Nuclear Magnetic Resonance Studies of Cyclodextrin Complexes of Linoleic Acid and Arachidonic Acid

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Structures of α- and β-cyclodextrin complexes of linoleic and arachidonic acids were investigated by detailed ^1H spin–lattice relaxation time (T_1) measurements and one-dimensional difference nuclear Overhauser enhancement (NOE) studies. On the basis of the observed NOE proximities and T_1 values, it is concluded that the carboxyl arms of both linoleic and arachidonic acids are present within the cyclodextrin cavity with double bonds partly exposed or buried. While the double bond at 9–10 in linoleic acid is buried, the one at 12–13 is exposed, the molecule from position 11 being present in an inclined position, with the methyl end pointing away from the cavity laterally. The double bond in arachidonic acid at position 5–6 is buried; the other at 8–9 lies exposed. While the carboxyl arm is present within the cavity, the methyl arm lies parallel to the carboxyl arm outside the cavity.

INTRODUCTION

Cyclodextrins are known to form inclusion complexes with various compounds (Bender and Komiyama, 1978; Cramer, 1952; Saenger, 1980; Luttringhaus et al., 1958). Guest compounds range from polar to highly apolar reagents (Wojcik, 1975). Water is usually used as solvent even though inclusion complex formation takes place in dimethyl sulfoxide and in dimethylformamide (Siegel and Breslow, 1975). The molar ratio of guest (cyclodextrins and their derivatives) to host is usually 1:1 in inclusion complexes formed in solution (Lammers and Van Diemen, 1972). Free fatty acids and their CoA derivatives are known to sequester in the hydrophobic inner cavity of cyclodextrins (Schlenk and Sand, 1961; Bergeron et al., 1975). It was shown that the inhibition of glucose-6-phosphate dehydrogenase (Kawaguchi and Bloch, 1974), fatty acid synthetase (Flick and Bloch, 1974), and phosphofructokinase (Ramadoss et al., 1976) caused by fatty acyl coenzyme A or free fatty acid could be relieved by trapping these compounds through the use of cyclodextrins. Also, unsaturated fatty acids included in cyclodextrin have been shown to be protected completely against oxidation even in pure oxygen (Szejtli and Bánky Elöd, 1975). Although the formation of inclusion complexes of fatty acids by cyclodextrin is known, little is known about the structure and the relative orientation of the fatty acids within the cyclodextrin cavity. Hence, detailed one-dimensional difference nuclear Overhauser enhancement (1D NOE) experiments and proton T_1 measurements were carried out to arrive at the structure of some of the unsaturated fatty acid complexes with cyclodextrin.

MATERIALS AND METHODS

α- and β-cyclodextrins were purchased from Sigma Chemical Co. Linoleic and arachidonic acids were purchased from Nu Chek Prep, Inc. For ^1H NMR both for NOE and for T_1 measurements, a 0.047 M solution of fatty acid and cyclodextrin in DMSO-d_6 were used. ^1H NMR spectra were recorded on a Brüker WH-270 MHz NMR instrument fitted with a Spectrospin magnet operating at 20 ± 1 °C and an Aspect 3000 computer. An equimolar solution of unsaturated fatty acid (linoleic and arachidonic acids) with α-cyclodextrin and β-cyclodextrin in 0.5 mL of DMSO-d_6 was used for obtaining the spectra. One-dimensional difference NOE experiments were carried out by accumulating typically 64 scans for off-resonance and the same number of scans for irradiation at a desired position to obtain the difference for each irradiation experiment. Irradiations were carried out for a duration of about 3 s to ensure that a sufficient amount of NOE built up during each accumulation (Divakar, 1990).

An inversion recovery method involving a 180°–τ–90° pulse sequence was employed for determining the T_1 values. About 10–15 τ values ranging from 0.1 to 5.0 s were used to obtain each set of data. A pulse delay equivalent to 5 times T_1 was employed between the pulses to allow all of the spins to relax completely. If A_α and A_τ are the intensities of the signals at an infinite delay time and for a given value of τ, respectively, ln $(A_\alpha - A_\tau)$ plotted against τ values gave a straight line with a negative slope equal to $1/T_1$ from which T_1 values were obtained. The line of best fit was obtained from least-squares analysis from which T_1 values were determined.

