

Molecular volumes of phospholipids and glycolipids in membranes

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ABSTRACT

Data on the molecular volumes of phospholipids and glycolipids in membranes are collected together in order to determine the contributions from the component groups, for as wide a range of lipids as possible, including sphingolipids. Wherever possible, the volumes of the methylene groups in the lipid chains are established from the dependence on chain length at fixed temperature in a given phase. In this way, it is also possible to determine the constant contribution from *cis* double bonds in the chains of monoenoic unsaturated phosphatidylcholines, and the volume of the branched methyl groups in isoacyl phosphatidylcholines. Issues concerning separation of contributions from the polar head groups from those of the chain terminal methyl groups are discussed. Molecular volumes of lipids in crystals are also analysed to provide information on head-group packing that can be compared with the situation in membranes, and used to set limits on the relative contributions from polar groups and terminal methyl groups. Comparisons are made with volumetric analyses based on diffraction studies of bilayers of single lipids. The parameters derived can be used to estimate molecular volumes of lipids for which dilatometric or densitometric data are lacking. Lipid volumes are determining parameters for lipid dynamics, membrane partitioning and permeation of solutes, and are essential quantities for the structural analysis of lipid membranes.

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1. Introduction

The volume that is occupied by each of the lipid molecules is one of the most fundamental properties of the lipid bilayer membrane. Its size, relative to the true van der Waals volume, determines the free volume of the membrane, which in turn regulates dynamic processes such as translational diffusion, the partitioning of hydrophobic molecules (including drugs and anaesthetics) into the membrane, and the permeability of the lipid membrane to solutes. In addition, the molecular volume is an essential parameter for structural determinations in membranes. It is required to obtain the bilayer thickness from X-ray measurements of the bilayer repeat distance in hydrated multilamellae (Luzzati, 1968). At a more detailed level of structural analysis, it determines electron or scattering-length densities that are needed for interpreting X-ray and neutron scattering experiments and for analysing form factors obtained by diffraction methods (Wiener et al., 1989; Kucerka et al., 2004, 2005a).

The basic quantity that is needed to determine the molecular volume of a lipid in a membrane is the partial specific volume, \bar{v}_1 , which is defined by the increase in volume V of an aqueous

dispersion on adding lipid:

$$\bar{v}_1 = \left(\frac{\partial V}{\partial m_1} \right)_{T,P,m_w} \quad (1)$$

where m_1 is the mass of lipid and m_w that of the water present. The change in volume of the dispersion also involves any apparent change in volume of the water which hydrates the lipid head groups. The apparent volume of a lipid molecule in a membrane is related to the lipid partial specific volume, \bar{v}_1 , by:

$$v_{\text{mol}} = \frac{M_1 \bar{v}_1}{N_A} \quad (2)$$

where M_1 is the molecular weight of the lipid and N_A is Avogadro's number. An alternative definition is the partial molar volume, which is given by: $V_m = M_1 \bar{v}_1$. As already noted, both quantities include any volume changes that are associated with hydration of the lipid head groups. For comparison, the volume of a lipid molecule in a crystal is related to the dimensions a , b and c of the unit cell by:

$$v_{\text{mol}} = abc \frac{\sqrt{1 - \cos^2 \alpha - \cos^2 \beta - \cos^2 \gamma + 2 \cos \alpha \cos \beta \cos \gamma}}{Z} \quad (3)$$

where α , β and γ are the angles between the unit cell axes and Z is the number of lipid molecules in the unit cell. Note that not all lipid crystals are orthorhombic (for which $\alpha = \beta = \gamma = 90^\circ$, and $v_{\text{mol}} = abc/Z$). The definition in Eq. (3) includes any solvent molecules of

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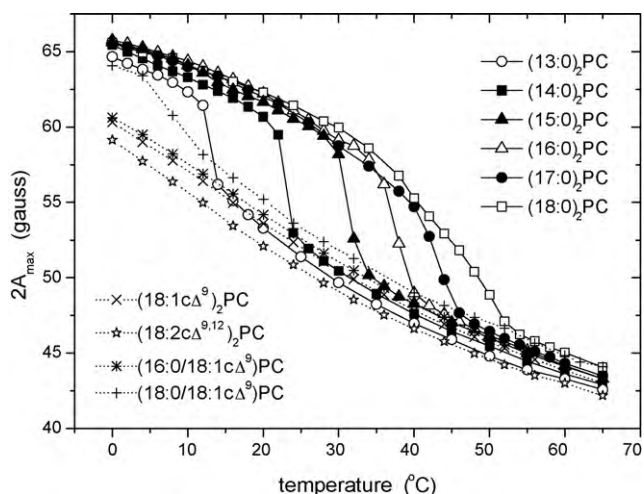


Fig. 1. Temperature dependence of the outer hyperfine splitting, $2A_{\max}$, of phosphatidylcholine spin-labelled on the 5 C-atom of the *sn*-2 chain (0.5 mol%) in hydrated bilayers of saturated diacyl phosphatidylcholines, $(n:0)_2\text{PC}$, and unsaturated diacyl phosphatidylcholines, $(n:1,2)_2\text{PC}$, $(n_1:0/n_2:1)_2\text{PC}$. Phospholipids were obtained from Avanti Polar Lipids (Alabaster, AL); spin label was synthesised as described (Marsh and Watts, 1982; Marsh, 2009). For details of sample preparation and EPR spectroscopy, see (Rama Krishna and Marsh, 1990; Bartucci et al., 1993; Schorn and Marsh, 1996a).

crystallisation, the volume of which must be subtracted to give the bare lipid volume that is comparable to the definition derived from the partial specific volume and given by Eq. (2).

2. Reference temperature

Combination of volumetric data from lipids of the same species but different chain lengths requires a choice of the appropriate reference temperature. Potential candidates are the same absolute temperature (see e.g., Nagle and Wiener, 1988), or comparable temperatures relative to the chain-melting transition, i.e., the same reduced temperature (see e.g., Small, 1986).

Fig. 1 shows the temperature dependence of the outer hyperfine splitting, $2A_{\max}$, from a phosphatidylcholine spin probe in hydrated bilayers of saturated diacyl phosphatidylcholines with different chain lengths in the range $n=13$ –18 C-atoms, and also of unsaturated diacyl phosphatidylcholines with symmetrical or mixed chains. The spin-label moiety is attached at C-5 of the *sn*-2 chain. The outer hyperfine splitting depends on the amplitude, and also rate, of angular motion of the spin-labelled chain segment (Lange et al., 1985; Moser et al., 1989; Schorn and Marsh, 1996b). It is therefore a useful parameter for characterising chain dynamics in both gel and fluid membrane phases (Rama Krishna and Marsh, 1990; Bartucci et al., 1993; Schorn and Marsh, 1996a), and depends directly on the available free volume within the lipid bilayer. Lower values of A_{\max} correspond to higher amplitudes and/or rates of chain rotation, and hence to larger free volumes. This is seen clearly from the abrupt decrease in A_{\max} that occurs on chain melting of the bilayers of the different-chainlength phosphatidylcholines at their corresponding gel-to-fluid transition temperatures. However, the sensitivity is much less in the gel phase than it is in the fluid phase, because the former corresponds to the slow-motion regime of conventional spin-label EPR spectroscopy, whereas the latter lies in the fast-motional regime.

Although the EPR splittings depend on other factors in addition to free volume, the results of Fig. 1 clearly indicate that the lipid bilayers are in equivalent states at the same absolute, rather than reduced, temperature. The values of A_{\max} are comparable for all chain lengths at the same temperature when in

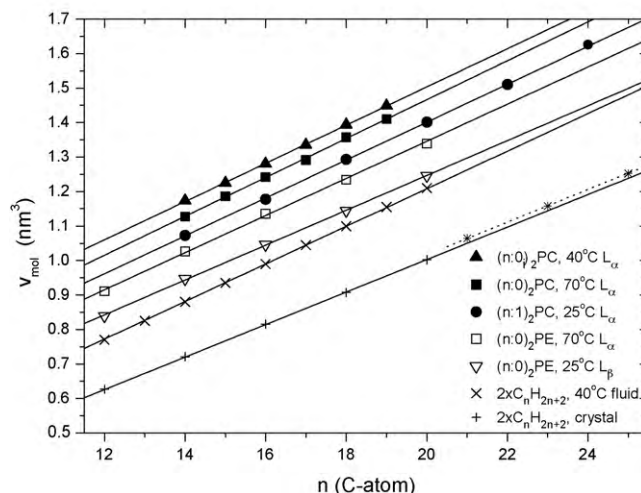


Fig. 2. Lipid molecular volume as a function of acyl chain length, n . Saturated straight-chain diacyl phosphatidylcholines $(n:0)_2\text{PC}$ in the L_α phase at 70 °C (solid squares; Schmidt and Knoll, 1985); monoenoic diacyl phosphatidylcholines $(n:1)_2\text{PC}$ in the L_α phase at 25 °C (solid circles; Uhríková et al., 2007); branched-chain di-isoacyl phosphatidylcholines $(n:0)_2\text{PC}$ in the L_α phase corrected to 40 °C (solid triangles; Yang et al., 1986); saturated straight-chain diacyl phosphatidylethanolamines $(n:0)_2\text{PE}$ in the L_α phase corrected to 70 °C (open squares; Koynova and Hinz, 1990; Seddon et al., 1984) and in the L_β phase corrected to 25 °C (open inverted triangles; Koynova and Hinz, 1990; Seddon et al., 1984); straight-chain alkanes $\text{C}_n\text{H}_{2n+2}$ in the liquid state at 40 °C (\times ; Rossini et al., 1953) and in the crystalline state (+ even n ; Nyburg et al., 1976; Müller and Lonsdale, 1948; Crissman et al., 1970; * odd n ; Smith, 1953). Note: n for di-isoacyl PCs excludes the branched methyl group.

a given phase (gel or fluid), but are larger for the lower-melting lipids at a given temperature difference from the chain-melting transition. This is not a phenomenon that is restricted to phosphatidylcholines; similar EPR results have been found with bilayers of biotinylphosphatidylethanolamines (Swamy and Marsh, 1994). Thus, the appropriate reference temperature for comparing data on lipid molecular volumes is the same absolute temperature, irrespective of chain-melting temperature (except that the lipids must be in the same phase).

