J|A|C|S
COMMUNICATIONS

Published on Web 03/20/2003

Rhodopsin Exhibits a Preference for Solvation by Polyunsaturated Docosohexaenoic Acid

Scott E. Feller,*,† Klaus Gawrisch,‡ and Thomas B. Woolf§

Department of Chemistry, Wabash College, 301 West Wabash, Crawfordsville, Indiana 47933, Laboratory of Membrane Biochemistry and Biophysics, NIAAA, National Institutes of Health, 12420 Parklawn Drive, Rockville, Maryland 20852, and Department of Physiology, Johns Hopkins University, School of Medicine, 725 North Wolfe Street, Baltimore, Maryland 21205

Received February 10, 2003; E-mail: fellers@wabash.edu

Numerous experimental studies suggest an important role for polyunsaturated lipids, specifically those containing a docosohexaneoic fatty acid chain, in the function of rhodopsin and other G-protein coupled receptors.[1−3] Additionally, the possibility that rhodopsin and other membrane proteins might control the lipid composition of their local environments, for example, through the formation of lipid rafts, has attracted considerable attention due to its importance in a wide range of processes occurring at membrane surfaces. In the following, we describe a molecular dynamics simulation of rhodopsin in a realistic environment, that is, with polyunsaturated lipids similar to retinal membranes. The atomic-level picture that emerges from this simulation reveals that rhodopsin has the ability to organize the lipid solvation structure through preferential interactions with the polyunsaturated docosohexaneoic acid (DHA, 22:6n3) chains.

The starting points for our simulation are the recently determined X-ray structure of dark-adapted rhodopsin[4] and the conformations of fluid phase (L_α) 1-stearoyl-2-docosohexaenoyl-*sn*-glycero-3-phosphatidylcholine (SDPC) from a previous molecular dynamics (MD) simulation.[5] For our calculations, the program CHARMM[6] was used to perform construction, equilibration, and production. Complete details of system construction are given in a submitted paper describing rhodopsin in a dioleoylphosphatidylcholine bilayer.[7] In this approach, similar to that employed for other lipid−protein systems,[8] the CHARMM potential is first used to build in missing regions (here residues 236−239 and 328−333) and to relax the X-ray structure into the CHARMM potential function with minimization. Reasonable lipid conformations are generated by initially simulating a 2D periodic system with van der Waals spheres (sized to estimate a PC headgroup) interacting with each other and with the fixed protein. The lipid conformations are then selected from a lipid library (consisting of conformations generated in the previous study[5]) and placed with the headgroup *x* and *y* positions defined by the sphere locations and the *z* coordinates determined by experimental estimates of the membrane thickness. This is followed by systematic translation and rotation to remove bad contacts and extensive relaxation with decreasing harmonic restraints. System construction was completed by adding water and salt and converting a lipid into the two fatty acids (palmitate) covalently linked to the protein, providing a system that is both electrically neutral and similar to experimental conditions. During equilibration, we gradually shifted from constant volume calculations to the constant normal pressure, constant cross-sectional area calculations that were subsequently used for the production run. The production run was performed on 16 nodes of a Beowulf cluster for 12.5 ns, with Coulombic interactions calculated using an Ewald summation technique. In sum, our system consisted of 41 896 atoms (the rhodopsin protein, 99 lipids, two palmitate chains, 14 sodium cations, 16 chloride anions, and 7400 water molecules) with three-dimensional periodic boundary conditions.

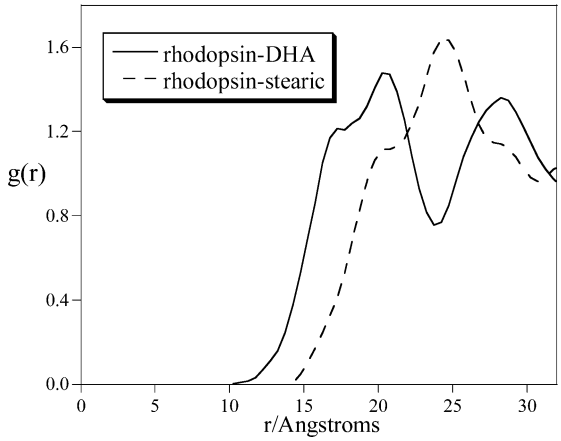

Figure 1. Two-dimensional radial distribution function, $g(r)$, based on the protein and fatty acid centers of mass. The solid and dashed lines give the results for the docosohexaneoic and stearic acid chains, respectively.