RESULTS AND DISCUSSION

The differences in chemical shift values observed between free α-cyclodextrin (ACD), β-cyclodextrin (BCD), and linoleic and arachidonic acids and the cyclodextrin–fatty acid complexes are shown in Table I. Immediately on complexation, the carboxyl protons from both linoleic and arachidonic acids around 12.0 ppm broadened out completely, indicating that the carboxyl groups are involved in hydrogen-bonding interaction with hydroxyl groups of cyclodextrins. Also, observation of two methylene signals for $-CH_2-$ at position 2 (2.17 and 1.04 ppm) in the arachidonic acid–ACD complex indicated that a certain amount of acid is present in deprotonated form as well. The other protons show little shifts on complexation. However, 1D difference NOE experiments and T_1 measurements have provided quite useful information regarding the geometry of these complexes.

1D Difference NOE Experiments. The protons from cyclodextrin can be classified into three groups. The protons from the wider end of the BCD and ACD cavity (H-2, H-3, 2-OH, and 3-OH) constitute the first group, the protons from the middle portion H-1 and H-4 constitute the second group, and the protons from the narrower end H-5, H-6a, H-6b, and 6-OH form the third group. Irradiation of different protons from fatty acid complexes of cyclodextrin affects mainly those protons either from the

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Table I. Chemical Shift Values of Linoleic Acid, Arachidonic Acid, and Cyclodextrin Complexes

	linoleic acid chemical shift				arachidonic acid chemical shift		
		complex				complex	
assignment	free	BCD[a]	ACD[a]	assignment	free	BCD[a]	ACD[a]
-COOH	11.99			-COOH	12.04		
-CH=CH- 9, 10, 12, 13	5.32	5.31	5.31	-CH=CH- 5, 6, 8, 9, 11, 12	5.32	5.31	5.34
$-CH_2-$ (11)	2.72	2.72	2.72	$-CH_2-$ 7, 10, 13	2.78	2.75	2.77
2	2.18	2.17	2.17	2	2.19	2.18	2.19 (1.04)[b]
8, 14	2.00	2.00	2.00	4, 16	2.02	2.02	2.02
17	1.47	1.46	1.44	19	1.55	1.53	1.53
3-7, 15, 16	1.25	1.26	1.26	3, 17, 18	1.29	1.25	1.25
18	0.85	0.84	0.85	20	0.85	0.84	0.85
BCD				BCD			
2-OH	5.75	5.75				5.75	
3-OH	5.70	5.70				5.70	
H-1	4.83	4.82				4.82	
6-OH	4.49	4.50				4.50	
H-4, H-5, H6a and b	3.63-3.54		3.62-3.53			3.62-3.53	
H-3 and H-2	3.40-3.31		3.45-3.30			3.43-3.30	
ACD		complex ACD				complex ACD	
2-OH	5.53	5.54				5.55	
3-OH	5.48	5.49				5.48	
H-1	4.81	4.80				4.83	
6-OH	4.51	4.53				4.55	
H-4, H-5, H6a and b	3.77-3.55		3.77-3.55			3.77-3.65	
H-3, H-2	3.40-3.28		3.40-3.28			3.48-3.23	

[a] ACD, α-cyclodextrin; BCD, β-cyclodextrin. [b] Two peaks were observed at 2.19 and 1.04 ppm for the $-CH_2$ adjacent to -COOH (see text).