The above conclusion was reached previously by Wiener and White (1992) from neutron diffraction experiments on fluid-phase dioleoyl phosphatidylcholine bilayers. The ratio of methyl to methylene volumes was found to be approximately two, a result that is obtained for liquid alkanes when comparison is made at the same absolute temperature, but not when made at the same reduced temperature. The ratio of two is also obtained in molecular dynamics simulations of dioleoyl phosphatidylcholine bilayers, demonstrating that this experimental feature can be reproduced by simulations (Armen et al., 1998). Additionally, Nagle and Wiener (1988) have argued on theoretical grounds that absolute temperature is the appropriate reference state. This is the approach that will be adopted here.

3. Phospholipids

3.1. Chain-length dependence and CH_2 volume

Fig. 2 shows the dependence of the lipid molecular volume, v_{mol} , on length, n , of the hydrocarbon chains. Data are given for the phospholipids phosphatidylcholine (PC) and phosphatidylethanolamine (PE), and for saturated straight-chain n -alkanes ($\text{C}_n\text{H}_{2n+2}$). For a given lipid, measurements either are at the same temperature in the same phase, or are corrected to the same temperature by using the volume expansion coefficient in

Table 1

Component volumes of molecular groups, obtained by linear regression to the chain-length dependence (Eqs. (4) and (5)) for phospholipids in membranes, and for *n*-alkanes (see Fig. 2).

| Lipid | Phase | <i>T</i> (°C) | v_{CH_2} | | $v_{\text{end}}^{\text{a}}$ | | Ref. ^b |
|--|-------------------------------------|---------------|--------------------|--------------|-----------------------------|----------------------|-------------------|
| | | | (nm ³) | (ml/mol) | (nm ³) | (ml/mol) | |
| (<i>n</i> :0) ₂ PC | L _α | 70 | 0.0282 ± 0.0004 | 17.0 ± 0.2 | 0.451 ± 0.012 | 272 ± 7 | 1 |
| (<i>n</i> :1) ₂ PC | L _α | 25 | 0.0275 ± 0.0002 | 16.6 ± 0.1 | 0.522 ± 0.005 | 314 ± 3 | 2 |
| (<i>n</i> :0) ₁ ₂ PC | L _α | 40 | 0.0277 ± 0.0003 | 16.7 ± 0.2 | 0.506 ± 0.007 ^c | 305 ± 4 ^c | 3 |
| (<i>n</i> :0) ₂ PE | L _α | 70 | 0.0269 ± 0.0007 | 16.2 ± 0.4 | 0.376 ± 0.017 | 227 ± 11 | 4 |
| | L _β | 25 | 0.0253 ± 0.0002 | 15.2 ± 0.1 | 0.337 ± 0.007 | 203 ± 4 | 4,5 |
| <i>n</i> -C _{<i>n</i>} H _{2<i>n</i>+2} | Liquid ^d | 40 | 0.02739 ± 0.00003 | 16.49 ± 0.02 | 0.1117 ± 0.0003 | 67.2 ± 0.2 | 6 |
| | | 70 | 0.02783 ± 0.00004 | 16.76 ± 0.03 | 0.1194 ± 0.0005 | 71.9 ± 0.3 | 6 |
| | Crystal, even <i>n</i> ^e | – | 0.0236 ± 0.0001 | 14.20 ± 0.06 | 0.0769 ± 0.0013 | 46.3 ± 0.8 | 7–9 |
| | Crystal, odd <i>n</i> ^f | – | 0.0237 ± 0.0001 | 14.29 ± 0.05 | 0.0810 ± 0.0018 | 48.8 ± 1.1 | 10 |

Note: For isoacyl lipids (*n*:0_{*i*}), *n* in the linear regression excludes the branched methyl group, which is included in v_{end} (17:0_{*i*})=(15Me-16:0): isoheptadecanoyl=15-methylhexadecanoyl.

^a Total volume of all molecular groups other than the chain methylenes: $v_{\text{end}}(\text{diacyl}) = v_{\text{pol}} + n_{\text{CH}}v_{\text{CH}} + 2v_{\text{CH}_3}$ (except for branched-chains, see footnote c), and $v_{\text{end}}(\text{alkane}) = 2v_{\text{CH}_3}$.

^b References: 1. Schmidt and Knoll, 1985; 2. Uhríková et al., 2007; 3. Yang et al., 1986; 4. Koynova and Hinz, 1990; 5. Seddon et al., 1984; 6. Rossini et al., 1953; 7. Nyburg et al., 1976; 8. Müller and Lonsdale, 1948; 9. Crissman et al., 1970; 10. Smith, 1953.

^c Includes the two branched methyl groups and H-atom deficit of the methylenes to which they are attached.

^d 14 values in the range *n* = 7–20.

^e Triclinic crystals: *n* = 10–20 (*N* = 6).

^f Orthorhombic crystals: *n* = 21–29 (*N* = 5).

that particular phase. Under these conditions, the molecular volume depends linearly on chain length, as would be expected if the volumes of the component molecular groups of a diacyl phospholipid are the same at the same temperature in a given phase:

$$v_{\text{mol}}(\text{diacyl}) = n_{\text{CH}_2}v_{\text{CH}_2} + n_{\text{CH}}v_{\text{CH}} + 2v_{\text{CH}_3} + v_{\text{pol}} \quad (4)$$

where n_{CH_2} and v_{CH_2} are the number and volume of methylene groups in the chains, n_{CH} and v_{CH} are the number and volume of methine groups (unsaturated C-atoms) in the chains, v_{CH_3} is the volume of a terminal methyl group, and v_{pol} is the volume of the polar head group, which includes the glycerol backbone and chain carbonyls. Eq. (4) applies to all diacyl (and dialkyl) phospholipids and glycolipids, except those with branched chains (see later). For *n*-alkanes, the corresponding expression for the molecular volume is simply:

$$v_{\text{mol}}(\text{alkane}) = n_{\text{CH}_2}v_{\text{CH}_2} + 2v_{\text{CH}_3} \quad (5)$$

Volumes of both methylene (v_{CH_2}) and terminal methyl (v_{CH_3}) groups can be determined from the linear dependence on chain length that is predicted by Eq. (5) for *n*-alkanes. However, this is not the case for the diacyl lipids. Although v_{CH_2} can be determined unambiguously for diacyl lipids, Eq. (4) indicates that the volume of the terminal methyl groups cannot be determined separately. Only the combined quantity $v_{\text{pol}} + 2v_{\text{CH}_3}$ can be obtained from the chain-length dependence of the molecular volume of saturated diacyl (or dialkyl) lipids.

The results of linear regressions to the chain-length dependences of the molecular volumes of various lipids (including those of Fig. 2), at constant temperature in the same phase, are given in Table 1. In the fluid L_α phase, the effective volume of a methylene group is $v_{\text{CH}_2} = 0.027 - 0.028 \text{ nm}^3$ depending on temperature and lipid, and is very similar to that in liquid *n*-alkanes. In the L_β gel phase of PEs, the volume of a methylene group ($v_{\text{CH}_2} = 0.025 \text{ nm}^3$) is less than in the fluid L_α phase, but is slightly larger than in crystalline hydrocarbons ($v_{\text{CH}_2} = 0.024 \text{ nm}^3$). It is close to that expected for a gel-phase chain, which has a cross-sectional area of 0.20 nm² and all-*trans* segment length of 0.127 nm (see, e.g., Marsh, 1990). The volume of the methyl groups in liquid *n*-alkanes ($v_{\text{CH}_3} = 0.0559 - 0.0597 \text{ nm}^3$) is 2.04–2.14 times that of a methylene group, and this ratio increases with increasing temperature. In crystalline *n*-alkanes, for comparison, the volume of the methyl groups ($v_{\text{CH}_3} = 0.038 - 0.041 \text{ nm}^3$) is 1.6–1.7 times that of

a methylene group, where the lower value corresponds to triclinic crystals for even chain lengths *n* = 10–20 and the higher value to orthorhombic crystals for odd chain lengths *n* = 21–29 (see Table 1).

It should be noted that the linear extrapolation works only if the methylene groups occupy the same volume, independent of chain length. For this it is important that the lipids of different chain length be in the same phase and at the same temperature. Even so, there are potential problems in the more condensed phases, if the packing mode changes with chain length. This is the case with the L_β phase of saturated phosphatidylcholines, for which the methylene group volume decreases with increasing chain length at constant temperature, an effect that correlates with increasing rhombicity of the chain packing (Sun et al., 1996).

