Molecular Behaviors of *n*-Fatty Acids in Liquid State

Makio IWAHASHI^{*1}, Yasutoshi KASAHARA^{*1}, Hideyuki MINAMI^{*1},
Hideyo MATSUZAWA^{*1}, Masao SUZUKI^{*2} and Yukihiro OZAKI^{*3}

^{*1} School of Science, Kitasato University
(Sagamihara 228-8555, JAPAN)

^{*2} Advanced Science and Technology Research Center, Kyushu University
(Kasuga, Fukuoka 816-8580, JAPAN)

^{*3} School of Science, Kwansei Gakuin University
(Nishinomiya 662-8501, JAPAN)

Edited by M. Yonese, Nagoya City Univ., and accepted November 20, 2001 (received for review August 27, 2001)

Abstract: Through near infrared spectroscopy and ¹³C-NMR spin-lattice relaxation time measurements it was revealed that the molecules of the normal fatty acids in the liquid state are strongly dimerized even at 363K by hydrogen bonding between their carboxylic groups. Thus, dimer molecules for the acids are the units in their intra- and intermolecular movements. The dynamic molecular and aggregate structures of the fatty acids (C8-C18) in the liquid state were estimated through the analyses of their self-diffusion coefficient *D*, viscosity η , molar volume and X-ray diffraction. The apparent hydrodynamic radius evaluated from *D* and η for the fatty acid was almost constant, irrespective of the hydrocarbon chain length. This suggests that only a longitudinal translation (translational movement along molecular axes) would be allowed for the dimer molecules of fatty acids. In addition, the distribution function curves obtained from the X-ray diffraction data suggest that, in the pure liquid state, rod-like fatty acid dimers highly aggregate in parallel and probably make clusters that would be randomly aligned.

J. Oleo Sci., **51**, 157-164 (2002).

Key words: *n*-fatty acid, self-diffusion coefficient, liquid structure, ¹³C-NMR spin-lattice relaxation time, near-infrared spectroscopy

1 Introduction

Fatty acids are characteristic building-block components of most lipids such as phospholipid and glycolipid that form biomembranes; they play important role in membrane functions such as flexibility, fluidity and material transfer. The functions seem to depend mainly on the property as a liquid rather than that as a solid, so that the study on the liquid of the fatty acid is considered to be very important. However, the dynamic molecular and liquid structures of the acids in the liquid state have been scarcely studied because their experimental determinations are very difficult and, in addition, the liquid state of the acids has hitherto been

believed to be merely isotropic irrespective of temperature or pressure. In the present study the dynamic molecular structures and the assembly structures of the normal fatty acids (C8-C18) in the liquid state have been studied by means of near-infrared (NIR) spectroscopy, and also density, viscosity, translational self-diffusion coefficient, ¹³C-NMR spin-lattice relaxation time and X-ray diffraction.

2 Experimental

2.1 Samples

Samples of tetradecane (99.9%, Wako Pure Chemical Industry Co.), octanoic, nonanoic, decanoic, undecanoic, dodecanoic, tetradecanoic, hexadecanoic and octadecanoic acids (>99.9%, Nippon Oil and Fats Co.), and the alkanoyl chlorides (>98%, Tokyo Kasei Co.)

Corresponding author: Makio IWAHASHI
E-mail: maki@kitasato-u.ac.jp

corresponding to the fatty acids were used without further purification. The purity of the samples of the acids was confirmed by gas-liquid chromatography (Shimadzu GC-14A with a capillary column of SP-2560).

2.2 Density Measurements

The densities for the samples of octanoic and nonanoic acids were measured on a vibration-type densimeter (Anton Paar Model DMA 58) in the temperature range $323.2 - 353.1 \pm 0.01$ K. Degassed distilled water was used for calibrating the densimeter.

2.3 Viscosity Measurements

The viscosity for the samples of octanoic and nonanoic acids was measured with an Ostwald capillary viscometer in the temperature range $323.2 - 353.1 \pm 0.05$ K. Distilled water was used for calibrating the viscometer.

2.4 NIR Spectroscopic Measurements

The NIR spectra for the samples of octanoic, nonanoic, decanoic and undecanoic acids were measured at a resolution of 1.0 nm on a Hitachi U-3500 spectrometer. A quartz cell having a 0.5-cm path length was used. A Hitachi temperature-regulated cell holder (No. 131-0030) was used to maintain the temperature of the sample within ± 0.1 K.

