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NOTE

Proton NMR Relaxation Times of Polyunsaturated Fatty Acids in Chloroform Solutions and Aqueous Micelles

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Abstract: The spin-lattice (T_1) and spin-spin (T_2) relaxation times of protons on linoleic acid (LA), arachidonic acid (AA), and DHA in $CDCl_3$ solutions and aqueous micelles were measured. Protons at hydrophobic sites on DHA and AA in micelles had a longer T_2 than those on LA, whereas little difference was found for proton relaxation times among fatty acids in $CDCl_3$ solutions. DHA molecules in micelles were thus more flexible than LA in micelles.

Key words: micelle, PUFA, lipid oxidation, spin-spin relaxation time

1 Introduction

The functions of polyunsaturated fatty acids (PUFAs) such as docosahexaenoic acid (DHA) in food have attracted consumer attention. However, PUFAs are easily oxidized to form compounds that act as active oxygen and are harmful to human health. PUFAs are contained in many foods in the form of emulsion. In food processing and preservation, it is important to prevent PUFA oxidation in food, which is why research on the oxidative stability of PUFAs in emulsions has attracted attention.

The key event in lipid peroxidation is the formation of a lipid radical by the abstraction of a hydrogen radical from one of the bisallylic positions on the substrate lipids (1). The resulting pentadienyl radical reacts at both ends with oxygen to form 2 conjugated diene monohydroperoxides. The physical properties of PUFAs in different environments affect the rate of hydrogen abstraction of specific bisallylic positions and/or cause oxygen to selectively oxygen at certain pentadienyl radicals, which presumably changes the isomeric distribution of monohydroperoxide (2).

The oxidizability of PUFAs and their sodium salts in aqueous micelle was found to decrease with the increase in the number of bisallylic positions (CH=CH-CH_2-CH=CH) in molecules (3), a trend also observed in liposomes of phosphatidylcholine (PC) with PUFAs (4). The order in oxidizability is the reverse in the bulk phase or in organic solvents (5). The physical properties of the liposome membrane produced by PC containing PUFA, such as order parameter, correlation time, and water solubility, differ from those of liposomes produced by PC with only saturated fatty acids (6-8), and this presumably affects the mechanism of their oxidation (2). Rajamoorthi and Brown showed in ^{2}H and ^{31}P NMR experiments that PC containing arachidonic acid (AA) had different structural and dynamic properties from that containing only saturated fatty acids (9). Studies on molecular dynamics and solid-state NMR of liposomes showed the DHA moiety in PC to be more flexible than the saturated fatty acid moiety (10,11).

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Several conformations, including helical and iron-angle suggested as 2 major conformations for DHA in liposomal PC by Applegate *et al*. (12) are also possible for PUFAs in PC forming liposomes (13).

Research on PUFA conformations in emulsions lags despite advanced conformational studies of PC containing PUFA in liposomes. We tried studied the conformation and dynamics of PUFAs in micelles, simpler model for emulsion, to clarify the physical properties related to the oxidative stability of emulsified PUFAs.

The conformation and kinetics of a molecule or part of a molecule are reflected in NMR relaxation time, i.e., spin-lattice (T_1) and spin-spin (T_2) relaxation time. We measured T_1 and T_2 of protons on DHA, AA, and linoleic acid (LA) molecules in micelles in D_2O and in $CDCl_3$ solution to compare the molecular dynamics of PUFAs in micelles to that the molecular dynamics in a solution.

2 Material and Methods

2·1 Materials

Sodium salts of DHA, AA, and LA were purchased from Sigma, Co. (Tokyo, Japan).

2·2 Preparation of Micelles

The sodium salt of each lipid (15 μmol) was dissolved in 1.0 mL of D_2O. To eliminate oxygen, the sample was vacuumed gently, air was purged with nitrogen gas, and the sample was sonicated. The series repeated 3 times.

2·3 Preparation of $CDCl_3$ Solution

Each lipid (15 μmol) was dissolved into 1.0 mL of $CDCl_3$. To eliminate oxygen in the solution, the sample was frozen in liquid nitrogen and vacuumed. Air was then purged with nitrogen gas. This series was repeated twice for each sample.

2·4 NMR Measurement

T_1 and T_2 of PUFAs in the solution and micelles prepared above were measured by inversion recovery and CPMG method by a DRX600 NMR spectrometer (Bruker Biospin, Karlsruhe, Germany) operated with XwinNMR software (Bruker Biospin) at a proton resonance frequency of 600.13 MHz. Results are the average ± SD of 3 replications.

