All n-3 PUFA are not the same: MD simulations reveal differences in membrane organization for EPA, DHA and DPA

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ABSTRACT

Eicosapentaenoic (EPA, 20:5), docosahexaenoic (DHA, 22:6) and docosapentaenoic (DPA, 22:5) acids are omega-3 polyunsaturated fatty acids (n-3 PUFA) obtained from dietary consumption of fish oils that potentially alleviate the symptoms of a range of chronic diseases. We focus here on the plasma membrane as a site of action and investigate how they affect molecular organization when taken up into a phospholipid. All atom MD simulations were performed to compare 1-stearoyl-2-eicosapentaenoylphosphatylcholine (EPA-PC, 18:0-20:5PC), 1-stearoyl-2-docosahexaenoylphosphatylcholine (DHA-PC, 18:0-22:6PC), 1-stearoyl-2-docosapentaenoylphosphatylcholine (DPA-PC, 18:0-22:5PC) and, as a monounsaturated control, 1-stearoyl-2-oleoylphosphatidylcholine (OA-PC, 18:0-18:1PC) bilayers. They were run in the absence and presence of 20 mol% cholesterol. Multiple double bonds confer high disorder on all three n-3 PUFA. The different number of double bonds and chain length for each n-3 PUFA moderates the reduction in membrane order exerted (compared to OA-PC, $\overline{S}_{CD} = 0.152$). EPA-PC ($\overline{S}_{CD} = 0.131$) is most disordered, while DPA-PC ($\overline{S}_{CD} = 0.140$) is least disordered. DHA-PC ($\overline{S}_{CD} = 0.139$) is, within uncertainty, the same as DPA-PC. Following the addition of cholesterol, order in EPA-PC ($\overline{S}_{CD} = 0.169$), DHA-PC ($\overline{S}_{CD} = 0.178$) and DPA-PC ($\overline{S}_{CD} = 0.182$) is increased less than in OA-PC ($\overline{S}_{CD} = 0.214$). The high disorder of n-3 PUFA is responsible, preventing the n-3 PUFA-containing phospholipids from packing as close to the rigid sterol as the monounsaturated control. Our findings establish that EPA, DHA and DPA are not equivalent in their interactions within membranes, which possibly contributes to differences in clinical efficacy.

1. Introduction

Long chain omega-3 polyunsaturated fatty acids (n-3 PUFA)[1] are a distinct class of fatty acids that have multiple double bonds, with the last double bond being located 3 carbons from the terminal methyl (n or ω) end of the chain [1]. They began to attract attention in the 1970's when a low incidence of cardiovascular disease was noted in the Inuit population who consumed a diet rich in oily fish that contain an abundance of n-3 PUFA [2]. Since then many more health benefits have been discovered including the treatment of neurological problems [3], relief of the symptoms of inflammatory disorders [4], improvements in whole body metabolism [5] and prevention of the progress of certain cancers [6]. The major source of long chain n-3 PUFA in the diet is oily cold-water fish and/or extracted oil supplements [1]. Eicosapentaenoic acid (EPA, 20:5) with 20 carbons and 5 double bonds at the 5, 8, 11, 14 and 17 positions and docosahexaenoic acid (DHA, 22:6) with 22 carbons and 6 double bonds at the 4, 7, 10, 13, 16 and 19 positions are the primary ones. Present in smaller concentration, and to which EPA often converts, is docosapentaenoic acid (DPA, 22:5) with 22 carbons and 5 double bonds at the 7, 10, 13, 16 and 19 positions. DPA has only recently begun to be the subject of research, in contrast to EPA and DHA that have been widely studied [7]. Although all three n-3 PUFA are

similar in chemical structure, whether they have similar, unique or complementary impact upon health remains a matter of debate [8].

A complete explanation of the mode of action for n-3 PUFA currently does not exist. Modulation of the organization of plasma membranes following the uptake of n-3 PUFA into phospholipids is among the potential mechanisms that have been identified [9–11]. The basic idea is a variation on the lipid raft concept [12]. According to this model, predominantly saturated sphingolipids and cholesterol segregate into tightly packed nanodomains that coalesce into functional microdomains (rafts) within a surrounding phospholipid environment that is less ordered. By incorporating into membrane phospholipids, highly disordered n-3 PUFA that have an aversion for cholesterol are then proposed to manipulate the composition and structure of the domains. One example of a scenario envisaged, for instance, has the introduction of n-3 PUFA-containing phospholipids into a raft displacing cholesterol and breaking apart the domain [11]. Detergent extraction assays and imaging studies performed on cells either treated with n-3 PUFA or obtained following a n-3 PUFA enriched diet support the general premise that n-3 PUFA are taken up into rafts and affect their size [13,14]. Changes in the levels of other fatty acids due to cellular metabolism, however, complicate interpretation. A series of studies on biomimetic membranes of controlled composition carried out in our laboratories have started to provide insight [15–18]. They established that the partitioning of an n-3 PUFA-containing phospholipid between more ordered raft-like and less ordered non-raft environments is sensitive to molecular structure. A much greater tendency for DHA- than EPA-containing phosphatidylcholine (PC) to infiltrate raft-like domains was revealed, which we attribute to a differential in the disorder associated with DHA relative to EPA and speculate may indicate a variance in bioactivity. To better understand this behavior and the possible role that the structure of an individual n-3 PUFA plays in determining biological activity, we have run atomistic MD simulations to make a direct comparison of EPA, DHA and DPA and their interaction with cholesterol.