2.5 NMR Measurements

The self-diffusion coefficient, D , for the samples of fatty acids was determined by means of the pulsed-gradient NMR method (1-4). All the measurements were made on protons at 399.65 MHz in the temperature range $323 - 353 \pm 0.5$ K on a JEOL NMR spectrometer (Model EX-400). The ^{13}C -NMR spin-lattice relaxation time, T_1 , for the samples was obtained by the inversion recovery method (3-5), employing a 180° - τ - 90° pulse sequence, with the NMR spectrometer in the temperature range $323 - 353 \pm 0.5$ K.

2.6 X-ray Diffraction Measurements

X-ray diffraction experiments for the liquid samples of octanoic, nonanoic and decanoic acids and tetradecane were carried out by using a diffractometer constructed especially for the liquid samples (6). The diffractometer has a two-dimensional detector, a so-called "imaging plate" (DIP-100 of MAC Science Co. Ltd.). A 1-mm-thick sample was sealed in a cell with 5-

μm -thick Mylar windows. AgK α (25 mA, 50 kV, $\lambda = 0.5608 \text{ \AA}$, λ : wavelength) radiation was used.

3 Results and Discussion

3.1 Dissociation of Dimer Molecules of Fatty Acid into Monomer Species

NIR spectra of the fatty acids in the liquid phase were measured in the 1100-1700 nm region over a temperature range of 283.3-352.9 K. **Figure 1** shows the spectra for nonanoic acid, as an example, in the liquid state at various temperatures. The intensity of the absorption band is expressed in terms of the molar absorptivity, ϵ . The band at 1445 nm (7), which is attributable to the OH vibration for free fatty acid, grows and becomes more intense with increasing temperature, that is, the dissociation of acid dimers into monomeric species occurs even in the liquid state and increases with increasing temperature. From the analysis of the 1445 nm band we are able to determine the degree of dissociation, α , by applying the procedure mentioned in the previous paper (7). In the analysis, however, it should be noticed that the band at 1445 nm is superimposed on the bands corresponding to the CH-vibration modes of the CH_3 and CH_2 groups. It is there-

Fig. 1 NIR Spectra for Nonanoic Acid in the Liquid State at 285.3, 285.9, 291.8, 296.2, 300.2, 304.5, 313.0, 321.7, 331.3, 340.5, 348.7 and 357.6 K. Intensity of the free OH band of monomeric nonanoic acid at 1445 nm increases with increasing temperature.

fore necessary to subtract all the contributions of the CH modes to obtain the net ϵ for the OH vibration mode. In the previous paper (7), the concentration of free *cis*-octadecenoic acid was assumed to be zero near its melting point (289.1 K for its β -form crystal) so that for the determination of the α of *cis*-octadecenoic acid, the spectrum of the acid obtained at 292 K was subtracted from the spectra at various temperatures. However, in the present study we have treated many kinds of fatty acids whose melting points are high and vary. Because it is doubtful that the concentration of the free monomeric acid is zero even at high melting temperatures, we used alkanoyl chlorides (C8-C18) to obtain difference spectra canceling the absorption band due to CH vibrations.

In **Fig. 2** α for nonanoic acid (open circle) is plotted against temperature. The α value is $\sim 1\%$ at 290 K and increases with the rise in temperature but even at 363 K only $\sim 3.2\%$ of the acid dimers dissociates into the monomeric species in the liquid state. Most molecules of nonanoic acid in the liquid state still remain as dimers even at such relatively high temperatures. The other fatty acids also gave similar results to the nonanoic acid as shown in **Fig. 2**. That is, the dimers for the normal fatty acids are apparently the units in their intra- or intermolecular movements.

Fig. 2 Temperature Dependence of Degree of Dissociation, α , of Acid Dimers into Monomeric Species. ▲ denotes octanoic acid; ○, nonanoic acid; □, decanoic acid; and ◇, undecanoic acid.

3·2 Intramolecular Segmental Movements of Fatty Acid

^{13}C -NMR spin-lattice relaxation time T_1 for the different carbon atoms along the hydrocarbon chain of nonanoic acid were measured at various temperatures. ^{13}C -NMR spin-lattice relaxation of a protonated carbon is overwhelmingly dominated by dipole-dipole interactions with the attached protons; T_1 for the carbon atom in a segment is related to the number of directly bonded hydrogen, N , and the effective correlation time, τ_c , for the rotational movement of the segments in the acid molecule (8), that is, the reciprocal of τ_c represents the magnitude of the segmental motion of the carbon atom. T_1 is given in terms of N and $1/\tau_c$

$$T_1 = \frac{r_{\text{CH}}^6}{N \left(\frac{h}{2\pi} \right)^2 \gamma_{\text{C}}^2 \gamma_{\text{H}}^2} \left(\frac{1}{\tau_c} \right) \quad (1)$$

where h is Planck's constant, and γ_{C} and γ_{H} are the gyromagnetic ratios of ^{13}C and ^1H , respectively. r_{CH} is the C-H distance usually about 0.109 nm, and $1/\tau_c$ represents the magnitude of the segmental motion of the carbon atom at different positions.

