorough drying of the organic layer yielded 2.2 g of crude product, which was used without further purification in the next reaction step. To a solution of 1.8 g (47 mmol) LiAlH4 in anhydrous diethyl ether was added 8.2 g (21 mmol) of the alkylated diethyl malonate at 0°C. After refluxing the solution for 1 h the mixture was worked up according to standard procedures, resulting in 5.9 g (90%) 2-hydroxymethylnonadecanol. 1H NMR: δ = 0.88 ppm (t, 3H, CH3), 1.25 ppm (m, 32H, CH2), 1.7 – 2.1 ppm (m, 3H, CH + OH), 3.75 ppm (m, 4H, CH3O).
1-Hexadecyl-2-deoxy-2-heptadecylglycerol

Alkylation was performed according to Yamauchi [24] with 1.8 g (6 mmol) alcohol and 3.5 g (9 mmol) hexadecyl tosylate [25]. Purification was obtained by recrystallization from ethyl acetate. "\( \delta = 0.88 \) ppm (t, 6H, CH3), 1.28 ppm (m, 48H, CH2), 1.56 ppm (m, 4H, /CH3), 2.18 ppm (m, 1H, CH), 3.3-3.8 ppm (m, 7H, CH2O + OH).

\((1,2\text{-sn-Dihexadecylglyceryl)silatrane 1A and (1-hexadecyl-2-deoxy-2-heptadecylglycerylsilatrane 1B.}\)

The silatranes were synthesized according to a procedure developed by Voronkov et al. [26] with slight modifications. Triethanolamine and tetracethyl orthosilicate were distilled before use. The alcohol was dried in vacuo over \( \text{P}_2\text{O}_5\). Equimolar amounts of the reagents and a catalytic amount of KOH were mixed in toluene. The mixture was stirred at 100°C for 24 h. During this time the formed ethanol was azeotropically removed. After removing the solvent the crude product was crystallized twice from hot hexane.

\( {^1H}\text{NMR: } \delta = 0.88 \text{ ppm (t, 6H, CH},_3\text{), 1.25 ppm (m, 48H, CH}_2\text{), 1.58 ppm (m, 4H, /CH}_3\text{), 2.84 ppm (t, 6H, SiOCH}_3\text{), 3.38-3.80 ppm (m, 9H, }\text{CH, CH}_2\text{O), 3.82 ppm (t, 6H, NCH}_2\text{).} \)

\( \text{Compound 1A. } {^1H}\text{NMR: } \delta = 0.88 \text{ ppm (t, 6H, CH}_3\text{), 1.25 ppm (m, 48H, CH}_2\text{), 1.58 ppm (m, 4H, /CH}_3\text{), 2.84 ppm (t, 6H, SiOCH}_3\text{), 3.38-3.80 ppm (m, 9H, }\text{CH, CH}_2\text{O), 3.82 ppm (t, 6H, NCH}_2\text{).} \)

\( \text{Compound 1B. } {^1H}\text{NMR: } \delta = 1.80 \text{ ppm (m, 1H, CH), 2.83 ppm (t, 6H, SiOCH}_2\text{), 3.27 - 3.68 ppm (m, 6H, CH}_2\text{O), 3.83 ppm (t, 6H, NCH}_2\text{).} \)

The solution \( ^{13}\text{C-NMR} \) and the solid-state \( ^{29}\text{Si-NMR} \) chemical shifts are compiled in Tables 1 and 3, respectively (vide infra).

\( ^{13}\text{C-NMR} \) chemical shifts for the silatranes (pentacoordinated silicon), phosphoranes (pentacoordinated phosphorus) and phosphates (tetracoordinated phosphorus) in \( \text{CDCl}_3 \)

The phosphates were prepared via the same procedure as outlined in [10].

