Physical effects of poly-unsaturated fatty acids on membranes

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Abstract

A brief review is given of our current understanding of the physical and physico-chemical effects of poly-unsaturated fatty acids on lipid bilayers as models of biological membranes. Results from a variety of different experimental and theoretical studies will be synthesized into a coherent picture in which the major effects are described in terms of changes in mechanical properties, permeability, lateral pressure profile, and lateral lipid-domain organization. It is pointed out that the addition of an extra double bond in the fatty-acid acyl chains can lead to major changes in the bilayers properties that may reflect on protein- and receptor function.

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Introduction

The lipid composition of biological membranes has remained an elusive problem in membrane science for decades and it is still a mystery why Nature has embarked on a strategy involving the use of a large diversity of lipids for each type of cellular membrane (Mouritsen 2005; Mouritsen and Andersen, 1998; Heimburg, 2007). Until recently, little was known regarding the actual lipid composition of membranes in terms of lipid species, but at the moment we witness an upsurge in the available data for the exact lipid composition of specific membranes and organelles, mainly obtained by the use of modern lipidomics techniques. Although basically unexplained, lipid composition and diversity have over the years been rationalized by the need for membranes to exert some kind of homeostatic control, e.g. by maintaining some more or less well-defined physico-chemical properties, such as fluidity, hydrophobic thickness, phase state, diffusivity, microviscosity, etc. Chemical lipid-specificity for protein function has been asserted in some cases, where specific binding sites at membrane proteins have been identified, but in the vast majority of cases, the putative requirement of certain lipids for the functioning of a specific membrane protein or receptor has turned out only to be apparent, and full or at least part of the function could be supported by an appropriate combination of other lipids.

The growing interest in determining the details of the trans-membrane structure (Cantor, 1999; 2003) and the small-scale lateral structure of membranes (Jacobson *et al.*, 2007) may be seen as an attempt to unravel in quantitative terms which physical and possibly unspecific mechanisms may be responsible for lipid-protein interactions in membranes. This viewpoint is that of the physical chemist, and the emergence of novel and powerful, quantitative experimental techniques, combined with large-scale computer simulations, has lead to new insight that eventually may give some clue to lipid diversity in membranes.

The effect of unsaturated and in particular poly-unsaturated fatty acids (PUFAs) on membrane properties and protein function holds a prominent position in this complex of problems. Except for very few cases, the surfactant lining of the lung alveoli being a well-known example, virtually all cell membranes of mammalian cells contain a substantial portion of unsaturated lipids. Neural membranes constitute an extreme case where more than half of the lipids are poly-unsaturated, notably docosahexaenoic acid (DHA), eicosapentaenoic acid, and arachidonic acid.

Traditionally, unsaturation has been explained as a need for membranes to stay fluid in the functional state, but it is obvious that fluidity can be controlled in many different ways and that by introducing unsaturation in lipids, other properties in addition to fluidity change at the same time, such as membrane thickness, trans-bilayer stress profile, permeability barrier, and membrane elasticity, just to name a few. Moreover, the concept of membrane fluidity is a best misleading and should be properly defined in each specific context.

Experimental and theoretical methods to study physical properties of model membranes

Quantitative studies of the various physical and physico-chemical effects of PUFAs on membranes proceed conveniently by means of well-defined lipid-bilayer models in the form of vesicles, liposomes, or supported bilayers. These model systems can be studied by experimental physical techniques, such as nuclear magnetic resonance spectroscopy, X-ray and neutron scattering, fluorescence microscopy, micromechanical experiments, and electrophysiological methods, or by theoretical and simulational techniques, such as molecular modeling and atomic-scale molecular dynamics simulation techniques. Interpretation of the experimental data are complicated by the fact, that even simple membranes composed by two or more lipid species have complex phase behavior, with the possibility of both large-scale phase separation as well as small-scale domain- or raft formation. An important regulator of this lateral organization is cholesterol which is abundant in all plasma membranes. The simulation of model systems is mostly restricted to small systems with few different molecular components, and detailed molecular dynamics simulations in particular are hampered by severe restriction in time.