In **Fig. 3** the $1/\tau_c$ values at various temperatures are plotted against the position of the carbon atoms in nonanoic acid. The $1/\tau_c$ value for each carbon atom obviously increases with increasing temperature. At any temperature the rotational movement of the carbon atom at the second position attached to the dimerized carboxylic group is considerably restricted, whereas that for other carbon atom increases, and especially that for the carbon atom beyond the sixth position increases rapidly toward the end of the hydrocarbon. That is, the methyl group positioned at the end of the acid molecule moves around most vigorously. The schematic illustration in **Fig. 3** demonstrates the rotational movements of the dimer molecule. Such dynamic dimers would form the liquid of the acid.

3·3 Density, Viscosity, Self-Diffusion Coefficient and Apparent Hydrodynamic Radius

In **Tables 1, 2** and **3**, data on density, viscosity, η , and self-diffusion coefficient, D , for all samples of fatty acids at various temperatures are summarized. The data for density and viscosity include our experimental results and reference ones (9-13). Apparent hydrody-

Fig. 3 Reciprocal Correlation Time, $1/\tau_c$, for the Carbon Atom at Different Position in Nonanoic Acid Molecule. Shadow part in upper schema imaginatively presents the intensity of the intramolecular-rotational movement of a dimer molecule of nonanoic acid.

namic radius (Stokes-Einstein radius), a , was calculated from the D and η values by using the following Stokes-Einstein formula under a slip boundary condition (14-18):

$$a = \frac{kT}{4\pi\eta D} \quad (2)$$

where k is Boltzmann's constant and T , the absolute temperature. In **Fig. 4** the a values at various temperatures for the normal fatty acids are plotted against their

Table 1 Density ($\rho/\text{g cm}^{-3}$) for Normal Fatty Acids.

T / K	323.1	333.1	343.1	353.1
C8	0.8860	0.8778	0.8701	0.8609
	0.8859 ^a	0.8780 ^a	0.8701 ^a	0.8605 ^b
C9	0.8807	0.8736	0.8658	0.8585
	0.8838 ^c	0.8744 ^c	0.8665 ^c	0.8571 ^c
	(322.0 K)		(344.2 K)	(355.3 K)
C10	0.8771 ^a	0.8699 ^a	0.8620 ^a	0.8520 ^b
	0.8788 ^c	0.8670 ^b	0.8615 ^c	0.8526 ^c
	(322.0 K)	0.8694 ^c	(344.2 K)	(355.3 K)
C12	0.8707 ^a	0.8629 ^a	0.8560 ^a	0.8483 ^a
		0.8634 ^c		0.8434 ^b
C14		0.8589 ^a	0.8514 ^a	0.8440 ^a
		0.8573 ^b		0.8434 ^b
C16				0.8414 ^b
C18				0.8377 ^b

a: reference (9); b: reference (10); c: reference (11)

Table 2 Viscosity ($\eta/\text{mPa s}$) for Normal Fatty Acids.

T / K	323.1	333.1	343.1	353.1
C8	2.96	2.42	2.02	1.69
	2.986 ^a	2.421 ^a	2.021 ^a	
C9	3.79	3.03	2.53	2.10
	3.92 ^c		2.48 ^c	2.02 ^c
	(322.0 K)		(344.2 K)	(355.3 K)
C10	4.578 ^a	3.72 ^c	3.00 ^c	2.52 ^c
C12	6.855 ^a	5.336 ^a	4.269 ^a	3.469 ^a
		5.42 ^c		
C14		7.452 ^a	5.984 ^d	4.791 ^d
		7.48 ^c	5.83 ^c	
			(344.2 K)	
C16			7.682 ^d	6.082 ^d
C18			9.87 ^b	7.68 ^c

a: reference (9); b: reference (10); c: reference (11); d: reference (12)

Table 3 Self-Diffusion Coefficient ($D/10^{-10}\text{m}^2\text{s}^{-1}$) for Normal Fatty Acids.