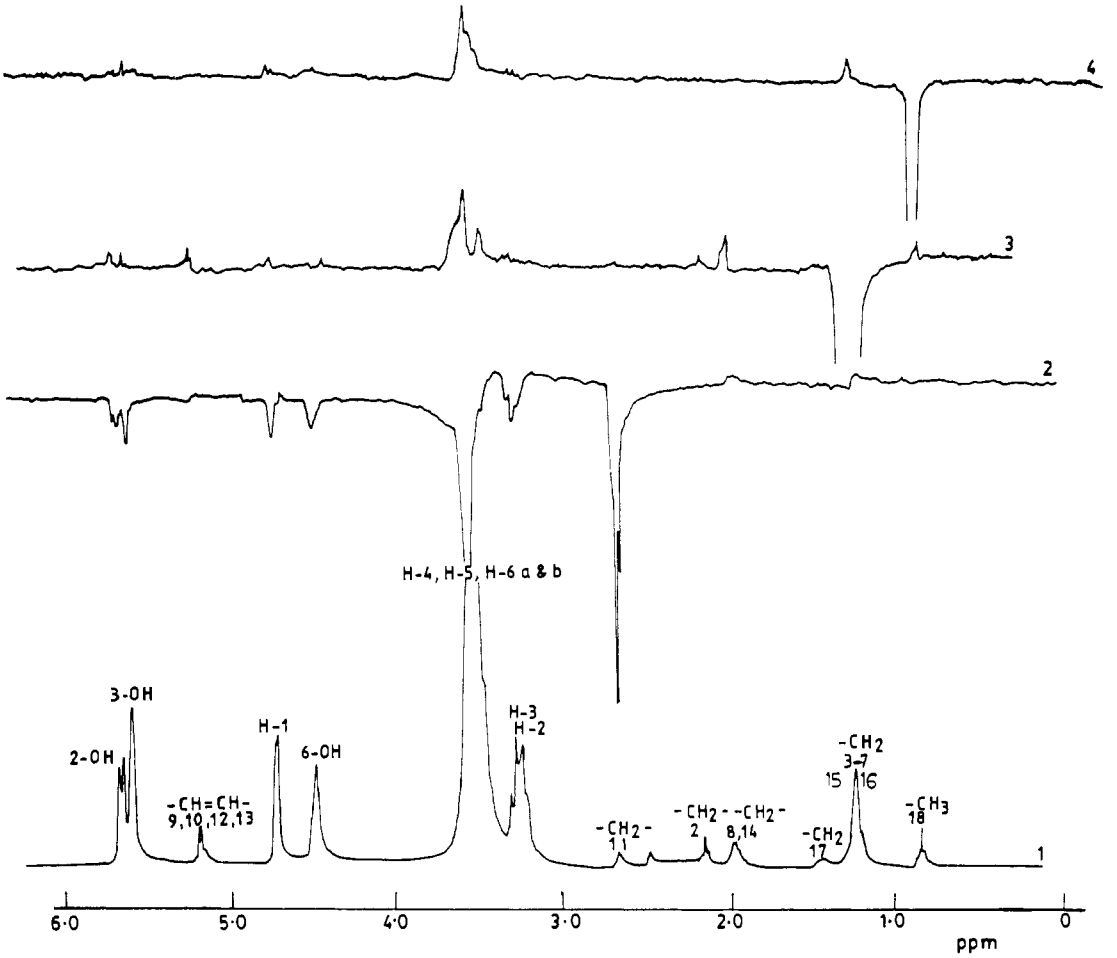

Figure 1. 1D difference NOE spectra at 270 MHz for linoleic acid–β-cyclodextrin complex (1) irradiated at off-resonance, (2) irradiated at $-CH_2-$ (11), (3) irradiated at $-CH_2-$ (3-7, 15, 16), and (4) irradiated at $-CH_3$ (18). Equimolar (0.047 M) solutions of fatty acid and cyclodextrin in DMSO-d_6 were used.

same molecule or in the other molecule that are proximal through either space or bond.

In the linoleic acid–BCD complex, irradiation of olefinic protons at 5.32 ppm from positions 9, 10, 12, and 13 affected the upper plane protons of BCD (Figures 1 and 3). Also, similar effects were observed when $-CH_2-$ protons (allylic) at positions 8, 11, and 14 were irradiated. When the $-CH_3$ at position 18 was irradiated, it affected 2-OH

