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Docosahexaenoic acid domains: the ultimate non-raft membrane domain

Stephen R. Wassall[a,*], William Stillwell[b]

[a] Department of Physics, Indiana University Purdue University Indianapolis, 402 North Blackford Street, Indianapolis, IN 46202-3273, United States
[b] Department of Biology, Indiana University Purdue University Indianapolis, 723 West Michigan Street, Indianapolis, IN 46202-5132, United States

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ABSTRACT

What distinguishes polyunsaturated fatty acids (PUFAs) from less unsaturated fatty acids is the presence of a repeating =CH–CH_2–CH= unit that produces an extremely flexible structure rapidly isomerizing through conformational states. Docosahexaenoic acid (DHA) with 6 double bonds is the most extreme example. The focus of this review is the profound impact that the high disorder of DHA has on its interaction with cholesterol when the PUFA is incorporated into membrane phospholipids. Results from a battery of biophysical techniques are described. They demonstrate an aversion of DHA for the sterol that drives the lateral segregation of DHA-containing phospholipids into liquid disordered (l_d) domains that are depleted in cholesterol. These domains are compositionally and organizationally the antithesis of lipid rafts, the much-studied liquid ordered (l_o) domain that is enriched in predominantly saturated sphingolipids and cholesterol. We hypothesize that the introduction of DHA-rich domains into the plasma membrane where they coexist with lipid rafts is the origin, in part, of the astonishing diversity of health benefits that accrue from dietary consumption of DHA. According to our model, changes in the conformation of signaling proteins when they move between these disparate domains have the potential to modulate cell function.

1. Introduction

The apparent structural simplicity of docosahexaenoic acid (DHA, 22:6) belies its tremendous biological versatility (Stillwell, 2006). At first glance, the molecule's simple stick structure (Fig. 1) might indicate that it functions in a manner similar to other common fatty acids. Indeed with 22 carbons and 6 double bonds, DHA is only a bit longer and more unsaturated than most fatty acids. Is this difference enough to account for the "special" biological properties of this polyunsaturated fatty acid (PUFA)? The answer requires a more sophisticated view than reflected by a simple stick structure, one that recognizes the dynamic complexity of the conformations adopted by DHA.

Because DHA has 22 carbons, membranes rich in the PUFA were initially expected to be thick and hence poorly permeable. Early measurements of the rod outer segment, where up to half of the acyl chains are DHA, surprisingly showed the opposite (Dratz et al., 1985)! These membranes are quite thin and leaky. Indeed, on the basis of NMR data for a series of model membranes bearing increasing levels of unsaturation, Gawrisch and co-workers (Holte et al., 1995) surmised that DHA behaves as if it is actually shorter than the 18-carbon fatty acid oleic (OA, 18:1) acid. A "molecular spring model" in which the DHA chain forms a helix was one possible explanation that attracted attention for some time (Dratz and Holte, 1992). An ensemble of many rapidly inter-converting conformations is the definitive picture that has subsequently emerged from a combination of NMR, X-ray diffraction and MD simulations (Eldho et al., 2003; Feller et al., 2002; Huber et al., 2002). This extreme flexibility of the PUFA chain accounts for the high "fluidity" (Holte et al., 1995; Salem and Niebylski, 1995), permeability (Huster et al., 1997), elasticity (Smaby et al., 1997), fusion (Ehringer et al., 1990; Kafrawy et al., 1998), enhanced flip-flop (Armstrong et al., 2003) and preference for non-lamellar phases (Gawrisch and Holte, 1996; Shaikh et al., 2001) associated with DHA in membranes. Therefore, DHA-rich regions in membranes are thin, have looser lipid packing, may prefer non-lamellar phases, can induce negative curvature strain to proteins (Slater et al., 1994) and are just more "dynamic" than the regions of membranes composed of other fatty acids.