T / K	323.1	333.1	343.1	353.1
C8	3.93	5.06	6.08	7.85
C9	3.08	4.10	5.06	6.13
C10	2.65	3.47	4.25	5.36
C12	1.80	2.45	2.97	3.78
C14		1.72	2.23	2.94
C16			1.72	2.26
C18			1.43	1.82

hydrocarbon-chain length, together with the hypothetical mean radius, a_0 , (open square) which was obtained by substituting the density (ρ) value at 353 K into Eq. 3:

$$a_0 = \left(\frac{3M}{4\pi\rho L} \right)^{\frac{1}{3}} \quad (3)$$

where M is the molar weight of fatty acid and L is the Avogadro number.

The a_0 value increases monotonously with the increase in chain length, whereas the apparent hydrodynamic radius a obtained from Eq. 2 is obviously smaller than a_0 and seems to be almost constant, irrespective of the hydrocarbon chain length and also temperature. Strictly speaking, the a value decreases very slightly

with increasing hydrocarbon chain length. The constancy of the a value for a normal fatty acid molecule would be ascribed to the longitudinal motion (translational movement along molecular axes) of an extended rod-like dimer: Both rotational and transverse motions of the rod-like dimer are hindered by other rods in its vicinity (19, 20).

3.4 Liquid Structure Determined by X-ray Diffraction

In order to get more direct information about the mode of assembly of the dimer molecules, we carried out X-ray diffraction measurement for the liquid samples of tetradecane and octanoic, nonanoic and decanoic acids. **Figure 5** shows the X-ray diffraction spectra obtained for the liquid sample of nonanoic acid at 293 and 347 K. The region of the scattering angle parameter s ($= 4\pi \sin\theta/\lambda$, 2θ is scattering angle) was $0.4 - 9\text{\AA}^{-1}$. A sharp band exists at $s = 1.2\text{\AA}^{-1}$ and a small and broad band, around $2.6\text{\AA}^{-1}$.

Figures 6a and **6b** show the radial distribution function curves obtained by the Fourier transformation of the X-ray diffraction spectra for nonanoic acid and tetradecane at 293 K (solid line) and 347 K (dotted line), respectively. The clear distribution patterns in both the curves mean that the acid molecules, as well as

Fig. 4 Stokes-Einstein Hydrodynamic Radius, a , of Normal Fatty Acid against the Number of Carbon Atom at 323.1 (○), 333.1 (◇), 343.1 (△) and 353.1 K (★). Open squares are hypothetical mean radii calculated from the densities of the homologous fatty acids at 353 K, according to Eq. 2.

Fig. 5 X-ray Diffraction Spectra for the Liquid Sample of Nonanoic Acid at 293 K (solid line) and 347 K (dotted line). s (scattering angle parameter) = $4\pi \sin \theta / \lambda$.

those of tetradecane, highly aggregate in parallel. A small peak at *ca.* 0.16 nm indicates the contribution of the neighboring carbon atoms in a molecule. At 293 K the distances between the molecules directly neighboring each other are 0.54 nm for nonanoic acid and 0.55 nm for tetradecane, respectively. The distribution patterns in **Figs. 6a** and **6b** are almost equal to each other except the slight difference in the peak position. In addition, octanoic and decanoic acids showed almost the same distribution function pattern as nonanoic acid, that is, rod-like molecules such as fatty acids or tetradecane tend to aggregate with each other, and may form clusters resembling bundles of straw. No optical anisotropy such as streaming birefringence has ever been observed for pure liquids of the fatty acids, so that the full sizes of the clusters are smaller than the wavelength of visible light, and would be randomly oriented and make a liquid. As the rotational (end-over-end), as well as transverse, motion of each rod in the clusters may be severely restricted, the rod-like dimers of the fatty acid seem to translate only longitudinally (along molecular axes) so that the fatty acids would give almost the same Stokes-Einstein radius a , irrespective of the chain length of the fatty acid.