Fluidity, acyl-chain order, phase state, diffusion, mechanics, permeability, and lateral pressure profile

Based on such quantitative experimental as well as theoretical model studies a coherent picture is gradually emerging of the effect of PUFAs on membrane structure and dynamics and how the membrane properties control protein function. Increasing the degree of unsaturation of the fatty acids in the lipids making up a bilayer membrane has a number of generic effects (Stillwell and Wassall, 2003). The most dramatic effect in physical properties occurs when two or more double bonds are introduced. In the case where nothing but the degree of unsaturation is changed, i.e. the acyl-chain length is kept constant and the polar

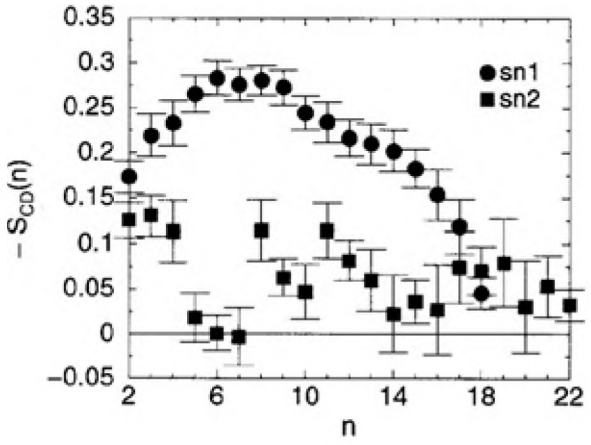

Fig. 1. Acyl-chain segmental order parameter, S_{CD} (n), along the two acyl chains of lipids composed of 18:0 and 22:6 DHA chains. The data derives from an atomic-scale molecular dynamics simulation of a lipid bilayer. Adapted from Saiz and Klein (2001).

head groups remain the same, these effects are as follows. The phase state of the bilayer changes towards a stabilization of fluid phases, specifically liquid-disordered phases, that is phases or phase-separated states of low melting points. The liquid-disordered phases have high lateral molecular mobility, i.e. large molecular diffusion constants and a substantial degree of acyl-chain disorder (Saiz and Klein, 2001; Elho *et al.*, 2003; Martinez-Seara *et al.*, 2007) as illustrated in Fig. 1. In addition, the liquid-disordered phase displays accelerated dynamics of the head groups and the glycerol backbone, as well as augmented acyl-chain rotational dynamics (Ollila *et al.*, 2007). The thickness of the bilayer is deceased in order to allow for the increased acyl chain disorder.

The liquid-disordered phases can be turned into liquid-ordered phases upon introduction of cholesterol, which however retains the diffusional characteristics of a liquid. Upon increasing the degree of unsaturation, the transbilayer passive permeability is diminished substantially (Huster *et al.*, 1997; Olbrich *et al.*, 2000) which is accompanied by a reduction in bilayer bending rigidity and an increase in lateral compressibility (Rawicz *et al.*, 2000; Olbrich *et al.*, 2000; Koenig *et al.*, 1997). These effects are illustrated in Fig. 2.

The lateral pressure profile of the bilayer, cf.