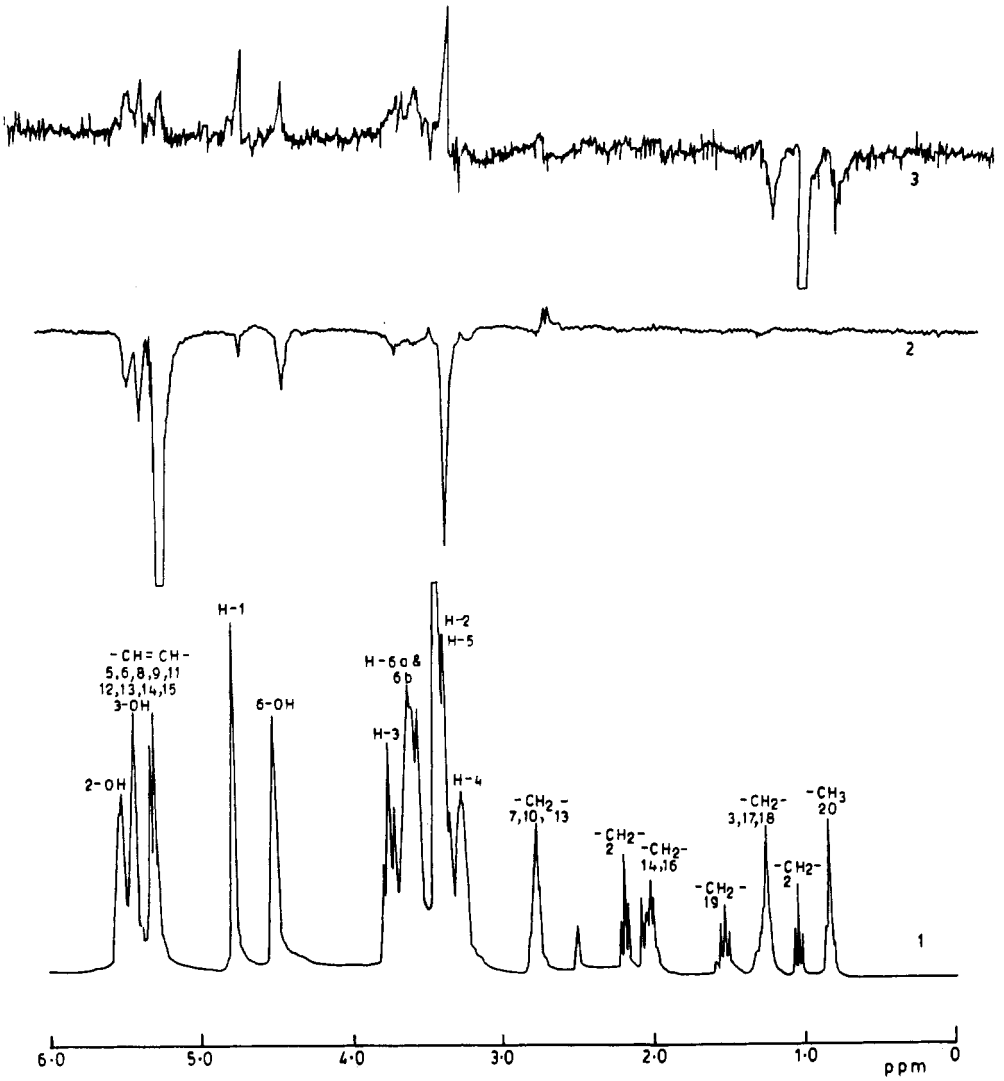

Figure 2. 270-MHz 1D difference NOE spectra for arachidonic acid–α-cyclodextrin complex (1) irradiated at off-resonance, (2) irradiated at –CH=CH–, and (3) irradiated at $-CH_2-$ (3, 17, 18). Other experimental conditions are given under Materials and Methods.

along with H-1 and some of the lower plane protons. The latter portion protons were affected even when $-CH_2-$ at position 17 was irradiated. Also, saturation of $-CH_2-$ at position 2 affected the allylic protons at positions 8 and 14. Similar effects were observed on these linoleic acid signals when the corresponding signals from cyclodextrin groups were irradiated. Since similar types of protons possess the same chemical shift values, such as the $-CH_2-$ at positions 3, 7, 15, and 16 and –CH=CH– at positions 9, 10, 12, and 13, each irradiation position represents more than one similar group except for a few signals. Hence, specific effects of irradiation could not be delineated accurately and the interpretation of the effects was circumstantial. Observed intramolecular proximities in linoleic acid in the complex are between positions 2 and 18, 8 and 11, 11 and 14, and 2 and 17 (Figure 3). The effects were much more pronounced and distinct when the above experiments were tried with the α-cyclodextrin–linoleic acid system.