3 Results and Discussion

Proton NMR signals of each PUFA (**Fig. 1**) were assigned using DQF-COSY, HMQC, and HMBC (**Table 1**). Some signals overlapped, making it difficult to obtain relaxation times of individual protons, but the magnetization decay of each peak derived from 2 or more overlapped proton signals showed almost a single exponential decay, i.e., relaxation times of protons were very similar when calculated by nonlinear analysis. We then calculated average relaxation times of protons overlapping at the same chemical shifts.

T_1 is listed in **Table 2** and T_2 in **Table 3**. Comparing protons in PUFAs forming micelles to those of the $CDCl_3$ solution, we found 2 typical trends. First, protons in PUFAs forming micelles had a shorter relaxation time than those in the $CDCl_3$ solution, except for methylene adjacent to the methyl terminal (c) of DHA. Second, T_2 of protons in micelles were much shorter than their T_1, whereas T_1 and T_2 were almost the same in the $CDCl_3$ solution. The decrease of T_2 by micelle formation indicated that lipid molecules in micelles are mutually rigidly associated so molecular motion is more restricted than in non-associated lipid molecules in the $CDCl_3$ solution. The shorter T_2 in micelles compared to T_1 suggested the system was closer to a solid state.

To compare the state of each position in PUFAs in a solution and micelles, we compared the T_2 of protons at

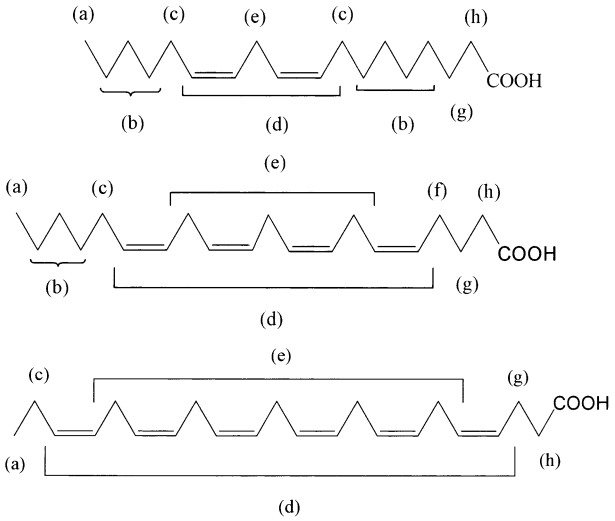

Fig. 1 PUFA Configuration. From above: linoleic acid (LA), arachidonic acid (AA), and docosahexaenoic acid (DHA).

Table 1 Chemical shift of PUFAs.

		(a)	(b)	(c)	(d)
$CDCl_3$ soln.	LA	0.90 (3H, t, 7.0Hz)	1.28-1.38 (14H, m)	2.04 (2H, m)*	5.30-5.45 (4H, m)
	AA	0.93 (3H, t, 6.9Hz)	1.29-1.41 (6H, m)	2.08 (2H, q, 7.1Hz)	5.34-5.46 (8H, m)
	DHA	0.97 (3H, t, 7.5Hz)	—	2.08 (2H, tqu, 0.6Hz, 7.5Hz)	5.29-5.45 (12H, m)
Aq. micelle	LA	0.88 (3H, t, 7.1Hz)	1.25-1.38 (14H, m)	2.05 (2H, m)***	5.24-5.41 (4H, m)
	AA	0.89 (3H, t, 7.0Hz)	1.25-1.38 (6H, m)	2.04 (2H, q, 7.2Hz)	5.30-5.47 (8H, m)
	DHA	0.94 (3H, t, 7.5Hz)	—	2.03 (2H, qu, 7.5Hz)	5.26-5.44 (12H, m)

		(e)	(f)	(g)	(h)
$CDCl_3$ soln.	LA	2.78 (2H, t, 8.2Hz)	2.04 (2H, m)*	1.63 (2H, qu, 7.2Hz)	2.34 (2H, t, 7.2Hz)
	AA	2.82-2.88 (6H, m)	2.17 (2H, q, 7.5Hz)	1.76 (2H, q, 7.5Hz)	2.39 (2H, t, 7.5Hz)
	DHA	2.80-2.86 (10H, m)	—	2.39-2.44 (2H, m)**	2.39-2.44 (2H, m)**
Aq. micelle	LA	2.71-2.74 (2H, m)	2.05 (4H, m)***	1.54 (2H, q, 7.1Hz)	2.17 (2H, t, 7.1Hz)
	AA	2.78-2.83 (6H, m)	2.07 (2H, q, 7.3Hz)	1.62 (2H, qu, 7.2Hz)	2.18 (2H, t, 7.9Hz)
	DHA	2.76-2.83 (10H, m)	—	2.31 (2H, td, 6.9Hz, 7.4Hz)	2.20 (2H, t, 6.9Hz)

* These peaks overlapped and were indistinguishable.

** These peaks overlapped and were indistinguishable.

*** These peaks overlapped and were indistinguishable.

Table 2 T_1 for PUFAs.