The slight decrease in a with increasing chain length

Fig. 6 (a) Radial Distribution Function Curves for Nonanoic Acid at 293 K (solid line) and 347 K (dotted line); (b) Radial Distribution Function Curves for Tetradecane at 293 K (solid line) and 347 K (dotted line).

would reflect the decrease in the segmentation motion (rotational motion of carbon atom) of the acid molecules with increasing chain length. In fact, as shown in Fig. 7, the relationships of $1/\tau_c$ vs. the position of carbon atoms for nonanoic and for octadecanoic acids indicate that the intramolecular segmental movements of nonanoic acid are obviously vigorous compared with those of octadecanoic acid. Especially the intensity of the movements (probably rotational movements) of the methylene parts (C16 and C17) near the end of the octadecanoic acid molecule is almost below 80% of that of the corresponding parts (C7 and C8) of the nonanoic acid molecule. In addition, as shown in Fig. 8, a plot of molar volume vs. the number of carbon atoms for fatty acid does not give a straight line: An increase in molar volume per methylene group slightly decreases with the increase in the number of carbon atoms. This means that an acid with a longer hydrocarbon chain has a more condensed packing mode in its liquid state.

In conclusion, the Stokes-Einstein formula under the slip boundary condition is generally applicable not only to a spherical or spheroid molecule of similar size in the order of magnitude to that for the surrounding solvent molecule (15, 20) but also to the rod-like molecules such as fatty acids or tetradecane with a relatively long hydrocarbon chain.

Fig. 7 Reciprocal-correlation time, $1/\tau_c$, at 353 K for the Carbon Atoms at Different Positions in Nonanoic (○) and in Octadecanoic (●) acid Molecules.

Fig. 8 Relationship between Molar Volume and Number of Carbon Atoms for Fatty Acid at 353 K. The plot deviates from the linear line especially above C10.

Acknowledgments

We express our deepest thanks to Professor Keiko Nishikawa of Chiba University for the measurements of X-ray diffraction of the liquid and to Miss Nami Taniguchi of Kitasato University for her assistance with the NIR measurements.

References

1. Stejskal, E. O. & Tanner, J. E. (1965) *J. Chem. Phys.*, **42**, 288-292.
2. Stejskal, E. O. (1968) *J. Chem. Phys.*, **49**, 1768-1777.
3. Iwahashi, M., Yamaguchi, Y., Kato, T., Horiuchi, T., Sakurai, I. & Suzuki, M. (1991) *J. Phys. Chem.*, **95**, 445-451.
4. Iwahashi, M., Hachiya, N., Hayashi, Y., Matsuzawa, H., Liu, Y., Czarnecki, M. A., Ozaki, Y., Horiuchi, T. & Suzuki, M. (1995) *J. Phys. Chem.*, **99**, 4155-4161.
5. Farrar, T. C. & Becker, E. D. (1971) *Pulse and Fourier Transform NMR*, Academic Press, New York, Chapter 2.
6. Nishikawa, K., Sakamoto, Y. & Iijima, T. (1991) *Jpn. J. Appl. Phys.*, **30**, 1303-1305.
7. Iwahashi, M., Suzuki, M., Czarnecki, M. A., Liu, Y. & Ozaki, Y. (1995) *J. Chem. Soc. Faraday Trans.*, **91**, 697-701.
8. Hertz, H. G. (1967) *Prog. Nucl. Magn. Reson. Spectrosc.*, **3**, 159-164.
9. Liew, K. Y., Seng, C. E., & Lan, E. K. (1991) *J. Am. Oil Chem. Soc.*, **68**, 488-492.
10. Small, D. M. (1986) *The Physical Chemistry of Lipids*.

- Plenum Press, New York, pp.593-594.
11. Nouredini, H., Teoh, B. C. & Clements, L. D. (1992) *J. Am. Oil Chem. Soc.*, **69**, 1184-1188.
 12. Nouredini, H., Teoh, B. C. & Clements, L. D. (1992) *J. Am. Oil Chem. Soc.*, **69**, 1189-1191.
 13. Fernandez-Martin, F. & Montes, F. (1976) *J. Am. Oil Chem. Soc.*, **53**, 130-136.
 14. Sutherland, G. B. B. M. (1905) *Philos. Mag.*, **9**, 781-785.
 15. Li, J. C. M. & Chang, P. (1955) *J. Chem. Phys.*, **23**, 518-522.
 16. Hu, C. M. & Zwanning, R. (1974) *J. Chem. Phys.*, **60**, 4354-4357.
 17. Espinosa, P. J. & de la Torre, J. G. (1987) *J. Phys. Chem.*, **91**, 3612-3616.
 18. Alms, G. R., Bauer, D. R., Brauman, J. I. & Pecora, R. (1973) *J. Chem. Phys.*, **58**, 5570-5578.
 19. Doi, M. & Edwards, S. F. (1978) *J. Chem. Soc. Faraday Trans. II*, **74**, 560-570.
 20. Teraoka, O. & Hayakawa, R. (1988) *J. Chem. Phys.*, **89**, 6989-6995.
-