How do proteins discriminate between polyunsaturated ω-6 and ω-3 fatty acids?

Seminar to the Polyunsaturated Lipid Function Interest Group
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Two mechanism of PUFA action on proteins must be considered: on the one hand, PUFA may influence function by altering biophysical properties of the lipid matrix. Such action most likely occurs in neural membranes with high PUFA content like synaptosomes and the retina. But even in membranes with lower PUFA content, the alteration of biophysical properties could be important as long as PUFAs accumulate in domains near membrane proteins. On the other hand, PUFA and their derivatives may act as ligands that interact with membrane proteins directly. In recent years, several examples of such interaction have been discovered. Both, aspects of PUFA action have been the subject of my talk.

Change of membrane elastic properties

There has been controversy regarding the mechanisms by which polyunsaturated fatty acids influence membrane bilayer properties. PUFA with double bonds in the cis-configuration have significantly fewer degrees of freedom than saturated hydrocarbon chains supporting the notion of rigid PUFA that perturb the lipid matrix because of their bulkiness. However, recent experiments conducted at LMBB and other laboratories support a different view of PUFA as highly flexible molecules existing in a multitude of conformations with rapid conformational transitions. The underlying cause for this flexibility are extremely low potential barriers for rotations about the C-C bonds between the double bonds that permit PUFA to rapidly change conformation without significant energetic penalties. Indeed, we have been able to demonstrate by NMR experiments conducted on model membranes that PUFA chain segments are exceptionally flexible. The PUFA segments near the carbonyl group move with motional correlation times of the order of 1 ns. The correlation times decrease and the motional amplitudes increase from double bond to double bond, reaching correlation times of the order of 10 ps at the methyl terminal end of the PUFA chain.

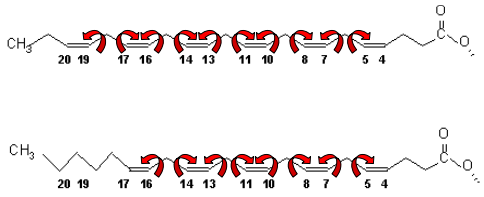

Fig. 1: The flexible links in docosahexaenoic acid (22:6n3, DHA) and docosapentaenoic acid (22:5n6, DPA).

The PUFA do not exist in a small number of permitted conformations, but convert rapidly between countless conformers. This applies particularly to the chain segments near the methyl terminal end. Comparison of PUFA flexibility with saturated and monounsaturated chains reveals faster motions and larger motional amplitudes, despite the rigidity of the cis-locked double bonds.

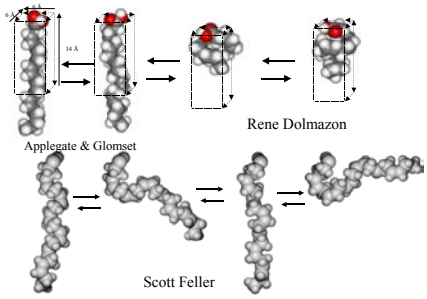

Fig. 2: PUFA convert rapidly between a large number of conformers.

Surprisingly, we have been able to detect significant differences in the distribution of PUFA chain density between the ω-3 docosahexaenoic acid (22:6n3, DHA) and the ω-6 docosapentaenoic acid (22:5n6, DPA) along the bilayer normal. The DHA tends to have higher density near the lipid water interface compared to the DPA. The difference is easily detectable as a difference in chain order parameters of saturated stearic acid that is paired with DHA or DPA in phosphatidylcholines, but also in DHA and DPA order parameters, motional correlation times along the chain, in the electron density profiles of lipid bilayers obtained in x-ray diffraction experiments that were conducted at the Nagle laboratory, and last but not least, the simulations conducted at the Feller lab.

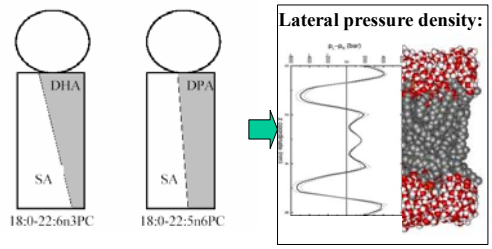

Fig. 3: The differences in distribution of chain densities between ω-6 and ω-3 PUFA are likely to trigger differences in lateral pressure densities.

