Lipid conformation in crystalline bilayers and in crystals of transmembrane proteins

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Abstract

Dihedral torsion angles evaluated for the phospholipid molecules resolved in the X-ray structures of transmembrane proteins in crystals are compared with those of phospholipids in bilayer crystals, and with the phospholipid conformations in fluid membranes. Conformations of the lipid glycerol backbone in protein crystals are not restricted to the gauche C1–C2 rotamers found invariably in phospholipid bilayer crystals. Lipid headgroup conformations in protein crystals also do not conform solely to the bent-down conformation, with gauche–gauche configuration of the phospho-diester, that is characteristic of phospholipid bilayer membranes. This suggests that the lipids that are resolved in crystals of membrane proteins are not representative of the entire lipid–protein interface. Much of the chain configurational disorder of the membrane-bound lipids in crystals arises from energetically disallowed skew conformations. This indicates a configurational heterogeneity in the lipids at a single binding site: eclipsed conformations occur also in some glycerol backbone torsion angles and C–C torsion angles in the lipid headgroups. Stereochemical violations in the protein-bound lipids are evidenced by one-third of the ester carboxyl groups in non-planar configurations, and certain of the carboxyls in the cis configuration. Some of the lipid structures in protein crystals have the incorrect enantiomeric configuration of the glycerol backbone, and many of the branched methyl groups in structures of the phytanyl chains associated with bacteriorhodopsin crystals are in the incorrect S-configuration.

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Keywords: lipid–protein interactions; headgroup conformation; chain conformation; torsion angles

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1. Introduction
A considerable and steadily growing number of endogenous lipids have now been resolved in the high-resolution X-ray structures of integral membrane proteins (for reviews see Fyfe et al., 2001; Pebay-Peyroula and Rosenbusch, 2001; Lee, 2003). Amongst these are phosphatidylincholine in association with cytochrome c oxidase from Paracoccus denitrificans (Iwata et al., 1995; Harrenga and Michel, 1999), cardiolipin and phosphatidylethanolamine associated with various bacterial photosynthetic reaction centres (McAuley et al., 1999; Fyfe et al., 2000; Nogi et al., 2000), and a variety of diphytanoyl lipid moieties in association with bacteriorhodopsin (Essen et al., 1998; Luecke et al., 1999; Belrhali et al., 1999; Takeda et al., 2000). Phosphatidylethanolamine, phosphatidylcholine, phosphatidylglycerol and cardiolipin moieties have also been reported in association with bovine cytochrome c oxidase (Tsukihara et al., 1996; Mizushima et al., 1999). More recently, phosphatidylglycerol and galactosyl-diacylglycerol have been resolved in association with photosystem I of a cyanobacterium (Jordan et al., 2001), and with a plant light-harvesting complex (Liu et al., 2004). The transmembrane domains of all the aforementioned proteins are α-helical. In addition, a molecule of lipopolysaccharide has been resolved in association with the β-barrel outer-membrane protein PhoA (Ferguson et al., 2000).

From the point of view of lipid–protein interactions, it is of direct interest to compare the above data with the structural information available from lipid bilayers. The X-ray structures of phospholipids in bilayer crystals provide a wealth of detail on the conformations and stereochemistry of phospholipids when close-packed in membranes (Hauser et al., 1981; Pascher et al., 1992, 1996). A detailed comparison with this lipid data will therefore delineate the perturbation of the molecular configuration by the protein–lipid interaction.

Here, we review analyses of the torsion angles of lipid molecules in crystals of transmembrane proteins and of the corresponding lipids in bilayer single crystals. Comparison of the two reveals stereochemical violations and possible conformational heterogeneity in the structures of the lipids associated with crystalline transmembrane proteins.

2. Definition of lipid torsion angles

Fig. 1a gives the notation for the torsion angles in a diacyl lipid, and explicit definitions are given in Table 1 (Sundaralingam, 1972). This notation is commonly used for describing the crystal conformations of glycerolipids and sphingolipids (Hauser et al., 1981; Pascher et al., 1992), except that the numbering of the glycerol backbone C-atoms is reversed to conform with the sn-convention (IUPAC-IUB Commission on Biochemical Nomenclature (CBN), 1977) for eucaryotic and bacterial glycerolipids. Fig. 1b gives the classification of the staggered and eclipsed rotamers used for conformational description of lipids, according to the range of torsion angle (Klyne and Prelog, 1960). Equiv.

| Table 1 |
| Definition of torsion angles with atom numbering of Fig. 1 |
| θ1 | C(11)-C(21)-C(31)-O(31) |
| θ2 | O(21)-C(21)-C(31)-O(32) |
| θ3 | O(11)-C(11)-C(21)-C(31) |
| θ4 | O(11)-C(11)-C(21)-O(21) |
| α1 | C(21)-C(31)-O(31)-P |
| α2 | C(31)-O(31)-P-O(32) |
| α3 | O(31)-P-O(31)-C(31) |
| α4 | P-O(31)-C(31)-O(32) |
| α5 | O(32)-C(31)-C(21)-N |
| α6 | C(31)-C(21)-C(22)-C(23) |
| α7 | C(21)-C(22)-C(23)-C(24) |
| α8 | C(21)-C(22)-C(23)-C(24) |
| γ1 | C(21)-C(11)-C(11)-C(11) |
| γ2 | O(11)-C(11)-C(12)-C(13) |
| γ3 | O(11)-C(11)-C(12)-C(13) |
| γ4 | C(11)-C(12)-C(13)-C(14) |
dent conformational designations frequently used are: trans, t (ap); gauche, g (sc); skew, s (ac); and cis, c (sp).

3. Lipid bilayers

3.1. Glycerol backbone configuration in bilayer crystals

The backbone conformation in crystals of glycerolipids has been studied by Pascher et al. (1992): it is characterised relative to the lipid chains by the $\theta_4$ and $\theta_2$ torsion angles. The torsion angle about the glycerol C(1)–C(2) bond, viz., $\theta_4$, specifies the relative orientation of the sn-1/sn-3 and sn-2 chains, and that about the C(2)–C(3) bond, viz., $\theta_2$, defines the orientation of the headgroup relative to the sn-2 chain. In addition, the corresponding chain stacking is characterised by which of the two chains is the leading or straight-extending chain.

This is designated by $\gamma$ for a leading sn-1 chain and by $\beta$ for a leading sn-2 chain. The chain backbone configuration in crystals is specified by the Pascher notation as: $\theta_4$/leading chain/$\theta_2$ (Pascher et al., 1992). That of the glycerol moiety itself is described by the corresponding $\theta_3$/ leading chain $\theta_1$ combination of torsion angles. In the sc/γ configuration, the glycerol backbone is oriented approximately parallel, and in the sc/β configuration, the glycerol moiety is oriented at approximately 45° to the bilayer normal. In both the −sc/γ and −sc/β configurations, however, the backbone of the glycerol moiety is oriented at approximately 45° to the bilayer normal.