\( \text{Compound 3A. } {^1H}\text{NMR: } \delta = 0.88 \text{ ppm (t, 6H, CH}_3\text{), 1.25 ppm (m, 52H, CH}_2\text{), 1.54 ppm (m, 4H, /CH}_3\text{), 3.77 ppm (d, 6H, POCH}_3\text{), 3.35 - 3.68 ppm (m, 7H, OCH}_2\text{ + CH), 3.98 - 4.24 ppm (m, 2H, POCH}_2\text{).} \)

\( \text{Compound 3B. } {^1H}\text{NMR: } \delta = 1.89 \text{ ppm (m, 1H, CH), 3.38 ppm (m, 4H, /CH}_3\text{), 3.78 ppm (d, 6H, POCH}_3\text{), 4.05 ppm (t, 2H, POCH}_2\text{).} \)

The solution \( ^{13}\text{C-NMR} \) chemical shifts are compiled in Table 1 (vide infra).

RESULTS

Conformational transmission in solution

The conformational analysis of the C2-C3 bond with \( ^1\text{H-NMR} \) spectroscopy is based on the Karplus equation, which relates vicinal coupling constants to the torsional angle between the coupling protons [27]. The measured coupling constants are a time-weighted average of the contributions made by the three staggered rotamers \( g, g', \) and \( g'' \) (see Fig. 3, vide supra). In our case a modified Karplus equation was used, in which the electronegativity of the substituents at the C-C bond and their relative positions have been taken into account [28]. The diastereotopic protons attached to C3 were assigned

Table 1. The solution \( ^{13}\text{C-NMR} \) chemical shifts for the silatranes (pentacoordinated silicon), phosphoranes (pentacoordinated phosphorus) and the phosphates (tetracoordinated phosphorus) in \( \text{CDCl}_3 \)

<table>
<thead>
<tr>
<th>Carbon atom</th>
<th>Solution ( ^{13}\text{C-NMR} ) chemical shift</th>
</tr>
</thead>
<tbody>
<tr>
<td>silatranene</td>
<td>phosphorane</td>
</tr>
<tr>
<td>1A</td>
<td>1B</td>
</tr>
<tr>
<td>( 2\text{oxo} )</td>
<td>( 2\text{deoxy} )</td>
</tr>
<tr>
<td>ppm</td>
<td></td>
</tr>
<tr>
<td>C3</td>
<td>62.74</td>
</tr>
<tr>
<td>C2</td>
<td>79.78</td>
</tr>
<tr>
<td>C1</td>
<td>72.41</td>
</tr>
<tr>
<td>x-sn-1</td>
<td>71.47</td>
</tr>
<tr>
<td>sn-2</td>
<td>70.46</td>
</tr>
<tr>
<td>Internal</td>
<td>29.81</td>
</tr>
<tr>
<td>( \alpha\text{-1} )</td>
<td>22.78</td>
</tr>
<tr>
<td>( \alpha\text{-2} )</td>
<td>14.18</td>
</tr>
<tr>
<td>POCH3</td>
<td>54.87 (10.8)</td>
</tr>
<tr>
<td>SiOCH2</td>
<td>57.83</td>
</tr>
<tr>
<td>NCH2</td>
<td>51.41</td>
</tr>
</tbody>
</table>

* Resonances could not be assigned properly.
according to the findings for the tetracoordinated or the pentacoordinated phosphorus derivative of 1,2-sn-dihexanoylglycerol [10]. Since the increase of electron density on O3 going from a tetracoordinated to a pentacoordinated state leads to the inducement of conformational transmission, 13C-NMR can be useful for probing the electron density on O3 indirectly via C3. A decrease (increase) of the 13C chemical shift means an increase (decrease) of the electron density on O3. The results are very well illustrated in Table 1.

Especially for silatrane 1A the 13C NMR was meaningful because the 1H-NMR analysis was not possible due to a near-equivalence of the H3 resonances. The 13C-NMR measurements indirectly show and confirm the expected conformational transmission based on the shielding of C3. The results of the corresponding deoxy lipid 1B obtained with 1H-NMR and 13C-NMR indicate the absence of conformational transmission. The conformational distributions around the C2-C3 linkage determined with 1H NMR (vide supra) for the various compounds is given in Table 2. In Table 2 we include the previously studied tetrahydrofurfuryl compounds [13, 19] as an integral reference system (see Fig. 4 for their rotamers). The deoxy lipid 3B also showed isochronous proton resonances, but comparison with a similar oxy/deoxy lipid combination showed that no conformational change occurs in the tetracoordinated situation by substitution of O2 for methylene (see Fig. 5).