3.2. Double-bond (v_{CH}) and isobranched (v_{iMe}) volumes

Comparison of the “end” contributions, v_{end} , in fluid-phase phosphatidylcholines gives estimates of the contributions of branched methyl groups, and of unsaturated bonds, to the lipid volume. Allowance can be made for the different temperatures of measurement by calculating an effective expansion coefficient from the volumes of a methylene group in the various bilayers (see Table 1). This turns out to be $\approx 6 \times 10^{-4} \text{ K}^{-1}$, for both (*n*:1)₂PC and (*n*:0)₁₂PC lipids, when referred to the (*n*:0)₂PC series. From the data for the (*n*:1)₂PC and (*n*:0)₂PC series, the volume of each CH group in the double bonds is then calculated to be $v_{\text{CH}} = 0.021 \text{ nm}^3$ at 25 °C. For comparison, a value of $v_{\text{CH}} = 0.0222 \text{ nm}^3$ at 25 °C was obtained from measurements on unsaturated lipids alone, but with the assumption of a fixed ratio between the volumes of methyl and methylene groups and/or a fixed polar group volume (Uhríková et al., 2007; Koenig and Gawrisch, 2005). In addition, expansion coefficients of 7.1 and $10.0 \times 10^{-4} \text{ K}^{-1}$ were found for the CH-group volume in the latter two references, respectively, i.e., reasonably close to the effective value used here. The effective volume of a CH group in liquid alkenes is also close to $v_{\text{CH}} = 0.021 \text{ nm}^3$ (Requena and Haydon, 1975), and that in (18:1)₂PC bilayers at 23 °C deduced by diffraction methods is $v_{\text{CH}} \approx 0.023 \text{ nm}^3$ (Wiener and White, 1992).

Applying a similar procedure to the end contribution for di-isoacyl phosphatidylcholines by using data from the (*n*:0)₁₂PC and (*n*:0)₂PC series, gives a volume contribution of $v_{\text{iMe}} = 0.031 \text{ nm}^3$ from each methyl branch. Note that v_{iMe} corresponds to the vol-

ume of a methyl group minus the single H-atom deficit produced by the methyl substitution, and is therefore formally equivalent to the volume of a methylene group. The volume contribution of methyl branching $v_{\text{imE}}/v_{\text{CH}_2} = 1.1$, relative to that of a methylene group, is therefore reasonable and compares well with the value of 1.2 that was obtained for this ratio by combining dilatometric and X-ray diffraction measurements solely on the isobranched lipids (Yang et al., 1986). Because, however, both methyl groups are formally equivalent in isoacyl chains, the effective volumes of the two should be averaged.

This brings one to the thorny problem of separating the contribution to v_{end} of the terminal methyl groups, $2v_{\text{CH}_3}$, from that of the polar groups, v_{pol} . Before attempting this, it is helpful to consider the molecular volumes in phospholipid crystals.

3.3. Phospholipid crystal volumes

Molecular volumes obtained from the unit cell dimensions of crystalline lipids are given in Table 2. Where solvent molecules co-crystallise with the lipid, the volumes of these molecules have been subtracted to give the net lipid volume, as indicated in the footnotes. It should be mentioned that not all structures correspond to the same conformation or packing of the lipid molecules (Pascher et al., 1992; Marsh and Páli, 2006). For instance, those of (12:0)₂PEMe,

Table 2
Molecular volumes in phospholipid crystals.

| Lipid ^a | $v_{\text{mol}}(\text{nm}^3)$ | $v_{\text{pol}}(\text{nm}^3)$ | Ref. ^b |
|---------------------------------------|-------------------------------|-------------------------------|-------------------|
| (14:0) ₂ PC ^c | 1.008 | 0.362 | 1, 2 |
| (O-18:0/O-1:0)PC ^d | 0.8513 ± 0.0007 | 0.3702 ± 0.0007 | 3 |
| (12:0)deoxyLPC ^d | 0.608 ± 0.005 | 0.332 ± 0.005 | 4 |
| (16:0/0:0)PC ^d | 0.7677 ± 0.0013 | 0.3970 ± 0.0013 | 1 |
| (O-16:0/0:0)PC ^e | 0.7146 ± 0.0011 | 0.3203 ± 0.0011 | 1 |
| (12:0) ₂ PE ^f | 0.814 ± 0.011 | 0.262 ± 0.011 | 5 |
| (16:0/0:0)PE | 0.645 ± 0.003 | 0.274 ± 0.003 | 6 |
| (12:0) ₂ PEMe | 0.8521 ± 0.0008 | 0.3000 ± 0.0008 | 1 |
| (12:0) ₂ PEMe ₂ | 0.8981 ± 0.0008 | 0.3460 ± 0.0008 | 7 |
| (16:0/0:0)PEMe ₂ | 0.6946 ± 0.0005 | 0.3240 ± 0.0005 | 1 |
| (14:0) ₂ PG ^g | 0.993 ± 0.002 | 0.346 ± 0.002 | 8 |
| (12:0) ₂ PA ^h | 0.7807 ± 0.0007 | 0.2287 ± 0.0007 | 1 |
| (14:0) ₂ PA ^g | 0.864 ± 0.003 | 0.217 ± 0.003 | 9 |
| (12:0/0:0)PA ⁱ | 0.4682 ± 0.0005 | 0.1921 ± 0.0005 | 10 |
| 14:0/PA/0:0 ^g | 0.5534 ± 0.0005 | 0.2301 ± 0.0005 | 1 |
| (O-16:0)deoxyLPA1 ^d | 0.5493 ± 0.0008 | 0.1549 ± 0.0008 | 11 |
| (O-16:0)deoxyLPA2 ^d | 0.5624 ± 0.0014 | 0.1681 ± 0.0014 | 11 |
| (12:0) ₂ DG | 0.708 ± 0.002 | 0.156 ± 0.002 | 12 |
| (16:0) ₂ DG | 0.893 ± 0.003 | 0.151 ± 0.003 | 13 |

^a (14:0)₂PC, 1,2-(14:0)₂PtdCho.2H₂O; (O-18:0/O-1:0)PC, 1-(O-18:0)-2-(O-1:0)PtdCho.H₂O; (16:0/0:0)PC, 1-(16:0)-2-lysoPtdCho; (12:0)deoxyLPC, 1-(12:0)-2-deoxyPtdCho.H₂O; (O-16:0/0:0)PC, 1-(O-16:0)-2-lysoPtdCho; (12:0)₂PE, 1,2-(12:0)₂PtdEtn.HAc; (16:0/0:0)PE, 1-(16:0)-2-lysoPtdEtn; (12:0)₂PEMe, 1,2-(12:0)₂Ptd-N-MeEtn; (12:0)₂PEMe₂, 1,2-(12:0)₂Ptd-N,N-Me₂Etn; (16:0/0:0)PEMe₂, 1-(16:0)-2-lysoPtd-N,N-Me₂Etn; (14:0)₂PG, 1,2-(14:0)₂PtdGro; (12:0)₂PA, 1,2-(12:0)₂PtdH; (14:0)₂PA, 1,2-(14:0)₂PtdH; (12:0/0:0)PA, 1-(12:0)-2-lysoPtdH; (O-16:0)deoxyLPA 1 and 2, 1-(O-16:0)-2-deoxyPtdH; 14:0/PA/0:0, 1-(14:0)-3-lysoPtdH; (12:0)₂DG, 1,2-(12:0)₂Gro; (16:0)₂DG, 1,2-(16:0)₂Gro.

^b References: 1. Pascher et al., 1992; 2. Pearson and Pascher, 1979; 3. Pascher et al., 1986; 4. Hauser et al., 1980; 5. Elder et al., 1977; 6. Pascher et al., 1981b; 7. Pascher and Sundell, 1986; 8. Pascher et al., 1987; 9. Harlos et al., 1984; 10. Pascher and Sundell, 1985; 11. Pascher et al., 1984; 12. Pascher et al., 1981a; 13. Dorset and Pangborn, 1988.

^c Corrected from dihydrate with $v_{\text{H}_2\text{O}} = 0.030 \text{ nm}^3$.

^d Corrected from monohydrate with $v_{\text{H}_2\text{O}} = 0.030 \text{ nm}^3$.

^e Corrected from chloroform solvate with $v_{\text{CHCl}_3} = 0.1038 \text{ nm}^3$ (Fourmé and Renaud, 1966).

^f Corrected from acetic acid solvate with $v_{\text{HAc}} = 0.07475 \text{ nm}^3$ (Nahringsbauer, 1970).

^g Corrected from monosodium salt with ionic radius $r_{\text{Na}} = 0.097 \text{ nm}$.

^h Corrected from monohydrated monosodium salt with $v_{\text{H}_2\text{O}} = 0.030 \text{ nm}^3$ and ionic radius $r_{\text{Na}} = 0.097 \text{ nm}$.

ⁱ Corrected from dihydrated disodium salt with $v_{\text{H}_2\text{O}} = 0.030 \text{ nm}^3$ and ionic radius $r_{\text{Na}} = 0.097 \text{ nm}$.

(12:0)₂PEMe₂, (16:0/0:0)PEMe₂, (16:0/0:0)PC, (O-16:0/0:0)PC and (O-18:0/O-1:0)PC have head groups that interdigitate between opposing layers. All chains of the lipids in Table 2 are straight and saturated. The volume of the polar group, v_{pol} , in the crystal can be estimated by subtracting the total volume of the methylene and methyl groups in the chains, using the values $v_{\text{CH}_2} = 0.0237 \text{ nm}^3$ and $v_{\text{CH}_3} = 0.0395 \text{ nm}^3$ that were obtained from crystalline alkanes (Table 1). This definition of the polar group is indicated schematically in Fig. 3; it includes the carbonyls of the acyl chains. The values obtained for v_{pol} are listed in Table 2. They follow the relative sizes expected for the various lipid species: PC > PEME₂ > PEME > PE > PA > DG.