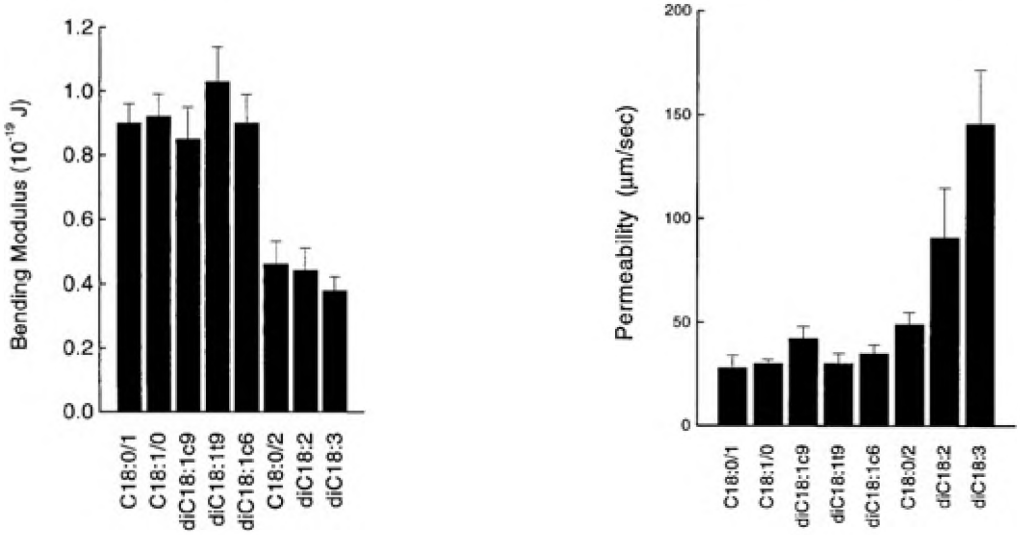

Fig. 2. Left: Elastic bending moduli for di-C_{18} phosphatidylcholine bilayers arranged in order of increasing unsaturation. Adapted from Rawicz *et al.* (2000). Right: Water permeability for di-C_{18} phosphatidylcholine bilayers arranged in order of increasing unsaturation. Courtesy by Evan Evans. Adapted from Olbrich *et al.* (2000).

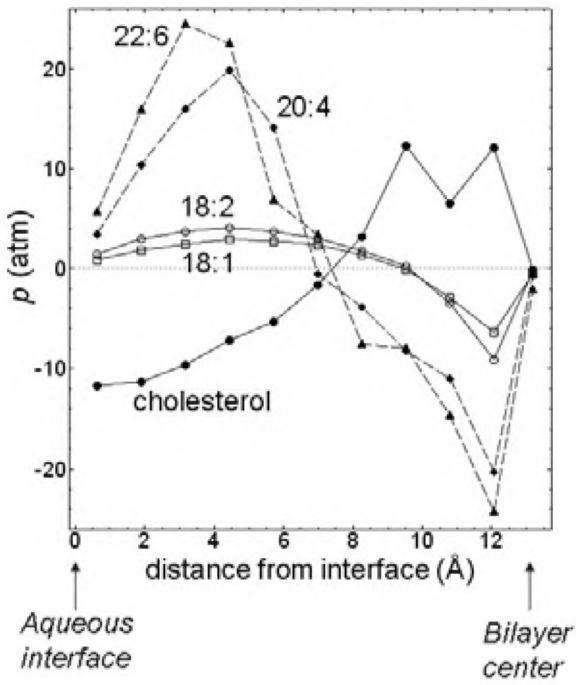

Fig. 3. Theoretical prediction of changes in the lateral pressure profile of lipid bilayers that become incorporated with 1 mol% unsaturated lipid into a 16:0 lipid bilayer. The data are compared to the opposite changes induced by cholesterol incorporation. Courtesy of R. S. Cantor.

Fig. 3, is changed by increasing unsaturation in a manner that shifts the lateral stresses towards the head-group region of the bilayer (Carillo-Trip and Feller, 2005; Michailescu and Gawrisch, 2006; Ollila *et al.,* 2007). This effect implies changes in the curvature stress of the bilayer and an increase in the flip-flop rate between the two monolayer sheets (Armstrong *et al.,* 2003). Possibly even more important, introduction of an extra double bond in the acyl chain, specifically from five to six, has a significant effect on the lateral pressure profile whereas it appears only to have a marginal effect on other bulk bilayer properties (Eldho *et al.,* 2003). Furthermore, the actual position of the double bonds has a significant effect on form of the lateral pressure profile (Ollila *et al.,* 2007).