Table 2. Conformational distributions

<table>
<thead>
<tr>
<th>Compound</th>
<th>Conformational distribution</th>
<th>Chemical shift of C3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x(g+) x(g') x(g-)</td>
<td>ppm</td>
</tr>
<tr>
<td>Pentacoordinated silicon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tetrahydrofurfuryl</td>
<td>0.30 0.18 0.52</td>
<td>66.4</td>
</tr>
<tr>
<td>Oxydihexadecyl 1A</td>
<td>0.33 0.44 0.23</td>
<td>62.7</td>
</tr>
<tr>
<td>Deoxydihexadecyl 1B</td>
<td>0.32 0.35 0.33</td>
<td>69.9</td>
</tr>
<tr>
<td>Pentacoordinated phosphorus tetrahydrofurfuryl</td>
<td>0.36 0.35 0.29</td>
<td>66.6</td>
</tr>
<tr>
<td>Oxydihexadecyl 2A</td>
<td>0.40 0.43 0.17</td>
<td>70.6</td>
</tr>
<tr>
<td>Deoxydihexadecyl 3B</td>
<td>0.44 0.41 0.15</td>
<td>67.2</td>
</tr>
<tr>
<td>2-Hexyl-3-hexanoylglycerol</td>
<td>0.40 0.37 0.23</td>
<td></td>
</tr>
<tr>
<td>2-Deoxy-2-heptyl-3-hexanoylglycerol</td>
<td>0.37 0.38 0.25</td>
<td></td>
</tr>
</tbody>
</table>

Conformational transmission in the solid state

Fig. 6 shows the CP-MAS 13C-NMR spectrum of the silatrane compound of 1,1,2-sn-dihexadecylglycerol (compound 1-1A) along with the 2-deoxy system (compound 2-1B) as counterpart. The solid-state chemical shifts of the silatrane and phosphate lipids are assigned according to the findings for the compounds in solution (Table 1, vide supra) and are compiled in Table 3.

Although the solid-state spectra are characterized by broad lines, some interesting differences in chemical shift appear when comparing compounds 1-1A and 2-1B, and compounds 1A and 1B. In compound 1-1A, the methylene carbons of the chains and the ultimate methyl carbon are deshielded by approximately 0.6 ppm with respect to the DL modification and resonate at 33.7 ppm and 15.8 ppm, respectively. A similar chemical shift for the inner chain carbons was found in compound 2-1B. It has been well established that hydrocarbon chains with a chemical shift in the range from 33.6 – 34.3 ppm are arranged in a triclinic lattice with...
parallel chain planes, whereas chains resonating at approximately 33.0 ppm are packed in one of the other crystallographic structures, i.e. orthorhombic, pseudohexagonal or monoclinic with perpendicular chain planes [29—31]. Therefore it is concluded that compounds 1-L A and 1-L B are packed in triclinic cells, whereas compound 1-L A, and the corresponding phosphates, are packed in a non-triclinic lattice (the exact type is of no importance, since the chemical shifts of the inner chain and terminal carbons are identical in all three cells). Hence the chemical shift differences between compounds 1-L A and 1-L A result from the different lattices in which the chains are packed.