Within a given species, only (12:0)₂PA and (14:0)₂PA with $v_{\text{pol}} = 0.223 \pm 0.006 \text{ nm}^3$, and (12:0)₂DG and (16:0)₂DG with $v_{\text{pol}} = 0.154 \pm 0.003 \text{ nm}^3$, should be directly comparable, and are so. All other crystalline phospholipids of a single head-group type differ in the chain linkage, in the presence of the second chain, and/or have propanediol instead of a glycerol backbone. The propanediol (i.e., 2-deoxy) lipids clearly have smaller polar volumes than do the diacyl species, although this is not unambiguously the case for lysolipids in which one H-atom replaces a carbonyl, nor for ether linkages which lack one carbonyl. If differences between the latter are ignored, mean values of $v_{\text{pol}} = 0.376 \pm 0.011$, 0.268 ± 0.006 , 0.335 ± 0.011 , 0.217 ± 0.009 and $0.162 \pm 0.007 \text{ nm}^3$ are obtained for PC, PE, PEME₂, PA and deoxylysoPA polar groups, respectively. For comparison, the molecular volumes of crystalline glycerylphosphorylcholine (GPC) and glycerylphosphorylethanolamine (GPE) are: 0.316 and 0.227 nm^3 , respectively (Abrahamsson and Pascher, 1966; DeTitta and Craven, 1973). For crystalline glycerol-2-phosphate (analogous to 14:0/PA/0:0), the molecular volume after correction for water of hydration and sodium ions is 0.126 nm^3 (Ul-haque and Caughlan, 1966). The polar groups in phospholipid crystals have comparable but larger volumes and therefore are less tightly packed than are the isolated headgroup moieties, GPC and GPE.

3.4. Polar group (v_{pol}) and terminal methyl (v_{CH_3}) volumes

Since the pioneering work of Reiss-Husson and Luzzati (1964), and see also Tardieu et al. (1973) and Nagle and Wilkinson (1978), it has been customary to estimate the volume of the polar group in lipid membranes by assuming that the volume of a terminal methyl group is twice that of a methylene group, as in liquid hydrocarbons. The latter are isotropic phases, whereas membranes are ordered liquid-crystalline phases (see e.g., Cevc and Marsh, 1987). Therefore, it is uncertain to what extent this approximation holds true. Note, in particular, that both the temperature expansion coefficient (Koenig and Gawrisch, 2005) and compressibility (Wiener and White, 1992) of the terminal methyl groups are much greater than those of the methylenes, which is not compatible with a fixed ratio between the two. Alternatively, an estimate for the polar-group volume is afforded by the crystal volumes of GPC and GPE, adjusted by the van der Waals volume of the carbonyl substitution for the H-atoms of the glycerol hydroxyls (Bondi, 1964). Combined with measured values of v_{end} , this value for v_{pol} then yields an estimate for the ratio of methyl and methylene volumes, $v_{\text{CH}_3}/v_{\text{CH}_2}$.

Table 3 gives the results of such calculations for the various phosphatidylcholines and for phosphatidylethanolamines. First are given the volumes of the polar group, v_{pol} , deduced with a fixed value of the methyl to methylene volume ratio. A value of $v_{\text{CH}_3}/v_{\text{CH}_2} = 2$ appropriate to fluid alkanes is assumed for the fluid L_α phases, and a value of $v_{\text{CH}_3}/v_{\text{CH}_2} = 1.7$ appropriate to orthorhombic crystalline alkanes for the L_β gel phase (see Table 1). For the unsaturated and isoacyl PCs, allowance must be made for the contribution of the CH methine groups and branched methyl groups, respectively, to v_{end} . This is done by using the values

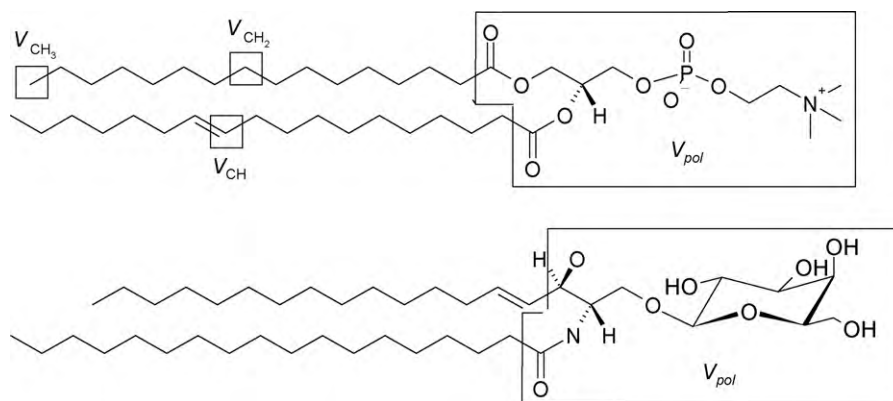


Fig. 3. Definition of the polar group in glycerolipids (top) and sphingolipids (bottom).

Table 3

Volumes of phospholipid polar groups, ν_{pol} , calculated from end contributions to the chain-length dependence (see Table 1) for a fixed ratio of methyl to methylene volumes, $\nu_{\text{CH}_3}/\nu_{\text{CH}_2}$, and vice-versa.

| Lipid | Phase | T ($^{\circ}\text{C}$) | $\nu_{\text{CH}_3}/\nu_{\text{CH}_2}$ ^a | $\nu_{\text{pol}}(\text{nm}^3)$ | $\nu_{\text{pol}}(\text{nm}^3)$ ^a | $\nu_{\text{CH}_3}/\nu_{\text{CH}_2}$ | Ref. ^b |
|--|--------------|----------------------------|--|---------------------------------|--|---------------------------------------|-------------------|
| ($n:0$) ₂ PC | L_{α} | 70 | 2 | 0.338 | 0.340 | 1.97 | 1 |
| ($n:1$) ₂ PC ^c | L_{α} | 25 | 2 | 0.329 | 0.340 | 1.78 | 2 |
| ($n:0$) ₂ PC ^d | L_{α} | 40 | 2 | 0.332 | 0.340 | 1.88 | 3 |
| ($n:0$) ₂ PE | L_{α} | 70 | 2 | 0.269 | 0.251 | 2.32 | 4 |
| | L_{β} | 25 | 1.7 | 0.251 | 0.251 | 1.70 | 4, 5 |

^a Maintained fixed.

^b References: 1. Schmidt and Knoll, 1985; 2. Uhríková et al., 2007; 3. Yang et al., 1986; 4. Koynova and Hinz, 1990; 5. Seddon et al., 1984.

^c Calculated with $\nu_{\text{CH}} = 0.021 \text{ nm}^3$.

^d Calculated with $\nu_{\text{Me}} = 0.031 \text{ nm}^3$ for the methyl branch.

obtained as described above and noted in the footnotes to Table 3. Consistent values for the volume of the PC polar group in fluid membranes $\nu_{\text{PC}} = 0.336 \text{ nm}^3$ must be obtained in this way, the differences corresponding only to the slight temperature dependence. This value is somewhat smaller than that deduced from phosphatidylcholine crystals ($\nu_{\text{PC}} = 0.376 \pm 0.011 \text{ nm}^3$), but similar to the volume of $\nu_{\text{PC}} = 0.340 \text{ nm}^3$ in crystals of the GPC headgroup alone, after adjustment for the carbonyl contribution.

For phosphatidylethanolamine, on the other hand, the volume of the PE polar group in fluid membranes, $\nu_{\text{PE}} = 0.269 \text{ nm}^3$ (Table 3) is very similar to that in the crystal ($\nu_{\text{PE}} = 0.268 \pm 0.006 \text{ nm}^3$, which is not much larger than the carbonyl-adjusted volume in crystals of the GPE headgroup alone ($\nu_{\text{PE}} = 0.251 \text{ nm}^3$). In the L_{β} gel phase, the PE polar-group volume is only slightly smaller than in the fluid L_{α} phase and is very close to the carbonyl-adjusted volume in GPE crystals. The accordance between polar-group volumes in fluid phospholipid membranes and those in crystals for PE, but not for PC, correlates with the smaller, less hydrated, hydrogen-bonded head groups in the former case (Cevc and Marsh, 1985), which results in a packing that is more similar to that in the crystal. In PC membranes, on the other hand, the head groups are extensively hydrated, which allows more efficient packing than is possible in crystals of the mono- or dihydrate (cf. Table 2).

The second pair of entries in Table 3 gives the estimates for the methyl/methylene volume ratio, which are based on the volume of the polar-group alone with carbonyl contributions added. For the fluid L_{α} phases, these values of $\nu_{\text{CH}_3}/\nu_{\text{CH}_2}$ are comparable to those found for liquid n -alkanes (viz., 2.0 and 2.1 at 40 and 70 $^{\circ}\text{C}$, respectively). For gel-phase PE, the value of $\nu_{\text{CH}_3}/\nu_{\text{CH}_2}$ is close to that for orthorhombic crystalline alkanes, but that for the fluid phase somewhat exceeds the ratio for liquid alkanes. Taken as a whole, these results suggest that a methyl/methylene ratio of $\nu_{\text{CH}_3}/\nu_{\text{CH}_2} \approx 2$ is reasonable for the fluid phase, as concluded by other authors (Nagle and Wiener, 1988; Wiener and White, 1992; Koenig and Gawrisch, 2005), and that a ratio somewhat less than two (i.e., approaching

that of orthorhombic crystalline hydrocarbons) is more appropriate for the gel phase of phosphatidylethanolamines.