The torsion angles of the glycerol backbone in lipid crystals correspond exclusively to staggered conformations (Pascher et al., 1992). In principle, all combinations of $\theta_4$/$\theta_2$ staggered torsion angles are represented in lipid crystal structures, but only those with $\theta_3 =$ +sc or $-$sc allow the parallel chain stacking charac-
Table 2

Torsion angles (°) of crystalline phospholipids, classified according to θ1/leading chain/θ2 and glycerol configuration θ3/θ4. Structures of phospholipids in fluid bilayers (L<sub>d</sub>) derived from NMR are included for comparison.

<table>
<thead>
<tr>
<th>Lipid&lt;sup&gt;a&lt;/sup&gt;</th>
<th>θ1</th>
<th>θ2</th>
<th>θ3</th>
<th>θ4</th>
<th>θ5</th>
<th>θ6</th>
<th>θ7</th>
<th>θ8</th>
<th>θ9</th>
<th>θ10</th>
<th>Ref.&lt;sup&gt;b&lt;/sup&gt;</th>
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<tr>
<td>sc/α, sc/β, φ = 90&lt;sup&gt;°&lt;/sup&gt; (27% of crystal structures)</td>
<td>DLPE</td>
<td>154 56 68 106</td>
<td>67</td>
<td>−52</td>
<td>65</td>
<td>−172</td>
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<td>97</td>
<td>179</td>
<td>−119</td>
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<td>177</td>
<td>178</td>
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<tr>
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<td>153</td>
<td>−54</td>
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<td>−179</td>
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<td>83</td>
<td>174</td>
<td>164</td>
<td>173</td>
<td>−173</td>
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<tr>
<td></td>
<td>sc/γ, sc/β, φ = 90&lt;sup&gt;°&lt;/sup&gt; (15% of crystal structures)</td>
<td>DLPEM</td>
<td>179</td>
<td>65</td>
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<td>159</td>
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<td>158</td>
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</table>

<sup>a</sup> DLPE, di(lauroyl)phosphatidylethanolamine; DMPS A, di(myrityl)phosphatidylglycerol; DMPC and B, dimyristoyl phosphatidylcholine; DMPS A and B, dimyristoyl phosphatidylethanolamine; DPPC and L, liquid-crystalline state of dipalmitoyl phosphatidylcholine; DPPC L<sub>d</sub>, liquid-crystalline state of dipalmitoyl phosphatidylethanolamine bilayers.


<sup>c</sup> Calculated from θ1 and θ2, respectively.

<sup>d</sup> Two interconverting enantiomers.

<sup>e</sup> α, β, γ, and δ are torsion angles of headgroup, glycerol backbone, α-2-chain and α-1-chain, respectively (see Hauser et al., 1981 and Fig. 3).

<sup>f</sup> sc/α, sc/β, φ = 90<sup>°</sup> and sc/β/α indicate anti-gauche (gauche−) and syn-gauche (gauche+), respectively.

<sup>g</sup> Trans (ap) conformation for θ1 is disfavoured relative to gauche (g).
gle fixed conformation, it is assumed that the $\theta_3$ torsion angle exchanges rapidly on the NMR timescale, between staggered conformers. High resolution NMR of monogalactosyldiacylglycerol in oriented bicelles has been interpreted in terms of a single predominant $\text{sc/} \text{st}$ configuration (Howard and Prestegard, 1995). On the other hand, high resolution NMR studies of phospholipid micelles were interpreted in terms of a rapid equilibrium between conventional, low-energy rotamers $\alpha_4 = \text{sc}$ and $\alpha_4 = -\text{sc}$ in an approximately 2:1 ratio (Hauser et al., 1988).

### 3.2. Headgroup configuration in bilayer crystals

The conformation of the headgroups of phospholipids in bilayer crystals has been reviewed by Pascher et al. (1992). In all cases, the headgroup lies preferentially parallel to the bilayer plane and its internal conformation is strikingly constant. Only the $\alpha_4$ torsion angle about the $\text{C}(32)-\text{C}(31)$ bond displays some limited variability. Two mirror image configurations are found in which the signs of the $\alpha_2/\alpha_3$ torsion angles are reversed, even for non-racemic systems.

For phosphatidylethanolamine and its $N$-methylated derivatives, including phosphatidylcholine, the headgroup configuration in bilayer crystals is specified by: $\alpha_2 = \text{ap}$, $\alpha_3 = \text{sc}$, $\alpha_4 = \text{ap}$ to $\pm\text{sc}$, and $\alpha_4 = \pm\text{sc}$, where the upper and lower signs represent the mirror images (see Table 2). The correlated $\alpha_2/\alpha_3 = \pm\text{sc}/\pm\text{sc}$ conformation is that expected on energetic grounds for a phosphate diester (Chandrasekhar et al., 2003). The $\text{sc/sc/} \pm\text{sc}$ combination is ca. 1 kcal/mol more favourable than the next lowest lying $\pm\text{sc/ap}$ combination, even after bond angle optimisation (Gorenstein and Kar, 1977). The $\text{C}(31)-\text{C}(32)$ torsion angle $\alpha_3$ is exclusively either $-\text{sc}$ or $+\text{sc}$, and its sign is correlated with the size of the $\alpha_4$ torsion angle. This configuration is determined by internal electrostatic attraction that directs the positively charged nitrogen to the phosphate oxygens. In lipid-protein complexes, interaction of the lipid nitrogen with the protein may be preferred over this internal interaction. In fluid lipid bilayers, the deuterium and phosphorus NMR spectral anisotropies for the polar headgroups of both dipalmitylophosphatidylcholine (DPPC) and dipalmitoyl phosphatidylethanolamine (DPPPE) can be described by a rapid interconversion between the two mirror-image configurations that are observed in the phospholipid crystals (Seelig et al., 1977; Seelig and Gally, 1976, and see Table 2).

In bilayer crystals of dimyristoyl phosphatidylglycerol, the headgroup configuration of the two optical enantiomers is specified by: $\alpha_1 = \pm\text{sc}$, $\alpha_2 = \pm\text{sc}$, $\alpha_3 = \mp\text{sc}$, $\alpha_4 = \text{ap}$, $\alpha_4 = \mp\text{sc}$, and $\alpha_4 = \mp\text{sc}$ (Pascher et al., 1992, 1987). This is because $\alpha_3 - \alpha_4$ is $+120^\circ$ for the $1-\text{sn}$ enantiomer and $-120^\circ$ for the $3-\text{sn}$ enantiomer, and vice versa for $\alpha_3 - \alpha_4 < 0$. The correlated $\alpha_2/\alpha_3$ conformations in dimyristoyl phosphatidylglycerol are again the $\pm\text{sc}/\mp\text{sc}$ combination expected for a phosphate diester. In contrast to the phosphocholine/ethanolamine headgroup, the $\alpha_2$ torsion angle in phosphatidylglycerol is $\text{ap}$ (i.e., trans) and it is $\alpha_4$ that is in the $\mp\text{sc}$ (i.e., gauche) conformation.