1D NOE experiments of arachidonic acid complexes of BCD and ACD provided some interesting details (Figures 2 and 3). As in the above case, olefinic protons showed proximity to protons from the upper plane of cyclodextrins. Also, the closeness of positions 2, 19, and 20 $-CH_2-$ and $-CH_3$ protons to the protons from the lower part of cyclodextrins (H-4, H-1, H-5, H-6a and b) was observed. Intramolecular proximities between positions 2 and 10, 4 and 7 (16 and 13), 2 and 19, and 2 and 20 were also observed. In spite of the magnitude of NOE effects being larger for ACD than BCD, the effects observed were similar and no characteristic differences were observed in the pattern of perturbation.

One of the significant features noticed was the sign of the NOE intensities in the cyclodextrin–fatty acid complexes. In the case of unsaturated fatty acid–cyclodextrin complexes, while the proximal protons (to the irradiated ones) showed negative NOE values, the distant ones exhibited positive values. For example, saturation of olefinic protons in the linoleic acid–ACD complex produced negative effects in cyclodextrin protons and positive effects within its own frame. Similarly, perturbation of 6-OH produced negative effects in ACD protons while it produced positive effects in the arachidonic acid signals. Also, signals like $-CH_2-$ at position 2 (3, 17, 18) in the arachidonic acid–BCD complex produced positive effects for the arachidonic acid protons. Other similar effects are shown in Figures 2 and 3.

While cyclodextrin protons may obey the condition $\omega^2\tau_c^2 \ll 1$ (at 270 MHz; ω is the larmor precision frequency, τ_c is the correlation time; $\omega = 1.696 \times 10^9$ rad S^{-1}), the groups from unsaturated fatty acids may follow the

