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Docosahexaenoyl Chains Isomerize on the Sub-Nanosecond Time Scale

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Docosahexaenoic acid (DHA) is essential to neuronal function. Lipids with DHA chains are known to facilitate conversion of the G-protein coupled receptor (GPCR) rhodopsin to its G-protein binding-competent state.[1] Therefore, understanding DHA dynamics in bulk liquid crystalline bilayers and near GPCR is of functional significance. NMR relaxation measurements on carbon atoms are a convenient tool to study dynamics on the time scale from pico- to microseconds.[2] Here, we measured natural abundance ^{13}C relaxation rates of polyunsaturated DHA chains in 18:0d35-22:6n3-PC membranes with and without bovine rhodopsin (lipid/rhodopsin molar ratio 250/1). The rates were analyzed with an ansatz similar to the model of Lipari and Szabo[3] for treatment of superimposed motions of proteins in liquids. It corresponds to the isotropic limit of the anisotropic diffusion model of lipids in bilayers by Brown.[4] This approach provided a carbon atom-specific description of DHA dynamics which shows that (i) the DHA acyl chains explore their full conformational space in tens of nanoseconds, and (ii) rhodopsin does not measurably alter rates of DHA vinyl methylene bond isomerization but instead affects collective modes of lipid motions with longer correlation times.

Experiments were conducted at 11.7 and 18.8 T, and results are reported in Figure 1. Spin−lattice, R_1, and spin−spin relaxation rates, R_2, generally decreased from the carbonyl to the terminal methyl end of DHA chains. The slope of data points in Figure 1A indicates that R_1 values are field independent and remain essentially unchanged after addition of protein (Figure 1A). The R_2 values exhibit a modest field dependence, in particular, the vinyl carbons that relaxed slightly faster at 18.8 T, as expected from a contribution of their significant chemical shift anisotropy (CSA) to spin−spin relaxation.[5] Interestingly, R_2 was higher in membranes containing rhodopsin, suggesting changes in spectral densities on a time scale stemming from slower collective lipid motions (Figure 1B). Hydrocarbon chain dynamics in lipid bilayers are characterized by a superposition of motions: individual bond flickering and isomerization, $10^{-12} < t < 10^{-10}$ s; collective lipid axial reorientation and tumble, $10^{-9} < t < 10^{-7}$ ns; and bilayer undulation, $10^{-7} < t < 1$ s.[6] Subsequently, we describe our model of extracting correlation times of fast internal bond motions and of bond order parameters declared on the 10^{-8}−10^{-7} s time scale. It was assumed that carbon relaxation rates are solely determined by modulation of ^1H−^{13}C dipole−dipole interactions from directly bonded protons. The influence of each motion on the nuclear relaxation rates is calculated via the time correlation functions of the H−C bond reorientation, $C(t)$. The Fourier transform of $C(t)$ yields the spectral density functions, $J(\omega)$, that determine R_1 and R_2 according to the following equations

$$R_1 = \frac{d^2}{4}[J(\omega_{^1H} - \omega_{^{13}C}) + 3J(\omega_{^{13}C}) + 6J(\omega_{^1H} + \omega_{^{13}C})] \quad (1)$$

$$R_2 = \frac{d^2}{8}[4J(0) + J(\omega_{^1H} - \omega_{^{13}C}) + 3J(\omega_{^{13}C}) + 6J(\omega_{^1H})] \quad (2)$$

where $d = \mu_0 h \gamma_{^1H} \gamma_{^{13}C} / 8\pi^2 r^3$ with $\gamma_{^{13}C}$ and $\gamma_{^1H}$, the magnetogyric ratios for carbon and protons, respectively, r, the distance between directly bonded ^1H and ^{13}C nuclei, $\omega_{^{13}C}$ and $\omega_{^1H}$, the resonance frequencies of carbons and protons, respectively.