### 3.3. Chain configuration in bilayer membranes

The $sp^3$-hybridized C-C single bonds in lipid bilayer crystals are restricted mostly to staggered conformations (Pascher et al., 1992). Eclipsed conformations carry an energy penalty that depends on the substituent atoms. For polymethylene chains, the rotational barrier that corresponds to the $\pm\text{sc}$ eclipsed positions is contributed almost exclusively by the intrinsic threefold rotational potential (Borisova and Vol’kenshtein, 1961; Abe et al., 1966). The dependence of the conformational energy on torsion angles, $\phi$, in the trans (ap) to gauche ($\pm\text{sc}$) range may therefore be approximated by (see, e.g., Flory, 1969): $E(\phi) \approx E_0 + \frac{1}{2}(E_{\text{ga}} - E_0)(1 + \cos 3\phi)$ (1)

where $E_0 \approx 3$ kcal/mol is the rotational barrier height and $E_0$ is the energy of the neighbouring potential minimum (see Fig. 2). For the trans (ap) potential minimum, $E_0 \approx E_0 = 0$, and for the gauche ($\pm\text{sc}$) minima, $E_0 \approx E_0 \approx 0.5$ kcal/mol. Eq. (1) can be used to estimate the conformational energies corresponding to the torsional angle ranges defined in Fig. 1b. The energy span over the $0-30^\circ$ range about the trans conformation is $E(\text{ap}) \approx 0-1.5$ kcal/mol, that for the gauche configuration is $E(\pm\text{sc}) \approx 0.5-1.75$ kcal/mol, and that for the $\pm\text{sc}$ eclipsed conformation is $E(\pm\text{sc}) \approx 1.5-3$ kcal/mol. The extremes assigned to the staggered conformational ranges therefore already encompass quite high energies, relative to thermal energies (even at room temperature). The $\pm\text{sc}$ eclipsed conformation, even at the $30^\circ$ extremes of its range is considerably higher in energy than the gauche minimum, $E_0$. Substitution of bulky groups in polymethylene chains will tend to make energies of the eclipsed conformations even greater. The $sp$
C double bonds, in unsaturated lipid chains. The C–C single bonds adjacent to a cis double bond are conformationally less restricted than those in a saturated chain. Two broad potential minima exist around torsion angles ±110° (extending approximately from ±85° to ±140°) that correspond to the skew (ac) conformations. Quasi straight-chain packing can be realised with conformational sequences about the double bond (Δ) of the type g°x°Δ° and equivalents (Li et al., 1994). Similar quasi-parallel packing of saturated chains can be achieved if the gauche conformations are contained in kink sequences, such as g°g°g° (Trouillet, 1971). Adjacent gauche conformations of the same sense (g°g°g° or g°g°g°) produce a right-angle bend in the all-trans chain direction, but are otherwise energetically allowed with E(g°g°g°) ~ 1 kcal/mol for the pair of bonds (cf. Marsh, 1974). Adjacent gauche conformations of opposite sense (g°g°g° or g°g°g°) are sterically forbidden for strictly ±60° torsion angles because of the pentane effect. Relatively high local potential minima, however, are found displaced from the g°g°g° configuration at torsion angle pairs ~65°, 103° and ~103°, 65° (Abe et al., 1966). These shallow conformational minima have an energy of E(g°g°g°) ~ 3 kcal/mol, relative to all-trans, E(0°), i.e., approximately half the value per bond of the ±ac eclipsed conformers, E(±ac).

4. Membrane protein crystals

4.1. Lipid backbone and headgroup torsion angles in protein crystals

Table 3 gives the torsion angles determined for a range of phospholipids or glycolipids in crystals of cytochrome c oxidase from P. denitrificans (Harrenga and Michel, 1999), Rhodobacter sphaeroides (Svensson-Ek et al., 2002) and bovine heart (Mizushima et al., 1999); of photosynthetic reaction centres from Rh. sphaeroides (McAuley et al., 1999; Fyfe et al., 2000; Camara-Artigas et al., 2002) and Thermochromatium tepidum (Nogi et al., 2000); of photosystem I from Synecococcus elongatus (Jordan et al., 2001); of light-harvesting complex II from spinach (Liu et al., 2004); of cytochrome c reductase from Saccharomyces cerevisiae (Lange et al., 2001) and chicken heart (Zhang et al., 1998); of the ADP/ATP exchange carrier from bovine heart mitochondria (Pebay-Peyroula et al., 2003); of the cytochrome b6f complex from Mastigocladus laminosus (Kurisu et al., 2003) and Chlamydomonas reinhardtii (Strobel et al., 2003); of the KcsA K+-channel from Streptomyces lividans (Valiyaveetil et al., 2002); and of formate and succinate dehydrogenases, and nitrate reductase, from Escherichia coli (Jormakka et al., 2002; Yankovskaya et al., 2003; Bertero et al., 2003).

In addition to the diacyl lipids phosphatidylcholine (PtdCho), phosphatidylethanolamine (PtdEtn), phosphatidylglycerol (PtdGro), phosphatidylinositol (PtdIns), phosphatidate (Ptd), diglyceride (acyl2Gro), galactosyldiglyceride (Gal[acyl]2Gro), digalactosyldiglyceride (Gal2[acyl]2Gro), glycosylgalactosyl diglyceride (GalGlc[acyl]2Gro) and sulfoquinovosyl diglyceride (SQacyl2Gro), data are presented in Table 3
| Lipid       | αC | αP | αC | αP | αC | αP | αC | αP | αC | αP | αC | αP | αC | αP | αC | αP | αC | αP |
|------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| Cytochrome c reductase (bovine) | PDB: 1V54 (Tsukihara et al., 2003) | 138 | 96 | 99 | 120 | 45 | 80 | 47 | 85 | 135 | 118 | 133 | 117 | 188 | 172 | 170 |
|            | PDB: 1QLE (Harrenga and Michel, 1999) | 151 | 97 | 127 | 177 | 89 | 176 | 82 | 159 | 98 | 127 | 174 | 145 | 114 | 112 | 129 | 168 | 163 |
|            | PDB: 1EYS (Nogi et al., 2000) | 106 | 61 | 178 | 73 | 186 | 73 | 178 | 76 | 125 | 116 | 125 | 116 | 125 | 116 | 125 | 116 |
|            | Reconstituted from Escherichia coli | 106 | 61 | 178 | 73 | 186 | 73 | 178 | 76 | 125 | 116 | 125 | 116 | 125 | 116 | 125 | 116 |