3.5. ω -cyclohexyl chain volumes (ν_{cyc})

Molecular volumes have also been determined for three chain lengths ($n = 13 - 15$) of diacyl phosphatidylcholines with ω -cyclohexyl chains (Blume et al., 1987). Here the terminal methyl group (not counted in n) is replaced by the cyclohexyl moiety. At 60 $^{\circ}\text{C}$ in the L_{α} phase, the methylene volume is $\nu_{\text{CH}_2} \approx 0.027 \pm 0.001 \text{ nm}^3$, and the volume contribution of each cyclohexyl group is estimated to be $\nu_{\text{cyc}} = 0.150 \pm 0.014 \text{ nm}^3$ by assuming a PC polar group volume of $\nu_{\text{pol}} = 0.340 \text{ nm}^3$ (see above). For comparison, the molecular volume in liquid cyclohexane is $\nu_{\text{mol}} = 0.180 \text{ nm}^3$ at 20 $^{\circ}\text{C}$ ($\rho = 0.7785 \text{ g/ml}$).

4. Glycoglycerolipids

4.1. Chain-length dependence and CH_2 volume

Fig. 4 shows the chain-length dependence of the molecular volume of the 1,2-dialkyl-3- β -D-glucosyl-*sn*-glycerol glycolipids (abbreviated (O- $n:0$)₂Glc β DG) in the crystalline (L_c), gel (L_{β}) and fluid (L_{α}) lamellar membrane phases, and in the fluid inverted hexagonal phase (H_{II}). A linear dependence on chain length at fixed temperature is found in each of the different phases. Table 4 gives the parameters of the linear regressions, ν_{CH_2} and ν_{end} , for the (O- $n:0$)₂Glc β DG series shown in Fig. 4, and more limited data for the corresponding series of galactosyl diacylglycerols, (O- $n:0$)₂Gal β DG. The crystalline L_c phases are characterised by a very low methylene group volume, $\nu_{\text{CH}_2} = 0.021 \pm 0.001 \text{ nm}^3$, which is even lower than that registered in crystalline n -alkanes ($\nu_{\text{CH}_2} = 0.024 \text{ nm}^3$). This could indicate a different chain packing mode in glycolipids from that in crystalline paraffins. The methylene group volume in the L_{β} gel phase of glycoglycerolipids is larger

Table 4
Component volumes of molecular groups, obtained by linear regression to the chain-length dependence (Eq. (4)) for glycolipids in membranes (see Fig. 4)^a.

| Lipid | Phase | T (°C) | v_{CH_2} | | $v_{\text{end}}^{\text{b}}$ | |
|-----------------------------|-----------------|--------------------|--------------------|------------|-----------------------------|----------|
| | | | (nm ³) | (ml/mol) | (nm ³) | (ml/mol) |
| (O-n:0) ₂ GlcβDG | L _c | 20 | 0.0214 ± 0.0008 | 12.9 ± 0.5 | 0.408 ± 0.022 | 245 ± 13 |
| | L _β | 20 | 0.0226 ± 0.0006 | 13.6 ± 0.3 | 0.411 ± 0.016 | 248 ± 9 |
| | H _{II} | 80 | 0.0265 ± 0.0007 | 16.0 ± 0.4 | 0.414 ± 0.022 | 250 ± 13 |
| (O-n:0) ₂ GalβDG | L _c | 20,65 ^c | 0.0207 ± 0.0013 | 12.5 ± 0.8 | 0.452 ± 0.032 | 272 ± 19 |
| | H _{II} | 80 | 0.0250 ± 0.002 | 15.1 ± 1.3 | 0.437 ± 0.060 | 263 ± 36 |

^a Densitometric data from Hinz et al. (1991).

^b Total volume of all molecular groups other than the chain methylenes: $v_{\text{end}} = v_{\text{pol}} + 2v_{\text{CH}_3}$.

^c Combines data for $n = 12, 14$ at 20 °C with that for $n = 16, 18$ at 65 °C (all in the crystalline state).

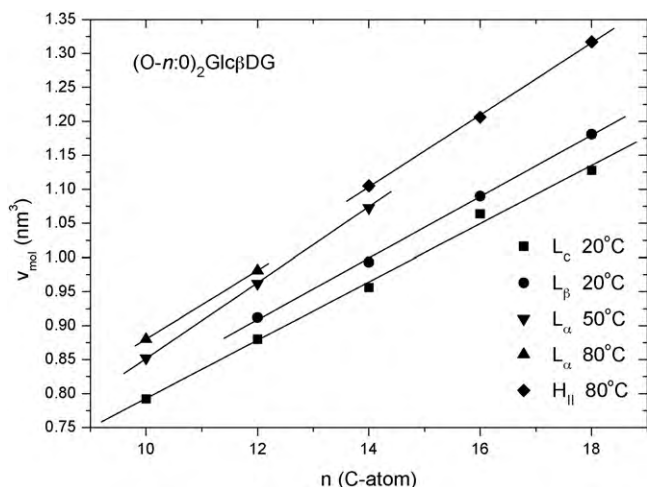


Fig. 4. Dependence of lipid molecular volume on alkyl chain length, n , for 1,2-dialkyl-3- β -D-glucosyl-*sn*-glycerols, (O- n :0)₂GlcβDG, in the L_c phase at 20 °C (squares), in the L_β phase at 20 °C (circles), in the L_α phase at 50 °C (downward triangles) and 80 °C (upward triangles), and in the H_{II} phase at 80 °C (diamonds). Data from Hinz et al. (1991). Lines are linear regressions.

than that in the crystalline phase, but still remains relatively small ($v_{\text{CH}_2} = 0.023 \pm 0.001 \text{ nm}^3$). In the fluid H_{II} phase, the methylene volume is larger: $v_{\text{CH}_2} \approx 0.026 \pm 0.001 \text{ nm}^3$, but somewhat lower than for phospholipids in the L_α phase, especially the phosphatidylcholines. On the whole, the glycolipid chains appear to pack more densely than do those of the phospholipids, particularly in the more condensed phases. This can be attributed to lower hydration and hydrogen bonding between the sugar headgroups.

4.2. Glycolipid polar group volumes

Table 5 presents the results of calculations of the volumes of the polar groups in the (O- n :0)₂GlcβDG and (O- n :0)₂GalβDG series. Values for the methyl to methylene volume ratio, $v_{\text{CH}_3}/v_{\text{CH}_2}$, are assumed as in Table 3 for the L_α and L_β phases of phospholipids, together with $v_{\text{CH}_3}/v_{\text{CH}_2} = 1.65$ (see crystalline alkane data in Table 1) for the L_c phases of the glycolipids. Comparable volumes are obtained for both hexose head groups, but with slightly larger values for the galactosyl dialkylglycerols than for the glucosyl dialkylglycerols. The effective polar-group volumes in the gel phase are comparable to those in the fluid phase. Note that all lipids in these series are dialkyl glycerols with ether-linked chains and therefore v_{pol} does not include the carbonyl groups that would be contributed by the chains of diacyl lipids.

Also included in Table 5 are data for a further monoglycosyl dialkylglycerol, (O- n :0)₂ManβDG, and for the diosyl and triosyl diacylglycerols, (O- n :0)₂MalβDG and (O- n :0)₂MtrβDG, which contain two and three hexose units, respectively, in the polar head group. Partial specific volumes are available for only one

Table 5

Volumes of glycolipid polar groups, v_{pol} , in membranes calculated from end contributions (see Table 4)^a for a fixed ratio of methyl to methylene volumes, $v_{\text{CH}_3}/v_{\text{CH}_2}$.

| Lipid | Phase | T (°C) | $v_{\text{CH}_3}/v_{\text{CH}_2}^{\text{b}}$ | $v_{\text{pol}}(\text{nm}^3)$ |
|---------------------------------|------------------------------|--------|--|-------------------------------|
| (O- n :0) ₂ GlcβDG | L _c | 20 | 1.65 | 0.337 |
| | L _β | 20 | 1.7 | 0.334 |
| | H _{II} | 80 | 2 | 0.308 |
| (O- n :0) ₂ GalβDG | L _c | 20,65 | 1.65 | 0.384 |
| | H _{II} | 80 | 2 | 0.337 |
| (O- n :0) ₂ ManβDG | L _β ^c | 20 | 1.7 | 0.316 |
| | H _{II} ^d | 80 | 2 | 0.335 |
| (O- n :0) ₂ MalβDG | L _β ^c | 20 | 1.7 | 0.512 |
| | L _α ^d | 80 | 2 | 0.513 |
| (O- n :0) ₂ MtrβDG | L _β ^c | 20 | 1.7 | 0.612 |
| | L _α ^d | 80 | 2 | 0.636 |

^a Densitometric data from Hinz et al. (1991).

^b Maintained fixed.

^c Calculated from v_{mol} with $v_{\text{CH}_2} = 0.0226 \text{ nm}^3$ from (O- n :0)₂GlcβDG.