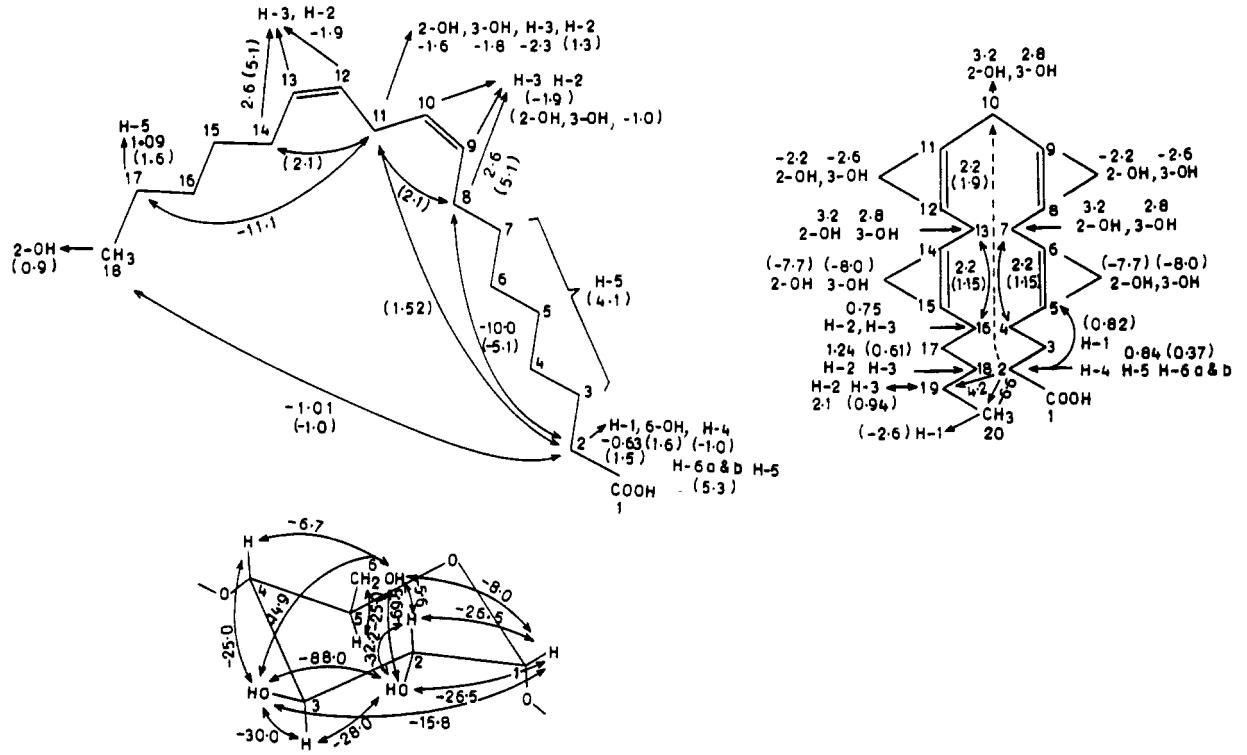

Figure 3. 1D difference NOE connectivities between α-cyclodextrin, β-cyclodextrin, and linoleic and arachidonic acid complexes. Those of free fatty acids are not shown. Values and assignments in parentheses are for α-cyclodextrin complexes. Values less than 1% are ignored. For cyclodextrins some maximum intramolecular NOE values are shown irrespective of α or β. Intramolecular NOEs are indicaterd by arrows; intermolecular NOEs are shown by specifying the group from host which show maximum NOE values when the particular groups in question from the guest are perturbed and vice versa. In arachidonic acid and linoleic acid intramolecular NOEs of vicinal protons are not shown.

condition $\omega^2\tau_c^2 \gg 1$ and vice versa. While the former gives rise to positive NOE, the latter gives rise to negative NOE (Roberts and Jardetzky, 1981). Also, inclusion of one portion of the guest molecule led to a situation where the portions buried and exposed exhibited two different orientations and hence different correlation times (Divakar, 1990). That the observed effects are due to NOE rather than spin diffusion can be clearly inferred by observing different signs for NOE intensities on irradiation of protons from different molecular frames. Use of DMSO-d_6, a viscous solvent, would also enhance τ_c considerably and hence might not lead to $\tau_c = \omega^{-1}$, a non-NOE condition (Wüthrich, 1986). However, the situation may be different in D_2O.

T_1 Measurements. It was found that the groups from both fatty acids on complexation with BCD showed lower T_1 values than those from free fatty acids (Table II). In both linoleic and arachidonic acid complexes, it was found that the $-CH_2-$ at position 2 showed maximum reduction in T_1 values, 40% reduction in linoleic acid, and 66.4% reduction in arachidonic acid, clearly indicating the insertion of carboxyl end in both. Similarly, the extent of reduction was also of slightly lesser magnitude than the former for $-CH_2-$ 17 (linoleic acid, 40%), $-CH_2-$ 19 (arachidonic acid, 65.8%), and $-CH_3$ 20 (arachidonic acid, 63.2%). The decrease in order of reduction in T_1 for linoleic acid was found to be $-CH_2-$ (3–7, 15, 16, 58.4%) > $-CH_2-$ (8, 14, 40.8%) > $-CH{=}CH-$ (9, 10, 12, 13, 30.8%) > $-CH_3$ (18, 23%) > $-CH_2-$ (11, 0%). Similarly for arachidonic acid, the decrease in order of reduction in T_1 was found to be $-CH{=}CH-$ (5, 6, 8, 9, 11, 12, 14, 15, 61.5%) > $-CH_2-$ (3, 17, 18, 58.2%) > $-CH_2-$ (4, 16, 45.5%) > $-CH_2-$ (7, 10, 13, 39.1%). While the olefinic double-bond protons in arachidonic acid showed about 61.5% reduction in T_1, that from linoleic acid showed only 30.89% reduction on complexation, implying that the double bond, especially the one at position 5 in arachidonic acid, is present within the cavity and that in linoleic acid is probably present at the periphery of the cavity. Among the allylic $-CH_2-$ protons, the $-CH_2-$ 11 in linoleic acid did not show any reduction in T_1. However, those at positions 7, 10, 13, 4, and 16 from arachidonic acid showed lowering of T_1 values.