### References

Table 3 (Continued)

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<thead>
<tr>
<th>Lipid</th>
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<th>$\alpha_2$</th>
<th>$\beta_1$</th>
<th>$\beta_2$</th>
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55
in crystals of four different transmembrane proteins. For \( n > 3 \), \( \gamma_2 \) and \( \beta_2 \) correspond to torsion angles of the purely hydrocarbon section of the chain, undisturbed by the ester or ether linkage. For the two phosphatidylcholine lipids associated with \( P. \ denticorum \) cytochrome \( c \) oxidase (Har enga and Michel, 1999), all four chains are 18 C-atoms in length and the torsion angles with \( n = 3 \) correspond to a predominantly \( \text{trans} \) (ap) conformation throughout the chain (Fig. 3A). The latter is especially the case for one of the two molecules, where the mean \( \text{trans} \) torsion angle is 182 ± 9° (S.D., \( N = 29 \)). The only exception to the quasi all-\( \text{trans} \) structure is the single \( cis \) (ap) conformation \( \beta_1 = 0° \), in the \( sn-2 \) chains of both lipids. This almost certainly corresponds to the presence of a double bond, although all bonds are modelled with bond lengths and bond angles appropriate to C-C single bonds. The bonds adjacent to the C11–C12 \( cis \) bond are, nevertheless, in the \( \text{trans} \) (ap) conformation, and not in an allowed \( \text{skew} \) conformation.

Fig. 3B gives the chain torsion angles of cardiolipin (diphasotyphal) glycerol, with four chains in association with a photosynthetic reaction centre mutant from \( Rb. \ sphaeroideas \). Not all chains were fully resolved in the electron density, nor could any double bonds be resolved specifically, although expected in the endogenous lipid (McAuley et al., 1999). For the AM260W mutant, the first 14 C-atoms in the \( sn-1 \) chain and the first 15 C-atoms in the \( sn-2 \) chain are resolved for one phosphatidyl moiety of diphasotyphal glycerol. Correspondingly, 15 C-atoms of the \( sn-1 \) chain and 9 C-atoms of the \( sn-2 \) chain are resolved for the other phosphatidyl moiety. The cardiolipin chain configuration is predominantly \( \text{trans} \) (ap) in this reaction centre, as for the phosphatidylcholine molecules associated with bacterial cytochrome \( c \) oxidase. However, the spread in torsion angles is considerably larger (the mean \( \text{trans} \) dihedral is 182 ± 16° S.D.) for the reaction centre cardiolipin, and a significant proportion of the torsion angles lies in the eclipsed (\( \pm \text{ac} \)) range. Only two torsion angles lie within the gauche (\( \pm \text{gc} \)) range of staggered conformations. None occurs in the \(cis\) (ap) range, although unsaturated chains are expected in \( Rb. \ sphaeroideas \) cardiolipins (Russell and Harwood, 1979). Qualitatively similar results (not shown) are found for the cardiolipin molecule associated with the FM197/RGM203D mutant reaction centre (Fyfe et al., 2000). This is of significance because the latter data set was collected at low temperature (100K), whereas that for the AM260W mutant was obtained at room temperature (298 K).

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<td>170 8</td>
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</table>

References: 1. Essen et al. (1998); 2. Luecke et al. (1999); 3. Belrhali et al. (1999); 4. Takeda et al. (1999).
the conformational flexibil-
ity arises from an increased population of gauche (±ac) rotamers; that of the eclipsed (±ac) rotamers is much lower, particularly for the monogalactosyl diglyceride.

Fig. 3D shows the torsion angles for the chains of three diphytanyl lipid molecules (six chains in total) associated with bacteriorhodopsin (Essen et al., 1998). Each chain is ether-linked to the glycerol backbone, instead of ester-linked as are the chains of the other lipids considered above. The phytanol chain is 16 C-atoms long and fully saturated, but with methyl branches at the 3, 7, 11 and 15 C-atom positions (i.e., a total of 20 C-atoms). Because of the methyl branch at the penultimate carbon of a phytanyl chain, there is ambiguity in defining the final main-chain torsion angle. For this reason, the torsion angles γα and βα in Fig. 3D are defined only up to n = 15. The spread in torsion angles of the phytanyl chains interacting with bacteriorhodopsin found in Fig. 3D is qualitatively similar to that found for the diacyl lipids associated with photosystem I. Again, the greater conformational flexibility relative to Fig. 3A and B arises from a greater number of staggered gauche (±ac) rotamers, rather than of energetically unfavoured eclipsed (±ac) rotamers. No cis (sp) conformers are found, and none are expected for saturated phytanyl chains.

Results from the other three datasets for diphytanyl lipids associated with bacteriorhodopsin are not shown in Fig. 3. These differ considerably from those shown in Fig. 3D in that they contain a considerably higher proportion of eclipsed conformers. For the PDB:1C3W series, the population of eclipsed (±ac) conformers equals that of the trans (ap) conformers, and the proportion of gauche (±ac) conformers is rather low. The PDB:1QHJ and PDB:1QMB series are both characterised by an appreciable population of eclipsed (±ac) conformers, and also of gauche (±ac) conformers. Again, cis (sp) conformers are almost absent.

5. Comparison of lipid conformations in membranes and protein crystals

Many of the lipid chain configurations in protein crystals are characterised by a relatively high proportion of energetically unfavourable eclipsed conformations (Marsh and Páli, in press). In principle, rather high rotational conformational energies, can be sustained in the lipid molecules if there is a compensating energetically favourable stabilisation by the lipid–protein interaction. In this scenario, the surface contour of the hydrophobic protein side chains would force the lipid into eclipsed conformations in order to optimise their mutual interactions. Measurements on the selectivity of lipid–protein
interactions with rhodopsin and other membrane proteins reveal only a weak dependence on lipid chain length (Ryba and Marsh, 1992; Marsh, 1995). This implies that the interaction of lipid chains with the hydrophobic surface of transmembrane proteins is only marginally more favourable than the interaction of lipid chains with themselves. In further support of this, little selectivity is found between spin-labelled lipids of the same headgroup but different numbers of chains in the interaction with cytochrome c oxidase and with the Na, K-ATPase (Powell et al., 1987; Esmann et al., 1988). Finally, the off-rates for exchange of non-selective lipids at the intramembranous surface of integral proteins, although significantly slower, are nevertheless comparable to diffusive lipid–lipid exchange rates in fluid lipid bilayers (Marsh and Horváth, 1998). This again implies no strong preferential interaction of the lipid chains with the protein rather than with themselves.