The procedure described above led to a stable system that reached equilibrium, as determined by monitoring the internal energy, after approximately 2.5 ns of the production run. The alpha helical structure of the transmembrane regions fluctuated around the initial (X-ray) structure, giving an average RMS deviation of the backbone atoms of 1.01 Å (details of the protein structure will be described in a subsequent publication). In this communication, we concentrate on lipid−protein interactions and their effect on membrane structure and dynamics.

The radial distribution of lipid fatty acid chains around the protein is given in Figure 1, demonstrating that rhodopsin has a dramatic preference for solvation by the polyunsaturated DHA chain. The DHA chains penetrate approximately 4 Å deeper into the protein interface than do the stearic acid chains. The lipids are sufficiently structured as to produce alternating layers of polyunsaturated and saturated hydrocarbon, suggesting very strong ordering forces are at work. The preferential solvation of rhodopsin by DHA is consistent with experimental measurements of the denaturation temperature as a function of lipid composition, which showed that the denaturation temperature was highest in the presence of polyunsaturated DHA chains.[9]

The mechanism by which the protein accumulates DHA in its immediate vicinity appears to be through favorable direct intermolecular interactions between rhodopsin and DHA. After the lipid−protein interaction energy is separated into contributions from

† Wabash College.
‡ NIAAA, National Institutes of Health.
§ Johns Hopkins University.

10.1021/ja0345874 CCC: $25.00

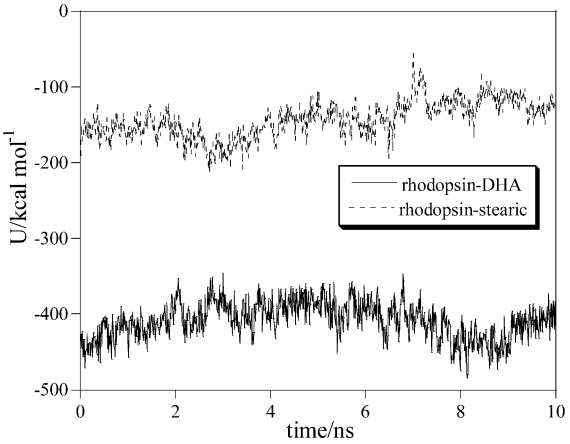

Figure 2. Interaction energy (sum of electrostatic and Lennard-Jones contributions to the potential energy) between rhodopsin and the fatty acid chains of the lipid as a function of time (after equilibration). The solid and dashed lines give the results for the docosohexaenoic and stearic acid chains, respectively.

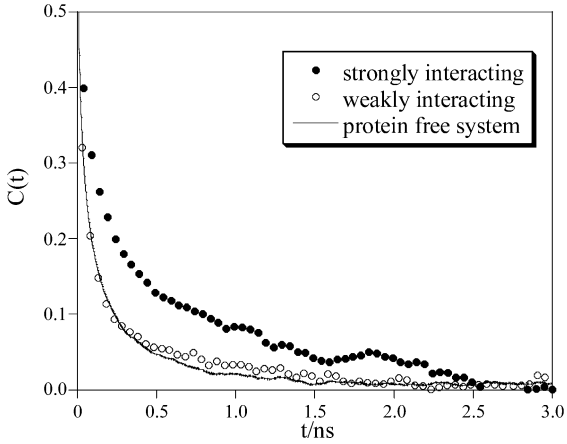

Figure 3. Reorientational correlation function, $C(t) = \langle P_2(\vec{\mu}(0)\vec{\mu}(t))\rangle$, for the C−H vector of the 10th carbon atom of the DHA chain. The averaging is over the 10 lipids with the strongest (●) and weakest (○) interactions with the protein.