We speculate that these differences in the distribution of lipid hydrocarbon chains alter lateral pressure density profiles of membranes. Integral membrane proteins perform work against the lateral pressure from the lipid matrix when undergoing structural transitions upon activation. Theoretical studies by Cantor and others confirmed that such changes in the pressure profiles may alter the free energy of protein conformers and therefore the probability of forming an activated state, e.g. the meta-II state of the GPCRs rhodopsin. Our observations clearly point toward a difference in biophysical properties of membranes rich in DHA or DPA.

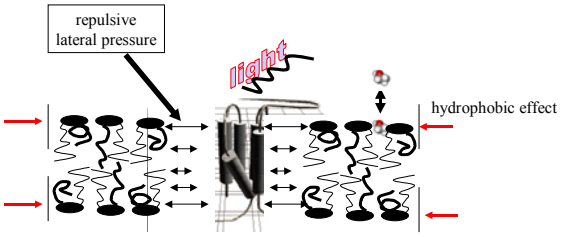

Fig. 4: Work by the protein against the lateral pressure in the bilayer is a contribution to free energy of protein conformers.

Specific interaction with membrane proteins

In the second part of my talk, I referred to literature on x-ray structural investigations of proteins with specificity towards interaction with polyunsaturated membranes. On the basis of these structures I developed a hypothesis on the causes of specificity to interaction with PUFA. I evaluated the productive conformation of arachidonic acid bound to prostaglandin synthase, the DHA conformation in the binding pocket of human fatty acid binding protein, the arachidonyl inhibitor conformation in the binding pocket of fatty acid amide hydrolase, the fatty acids in the ligand binding domain of the retinoic acid-related orphan receptor ß (RORß), and the retinal conformation in the binding site in rhodopsin. In all these proteins, the carboxyl groups of fatty acids, or equivalent polar groups, are bound to the protein by strong, non-covalent interactions like salt bridges, direct hydrogen bonding to amino acids, or indirect hydrogen bonds via water. The analysis of interaction energies, when available, revealed that at least half of the total interaction enthalpy stems from those polar interactions. They do not appear to vary as a function of the length of hydrogen carbon chains and their degree of unsaturation.

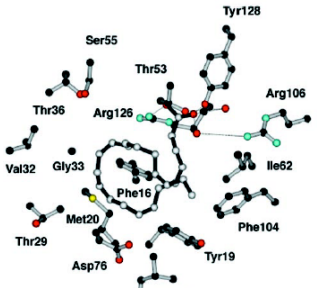

Fig. 5: Polyunsaturated DHA in the binding pocket of human fatty acid binding protein. (Reproduced from Balendiran at al. J. Biol. Chem. 275 (2000) 27045).

However, association constants to proteins do reflect a certain degree of specificity in most proteins. One contributing factor could be the size of binding pockets. Indeed, modifications of the proteins that causes steric hindrance to locating fatty acids methyls

in the binding pocket is known to interfere with fatty acid binding. But such sensitivity may not explain specificity to the number of double bonds per chain. It has been proposed that polyunsaturated hydrocarbon chains with a helical arrangement of double bonds may fit the structure of α-helical segments of a protein particularly well. The interaction strength may increase because the chains gain in Van der Waals interaction between protein and fatty acid. However, the analysis of known binding sites remains inconclusive on this point. There does not appear to be a strong interaction of PUFA chains with one particular helix. The fatty acid binding epitopes are noncontiguous; the fatty acids interact with interfaces of several helices as well as β-sheets.

There must be an additional, pointed interaction between fatty acids and proteins to achieve specificity to the number and location of double bonds per chain. I propose the existence of an interaction that fixes the location of double bonds to a particular region of the binding site. A candidate for such interactions are the so-called π/π interactions between the double bonds of PUFA and the aromatic residues of proteins. Indeed, it is remarkable that the fatty acid binding pockets in the proteins above are lined by a high density of the aromatic amino acids phenylalanine, tyrosine, and tryptophan.