For a proper evaluation of the observed 13C chemical shift differences one has to compare lipids within one type of crystallographic cell. It is very unlikely that the mere substitution of O2 for methylene would bring about a different crystallographic form (the bond length and angle are quite similar in both compounds). This supposition is supported by recent X-ray and electron diffraction studies in which no change in the type of chain (sub)cells was found when the ester linkage in 1,2-di-palmitoylglycerophosphocholine was replaced by ether bonds [32, 33]. Thus one can compare compounds 1-L A and 1-L B, since both have triclinic lattices. A marked shielding of approximately 40 ppm difference is seen for C2 between solid compounds 1-L A and 1-L B. This difference is mainly due to the short-range substitution effect (CH2 replacing O), and appears also between the oxy and deoxysilatrane lipids 3A and 3B, respectively. The C3 nucleus, which is further away, is deshielded by 7.3 ppm on going from the oxysilatrane to the deoxysilatrane. At the end of the lipid chains, where effects of the substitution in the glyceryl backbone are negligible, a deshielding of 0.9 ppm occurs for the ultimate methyl groups in the oxysilatrane with respect to the deoxy counterpart. Such a large shift difference has never been observed within one crystallographic class. This Van der Waals deshielding effect originates from an increase in packing density in this area, since it is well documented that the 13C nuclei of two approaching methyl groups become less shielded due to the mutual depolarization effect of the C-H bonds of the respective groups [31, 34, 35]. In addition, the optimum cross-polarization contact time for the terminal methyl groups decreases from 1.5 ms to 1.0 ms on going from the deoxysilatrane to the oxysilatrane (see Table 4). This result also indicates that in compound 1-L A the methyl groups are less mobile than in compound 1-L B, since the resonance intensities of carbons in the more mobile regions reach their maximum at longer contact times compared to the signals of carbons in restricted areas [22].

In contrast with the chemical shift differences found in the silatrane, no differences in the chain region are observed between the oxysilatrane and deoxysilatrane 3A and 3B, respectively, both in a non-triclinic arrangement. Apparently, the replacement of O2 by methylene has no influence on the chain packing in the phosphate lipids. Consistent with

![Fig. 6. CP-MAS 13C-NMR spectra of compounds 1-L A and 1-L B](image-url)

**Table 3. The solid-state 13C chemical shift for the enantiomerically pure and racemic silatrane and phosphates**

The experimental conditions are compiled in the experimental section. 2 and 3 indicate the first and terminal chain carbons, respectively. Estimated accuracy of δ is ±0.05 ppm. Values for SiOCH3, NCH2 and 29Si are in agreement with those reported in [42, 43].

<table>
<thead>
<tr>
<th>Carbon atom</th>
<th>Solid-state 13C-NMR chemical shift</th>
<th>Phosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>silatrane</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-oxys</td>
<td>2-deoxy</td>
</tr>
<tr>
<td></td>
<td>non-triclinic</td>
<td>non-triclinic</td>
</tr>
<tr>
<td></td>
<td>1-L A</td>
<td>1-L B</td>
</tr>
<tr>
<td>C3</td>
<td>61.80</td>
<td>61.53</td>
</tr>
<tr>
<td>C2</td>
<td>83.29</td>
<td>81.14</td>
</tr>
<tr>
<td>C1</td>
<td>73.61*</td>
<td>70.87*</td>
</tr>
<tr>
<td>a-sn-1</td>
<td>73.61*</td>
<td>70.87*</td>
</tr>
<tr>
<td>sn-2</td>
<td>75.63*</td>
<td>73.42*</td>
</tr>
<tr>
<td>Internal</td>
<td>33.12</td>
<td>33.67</td>
</tr>
<tr>
<td>ω-1</td>
<td>24.83</td>
<td>24.62</td>
</tr>
<tr>
<td>POCH3</td>
<td>58.05</td>
<td>57.67</td>
</tr>
<tr>
<td>SiOCH2</td>
<td>51.45</td>
<td>51.20</td>
</tr>
<tr>
<td>NCH2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29Si</td>
<td>-95.11</td>
<td>-95.19</td>
</tr>
</tbody>
</table>

* Resonances could not be assigned properly.
this result is the observation that both phosphates have the same optimum contact times for the methyl groups, indicating comparable mobilities.