The results of spectroscopic thermodynamic studies therefore suggest that lipid chains have affinities among themselves that are energetically similar to those for the lipid–protein interface. Typically chain cohesions in fluid lipid bilayers correspond to interaction free energies of \( \Delta G \sim -0.6 \text{kcal/mol} \text{CH}_2 \) (Cevc and Marsh, 1987; King and Marsh, 1987). The preferential selectivities for the lipid–protein interface, determined as mentioned above, are approximately one-tenth of this (Marsh and Horváth, 1998). A possible exception is cardiolipin interacting with the ADP–ATP carrier, for which the off-rate of lipid exchange is considerably lower than would be predicted from the average relative association constant (Horváth et al., 1990). On balance, these measurements therefore suggest that lipid chain interactions with integral proteins in membranes are unlikely to produce sufficient stabilisation energy to compensate the formation of energetically unfavourable eclipsed conformations.

It is possible that the energetic considerations above regarding generalised lipid–protein interactions in membranes may not apply to the lipids that are resolved specifically in the X-ray structures of membrane protein crystals. Much stronger stabilisation of the lipid–protein interaction may be required or indicated in the latter case. This cannot, however, apply universally to cases in which all lipids at the protein interface are resolved (e.g., Belrhali et al., 1999). Further, very few eclipsed conformations are present in structures for which the mean temperature factors (B-values) of the lipids are comparable to those of the protein. This is also true for the protein side chains in high-resolution crystal structures (Ponder and Richards, 1987). Both these facts suggest rather strongly that the conformational violations in the lipids may well be the result of configurational disorder.

### Table 5

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<th>( \beta_2 \beta_4 )</th>
<th>( \theta_1 \gamma_1 )</th>
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* For definition of torsion angles see Fig. 1.

**Table 5**

Classification of glycerolipid structures in membrane protein crystals according to backbone configuration (Marsh and Pálí, in press). The results of spectroscopic thermodynamic studies thus indicate no strong preferential interaction of the lipid chains with the protein rather than with themselves. The classification of lipid interactions in membrane protein crystals according to their \( \theta_1 \gamma_1 \) torsion angle configurations. Combinations of these complementary torsion angle pairs are given appropriately to the expected optical enantiomer for non-archaea and archaea. Because the interaction of the lipid chains with the hydrophobic protein side chains removes the necessity for the strictly parallel chain stacking that is found in lipid crystals, a leading chain cannot always be identified unambiguously. Therefore, this classification is not attempted. Thirty-five percent of the structures have staggered configurations that are found with parallel chain stacking in the crystals of phospholipids (cf. Table 2). A further five structures of archaeal lipids have corresponding configurations but of the opposite optical enantiomer. Seven of the structures have staggered conformations that would not give rise to parallel chain stacking in bilayers and therefore are not represented in the phospholipid crystals. All of the remaining structures have at least one eclipsed conformation and 42% of these have both torsion angles in the eclipsed range.

The staggered, parallel-chain structures for non-archaeal lipids in the protein crystals comprise 26% sc/sc, 23% sc/sc, 28% sc/ap, 5% sc/ap and 13% sc/sc configurations, together with 5% of the sc/sc...
enantiomer. In the lipid crystals, the relative occurrences are, for comparison: 36% sc/sc, 18% sc/–sc, 6% sclip, 9% –sc/–sc and 30% sc/–sc (Table 2). Thus, the protein-interacting lipids are relatively enriched in conformations with both sc and –sc conformations with the protein-interacting lipids are relatively enriched in gauche conformations with the protein-interacting lipids are relatively enriched in the dynamic configuration of glycerolipids (Thunnissen et al., 1990). As already mentioned, lipids at the protein interface must not necessarily display the parallel chain packing that is essential in bilayer crystals. Therefore, the ap/-sc configurations in Table 5 are not automatically disfavoured sterically. In fact, departures from parallel chain packing may be required in some instances if both chains are to be accommodated in grooves in the protein surface. There are examples of nonparallel-chain structures also for phospholipids in the binding sites of soluble proteins (Pascher, 1996; Marsh, 2003). Phosphatidylcholine bound as an inhibitor of an inhibitor complex with cobra venom phospholipase A2 (Plesniak et al., 1995). It has been suggested, on the basis of evidence summarised earlier above, that the sc/y conformer predominates in the dynamic configuration of glycerolipids in hydrated bilayer membranes (Pascher, 1996). This permits all three θ2 rotamers of the headgroup. The lipids associated with membrane proteins in their crystals apparently sample a wider conformational space. Of the conformations found in lipid bilayer crystals, all are fully represented by lipids in the crystals of membrane proteins.

5.2. Lipid chain configuration

The eucaryotic and bacterial lipids contain a rather large proportion of eclipsed skew conformers, at the expense of gauche conformers (see, e.g., Fig. 3A–C). For those chains with appreciable gauche populations, only six classical g+tg- kink sequences are found (Fig. 3C): 19 g+ sequences appear in the complete range of lipids surveyed in Tables 3 and 4, and five g- sequences occur at the chain ends (where they are sterically allowed). Interestingly, several of these coupled sequences (6 out of 30) appear in just two lipids: both are diolipin and diolipin have C9–C10 and C12–C13 associations with double bonds. All four chains of each cardiolipin, phosphatidylethanolamine, phosphatidylcholine and phosphatidylglycerol associated with bovine cytochrome c oxidase (Tsukihara et al., 2003) contain cis (sp) conformers, presumably associated with double bonds. All four chains of each cardiolipin have C9–C10 and C12–C13 cis rotamers corresponding to the C18:2Δ9,12 configuration of linoleic acid which constitutes nearly all chains of cardiolipin associated with bovine cytochrome c oxidase (Powell et al., 1985). The sn-2 chains of phosphatidylethanolamine have cis torsion angles, β7, β10, β13 and β16, about the C5–C6, C8–C9, C11–C12 and C14–C15 bonds which correspond to the C20:4Δ3,6,9,12 configuration of arachidonic acid. The sn-2 chains of phosphatidylglycerol have a cis configuration about the C11–C12 bond, which corresponds to C18:1Δ11, i.e., the cis configuration. The single cis conformation of phosphatidylcholines associated with P. denitrificans cytochrome c oxidase (Harrenga and Michel, 1999) (see Fig. 3A) is in the sn-2 chain, as expected for a cis double bond. The location of the putative unsaturated cis bond is at the C11–C12 position, rather than at the C9–C10 position as in oleic acid, the common monounsaturated fatty acid. Flanking skew conformations such as might facilitate straight-chain packing are also absent. Otherwise, the phosphatidylcholine chains associated with P. denitrificans cytochrome oxidase are predominantly in the trans configuration, corresponding to a rather pronounced ordering at the lipid–protein interface. Gauche conformations apparently are absent and the proportion of energetically unfavourable eclipsed conformations (which might indicate the presence of non-identified gauche conformations) is rather low, especially for one of the two molecules. For phosphatidylcholine associated with Rb. sphaeroides reaction centre (Camara-Artigas et al., 2002), the sn-2 cis bond is in the position expected for oleic acid; but for glucolyl galactosyl diglyceride also associated with the reaction centre, the cis bond is at position C8–C9 of the lipid chain and is flanked by trans rather than by skew conformers. The chains of these two lipids are also almost devoid of gauche rotamers, but disorder is introduced by a limited number of skew conformations. This is also the case for the resolved chain segments of phosphatidylcholine associated with S. cerevisiae cytochrome c reductase (Lange et al., 2001).