		(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)
$CDCl_3$ soln.	LA	3.6 ± 0.2	2.0 ± 0.2	2.0 ± 0.3*	3.5 ± 0.3	1.9 ± 0.1	2.0 ± 0.3*	2.0 ± 0.3	1.8 ± 0.2
	AA	3.3 ± 0.2	2.1 ± 0.3	2.2 ± 0.3	3.2 ± 0.5	1.6 ± 0.1	2.2 ± 0.3	1.8 ± 0.2	2.0 ± 0.4
	DHA	3.3 ± 0.4	—	1.5 ± 0.1	2.1 ± 0.3	3.5 ± 0.4	—	2.1 ± 0.2**	2.1 ± 0.2**
Aq. micelle	LA	1.6 ± 0.4	1.0 ± 0.3	0.91 ± 0.42***	1.2 ± 0.3	0.92 ± 0.1	0.91 ± 0.42***	0.82 ± 0.21	0.76 ± 0.11
	AA	1.6 ± 0.2	1.3 ± 0.2	1.0 ± 0.6	1.7 ± 0.3	0.95 ± 0.15	1.0 ± 0.2	0.95 ± 0.22	0.97 ± 0.10
	DHA	2.3 ± 0.3	—	2.1 ± 0.3	1.0 ± 0.2	1.9 ± 0.3	—	0.89 ± 0.10	1.1 ± 0.2

* These peaks overlapped and were indistinguishable.

** These peaks overlapped and were indistinguishable.

*** These peaks overlapped and were indistinguishable.

Table 3 T_2 for PUFAs.

		(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)
$CDCl_3$ soln.	LA	3.4 ± 0.3	1.8 ± 0.2	1.7 ± 0.3*	3.3 ± 0.4	1.8 ± 0.2	1.7 ± 0.3*	1.7 ± 0.3	1.6 ± 0.3
	AA	3.0 ± 0.3	1.8 ± 0.2	2.1 ± 0.2	3.0 ± 0.4	1.4 ± 0.1	2.0 ± 0.3	1.5 ± 0.2	1.8 ± 0.3
	DHA	3.2 ± 0.5	—	1.1 ± 0.1	2.1 ± 0.2	3.4 ± 0.4	—	2.0 ± 0.3**	2.0 ± 0.1**
Aq. micelle	LA	0.25 ± 0.10	0.21 ± 0.10	0.15 ± 0.08***	0.39 ± 0.11	0.10 ± 0.05	0.15 ± 0.08***	0.19 ± 0.04	0.48 ± 0.12
	AA	0.65 ± 0.18	0.64 ± 0.15	0.68 ± 0.24	0.55 ± 0.09	0.49 ± 0.07	0.70 ± 0.18	0.25 ± 0.10	0.19 ± 0.06
	DHA	1.4 ± 0.3	—	1.3 ± 0.2	0.86 ± 0.12	1.1 ± 0.2	—	0.28 ± 0.07	0.28 ± 0.09

* These peaks overlapped and were indistinguishable.

** These peaks overlapped and were indistinguishable.

*** These peaks overlapped and were indistinguishable.

corresponding positions in PUFA molecules. We found little difference between the T_2 of corresponding protons on PUFAs in the $CDCl_3$ solution, but methyl protons (a) of DHA in micelles were much larger value than those of LA, and that of AA were intermediate. Olefin protons (d) and bisallylic protons (e) showed a similar tendency as for methyl protons (a). For the proton on the carboxyl terminal (h), LA had a slightly longer T_2 than DHA. The mobility of the hydrophobic part of the DHA molecule is thus considered higher than that of LA when forming micelles. DHA molecules in micelles may be packed more loosely than in LA, and the hydrophobic moiety of DHA may move more freely in micelles. Micelles of AA appear to have flexibility in between those of DHA and LA.

Liposomes of PC with highly unsaturated fatty acids, such as AA and DHA, are more permeable and flexible in fatty acid chains than those of PC containing less unsaturated fatty acids such as LA (6, 7, 9-11, 13). Our result for micelles coincided with those reported for liposome systems, indicating the flexibility of hydrophobic parts of highly unsaturated fatty acids in micelles. This flexibility may allow water molecules to permeate micelles. The presence of water near acyl moieties of fatty acids should decrease the density of bisallylic positions in micelles, where radical hydrogen abstraction occurs at the start of peroxidation. Since the velocity of radical reaction is proportional to the matrix density, the presence of water near bisallylic positions affects the velocity of peroxidation, which may account for the oxidative stability of PUFA with more bisallylic positions in micelles (4) as suggested for liposomes (2). This sheds light on the oxidation mechanism of emulsions, which are the major status of lipids and oil in foods but more complicated than micelles.

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