Between compounds DL-1A and DL-3A (both non-triclinic) a chemical shift difference for the ultimate methyl groups is observed similar to the one between the triclinic forms of the compounds L-1A and DL-1B. The silatranes and phosphates have mutually comparable headgroup areas as indicated by Dreiding models (approximately 0.46 nm² for the silatrane and 0.41 nm² for the phosphate) and the alkyl chains are arranged in non-triclinic lattices. Therefore, the comparable chemical shift differences for the terminal methyl groups within both sets suggest a similar packing difference between compounds L-1A and DL-1B on the one hand and between compounds DL-1A and DL-3A on the other hand.

DISCUSSION

The combined results in the solution and condensed phases show that substantial changes in the chain-end region can be brought about by changing the conformational state of the glyceryl backbone. The close resemblance for the C3 chemical shifts of the oxysilatrane in the solid state and in solution seems indicative for a similar conformation of the molecule in both phases. In the solid phase a difference in the rotational state of the backbone between compounds L-1A and the DL-1B clearly appears from the pronounced de-shielding of the C3 nucleus in compound DL-1B with respect to compound L-1A (7.3 ppm). This deshielding is only partly due to a short-range substitution effect. From a comparison of the C3 chemical shifts in the phosphates 3A and 3B in solution (67.25 ppm and 68.30 ppm, respectively) with similar backbone conformations, it is estimated that this effect contributes about 1 ppm to the deshielding of C3. The remaining part must then be brought about by conformational differences. However, the conformational change around the C2-C3 bond from a trans arrangement (g⁻) in the oxysilatrane to a gauche orientation (g⁺, g') in the deoxy form cannot be the main cause for the observed downfield shift for C3 since this conformational effect is relatively small (about 1 ppm) and is known to produce an upfield shift [36]. On the other hand, it is well established that such a large deshielding of C3 can originate from a conformational change around the C1-C2 bond. When the normal C3,Ol trans orientation changes into a C3,Ol gauche conformation, then C3 is strongly shielded [36]. Therefore, in the oxysilatrane, a changed C1-C2 conformation must be present to explain the strong shielding effect. This, in addition to the findings regarding the preferred conformation in solution (vide supra), is related to the observed different chain-end packing in the t-oxysilatrane and dl-deoxysilatrane in the solid phase as visualized in Fig. 7.

In the solid state a g⁻ conformation around the C2-C3 bond in the oxysilatrane is expected, which is controlled by the electrostatic repulsion between O3 and O2. As a result of this head group conformation, a packing for the lipid chains similar to that found in the deoxysilatrane would result in steric repulsions between the silatrane cage and the sn-2 chains of neighbouring lipids. The C2-C3 g⁻ conformation can only be accommodated when the glyceryl backbone is more parallel to the bilayer plane. In order to restore the parallel alignment of the lipid chains, a conformational change around the C1-C2 bond from a C3,Ol trans to a C3,Ol gauche orientation is necessary. Indeed, the large shielding effect on C3 (7.3 ppm, vide supra) can be rationalized in a reasonable way with this conformational change [36, 37]. It should be emphasized, that the C1-C2 conformation in the solid state is governed completely by packing effects, in contrast to the monomeric phase [10]. The conformational changes as described now results in an elimination of the characteristic bend at the beginning of the sn-2 chain. This leads to a reduction in the effective chain length difference between the sn-1 and sn-2