^d Calculated from v_{mol} with $v_{\text{CH}_2} = 0.0258 \text{ nm}^3$ from mean of (O- n :0)₂GlcβDG and (O- n :0)₂GalβDG.

or two chain lengths of these lipids. Therefore, values for the methylene group volume could not be determined from a chain-length dependence. Instead, the mean values of v_{CH_2} obtained from the (O- n :0)₂GlcβDG and (O- n :0)₂GalβDG series are assumed for the fluid phase of (O- n :0)₂ManβDG, (O- n :0)₂MalβDG and (O- n :0)₂MtrβDG, and the single value from the (O- n :0)₂GlcβDG series is taken for the gel phase. The total contribution of the methylene and methyl groups is then subtracted from the molecular volume of the whole lipid to obtain v_{pol} . This method has often been applied previously to estimate the polar head group volume from the partial specific volume of a single lipid (Tardieu et al., 1973; Nagle and Wilkinson, 1978; Gulik et al., 1995). The values of v_{pol} obtained for (O- n :0)₂ManβDG are comparable to those for the other two monohexosyl diacylglycerols, (O- n :0)₂GlcβDAG and (O- n :0)₂GalβDG, and those obtained for the diosyl and triosyl diacylglycerols are progressively larger. The increment in v_{pol} between (O- n :0)₂ManβDG and (O- n :0)₂MalβDG is $\Delta v_{\text{pol}} = 0.196$ and 0.178 nm^3 in the gel and fluid phases, respectively. This compares well with the volume of a single hexose unit which is $\sim 0.192 \text{ nm}^3$, as estimated from the density of glucose (1.5620 g/cm^3). On the other hand, the increment between (O- n :0)₂MtrβDG and (O- n :0)₂MalβDG, corresponding to addition of a third hexose unit, is considerably smaller: $\Delta v_{\text{pol}} \sim 0.100$ and 0.123 nm^3 in gel and fluid phases, respectively. This attenuating effect is seen in Fig. 5, in which the molecular volumes of (O-18:0)₂ glycosyl diacylglycerols with a common chain length are plotted against the number of hexose units in the polar head group, both for gel-phase membranes and for L_α or H_{II} fluid phases. In the two cases, the volume increment between monoglycosyl ($n_{\text{glyc}} = 1$) and diosyl ($n_{\text{glyc}} = 2$) lipids is considerably larger than that

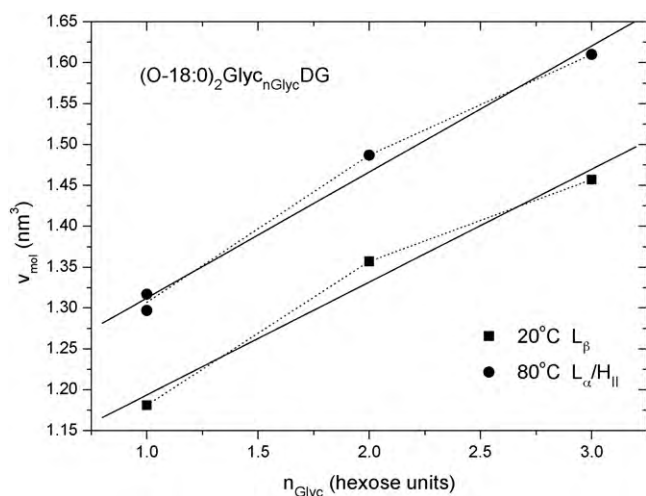


Fig. 5. Dependence of lipid molecular volume on number of glycosyl units, n_{Glyc} in the lipid polar head group for 1,2-(O-18:0)₂ monoglycosyl, ($n_{\text{Glyc}} = 1$), diosyl ($n_{\text{Glyc}} = 2$), and triosyl ($n_{\text{Glyc}} = 3$) *sn*-3-glycerols in the L_{β} phase at 20 °C (squares) and in the L_{α} or H_{II} phase at 80 °C (circles). Data from Hinz et al. (1991). Lines are linear regressions.

between triosyl ($n_{\text{glyc}} = 3$) and diosyl lipids. A linear regression to the combined data for the three lipid classes yields gradients of 0.138 ± 0.021 and $0.154 \pm 0.012 \text{ nm}^3/\text{hexose}$ in gel and fluid phases, respectively.

5. Glycosphingolipids

Systematic chain-length dependences of the partial specific volume are not available for sphingolipids. Therefore, component polar-group volumes may only be deduced by assuming values for v_{CH_2} and v_{CH_3} . This can be done most directly for the crystalline states, by using the values from crystalline *n*-alkanes as was done in Table 2 for phospholipids.

5.1. Glycosphingolipid crystal volumes

Table 6 gives the lipid molecular volumes in crystals of glycosphingolipids, ceramides and the sphingosine base. The contributions of any solvent molecules and/or counterions have been subtracted to yield the molecular volume, v_{mol} , of the sphingolipid. Correspondingly, the total volume of chain methylene and methyl groups has been subtracted from v_{mol} to yield v_{pol} , the volume of the polar group, as was done in Table 2 for the phospholipid crystals. The polar group so-defined includes, in addition to the sugar group, also the carbonyl of the *N*-acyl chain and all atoms of the sphingoid base up to and including the OH-bearing C-3 atom (see Fig. 3). For sphingosine (sphing-4-enine), this definition includes the 2H-atom deficit, relative to CH_2 , that is contributed by the two CH groups of the 4–5 *trans* double bond, which lies immediately outside the polar-group region.

The polar group volumes of both cerebroside in Table 6 contain an additional contribution from hydroxylation: of the *N*-acyl chain for Gal β 1Cer(d18:0/2(OH)18:0) and of the sphingoid chain for Glc β 1Cer(t18:0/0:0). Subtracting v_{pol} for the ceramide Cer(t18:0/24:0) (which also includes an additional contribution from a single-OH substitution) from that of Gal β 1Cer(d18:0/2(OH)18:0) yields a value of $v_{\text{Gal}} = 0.201 \text{ nm}^3$ for the hexose unit. This value corresponds reasonably well with the hexose volume $v_{\text{Glc}} = 0.193 \text{ nm}^3$ that is obtained directly for solid glucose (see above). Subtracting instead the value of v_{pol} for the single-chain glucosyl phytosphingosine Glc β 1Cer(t18:0/0:0) from that of Gal β 1Cer(d18:0/2(OH)18:0) yields a difference of 0.046 nm^3

Table 6
Molecular volumes in glycosphingolipid crystals.

| Lipid ^a | $v_{\text{mol}} (\text{nm}^3)$ | $v_{\text{pol}} (\text{nm}^3)^b$ | Ref. ^c |
|--|--------------------------------|----------------------------------|-------------------|
| Gal β 1Cer(d18:0/2(OH)18:0) ^{d,e} | 1.153 ± 0.002 | 0.364 ± 0.002 | 1 |
| Glc β 1Cer(t18:0/0:0) ^{f,g} | 0.688 ± 0.006 | 0.318 ± 0.005 | 2 |
| Cer(t18:0/24:0) ^g | 1.094 ± 0.004 | 0.163 ± 0.004 | 3 |
| Cer(t18:0/2,3(OH)2 18:0) ^{e,g} | 0.9558 ± 0.0008 | 0.1672 ± 0.0008 | 4 |
| Cer(d18:1/0:0) ^{h,i} | 0.4808 ± 0.0008 | 0.1101 ± 0.0008 | 5 |

^a Gal β 1Cer(d18:0/2(OH)18:0), 1- β -D-galactosyl-*N*-(2-D-hydroxyoctadecanoyl)-sphinganine; Glc β 1Cer(t18:0/0:0), 1- β -D-glucosyl-4-hydroxysphinganine; Cer(t18:0/24:0), *N*-tetracosanoyl-4D-hydroxy-sphinganine (*N*-tetracosanoyl-phytosphingosine); Cer(t18:0/2(OH)18:0), *N*-(2D,3D-dihydroxyoctadecanoyl)-4D-hydroxy-sphinganine (*N*-(2,3-dihydroxystearoyl)phytosphingosine); Cer(d18:1/0:0), sphing-4-enine (sphingosine). Note: d18:1 = sphing-4-enine (sphingosine); d18:0 = sphinganine (dihydroxysphingosine); t18:0 = 4D-hydroxysphinganine (phytosphingosine).

^b Includes C1-C3 and substituents of the sphingoid, and carbonyl of the amide-linked fatty acid.

^c References: 1. Pascher and Sundell, 1977; 2. Abrahamsson et al., 1977; 3. Dahlen and Pascher, 1972; 4. Pascher and Sundell, 1992; 5. Abrahamsson et al., 1972.

^d Corrected from ethanol solvate (1/2EtOH) with $v_{\text{EtOH}}/2 = 0.037 \text{ nm}^3$ (Jönsson, 1976).

^e v_{pol} includes contribution of the O-atom from the (OH) substitution at C-2 in the *N*-acyl chain, which is otherwise counted as an unsubstituted CH_2 group.

^f Corrected from hydrochloride monohydrate with $v_{\text{H}_2\text{O}} = 0.030 \text{ nm}^3$ and ionic radius $r_{\text{Cl}} = 0.181 \text{ nm}$.

^g v_{pol} includes contribution of the O-atom from the (OH) substitution at C-4 in the (t18:0) sphingoid (phytosphingosine) chain, which is otherwise counted as an unsubstituted CH_2 group.

^h Corrected from hydrochloride with ionic radius $r_{\text{Cl}} = 0.181 \text{ nm}$.

ⁱ v_{pol} includes volume deficit, relative to v_{CH_2} , of the two CH groups of the (d18:1) sphingosine.

which corresponds to the missing carbonyl in the single-chain derivative. Molecular dynamics simulations have suggested a contribution of $v_{\text{C=O}} = 0.039 \pm 0.001 \text{ nm}^3$ from each chain carbonyl in fluid phosphatidylcholine bilayers (Armen et al., 1998). The carbonyl volume in crystals might be expected to be smaller than this.

Subtracting the value of v_{pol} for Cer(t18:0/24:0) from the cerebroside values, as above, but then adding back that for sphingosine Cer(d18:1/0:0), produces polar-group volumes appropriate to cerebroside with sphingosine chains and no additional hydroxylation. These corrected values are $v_{\text{pol}} = 0.311$ and 0.265 nm^3 for the notional Gal β 1Cer(d18:1/0:0) and Glc β 1Cer(d18:1/0:0) cerebroside, respectively.