All atom MD simulation opens up a perspective on molecular structure and dynamics within lipid membranes that is unprecedented in detail [19]. The reliability of this computational method has been ascertained in tests on well-characterized systems that successfully reproduce experimental measurements. It is an approach that has been tremendously influential in developing a comprehension of the physical properties of PUFA-containing phospholipids. Simulations published for DHA-containing PC bilayers more than a decade ago were instrumental in showing that the polyunsaturated chain is highly flexible, rapidly undergoing isomerization between conformations that include extended and bent configurations [20–22]. A reduced energy barrier for rotation about the single bonds in the recurring =C−C−C= unit possessed by PUFA is responsible, debunking an alternative view that the rigidity of multiple bonds would produce low flexibility. Subsequent analysis of simulations on a 1-stearoyl-2-docosahexaenoylphosphatidylcholine (18:0-22:6PC, DHA-PC) bilayer in the presence of cholesterol showed that the steroid moiety prefers close proximity to the saturated stearic acid (SA) chain over the polyunsaturated DHA chain [23]. This finding corroborates an arrangement inferred from experimental measurements of the orientation of cholesterol and its solubility in polyunsaturated membranes [24]. Phenomenal disorder that pushes cholesterol away is what distinguishes PUFA and renders membrane architecture particularly responsive to their presence.

An investigation of how molecular organization in lipid bilayers is affected by differences in the structure of EPA, DHA and DPA is presented here. To this end, we performed atomistic MD simulations comparing 1-stearoyl-2-eicosapentaenoylphosphatidylcholine (18:0-20:5PC, EPA-PC), 1-stearoyl-2-docosahexaenoylphosphatidylcholine (18:0-22:6PC, DHA-PC), 1-stearoyl-2-docosapentaenoylphosphatidylcholine (18:0-22:5PC, DPA-PC) and 1-stearoyl-2-oleoylphosphatidylcholine (18:0-18:1PC, OA-PC) bilayers. Their molecular structure, together with cholesterol, is shown in Fig. 1. DHA-PC, EPA-PC and DPA-PC are representative of a phospholipid into which n-3 PUFA have been taken up in the plasma membrane, while OA-PC serves as a monounsaturated control [25]. In a structural motif that is typical of phospholipids found in animal membranes, they all have SA, a common saturated fatty acid, at the *sn*-1 position and an unsaturated fatty acid at the *sn*-2 position. The simulations were run on single component membranes and in the presence of 20 mol% cholesterol to characterize interaction with the sterol for each n-3 PUFA. Supplementary solid state ^{2}H NMR experiments employing analogs of the phospholipids perdeuterated in the *sn*-1 chain were conducted for validation.

2. Materials and methods

2.1. MD simulations

Atomistic MD simulations were performed on DHA-PC, EPA-PC, DPA-PC and OA-PC bilayers in the absence and presence of 20 mol% cholesterol. They were run in the constant particle number, pressure and temperature (NPT) ensemble. There were 98 PC molecules in the single component membrane simulations, and 80 PC molecules and 20 cholesterol molecules in the two-component membrane simulations. In each case, the membrane was hydrated with 2000 water molecules. The initial structure of OA-PC, OA-PC + cholesterol, DHA-PC and DHA-PC + cholesterol bilayers was assembled with the CHARMM-GUI Membrane Builder [26]. Then, by modifying the DHA chain on DHA-PC molecules, the initial structure of EPA-PC + cholesterol and DPA-PC + cholesterol bilayers was generated from our assembled DHA-PC + cholesterol bilayer. Locating the corresponding lipid molecule into a bilayer grid with a customized script created the initial structure of EPA-PC and DPA-PC bilayers. This approach avoided atom/chain conflicts that appear in the initial structure when it is directly modified from the assembled DHA-PC bilayer. All simulations were run using the CHARMM C36p force field [27]. Non-bonded (van der Waals and short-range electrostatic) interactions were gradually switched off at 10 Å, and the long-range electrostatic interactions were calculated using the particle-mesh Ewald method [28]. The membranes were equilibrated with the standard CHARMM-GUI six-step process over 200 ps [26] during which constraints on lipids were gradually released [29].

Production runs of our simulations were performed with NAMD [30] on the Big Red II super computer at Indiana University. Each one was run over 200 ns using a time step of 2 fs, with the first 20 ns considered to be equilibration. In all simulations, periodic boundary conditions were applied to a rectangular box on which the x and y dimensions (in the plane of the membrane) were kept equal while the z dimension (in the direction of the membrane normal) was varied independently. The temperature was kept at 37 °C and the pressure was maintained at 1 atm by the Langevin dynamics piston method [31]. Analyses of simulations, including calculating order parameters and densities of atoms in 1- and 3-dimensions were achieved with customized Tcl script executed in the visual molecular dynamics (VMD) program [32]. Snapshots of bilayers and illustrations were also created with VMD, and rendered by blender.

2.2. Solid state ^{2}H NMR

Details of solid state ^{2}H NMR experiments are provided in Supporting information.

3. Results and discussion

Biological membranes contain a diverse array of lipids. Phospholipids vary in head group and chain composition – number of carbons and number and location of double bonds. These variations in molecular structure modify physical properties and molecular organization within a membrane in a complex manner. The aim of our study is