Lateral molecular organization

Several studies have shown that PUFAs, in particular DHA, participate in lipid-domain formation in membranes and that cholesterol plays a key role in this type of lateral organization. In membranes containing both mono-unsaturated and poly-unsaturated lipids, cholesterol induces a sequestering of the PUFAs into domains depleted of cholesterol and mono-unsaturated lipids (Brzustowicz *et al.*, 2001; Niu and Littmann, 2002; Shaik *et al.,* 2004).

Lipid-protein interactions

Turning then to lipid-protein interactions, two types of quantitative model studies of the effect of PUFAs on the functioning of membrane proteins deserve mention. One type is based on using gramicidin A as a model protein to gauge the relationship between channel function and membrane elastic properties (Bruno *et al.,* 2007). By comparing the effect of oleic acid (OA) and DHA, it is found using single-channel electrophysiological techniques, that although OA partitions more

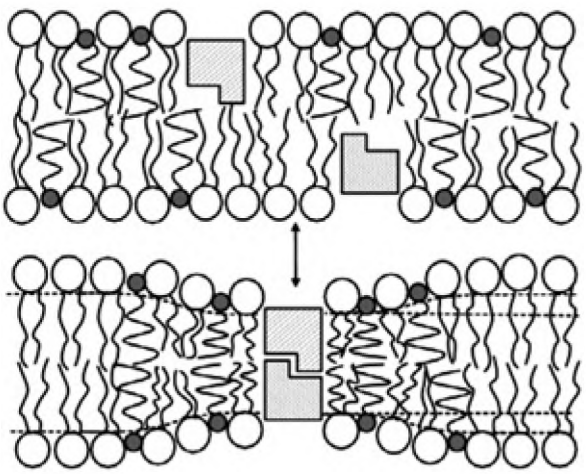

Fig. 4. Schematic illustration of local softening of a lipid bilayer by incorporation of DHA that supports local curvature and formation of a channel dimer of gramicidin A. Courtesy of Olaf Sparre Andersen. Adapted from Bruno *et al.* (2007).

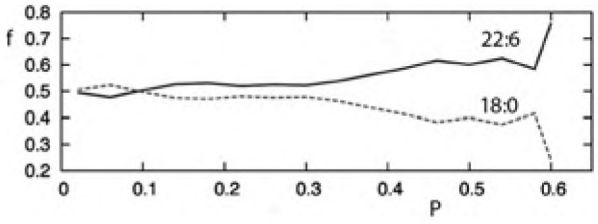

Fig. 5. Difference in packing properties of DHA (22:6) and saturated lipids 18:0 near rhodopsin incorporated in a lipid bilayer as determined from molecular dynamics simulations. The figure shows the fraction, f, of the two lipid species that have a particular packing score, P, at the lipid-protein interface. It appears that DHA packs in a more localized way with rhodopsin. Adapted from Grossfield *et al.* (2006).

strongly into the bilayers than DHA, DHA has a much larger effect than OA on channel function. Since DHA strongly softens the bilayer, it was concluded that bilayer elasticity is a key to understanding the influence of PUFAs like DHA on membrane protein function. DHA does not bind to the channel but is statistically accumulated locally around the channel whereas OA is not. This is illustrated schematically in Fig. 4.

The other type of study is concerned with rhodopsin reconstituted into model membranes (Feller and Gawrisch, 2005) where it was found that the effect of DHA on the structural transitions in rhodopsin could be mimicked by other non-lamellar-forming lipids such as phosphatidylethanolamine (PE) lipids that induce a similar propensity for forming curved structures (Brown, 1997). Furthermore it was found that rhodopsin segregates and packs tightly with the PUFAs, cf. Fig. 5, and this segregation is augmented by the presence of cholesterol (Grossfield *et al.*, 2006; Polozova and Littman, 2000).

Some conclusions

Global effects: Increasing the degree of unsaturation in the lipid-acyl chains softens bilayer membranes and increases the permeability.

Local effects: Unsaturation shifts lateral pressures towards the head-group region and induces small-scale lipid-domain formation organization of lipid membranes. Adding an extra double bond has a big effect and depends on the location of the double bond.

Protein function: The functioning of membrane receptors and channels may depend on the lateral pressure profile and the local domain formation.

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