Motions with correlation times on the order of a nanosecond are most effective in raising R_1 and R_2 via the $J(\omega)$ terms (eqs 1 and 2). Spin−spin relaxation rates R_2 have additional sensitivity to collective lipid motions with much longer correlation times via the $J(0)$ term (eq 2). Those slower collective modes of motion are solely responsible for the significantly higher R_2 rates compared to those of R_1.

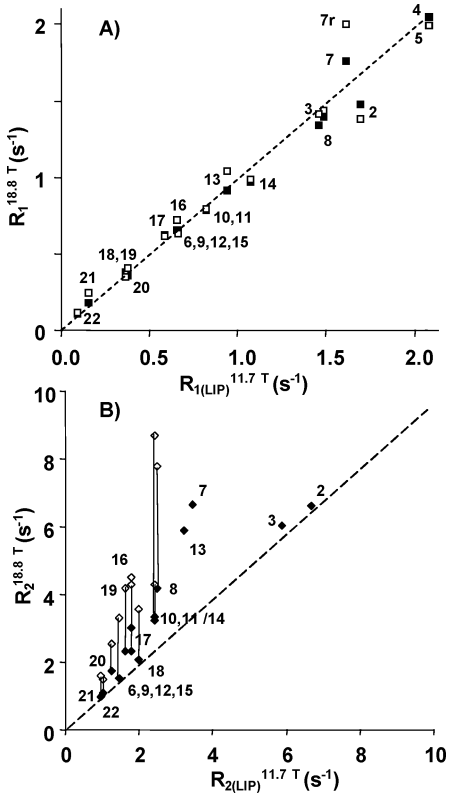

Figure 1. Plot of spin−lattice (A) and spin−spin (B) carbon relaxation rates of the DHA chain in 18:0(d35)-22:6n3PC measured at 11.7 T (x-axis) and 18.8 T (y-axis) with (open symbols) and without rhodopsin (filled symbols). Carbon chemical shifts were assigned as in ref 7. Experimental details are provided as Supporting Information.

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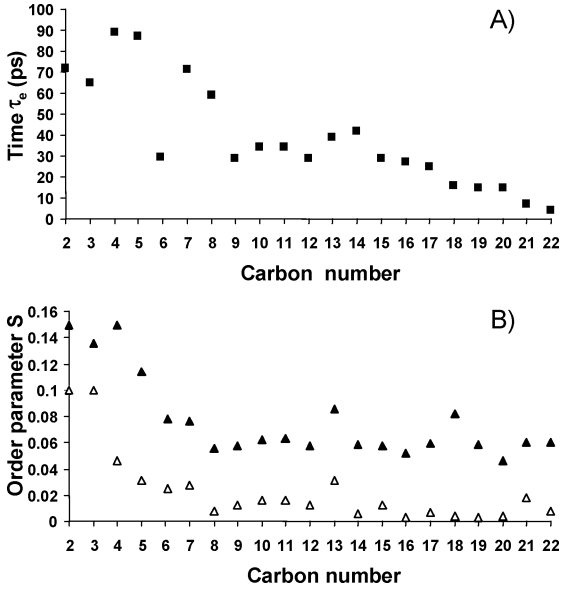

Figure 2. (A) Dependence of the correlation time of fast motions on the DHA chain carbon position. (B) Order parameter profile extracted from the relaxation analysis (▲) and from direct $^2H/^{13}C$ NMR order parameter measurements (△) that we reported previously.[7]

The correlation function describing fast reorientation of individual bonds and a superimposed collective reorientation of an entire molecule is conveniently approximated as

$$C(t) = S_{C-H}{}^2 \cdot e^{-t/\tau_c} + (1 - S_{C-H}{}^2) \cdot e^{-t/\tau_e}$$

where the correlation time τ_e describes the fast decay of $C(t)$ to a value proportional to the square of the bond order parameter S_{C-H} after fast conformational isomerization, collective lipid axial reorientation, and tumble. While in solution, the τ_c represents an isotropic reorientation which reduces order parameters to zero, motions of lipids in bilayers remain anisotropic, and the order of C−H bonds decays to low but finite values, for example, the bond order parameters measured by ^2H NMR on a time scale of $\sim 10^{-4}$ s. Here, the decay time τ_c represents slower, collective reorientation of lipids.