Membrane DHA-levels have been significantly enhanced in animals through diet (most commonly using appropriate fish oil supplements), by adding un-esterified fatty acid to cell cultures using as carriers organic solvents or serum albumin, or by fusion with DHA-containing liposomes (Salem et al., 1986; Stillwell, 2006; Stillwell and Wassall, 2003; Stillwell et al., 2005, 2006). The PUFA is

Abbreviations: DHA, docosahexaenoic acid; OA, oleic acid; PUFA, polyunsaturated fatty acids; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; SFA, saturated fatty acids; SM, sphingomyelin.

* Corresponding author. Tel.: +1 317 274 6908; fax: +1 317 274 2393.
E-mail address: swassall@iupui.edu (S.R. Wassall).

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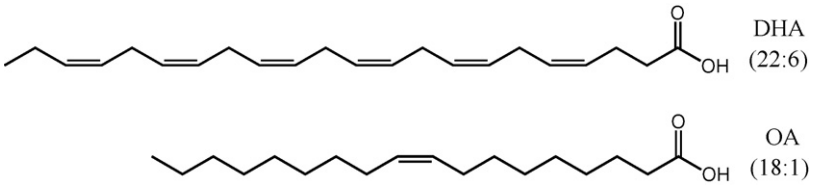

Fig. 1. Molecular structure of docosahexaenoic (DHA, 22:6) and oleic (OA, 18:1) acids.

known to readily incorporate into membrane phospholipids where it accumulates primarily into the *sn*-2 position while saturated palmitic (16:0) and stearic (18:0) acids predominantly occupy the *sn*-1 position (Anderson and Sperling, 1971). This hetero-acid motif is the most common for DHA-containing phospholipids. In most membranes, DHA has been reported to preferentially accumulate into the major structural phospholipids phosphatidylethanolamine (PE) and, in lesser amount, phosphatidylcholine (PC) (Emmelot and van Hoeven, 1975; Robinson et al., 1993; Zerouga et al., 1996). But it is not a universal rule as in neuronal membranes high concentrations of the PUFA have been found in the non-structural phospholipid phosphatidylserine (PS) (Salem et al., 1986). It is of potential interest that the two primary amine phospholipids PE and PS are not only enriched in DHA (Knapp et al., 1994) but also both reside primarily in the inner leaflet of membranes. DHA levels are not homogeneously distributed throughout an animal. There are two distinct categories, select tissues containing large amounts of DHA (often approaching 50 mol% of the total acyl chains) (Salem et al., 1986) and the remaining tissues with normally less than a few mol% (Stillwell and Wassall, 2003). High-DHA tissues, the rod outer segment (Wiegand and Anderson, 1983), sperm (Neill and Masters, 1973) and neurons (Breckenridge et al., 1972), have so much of this fatty acid that dipolyunsaturated species (DHA in both the *sn*-1 and *sn*-2 chains) are common (Miljanich et al., 1979). In these tissues DHA levels are not affected by the diet. In the remaining, low-DHA tissues, the PUFA-content can be severely impacted by the diet and the fatty acid is primarily found only in the *sn*-2 position of the phospholipids (Anderson and Sperling, 1971). Since the low-DHA tissues are the ones affected by DHA, it is here where most health benefits must originate.

Understanding the relationship between membrane complexity, structure and function remains one of the major outstanding unsolved problems in life sciences. It has been estimated that a "typical" membrane may contain ~1500 different lipid molecular species and ~100 different proteins co-existing in as yet poorly defined, heterogeneous lateral and trans-membrane patches known as domains. The domains are believed to range in size from very small (1–10 nm), probably with very short half-life and in constant flux, to macroscopic (μm) (Edidin, 2001). Their stability must reflect lipid–lipid and lipid–protein affinity and aversion (Stillwell and Wassall, 2003). Of special interest to us, are domains that we hypothesize form enriched in DHA-containing phospholipids (Stillwell, 2006; Stillwell and Wassall, 2003; Stillwell et al., 2005, 2006; Wassall et al., 2004). They are the opposite of lipid rafts (Brown and London, 2000; Pike, 2006; Simons and Ikonen, 1997), a reputed domain that recently has captured the attention of the membrane world. Rafts were first identified as the membrane fraction that is insoluble in cold non-ionic detergents such as Triton X-100 and, thus, historically are also defined as detergent resistant membranes (DRM) (Ahmed et al., 1997; Brown and London, 1997). They are phase separated, liquid ordered (l_o) domains enriched in predominantly saturated fatty acid (SFA)-containing sphingolipids and cholesterol that serve as the platform for characteristic lipid-linked signaling proteins. "Glued" together by the sterol, rafts float in a surrounding non-raft milieu of liquid disordered (l_d) lipids.