individual fatty acid chains, it is observed that the strength of the rhodopsin−DHA attraction is approximately 3 times greater than the rhodopsin-stearic acid potential energy. Figure 2 displays these energies as a function of time, demonstrating both the strong rhodopsin−polyunsaturate interactions and the stability of the energy difference with time. The favorable energetics of rhodopsin with the polyunsaturated chains appears to be quite general, with each transmembrane helix showing a similar magnitude of favorable interaction, although the ratio of the energies is particularly high for helix 3. An analysis by residue type shows that the interactions are not particularly side chain specific. All hydrophobic residues showed a stronger attraction to the polyunsaturated chain with a ratio ($U_{DHA}/U_{stearic}$) that averaged 2.7 with a range from 1.8 to 4.2. Decomposition of the interaction energy into electrostatic and dispersion contributions shows that the attraction between protein and fatty acid chain arises almost entirely from the latter. Although vinyl groups are more polar than methylene groups, the added stability of the polyunsaturated chain seems to come from large numbers of favorable contacts occurring at short distances. The ability of the DHA chain to adopt such favorable conformations was predicted by a combined experimental/computational study that we have published previously.[5] Rotational barriers for isomerization around methylenes connecting vinyl groups are extremely low (<1 kcal/mol) as compared to those in a saturated chain (∼3.5 kcal/mol). Additionally, the torsional minima for the rotatable bonds in DHA are very broad. The extreme flexibility of the DHA chain allows it to adopt the rugged surface of α-helical chains and to penetrate between them, at little or no intramolecular energy cost.

Strong interactions between lipid and protein are expected to alter motional correlation times of lipid segments. To investigate this possibility, we analyzed the reorientational relaxation of the C−H vectors along the DHA chain. The 10 lipids with the strongest protein−chain interactions were chosen along with the 10 lipids having the weakest interactions. The reorientational correlation function, which is related to the experimentally measurable ^{13}C spin−lattice (T_1) relaxation rate, is shown in Figure 3 for the strongly and weakly interacting sets. The chains near the protein surface are slowed considerably, showing mean correlation times that are twice as long as the chains on more distant lipids. The weakly interacting chains have correlation functions that are unchanged (within statistical error) from those calculated from our earlier simulation of a pure SDPC bilayer.[5] The changes in relaxation behavior caused by the protein may be measurable by NMR. For example, at the ^{13}C NMR resonance frequency of 125 MHz, the simulated correlation functions for carbon number 10 of the DHA chain correspond to T_1 values of 0.62 and 1.01 s for strongly and weakly interacting lipids, respectively.

In summary, we have shown through all-atom MD simulation that rhodopsin is preferentially solvated by polyunsaturated DHA. Starting from random lipid orientations, the affinity between protein and DHA led to a pronounced structuring of the lipid matrix through rotational reorientation of the lipids. The present simulation is consistent with numerous experimental studies suggesting such an affinity and in addition predicts that NMR relaxation measurements on DHA segments could be a valuable probe of lipid−protein interactions. The present work, while not contradicting models based on material properties of the membrane,[10] supports a view that at least some of the MII changes with lipid type that have been measured experimentally[11] arise from a direct molecular interaction that is an important aspect of rhodopsin function. While DHA stabilizes rhodopsin against thermal denaturation, it also eases the transition into the meta-II state that activates G-proteins,[2] a transition related to a rearrangement of transmembrane helices.[3] The thought that a deeper penetration of DHA chains into rhodopsin facilitates such structural changes is extremely interesting.

Acknowledgment. S.E.F. thanks the National Science Foundation for support under award MCB-0091508.

References

(1) Salem, N.; Litman, B.; Kim, H. Y.; Gawrisch, K. *Lipids* **2001**, *36*, 945−959.
(2) Mitchell, D. C.; Straume, M.; Litman, B. J. *Biochemistry* **1992**, *31*, 662−670.
(3) Kuhlbrandt, W. *Nature* **2000**, *406*, 569−570.
(4) Palczewski, K.; Kumasaka, T.; Hori, T.; Behnke, C. A.; Motoshima, H.; Fox, B. A.; Le Trong, I.; Teller, D. C.; Okada, T.; Stenkamp, R. E.; Yamamoto, M.; Miyano, M. *Science* **2000**, *289*, 739−745.
(5) Feller, S. E.; Gawrisch, K.; MacKerell, A. D. J. *J. Am. Chem. Soc.* **2002**, *124*, 318−326.
(6) Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. *J. Comput. Chem.* **1983**, *4*, 187−217.
(7) Crozier, P. S.; Stevens, M. J.; Forrest, L.; Woolf, T. B., submitted.
(8) Woolf, T. B.; Roux, B. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 11631−11635.
(9) Polozova, A.; Litman, B. J. *Biophys. J.* **2000**, *79*, 2632−2643.
(10) Botelho, A. V.; Gibson, N. J.; Thurmond, R. L.; Wang, Y.; Brown, M. F. *Biochemistry* **2002**, *41*, 6354−6368.
(11) Gibson, N. J.; Brown, M. F. *Biochemistry* **1993**, *32*, 2438−2454.

JA0345874