Overall, it is clear from T_1 values that the relative mobilities of groups from fatty acids are affected by complexation with BCD. From the extent of reduction in T_1 values it is also shown that in linoleic acid the carboxyl end is present within the cavity and the methyl end is also

Table II. T_1 Values of Linoleic and Arachidonic Acid Complexes of β-Cyclodextrin[a]

group	T_1, s free	T_1, s β-CD complex	percentage of reduction in T_1
linoleic acid			
$-CH_3$ 18	1.00	0.77	23.00
$-CH_2-$ 3–7, 15, 16	0.77	0.32	58.40
$-CH_2-$ 17	0.48	0.29	39.60
$-CH_2-$ 8, 14	0.71	0.42	40.80
$-CH_2-$ 2	0.55	0.33	40.00
$-CH_2-$ 11	0.63	0.63	00.00
$-CH{=}CH-$ 9, 10, 12, 13	0.91	0.63	30.80
arachidonic acid			
$-CH_3$ 20	1.82	0.67	63.20
$-CH_2-$ 3, 17, 18	0.91	0.38	58.20
$-CH_2-$ 19	1.11	0.38	65.80
$-CH_2-$ 4, 16	0.77	0.42	45.50
$-CH_2-$ 2	1.25	0.42	66.40
$-CH_2-$ 7, 10, 13	0.69	0.42	39.10
$-CH{=}CH-$ 5, 6, 8, 9, 11, 12, 14, 15	1.43	0.55	61.50

[a] An equimolar solution of β-cyclodextrin–fatty acid complex (0.047 M in DMSO-d_6) was used for measuring T_1 values. Error in measuring T_1 values will be about ±10–15%.

affected by complexation. This was found to be true for arachidonic acid also. The presence of free glucose along with linoleic acid did not reduce T_1 values of linoleic acid (not shown), indicating once again that only complexation is responsible for reduction in T_1.

CONCLUSIONS

Both BCD and ACD have deep cavities of the order of 7.0 and 6.7 Å, respectively. It is quite clear that the carboxyl arms of both fatty acids are present inside the cavity. In linoleic acid, the olefinic group is present at the edge of the wider end and the $-CH_2-$ groups (from positions 14 to 17) are located away from the cavity. This will result in olefinic groups (at least the 9–10 double bond) being partly buried and may explain how unsaturated fatty acids included in cyclodextrin are protected against oxygen. However, the proximity of 2, 17, and 18 $-CH_2-$ and $-CH_3$ groups to one another and also to the groups from the lower portion of cyclodextrin indicates that the portion comprising positions 14–18 may assume an angular disposition to the linear axis of the fatty acid molecule. The angular disposition of the methyl end probably leads to steric factors being responsible for reduction in T_1 values. The carboxyl group within the cyclodextrin cavity can be stabilized by hydrogen bonding to the 6-OH groups. However, the hydrogen-bonding interaction of the carboxyl group to the primary hydroxyl protons does not contribute to any significant change in pK_a values of the carboxyl group in the presence of cyclodextrins.

Because of the geometry of cis double bonds at positions 5, 8, 11, and 14 in arachidonic acid, both arms of the molecule, one with the carboxyl end and the other with the methyl end, lie close to one another. While the carboxyl arm is present deeper within the cavity, the methyl arm may lie parallel to the carboxyl arm outside the cavity. Such a disposition may also lead to reduction in mobility of the methyl end and hence reduction in T_1 values. The probability of both the arms being present within the cavity is rather remote.

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Registry No. ACD, 10016-20-3; ACD–arachidonic acid complex, 136762-78-2; ACD–linoleic acid complex, 103597-64-4; BCD, 7585-39-9; BCD–arachidonic acid complex, 136762-79-3; BCD–linoleic acid complex, 103597-65-5; arachidonic acid, 506-32-1; linoleic acid, 60-33-3.