It is the purpose of our investigations to determine how the incorporation of DHA affects membrane architecture, particularly the sequestration of lipids into raft and non-raft domains. In the membrane, DHA will encounter a vast array of lipids and proteins and the fatty acid's interactions with these components, both positive and negative, should alter membrane structure and function. Here we focus on the interaction of DHA-containing phospholipids with cholesterol.

2. Cholesterol has poor affinity for PUFA

The domains of DHA-containing phospholipid that we hypothesize form depleted of cholesterol, are the antithesis of rafts enriched in SFA-containing sphingolipids and cholesterol. Poor affinity of the sterol for PUFA is at the heart of our hypothesis (Wassall et al., 2004). The highly disordered nature of PUFA chains is responsible. This disorder is due to the low activation energy for rotation about the single C–C bonds that separate the unsaturated carbon atoms in the recurring =CH–CH_2–CH= pattern (Feller et al., 2002; Huber et al., 2002). A tremendously flexible chain results undergoing rapid inter-conversion between conformational states that range, as visualized in MD simulations of PUFA-containing phospholipids bilayers (Feller et al., 2002), from extended towards the center to bent conformers where the terminal methyl approaches the surface (Eldho et al., 2003; Mihailescu and Gawrisch, 2006; Soubias and Gawrisch, 2007). The rough fluctuating surface thus presented by a PUFA chain to the rigid steroid moiety of a neighboring cholesterol molecule deters close approach (Wassall et al., 2004). In contrast, the predominantly all-trans configuration adopted by a SFA chain presents a smooth façade that is compatible with intimate proximity to the sterol (Wassall et al., 2004).

There are a growing number of experimental observations that demonstrate the aversion cholesterol has for PUFA. Partition coefficients measured for cholesterol using a cyclodextrin assay are smaller in unilamellar vesicles composed of PC with DHA than less unsaturated chains (Niu and Litman, 2002). Closer contact with the 18:0 *sn*-1 chain in 18:0-22:6PC/[25,26,26,27,27,27,27-$^2H_7$] cholesterol (1:1 mol) is revealed by a higher rate of chain-to-sterol nuclear Overhauser enhancement spectroscopy (NOESY) in ^{1}H magic angle spinning (MAS) NMR experiments (Huster et al., 1998). This result is reproduced in MD simulations on an 18:0-22:6PC/cholesterol (3:1 mol) bilayer that corroborate the sterol favors solvation by saturated over polyunsaturated chains (Pitman et al., 2004). A similar inference may be drawn from ^{2}H NMR data showing that the introduction of cholesterol elicits essentially the same increase in membrane-ordering for arachadonic (20:4) acid-containing ([$^2H_{31}$]16:0-20:4PC) (Jackman et al., 1999) as for linoleic (18:2) acid-containing [$^2H_{31}$]16:0-18:2PC (Morrow et al., 1996), [$^2H_{31}$]16:0-18:1PC (Thewalt and Bloom, 1992) or [$^2H_{31}$]16:0-16:0PC (Vist and Davis, 1990). That the response as probed by a saturated [$^2H_{31}$]16:0 *sn*-1 chain changes little in the presence of PUFA at the *sn*-2 position that is consistent with the sterol exhibiting preferential affinity for SFA.

Unequivocal substantiation of poor affinity for cholesterol is provided by the greatly reduced solubility that we have measured