The chains of cardiolipin, phosphatidylethanolamine, phosphatidylcholine and phosphatidylglycerol associated with bovine cytochrome c oxidase (Tsukihara et al., 2003) contain cis (sp) conformers, presumably associated with double bonds. All four chains of each cardiolipin have C9–C10 and C12–C13 cis rotamers corresponding to the C18:2Δ9,12 configuration of linoleic acid which constitutes nearly all chains of cardiolipin associated with bovine cytochrome c oxidase (Powell et al., 1985). The sn-2 chains of phosphatidylethanolamine have cis torsion angles, β7, β10, β13 and β16, about the C5–C6, C8–C9, C11–C12 and C14–C15 bonds which correspond to the C20:4Δ3,6,9,12 configuration of arachidonic acid. The sn-2 chains of phosphatidylglycerol have a cis configuration about the C11–C12 bond, which corresponds to C18:1Δ11, i.e., the cis rather than the usual trans double bond of v vaccenic acid. The sn-2 chains of phosphatidylcholine have cis β7, β10, β13 and β16 torsion angles that would correspond to a C18:2Δ9,12 configuration, although the PDB:1V54 file describes this as a linoleoyl chain. Of the 34 double bonds in the phospholipids associated with bovine cytochrome...
cytochrome c oxidase, 65% have at least one adjacent skew (ac) conformation, and 35% have two adjacent skew conformers. These, however, account for only 24% of the total skew conformations in the chains of these lipids. The chains of one phosphatidylglycerol, and of galactosyl diglyceride, associated with S. elongatus PS I (Jordan et al., 2001) contain no cis bonds (see Fig. 3C). Conformational disorder is restricted mostly to gauche rotamers, with the population of eclipsed conformers being very low. The chains of phosphatidylglycerol and of digalactosyl diglyceride associated with spinach LHC II (Liu et al., 2004) also contain no cis bonds. However, the population of skew conformers in these chains is very high.

The cis (sp) conformers that appear in phosphatidylethanolamines associated with Rh. sphaeroides cytochrome c oxidase (Svenson-Ek et al., 2002) are confined to the sn-1 chain and largely appear in unconventional positions. They therefore likely correspond to energetically forbidden eclipsed conformers. Both sn-1 and sn-2 chains contain a high proportion of the energetically disallowed skew (ac) conformers that are not associated with double bonds. Similar considerations apply to phosphatidylethanolamine associated with the Th. tepidum reaction centre (Nogi et al., 2000), although here the γ9 = sp cis conformer is flanked by skew (−ac) conformers. The only cis conformer in the phosphatidylethanolamines associated with S. cerevisiae cytochrome c reductase (Lange et al., 2001) is γ9 = sp in a single sn-1 chain. The population of eclipsed skew conformers is rather high, however, 44% ±ac for both phosphatidylethanolamines. That for phosphatidylinositol, which contains no cis conformers is less: 30% ±ac, although still considerable. Phosphatidylethanolamine associated with E. coli succinate dehydrogenase (Yankovskaya et al., 2003) also conforms to this general pattern: 36% ±ac and no sp.

Although the four lipids associated with the M. laminosus dimer cytochrome hifr complex are designated as dioleoyl phosphatidylcholine (used in the crystallisation; Kurissu et al., 2003), only two of the eight chains contain a cis rotamer. Further, these two cis conformers are at the C8–C9 position of the chain, rather than at the C9–C10 position of the cis double bond in oleic acid. The chain disorder of these phosphatidylcholines is characterised by a rather large population of gauche conformers (21%), relative to the energetically disallowed skew conformers (7%). All four lipids, however, contain an unconventional methyl substitution at the C2 position of the glycerol backbone. This additional methyl group is not present in dioleoyl phosphatidylcholine, nor in any known naturally occurring glycerolipid.

The cardiolipsins associated with Rh. sphaeroides reaction centre (McAuley et al., 1999; Fyfe et al., 2000; Camara-Artigas et al., 2002), E. coli formate dehydrogenase (Jormakka et al., 2002), and E. coli succinate dehydrogenase (Yankovskaya et al., 2003), contain no cis conformers in their chains and also very few, or no, gauche rotamers. Cardiolipin associated with the reaction centre contains a large proportion (ca. 40%) of eclipsed ±ac conformers, as to a lesser extent that does associated with formate reductase (30% ±ac), whereas that associated with succinate dehydrogenase is characterised by a high trans population (76%) and 19% ±ac. On the other hand, cardiolipin associated with S. cerevisiae cytochrome c reductase (Lange et al., 2001) has two cis conformers, at C5–C6 in one phosphatidyl moiety and at C9–C10 in the other, but both in the sn-1 chain. This cardiolipin is also characterised by a relatively high gauche rotamer population (22% ±ac) and a similar proportion of eclipsed conformers (22% ±ac).

5.3. Phytanyl chain configurations

The branched methyl groups in the phytanyl chains of the archaeabacteria have several effects on the stability of the C-C rotamers (see e.g., Flory, 1969). The barrier height associated with the eclipsed conformers is increased. The trans configuration of an adjacent bond is increased in energy to be comparable to that of a gauche conformer in an unsubstituted chain. The energy of one of the two gauche rotamers is increased, relative to that in the unsubstituted chain, by a factor of two or more. For the R-configuration of the methyl branches, the most stable configuration for the bonds flanking the substituted C-atom is: g + t or tg-, as evidenced by the helical configuration of crystalline polypropylene. The presence of the methyl branches is therefore expected to increase the gauche population.