<table>
<thead>
<tr>
<th>Carbon atom</th>
<th>Optimum contact time (estimated accuracy ±0.2 ms) in CP-MAS ¹³C-NMR for the solid silatranes and phosphates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Silatrane</td>
</tr>
<tr>
<td>oxy</td>
<td>triclinic</td>
</tr>
<tr>
<td>dl-form</td>
<td>triclinic</td>
</tr>
<tr>
<td>Internal</td>
<td>0.7</td>
</tr>
<tr>
<td>α</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Fig. 7. Conformational transmission in the condensed phase. (A) The silatrane cage in a g⁻ (dashed lines) and g⁺ (solid lines) conformation. This latter arrangement induces steric repulsion between the head group and the sn-2 chains of neighbouring molecules. (B) Oxysilatrane lipid with the glyceryl backbone in a layer-parallel orientation and the O2 and O3 vicinal oxygens oriented trans. Due to a C1-C2 conformational change, the chain methyl groups are clearly more densely packed than in the case depicted in (A).
chain, and therefore the average packing densities of the end methyl groups will increase. In line with the conformational transmission model is the observation that between compounds 1-IA and 1-3A a comparable difference in packing of the ultimate methyl groups exists, as between compounds 1-1A and 1-1B. The higher electron density on O3 in the oxysilatrane relative to the oxypentane results in an increased population of the g' conformation around the C2-C3 bond in the oxysilatrane. According to the mechanism outlined before, this conformational difference is the origin of the changed packing of the ultimate methyl groups in both compounds.

Finally, support for this model arises from the 13C-NMR data for compounds 3A and 3B, with similar rotational equilibria in solution [10]. Consequently, virtually the same mersic model compounds. In the present paper evidence is presented for these conformational transmission effects in lipid models in the solid state. We obtained stable, solid lipids closely resemble each other.

Thus, it is demonstrated that conformational transmission effects originating in the head group can be transferred to the chain-end region by a reorientation of the glyceryl backbone. Such a change in the glyceryl backbone orientation from a layer-parallel to a more layer-perpendicular arrangement was also assumed in the high-pressure-induced transition in dioleylglycerolphosphocholine, thereby allowing an optimal packing of the cis double bonds [38]. As a result of the rearrangement of the glyceryl backbone, a condensing effect on the chain-end methyl groups was found with infrared spectroscopy, analogous to our observations for the silatrane.

Concluding remarks

It has already been demonstrated that a transition from tetra- to pentacoordinated phosphorus in lipid head groups can induce conformational changes in monomeric model compounds. In the present paper evidence is presented for these conformational transmission effects in lipid models in the solid state. We obtained stable, solid lipids with the same electronic properties as in the glyceryl backbone of the pentacoordinated phosphorus when phosphorus is replaced by pentacoordinated silicon (silatrane). Remarkable differences were observed in the chain-end packing between L-oxysilatrane and DL-deoxysilatrane and between DL-oxysilatrane and DL-oxypentane. In both sets of lipid models the differences can be attributed to conformational transmission effects. A model is proposed, in which the differences in chain packing are related to an enhanced electron density on O3 in the silatrane compounds, which leads to a preference for a g' conformation around the C2-C3 bond. Steric restrictions in the condensed phase prevent this conformation without a simultaneous reorientation of the glyceryl backbone. This reorientation is accompanied by C1-C2 conformational changes, which lead to a more dense packing of the end-chain methyl groups.

Recently, de Keijzer et al. [39, 40] found that the solvolysis of phosphates is accelerated in systems where conformational transmission is possible. They showed that this is due to a stabilization of the transient trigonal bipyramid that allows conformational changes in the leaving group located in the axis of the trigonal bipyramid. From the present results we conclude that the formation of a transient trigonal bipyramid may influence the membrane fluidity via the conformational transmission effect. This change in lipid packing can activate transport proteins, such as gramicidin A, as channel protein in the membrane. A model description for the lipid-protein interaction has been formulated recently [41] on the basis of molecular mechanics calculations, and involves a selective hydrogen bonding between a carbonyl group in the lipid molecule and a tryptophan residue in gramicidin A. A conformational change in the lipid then induces a change in protein structure to conserve this bonding, leading to a widening of the channel opening in the gramicidin. The findings of the theoretical calculations have been checked by a preliminary experimental study, in which an increase of Na⁺ efflux through gramicidin could be observed going from vesicles containing ether lipids to those containing ester lipids [41].

We wish to express our gratitude to Mr L. J. M. van de Ven for his valuable advise in obtaining the CP-MAS NMR results. Dr L. H. Koole is acknowledged for the stimulating discussions. The 500-MHz spectra were recorded at the Dutch National NMR Facility in Nijmegen.

REFERENCES