5.2. Glycosphingolipid polar group volumes

Considering now hydrated glycosphingolipids, the molecular volumes deduced from the partial specific volumes of the lipids dispersed in water are given in Table 7. The accuracy of these determinations is limited by the fact that all lipids are derived from natural sources and have heterogeneous chain composition. The molecular volumes are deduced from Eq. (2) by assuming that the chains of the predominant species correspond to Cer(d18:1/18:0). The volumes of the polar groups are also deduced with this same assumption, which is therefore to some extent self-correcting. Volumes for chain methylene groups are, by default, taken to be those in fluid glycosyl diacylglycerols (see Table 4) and the approximation $v_{\text{CH}_3}/v_{\text{CH}_2} \approx 2$ is also used. The volume for a CH group is taken as that deduced above for phosphatidylcholines ($v_{\text{CH}} = 0.021 \text{ nm}^3$). The volume deduced for the polar group of the monoglycosyl sphingolipid GalCer is somewhat smaller than that found for fluid monoglycosyl glycolipids (see Table 5) and that calculated above for crystalline Gal β 1Cer(d18:1/0:0) from the data in Table 6. It is, however, consistent with the relatively small increment in volume on increasing the number of hexose units in the sphingolipid head group.

Table 7

Volumes of glycosphingolipid polar groups, ν_{pol} , in membranes or micelles calculated from molecular volumes at 20 °C, assuming a Cer(d18:1/18:0) ceramide portion^a.

| Lipid ^b | $\nu_{\text{mol}}(\text{nm}^3)$ | $\nu_{\text{pol}}(\text{nm}^3)$ | Ref. ^c |
|---------------------|---------------------------------|---------------------------------|-------------------|
| GalCer | 1.122 | 0.257 | 1 |
| LacCer | 1.311 | 0.446 | 1 |
| Gb ₃ Cer | 1.52 | 0.655 | 1 |
| Gg ₃ Cer | 1.527 | 0.662 | 1 |
| Gg ₄ Cer | 1.6971 | 0.8325 | 1 |
| GM4 | 1.462 | 0.597 | 1 |
| GM3 | 1.638 | 0.773 | 1 |
| GM2 | 1.876 | 1.011 | 1 |
| GM1 | 2.065 | 1.200 | 1 |
| | 2.0487 | 1.1841 | 2 |
| GD3 | 2.054 | 1.189 | 1 |
| GD1a | 2.378 | 1.513 | 1 |
| | 2.3395 | 1.4749 | 2 |
| GD1b | 2.362 | 1.497 | 1 |
| GT1b | 2.659 | 1.794 | 1 |
| | 2.6307 | 1.7661 | 2 |

^a Lipids are from natural sources. Predominant ceramide species is assumed to be Cer(d18:1/18:0), where d18:1 = sphing-4-ene (sphingosine). Hydrocarbon volumes in ceramide chains are taken as: $\nu_{\text{CH}_2} = 0.0257 \text{ nm}^3$; $\nu_{\text{CH}} = 0.021 \text{ nm}^3$ and $\nu_{\text{CH}_3}/\nu_{\text{CH}_2} = 2$ (see Tables 4 and 1).

^b Lac, Gal β 1-4Glc; Gb₃, Gal α 1-4Gal β 1-4Glc; Gg₃, GalNAc β 1-4Gal β 1-4Glc; Gg₄, Gal β 1-3GalNAc β 1-4Gal β 1-4Glc; GM4, I³NeuAc-Gal-Cer; GM3, I³NeuAc-Lac-Cer; GM2, I³NeuAc-Gg₃-Cer; GM1, I³NeuAc-Gg₄-Cer; GD3, I³NeuAc₂-Lac-Cer; GD1a, IV³NeuAc-I³NeuAc-Gg₄-Cer; GD1b, I³NeuAc₂-Gg₄-Cer; GT1b, IV³NeuAc-I³NeuAc₂-Gg₄-Cer.

^c References: 1. Ulrich-Bott and Wiegandt, 1984; 2. Corti et al., 1991.

Fig. 6 shows the dependence of molecular volume on head group size for cerebrosides and asialogangliosides. The linear gradient is $0.195 \pm 0.009 \text{ nm}^3$ per hexose unit, which is similar to the larger of the two values obtained for glycosyl diacylglycerols (cf. Fig. 4). As seen from Fig. 6, essentially the same gradient ($0.201 \pm 0.006 \text{ nm}^3$ per hexose) is also obtained with increasing headgroup size of the monosialogangliosides from GM4 to GM1. On the other hand, a steeper gradient is found for the dependence on the number of sialic acid residues in the head groups of gangliosides analogous to GM1. Molecular volumes for the series from asialoGM1

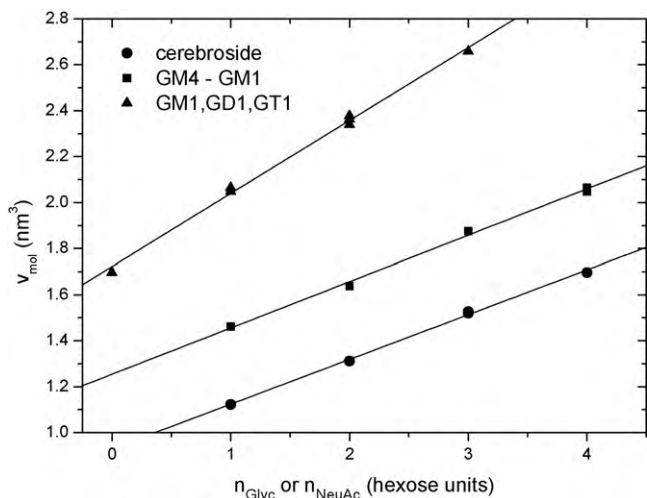


Fig. 6. Dependence of lipid molecular volume on number of glycosyl or N-acetylneuraminic acid units, n_{Glyc} or n_{NeuAc} , in the lipid polar head group of glycosphingolipids for aqueous dispersions at 20 °C. Cerebrosides and asialogangliosides (circles); monosialogangliosides GM4 to GM1 (squares); polysialogangliosides asialoGM1, GM1, GD1 and GT1 (triangles). Data from Ulrich-Bott and Wiegandt (1984). Lines are linear regressions.

(Gg₄Cer) to GT1b are shown in Fig. 6, from which a linear gradient of $0.301 \pm 0.009 \text{ nm}^3$ per NeuAc unit is obtained for the volume of a sialic acid residue in the fluid micellar state.

6. Conclusion

Examining the chain-length dependence of lipid volumes in membranes gives rise to a consistent description in terms of component molecular groups, without recourse to diffraction results on bilayers. Originally, component values for the fluid L_α phase were taken from liquid alkanes (Reiss-Husson and Luzzati, 1964): $\nu_{\text{CH}_2} = 0.0270 \text{ nm}^3$, $\nu_{\text{CH}_3} = 0.0540 \text{ nm}^3$, $\nu_{\text{CH}} = 0.0205 \text{ nm}^3$, together with the partial specific volume of egg phosphatidylcholine to estimate: $\nu_{\text{pol}} = 0.0360 \text{ nm}^3$ for the PC polar group at 25 °C (Tardieu et al., 1973). These values are reasonably close to those obtained here from densitometric measurements on PC bilayers alone (see Tables 1 and 3). The value of ν_{pol} for PC in Table 3 is somewhat lower than the above value, and also than early estimates from X-ray diffraction (quoted in Nagle and Tristram-Nagle, 2000). However, more recent X-ray analyses have produced values that are in rather good accord with the present work, both for PC and PE (see Appendix, Table A.1). For instance, the latest high-resolution refinement yields a value of $\nu_{\text{pol}} = 0.331 \text{ nm}^3$ for the L_{β'} phase of (14:0)₂PC at 10 °C (Tristram-Nagle et al., 2002). This value of ν_{pol} has then been assumed in subsequent refinements for fluid-phase PC bilayers (Kucerka et al., 2005a,b; 2006).

Volumetric data for the L_β gel phase of phosphatidylethanolamine have been deduced previously from a combination of X-ray diffraction and density measurements on (12:0)₂PE (see Table A.1). Values of $\nu_{\text{CH}_2} = 0.0260 \text{ nm}^3$ and $\nu_{\text{pol}} = 0.252 \text{ nm}^3$ were obtained at 20 °C (Nagle and Wiener, 1988), which are in good agreement with the present results from the chain-length dependence of phosphatidylethanolamine volumes (see Tables 1 and 3). The methyl group volume assumed ($\nu_{\text{CH}_3}/\nu_{\text{CH}_2} = 1.75$) is rather similar to that deduced here from data on crystalline alkanes. For the L_α phase of (12:0)₂PE, the gel-phase value of ν_{pol} was assumed, together with $\nu_{\text{CH}_3}/\nu_{\text{CH}_2} = 2$ from liquid *n*-alkanes. The methylene group volume was then estimated as $\nu_{\text{CH}_2} = 0.0273 \text{ nm}^3$ at 35 °C (Nagle and Wiener, 1988), which is reasonably close to the value found here.

In view of this measure of agreement, it seems reasonable to use the values established here for the chain volumes in phospholipid bilayers to estimate polar-group volumes for phospholipids for which volumetric data are available only for a single chain length. This approach has been used already for certain of the glyco-glycerolipids, in Table 5. Estimates for the polar-group volumes of dialkyl phosphatidylcholines and phosphatidylethanolamines, of diacyl phosphatidylglycerol and phosphatidic acids, and of sphingomyelin, that are obtained in this way are given in Table 8. Of these, only ν_{pol} for dialkyl phosphatidylcholines has been obtained by other means, and the present value for the fluid phase is found to lie within the range estimated from X-ray methods (see Table A.1). This, therefore, gives some confidence in the values for other lipids reported in Table 8, and correspondingly for the diosyl and triosyl glycerolipids in Table 5. Finally, it should be noted that the same method is also used to obtain estimates of the polar-group volumes of glycosphingolipids (for which volumetric data are much sparser) in Table 7.