The chains of the lipids associated with bacteriorhodopsin in PDB file 1BRR (Essen et al., 1998) have a high proportion of gauche rotamers (35% ±ac), consistent with the expectation for phytanyl chains (see Fig. 3D). The population of skew ±ac conformers is also relatively low. Not all gauche rotamers are associated with the methyl-substituted C-atoms (i.e., βs, βs, βs, βs, βs, βs, βs, and similarly for y), however, and the sense of the gauche rotation is not always consistent with the enantiomeric configuration. (Note that all of the PDB:1BRR lipids have the incorrect S-configuration.) It is probable that steric interactions of the branched methyl groups with the protein may also tend to increase the population of gauche rotamers. The other phytanyl-chain lipids associated with bacte-
rhodopsin (PDB:1C3W, 1QH and 1QM8; Luecke et al., 1999; Belthahm et al., 1999; Takeda et al., 2000) have a considerably higher population of the disallowed, skew eclipsed conformations than do the PDB:1BRR lipids. With the exception of the PDB:1C3W lipids, however, they do also have a relatively high gauche population, in line with expectation for methyl-branched chains. The PDB:1QM8 series of lipids have the correct R-configuration for the branched methyl group. However, only one out of six flanking gt/tg combinations (from a total of 23) has the correct sense, viz., \( g_α/γ = g^t \). For the PDB:1BRR lipid chains, the high gauche population has associated with it neighbouring gauche pairs: four \( g^g \), but also two sterically forbidden \( g^g \) combinations (see Fig. 3D). The latter is not the case for the other lipid series associated with bacteriorhodopsin, although \( g^g \) pairs are relatively abundant in the PDB:1QHJ lipids.

5.4. Headgroup configuration

Of the various headgroup conformers in Tables 3 and 4, only that for the B moiety of cardiolipin associated with the Rh. sphaeroides photosynthetic reaction centre (PDB:1QOV, 1MX; McAuley et al., 1999; Camara-Artigas et al., 2002) conforms completely to the configuration found in single crystals of phospholipids. At the protein interface, the range of polar group orientations and conformations is much wider than in lamellar crystals of diacyl phospholipids or in fluid phospholipid bilayer membranes (compare Tables 3 and 4 with Table 2). In nearly all cases, the lipid phosphate is anchored by hydrogen bonding to protein residues. For all protein-associated phospholipids, with the exception of one cardiolipin and one phosphatidylglycerol associated with bovine cytochrome c oxidase (PDB:1V54, Tsukihara et al., 2003), the \( ω_1 \)-torsion angle is either ap or \( τ_1 \), as found in phospholipid crystals. Only eight headgroup structures have the energetically most favourable \( ω_2/a_2 = +/tsc/αtsc \) configuration for the phosphate diester, and a further 10 have the next most favourable \( ω_2/a_2 = 0tsc/αtsc \) configuration. Several of the lipids have an energetically disfavoured eclipsed conformation for the \( ω_3 \)-torso angle (Tables 3 and 4).

One of the two distearoyl phosphatidylcholine molecules associated with \( P. denitrificans \) cytochrome c oxidase is in the energetically unfavourable \(-ac\) eclipsed conformation (see Table 3), because of interaction with Asp 124(C). The headgroup configuration of the other phosphatidylcholine molecule, by contrast, is very different. It extends away from the acyl chains, with all torsion angles except \( α_4 \) in the ap conformation, presumably because of steric and hydrophobic interactions with the protein side chains. Thus, the phosphate diester \( ω_2/a_2 = ap/tap \) does not have the energetically preferred \(-tsc/αtsc \) configuration, but \( α_4 \) is staggered rather than ap. Phosphatidylcholine associated with the Rh. sphaeroides reaction centre, and also with \( S. cerevisiae \) cytochrome c reductase, has one phosphate diester torsion angle in the energetically unfavourable ac configuration. Otherwise, the torsion angles conform to those found in phospholipid crystals, but the headgroup deviates somewhat from the fully bent-down orientation. None of the four phosphatidylcholines associated with the cytochrome b–f complex have the preferred \( \pm αtsc/αtsc \) configuration of the phosphate diester. The headgroups of these lipids are extended away from the chains in two cases, or are extended in a direction perpendicular to the chains in the other two cases. All four lipids are located in cavities at the dimer interface.

Phosphatidylethanolamine associated with the \( T. tepidum \) photosynthetic reaction centre has a headgroup configuration that departs from the energetically preferred conformations. The torsion angle \( α_2 \) of the phosphate diester is \(-ac\) rather than \(+sc\), and \( α_5 = τacC–C–C \) torsion angle is eclipsed. The headgroup is bent down towards the chains with the N-atom hydrogen-bonded to the backbone of Gly 256(M).

One of the two phosphatidylethanolamines associated with \( S. cerevisiae \) cytochrome c reductase has the second most favourable \( sc/αtsc \) configuration for the phosphate diester. The torsion angle \( α_3 = −sc \) is gauche and that of \( α_5 = ac \) is eclipsed. This brings the headgroup N-atom within hydrogen bonding distance of Glu 24(G). The other resolved phosphatidylethanolamine, has an \( ω_2 = ac \) configuration, instead of \(-sc\). Otherwise, torsion angles are in the ranges found in phospholipid crystals. For both phosphatidylethanolamine molecules, the headgroup is directed away from the chains. Phosphatidylethanolamine associated with \( E. coli \) succinate dehydrogenase has an ap\( \leftarrow sc \) configuration of the phosphate diester and torsion angles, otherwise, are those found in phospholipid crystals. The headgroup is bent down over the chains in a lipid-bilayer compatible configuration.

The six phosphatidylethanolamines associated with Rh. sphaeroides cytochrome c oxidase display a variety of different headgroup configurations that are directed
away from the chains, to a greater or lesser extent, in different orientations. The torsion angles differ considerably between the different molecules, and from those in phospholipid crystals, although the $\alpha_1$ torsion angle is $ap$ (with one exception), and predominantly the $\alpha_4$ torsion angle is also $ap$. None of the phosphatide diester torsion angles correspond to the energetically favourable configurations. The phosphatidylethanolamines associated with bovine cytochrome c oxidase also display a variety of headgroup conformations. Two equivalent headgroups are extended away from the chains and are involved in H-bonding to the protein, whereas those of the other two pairs are bent down, and one of these is also involved in H-bond formation.

The three dipalmitoyl phosphatidylglycerol molecules associated with photosystem I have an $ap_2$ torsion angle as for phosphatidylcholine/ethanolamine in bilayer crystals, rather than the $ac$ conformation found in crystals of dimyristoyl phosphatidylglycerol. Also, the second torsion angle of the phosphatide diester is in the $ap$-$ac$ conformation, rather than the expected $ac$-$ac$ conformation (compare Tables 2 and 3). The $\alpha_4$ torsion angles of two of the phosphatidylglycerol molecules are $ac$, as in the lipid crystals, whereas that of the third is the allowed $ap$ conformation. The $\alpha_5$ C–C torsion angle of one phosphatidylglycerol has the $ap$ conformation as in the lipid crystal, that of another is the staggered $sc$ conformer, whereas the remaining one has an eclipsed conformer. The oxygen torsion angles $\omega_{122}$ and $\omega_{55}$ in the three molecules, respectively. Thus, two headgroups have the natural $1-\alpha_1$ configuration, but the third has the incorrect $3-\alpha_1$ headgroup configuration. All other non-archaearial phosphatidylglycerol headgroups are the correct $1-\alpha_1$ configuration with mean values of $\omega_{12} = 122 \pm 2^\circ$ and $\omega_{55} = -122 \pm 2^\circ$ ($N = 21$).