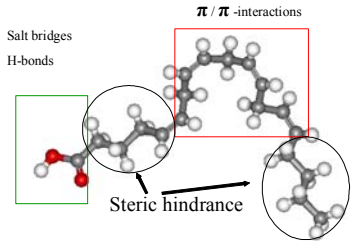

Fig. 5: I propose that the location of double bonds in binding pockets is fixed by π/π interactions. The specificity to ω-6 and ω-3 fatty acids may result from steric hindrance related to the size of binding pockets.

Recently, the interactions of aromatic residues in proteins has attracted interest. It was established that aromatic residues are attracted to positive charges in a hydrophobic environment (cation-π interactions), and that aromatic rings are capable of formation of weak hydrogen bonds with proton-donating groups. More recently, attention has focused on the much weaker CH/π interactions. But little is known about π/π-interactions between double bonds of PUFA and the aromatic residues on proteins. They have features that may result in specificity. In particular, the π/π-interactions comprise electrostatic interactions that are specifically pointed to the double bonds. The interactions are likely to favor a particular mutual orientation of double bonds and aromatic side chains. Unfortunately, the determination of the strength of such interactions is very challenging, experimentally as well as theoretically. The NMR experiments, x-ray structural studies, and the quantum chemical calculations must be conducted on simplified model systems. Hopefully, such experiments and simulations will result in effective interaction potentials that could be used in popular molecular mechanics simulation packages like CHARMM, AMBER, or GROMOS to calculate structure and dynamics of PUFA-protein complexes.

Summary

Recent experiments at our laboratory established the existence of differences in lipid order and dynamics between membranes rich in DHA or DPA. This supports the notion that integral membrane protein function could be altered via differences in membrane elastic properties between ω-6 and ω-3 fatty acids. Furthermore, recent reports in the literature suggest existence of specific interactions between PUFA or their derivatives and proteins. The specificity to ω-6 or ω-3 fatty acids may result from pointed interactions to double bonds in combination with steric hindrance related to the size of binding pockets. I propose that the location of double bonds at the binding sites of proteins is fixed by π/π-interactions.

Discussion

I would like to thank the audience for the many questions and comments throughout the talk and during the discussion period. The following points were particularly interesting to me:

Several participants asked if differences in lateral tension of membranes related to PUFA content may influence function of enzymes like PLA_2 or PKC.

I responded that studies which confirm sensitivity of enzyme function to lateral tension have been conducted as early as the 1970's. The PUFA appear to be the most potent modulators of membrane lateral tension. However, the differences between ω-3 DHA and ω-6 DPA are not necessarily expressed as differences in overall lateral tension, but as differences in the lateral tension profile. The latter is related to what has been called "curvature stress of membranes", the tendency of lipid monolayers to curl. It has been demonstrated that membrane proteins that change their shape, like rhodopsin, or proteins that require incorporation into the lipid matrix for activation, like certain isoforms of PKC, are sensitive to curvature stress.

Bill Lands pointed out a potential conflict between my notion of PUFA as highly flexible molecules and the binding of PUFA to pockets in proteins. He asked if my perception of PUFA bound to proteins by specific interactions is too static.

I expressed agreement with Bill's vision of flexible PUFA, even for PUFA at binding sites of proteins. Proteins are machines with moving parts, not static objects with open binding pockets that wait to be filled. The PUFA are likely to approach the integral membrane proteins from the hydrophobic core of the membrane and may slip by the transmembrane helices until they settle at the preferred site of interaction, triggering structural transitions within the protein. This requires flexibility from both peptide and protein. There may not be a binding pocket without the arrival of the PUFA. Bill added that enzymatic conversion of PUFA is inherently linked to protein flexibility.

Several attendees raised questions regarding specificity of proteins to interactions with particular PUFA.

I emphasized that specificity expressed in units of interaction energy could be a difference as small as 1-2 kcal/mol which is hard to measure and to assign to a particular interaction. The attendees pointed out that conducting studies on protein affinity to PUFA at this level of precision is notoriously difficult. Furthermore, we agreed that high affinity may not be the only reason for specificity. Many processes are controlled by rates of exchange (activation energies) rather than affinity.

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