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Table 8Volume of phospholipid polar groups, v_{pol} , in hydrated bilayer membranes, deduced from measurements of molecular volume, v_{mol} , for a single lipid chain length.^a

| Lipid | Phase | T (°C) | v_{mol} (nm ³) | v_{CH_2} (nm ³) ^b | $v_{\text{CH}_3}/v_{\text{CH}_2}$ | v_{pol} (nm ³) | Ref. ^c |
|--------------------------|-----------------------------|----------|-------------------------------------|---|-----------------------------------|-------------------------------------|-------------------|
| (O-16:0) ₂ PC | L _β ⁱ | 20 | 1.124 | 0.0241 | 1.7 | 0.319 | 1 |
| | L _α | 45 | 1.219 | 0.0275 | 2 | 0.284 | 1 |
| (O-12:0) ₂ PE | L _c | 25 | 0.837 | 0.0241 | 1.65 | 0.227 | 2 |
| | L _β | 24 | 0.868 | 0.0253 | 1.7 | 0.225 | 2 |
| | L _α | 59 | 0.914 | 0.0266 | 2 | 0.222 | 2 |
| | H _{II} | 135 | 0.983 | 0.0286 | 2 | 0.239 | 2 |
| (16:0) ₂ PG | L _β ⁱ | 25 | 1.098 | 0.0253 | 1.7 | 0.304 | 3 |
| | P _β ⁱ | 37 | 1.136 | 0.0260 | 1.7 | 0.320 | 4 |
| | L _α | 45 | 1.178 | 0.0275 | 2 | 0.298 | 3 |
| (14:0) ₂ PA | L _β | 25 | 0.943 | 0.0253 | 1.7 | 0.250 | 5 |
| | L _α | 50 | 1.004 | 0.0269 | 2 | 0.250 | 5 |
| EYSM ^d | L _β | 30 | 1.160 | 0.0261 | 1.7 | 0.350 | 6 |

^a From additivity of volumes: $v_{\text{pol}} = v_{\text{mol}} - n_{\text{CH}_2} v_{\text{CH}_2} - 2v_{\text{CH}_3}$.^b From Table 1, with adjustments for temperature by using an expansion coefficient: $\alpha_v = 0.001 \text{ K}^{-1}$.^c References: 1. Laggner et al., 1987; 2. Seddon et al., 1984; 3. Pabst et al., 2008; 4. Wilkinson et al., 1987; 5. Schmidt and Knoll, 1985; 6. Epanand and Epanand, 1980.^d Sphingomyelin from hen egg yolk, assuming that the predominant molecular species is SM(d18:1/16:0).

Appendix A. Group contributions from X-ray diffraction

It is useful, for comparison purposes, to collect together determinations of molecular group volumes that are based on refinement of electron density profiles from X-ray diffraction and scattering studies of fully hydrated bilayer membranes. In the liquid crystallographic method developed by Nagle, Tristram-Nagle and co-workers (Nagle and Tristram-Nagle, 2000; Tristram-Nagle et al., 2002), the electron density profile is fitted with the polar and hydrocarbon group contributions. In this way, it is possible to determine the position of the Gibbs dividing surface between hydrocarbon and

polar regions, and hence to define the lipid hydrocarbon thickness, d_{HC} .

The area per chain, A_{ch} , in the gel phase is obtained from the wide-angle X-ray reflections, which give the chain spacings. This is then used together with the hydrocarbon thickness, and chain tilt (θ_t) determined with aligned samples, to yield the volume of the hydrocarbon layer: $v_{\text{HC}} = 2d_{\text{HC}}A_{\text{ch}}/\cos\theta_t$ per lipid. The volume of the lipid polar group is then simply: $v_{\text{pol}} = v_{\text{mol}} - v_{\text{HC}}$. The methylene volume in the gel phase is estimated as $v_{\text{CH}_2} = A_{\text{ch}} \times 0.127 \text{ nm}^3$, where 0.127 nm is the length per CH₂ group along the axis of an all-*trans* chain. Thence, the volume of the terminal methyl

Table A.1

Group volumes from X-ray diffraction and densitometry.

| Lipid | Phase | T (°C) | v_{mol} (nm ³) | v_{CH_2} (nm ³) | $v_{\text{CH}_3}/v_{\text{CH}_2}$ | v_{pol} (nm ³) | Ref. ^a |
|--------------------------|-----------------------------|----------|-------------------------------------|--------------------------------------|-----------------------------------|-------------------------------------|-------------------|
| (12:0) ₂ PC | L _α | 30 | 0.991 | 0.0277 ^b | 1.9 | 0.331 ^c | 1 |
| (14:0) ₂ PC | L _β | 10 | 1.041 | 0.0252 ^b | 2.06 | 0.331 ^d | 2 |
| | L _α | 30 | | 0.0277 ^b | 1.9 | 0.331 ^c | 1 |
| (16:0) ₂ PC | L _β | 24 | 1.148 ± 0.002 | 0.02591 ± 0.00005 ^b | 2.05 ± 0.05 | 0.319 ± 0.006 ^d | 3 |
| | L _α | 50 | 1.2285 | 0.0280 ^b | 2 | 0.331 ^c | 4 |
| (18:1c) ₂ PC | L _α | 23 | 1.2956 ± 0.0007 | 0.027 ± 0.001 ^e | 2.12 ± 0.13 | 0.333 ± 0.028 ^f | 5 |
| | L _α | 30 | 1.303 | 0.0277 ^{b,g} | 1.92 | 0.333 ^c | 6 |
| (22:1c) ₂ PC | L _α | 30 | 1.522 | 0.0276 ^{b,g} | 1.94 | 0.330 ^c | 6 |
| (16:0/18:1c)PC | L _α | 30 | 1.256 | 0.0276 ^{b,g} | 1.94 | 0.331 ^c | 6 |
| (18:0/18:1c)PC | L _α | 30 | 1.3113 | 0.0276 ± 0.0007 ^{b,g} | 1.9 | 0.333 ^c | 7 |
| (O-16:0) ₂ PC | L _β ⁱ | 20 | 1.126 | 0.0245 | 2 | 0.291 ^h | 8 |
| | L _α | 48 | 1.223 | 0.0280 ⁱ | 2 ⁱ | 0.270 ^h | 8 |
| (12:0) ₂ PE | L _β | 20 | 0.863 | 0.0260 ^b | 1.75 ^j | 0.252 ^d | 9; 10 |
| | L _α | 35 | 0.907 | 0.0273 ^b | 2.0 ^e | 0.252 ^c | 9–11 |
| (14:0) ₂ PS | L _β | 20 | 0.979 ^k | 0.0259 ^b | 2.16 | 0.244 ^m | 12 |
| (18:1c) ₂ PS | L _α | 30 | 1.228 | 0.0280 ^{b,g} | 2 ^e | 0.244 ⁿ | 12 |

^a References: 1. Kucerka et al., 2005a; 2. Tristram-Nagle et al., 2002; 3. Sun et al., 1994; 4. Kucerka et al., 2006; 5. Wiener and White, 1992; 6. Kucerka et al., 2005b; 7. Greenwood et al., 2008; 8. Guler et al., 2009; 9. McIntosh and Simon, 1986; 10. Nagle and Wiener, 1988; 11. McIntosh, 1986; 12. Petrache et al., 2004.^b From additivity of volumes: $v_{\text{pol}} = v_{\text{mol}} - n_{\text{CH}_2} v_{\text{CH}_2} - 2v_{\text{CH}_3}$.^c Assumed equal to gel-phase value.^d From X-ray refinement of hydrocarbon thickness, d_{HC} , and lipid cross-sectional area, A_1 : $v_{\text{pol}} = v_{\text{mol}} - A_1 d_{\text{HC}}$.^e Assumed from liquid *n*-alkanes.^f Volume of CH group in double bond estimated as $v_{\text{CH}} = 0.023 \text{ nm}^3$ ($v_{\text{CH}}/v_{\text{CH}_2} = 0.85$).^g A fixed ratio of $v_{\text{CH}}/v_{\text{CH}_2} = 0.80$ is assumed for the double-bond CH groups.^h Corrected for two chain CH₂ groups included in head group in Guler et al. (2009).ⁱ Assumed equal to values for (16:0)₂PC (Guler et al., 2009).^j Assumed fixed.^k Deduced from v_{pol} (see Petrache et al., 2004).^m Assumed equal to (18:1c)₂PS fluid-phase value.ⁿ Deduced from comparison with (18:1c)₂PC (see Petrache et al., 2004).

groups in the gel phase is determined by subtracting the CH₂ contribution from ν_{HC} .

It is assumed that the volume occupied by the polar group in the fluid L_α phase is the same as that determined in the gel phase. The hydrocarbon volume is then obtained by subtracting this from ν_{mol} . The volume per CH₂ group in the fluid phase is then calculated from ν_{HC} by using the ratios $\nu_{\text{CH}_3}/\nu_{\text{CH}_2} = 1.9 - 2$ and $\nu_{\text{CH}}/\nu_{\text{CH}_2} = 0.80$ for the contribution of the terminal methyls and unsaturated carbons, respectively. In practice, greater precision is achieved by global fitting of the electron density profiles, with the number of constraints required depending on the resolution and amount of experimental data available (including in some cases neutron scattering) (Kucerka et al., 2008).

These values for gel and fluid phases are collected together in Table A.1. Interestingly, the $\nu_{\text{CH}_3}/\nu_{\text{CH}_2}$ ratios that are obtained for the gel phase by this method are close to 2.

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