Seven out of the 10 phosphatidylglycerol molecules associated with spinach LHC II have a more extended headgroup conformation than in bilayer crystals. The concensus structure is: $\alpha_1 = ap$, $\alpha_2 = ap$, $\alpha_3 = ap$, $\alpha_4 = ap$, $\alpha_5 = sc$ and $\alpha_6 = ac$. The remaining three phosphatidylglycerol molecules have a different concensus structure: $\alpha_1 = ap$, $\alpha_2 = ac$, $\alpha_3 = sc$, $\alpha_4 = ap$, $\alpha_5 = ac$ and $\alpha_6 = sc$, that approximates somewhat more to the bilayer configuration. The phosphatidylglycerols associated with bovine cytochrome c oxidase display a variety of headgroup conformations. With the exception of one equivalent pair, the headgroups are directed away from the hydrocarbon chains, to a greater or lesser extent. Those of two pairs of equivalent molecules are involved in hydrogen bonding to the protein.

The single diphytanoyl phosphatidylglycerol and diphytanoyl phosphatidylglycerolphosphate molecules associated with bacterial photosynthetic P700 structure (Takeda et al., 2000) have headgroup configurations that bear no similarity to those in dimyristoyl phosphatidylglycerol crystals (compare Tables 2 and 4). In both molecules, the $\alpha_1$ C–C torsion angle is in the energetically unfavourable $ac$ eclipsed conformation, and neither have the energetically favourable $sc/ac$ combination for the $\omega_{12}/\omega_{55}$ torsion angles. The oxygen torsion angles $\omega_{12}$ and $\omega_{55}$ are $ac$ for both, corresponding to the $3-\alpha_1$ headgroup configuration, as expected for phosphatidylglycerol and phosphatidylglycerol phosphate from archaeariae (Job and Kates, 1969).

In the diphosphatidylglycerol molecule associated with the Rh. sphaeroides reaction centre, the torsion angle $\omega_{12}$ is $ap$ for the A phosphatidyl moiety and $ac$ for the B phosphatidyl moiety. Thus, the headgroup glycerol is the $m-1$ enantiomer with respect to the A moiety and $m-3$ enantiomer with respect to the B moiety of the cardiolipin molecule. Otherwise, the headgroup configuration of the B-phosphatidyl moiety more closely resembles that of DMPG A in lipid crystals (see Tables 2 and 4). The $\alpha_1/\alpha_5 = ac/sc$ combination for the B-phosphodiester is energetically more favourable than the $sc/\omega_{55}$ combination for the A phosphodiester. For both A and B sections of the glycerol moiety: $\alpha_4 = ap$ and $\alpha_6 = sc$. Of the other diphosphatidylglycerol molecules, only the A moiety of one pair associated with bovine cytochrome c oxidase has the energetically favourable $ac/sc$ configuration for the phosphatide diester, and none have the next most favourable $ac/sc$ configuration. Otherwise, the cardiolipin headgroup torsion angles conform mostly with those found in phospholipid crystals, but with certain exceptions, which have an $\alpha_5 = ac$ eclipsed conformation. The headgroup conformation of all cardiolipins in membrane protein crystals is such that the chains of the two diacyl moieties are roughly parallel, as required in a bilayer membrane.

6. Conclusion

The lipids associated with integral proteins in crystals have survived both the solubilisation and purification processes, as well as crystallisation procedures. Routinely, integral proteins solubilised in non-ionic detergents retain part of the endogeneous lipid in the protein-containing mixed micelles. Only after exhaustive exchange against a large excess of exogeneous lipid in a mild detergent such as cholate can all endogeneous lipids be replaced (Warren et al., 1974; Hesketh...
et al., 1976). In this way, for instance, it was possible to replace the endogenous cardiolipin that co-purifies with cytochrome c oxidase (Powell et al., 1985; Watts et al., 1978), even though this mitochondria-specific lipid is found in crystals of the purified bovine protein (see Table 3) and is able to enhance the activity of the lipid-substituted enzyme (Abramovich et al., 1990).

By contrast, NMR experiments with deuterated lipids reveal that the major lipid population contacting the protein exchanges rapidly with the fluid bilayer pool (Oldfield, 1982; Bienvenue et al., 1982; Meier et al., 1987; Seelig et al., 1982). Electron spin resonance experiments with spin-labelled lipids put the off-rates for exchange in the region of ca. 1–10 MHz, depending on the affinity of the protein for a particular lipid (Marsh and Horváth, 1998). There are, therefore, grounds to expect that the lipids that are resolved in protein crystals might correspond to special structures that are not necessarily representative of the overall population of first-shell lipids at the protein interface. This interpretation is supported by the occurrence of headgroup configurations that differ widely from those in bilayer membranes.

Examination of the locations of the lipids at the protein interface reveals certainly that some, but not all, of the lipids in protein crystals are found in specialised sites. For example, the six unique lipids resolved in crystals of 

Rh. sphaeroides cytochrome c oxidase are located at well-defined positions, in a cleft formed by the two-helix bundles of subunit III, or at the interface between subunits IV and subunits III/IV (Svensson-Ek et al., 2002). One pair of cardiolipins associated with the bovine cytochrome c oxidase dimer is trapped within the contact sites between the two monomers. In yeast cytochrome c reductase, one of the phosphatidylethanolamines is located at the dimer interface, and the phosphatidylserine occupies a unique interhelical position (Lange et al., 2001). On the other hand, the several lipids resolved in crystals of bacteriorhodopsin are not trapped between helices or within the protein, rather they are positioned around the protein perimeter (Belrhali et al., 1999). Also, the cardiolipin molecules that are resolved in the crystals of bacterial reaction centres are located at the surface of the protein (Fyle et al., 2001), as are the cardiolipins and one phosphatidylethanolamine associated with yeast cytochrome c reductase (Lange et al., 2001).

Stereochemical violations and the occurrence of energetically disallowed rotamers for many lipid structures in the PDB remain a cause for concern. Whereas the latter could be indicative of conformational heterogeneity (see also DePristo et al., 2004), incorrect enantiomeric configurations point to deficiencies in the refinement procedure that most probably result from the use of inadequate restraint libraries (Kleywegt et al., 2003).

References


Ab, A., Mark, I.E., 1976. Conformational energies and the random coil dimensions and dipole-moments of the polyoxides CHyO(CH2)nO(CHy)2, CHyO(CH2)nO(CHy). J. Am. Chem. Soc. 98, 6406–6416.


Fyle, P.K., McAuley, K.E., Rofsman, M., Isaacs, N.W., Cogdell, B.J., Jones, M.R., 2001. Probing the interface between membrane...