In the framework of the above model, a lack of field dependence of R_1, as observed experimentally (Figure 1), requires $\tau_c \geq 10$ ns and $S_{C-H} \leq 0.2$. Consequently, R_1 depends solely on the initial decay of the correlation function $C(t)$ with correlation times τ_e, in the range from pico- to nanoseconds. They are directly calculated from R_1 using eq 1. The τ_e values decrease from 80 ps near the carbonyl group to 8 ps near the terminal methyl group, independent of the presence of rhodopsin (see Figure 2A). This clearly shows that the decay of the correlation function $C(t)$ is dominated by rapid motions from chain isomerization. Furthermore, the presence of rhodopsin does not alter correlation times of chain isomerization for the great majority of lipids. We may not exclude immobilization of a few DHA chains by rhodopsin. However, if there is any restriction on chain isomerization from lipid−protein interaction, it most certainly involves less than the ∼30 lipids that surround every rhodopsin molecule as a first layer.

A correlation time of fast collective DHA motions in the range from 10 ns $< \tau_c <$ 100 ns yielded reasonable order parameters S_{C-H} that fitted R_2 values at both magnetic field strengths within limits of experimental error (10−15%). Figure 2B shows calculated DHA bond order parameters assuming $\tau_c = 50$ ns. These values were extracted from the R_2 data at 11.7 T since the contribution to R_2 from the modulation of CSA was deemed to be negligible at the lower field. Remarkably, those values are up to a scaling factor in excellent agreement with order parameters measured by ^2H NMR and $^1H-^{13}C$ cross-polarization experiments.[7] The difference between order parameters obtained by relaxation and order parameters measured via static quadrupolar or dipolar interactions is their time scale, $\sim 10^{-8}$ s for relaxation and $\sim 10^{-4}$ s for quadrupolar and dipolar splittings. Therefore, it can be concluded that DHA chain isomerization on the sub-nanosecond time scale is responsible for the principle features of the order parameter profile. Order parameters are further reduced by slower collective motions. Their effect is equivalent to multiplying all bond order parameters by a constant factor, $S_{CM} < 1$, to reach the values defined on the much longer time scale. Some deviations for carbons C_2-C_5 indicate that upper chain segments of DHA may undergo additional, slower chain isomerization that is not perfectly randomized after 50 ns.

Addition of rhodopsin increased spin−spin relaxation rates R_2 by about a factor of 2 for carbons C_8-C_{22} and even more for chain segments near the carbonyl. Since no corresponding change of R_1 was measured, this proves that collective lipid motions with correlation times ≥10 ns are responsible for the change. It can be concluded that DHA chains isomerize on the sub-nanosecond time scale, resulting in low bond order parameters. For almost all lipids in the bilayer, those rapid motions are not modified by the presence of rhodopsin. Instead, rhodopsin alters much slower collective modes of motions that influence the entire chain.

The increase of R_2 could have resulted from an increase of membrane curvature in combination with lateral diffusion of lipids over curved bilayers, but while we observed an increase of mosaic spread of bilayer orientation after protein incorporation that indicates induction of membrane curvature, we did not detect the significant reduction of lipid order that would have accompanied it.[8] Therefore R_2 was probably raised due to an increase of correlation times of lipid motions in the range from 10^{-8} to 10^{-5} s. Such an increase was reported for spin-labeled lipids near rhodopsin.[9] Short-lived associations of DHA chains that increase motional correlation times of slower DHA chain reorientation by 1 order of magnitude in the first lipid layer surrounding the protein would explain observations.

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Supporting Information Available: Table of relaxation rates, materials, and methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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