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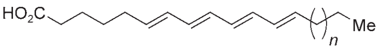
Fatty Acid Aggregation

Aggregation Behavior of Tetraenoic Fatty Acids in Aqueous Solution**

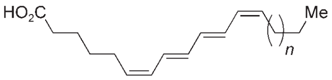
*Yonghui Wang, Jianguo Ma, Hwan-Sung Cheon, and Yoshito Kishi**

In connection with an ongoing program in this laboratory, we synthesized tetraenoic fatty acids (TE-FAs)[1] as a probe[2] for the study of association/dissociation events between fatty acids (FAs) and synthetic 3-*O*-methyl-D-mannose-containing polysaccharides (sMMP)[3,4] and synthetic 6-*O*-methyl-D-glucose-containing polysaccharides (sMGP).[4,5] During spectroscopic characterization of TE-FAs, we observed an interesting phenomenon: C_{20}-*t,t,t,t*-TE-FA (**1c**) exhibits an expected UV absorption at 303 nm with fine structure in methanol but a dramatically different UV absorption at 250 nm without fine structure in aqueous solution (Figure 1 A).



t,t,t,t-TE-FA series

1a: C_{16}-TE-FA (*n*=1); **1b**: C_{18}-TE-FA (*n*=3); **1c**: C_{20}-TE-FA (*n*=5)
1d: C_{22}-TE-FA (*n*=7); **1e**: C_{24}-TE-FA (*n*=9); **1f**: C_{26}-TE-FA (*n*=11)



c,t,t,c-TE-FA series

2a: C_{16}-TE-FA (*n*=1); **2b**: C_{20}-TE-FA (*n*=5); **2c**: C_{24}-TE-FA (*n*=9)

The observed blue shift in the UV absorption appeared to be associated with aggregation of **1c** in buffer solution. Aggregate formation is driven by hydrophobic interaction and is dependent on the polarity of solvents. Figure 1 B shows overlaid UV spectra of **1c** in buffer containing an increasing amount of methanol. As expected, with the increase of methanol content the unique UV absorption at 250 nm changed to the normal absorption of tetraene with fine structure. Similarly, the UV experiment demonstrated that aggregate formation of **1c** is concentration-dependent, with the estimated threshold concentration of aggregation being approximately 0.3 μM.[6] Lastly, sMMP and sMGP were shown to form a 1:1 complex with **1c** in aqueous solution, resulting in disaggregation and bringing the blue-shifted UV absorption back to the normal absorption of tetraenes.[6,7] These results support the view that the blue-shifted UV absorption of **1c** in aqueous solution is indeed associated with aggregation.

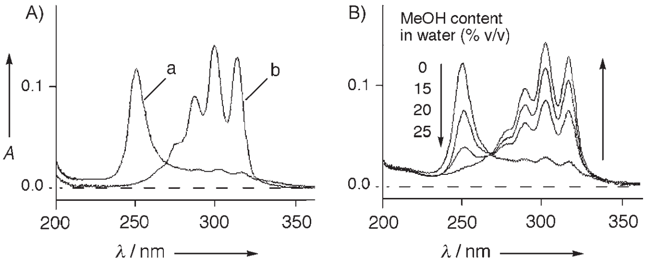


Figure 1. UV absorption spectra of C_{20}-*t,t,t,t*-TE-FA (**1c**; 2×10^{-6} M) at (23 ± 2) °C. A) UV spectra in a) aqueous solution (0.05 M sodium phosphate buffer, pH 7) and in b) methanol. B) UV spectra in aqueous solution with increasing methanol content (0→25% v/v).

The examples known in the literature suggest that the observed blue-shifted UV absorption may be attributed to an H-aggregation of **1c** in buffer solution. An H-aggregate contains a card-pack orientation of **1c**, and according to exciton theory[8] the H-aggregate excitonic splitting results in a high-energy allowed transition and a low-energy forbidden transition (Figure 2). Thus, the UV absorption of **1c** is blue-shifted. Related to this work, we should specifically mention that Whitten and co-workers have shown that FAs which incorporate various chromophores form H-aggregates characterized by a blue-shifted UV absorption.[9,10]

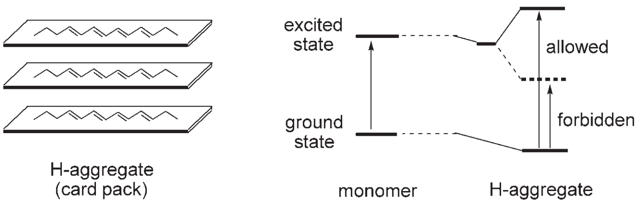


Figure 2. Explanation for the blue-shifted UV absorption of H-aggregates according to exciton interaction theory.

With the unique observations on C_{20}-*t,t,t,t*-TE-FA (**1c**), we selected TE-FAs **1a**–**f** and **2a**–**c** systematically to study the effects of chain length and tetraene geometry on aggregation behaviors.[1] All the TE-FAs exhibited the expected UV absorption with fine structure in methanol (**1a**–**f**: $\lambda_{max} \approx 300$ nm; **2a**–**c**: $\lambda_{max} \approx 304$ nm). The unique blue-shifted UV absorption was observed for the C_{20} (**1c**), C_{22} (**1d**), C_{24}

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[*] Dr. Y. Wang, Dr. J. Ma, Dr. H.-S. Cheon, Prof. Dr. Y. Kishi
Department of Chemistry and Chemical Biology
Harvard University
12 Oxford Street, Cambridge, MA 02138 (USA)
Fax: (+1) 617-495-5150
E-mail: kishi@chemistry.harvard.edu

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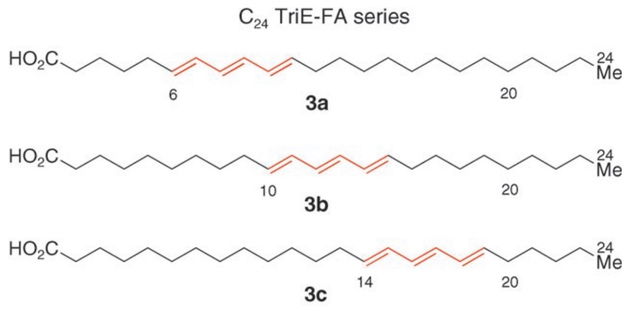
(**1e**), and C_{26} (**1f**) fatty acids in the *t,t,t,t*-TE-FA series, but only for the C_{24} fatty acid (**2c**) in the *c,t,t,c*-TE-FA series,[6] thereby demonstrating that both chain length and tetraene geometry affect aggregate formation.[11]

To gain insight into the aggregate structure, we studied the UV spectral behavior of three C_{24} trienoic fatty acids (TriE-FAs, **3a–c**) with the chromophore located at a different position of the FA backbone.[1] Regardless of the location of the chromophore, all the C_{24}-TriE-FAs exhibited a characteristic blue-shifted UV absorption, thereby demonstrating that the aggregate in question is formed in a head-to-head manner but not a head-to-tail manner.[12]



During the UV spectroscopic study, we noticed an intriguing behavior of C_{24}-*t,t,t,t*-TE-FA (**1e**) in aqueous solution: the sample revealed a UV absorption maximum at 248 nm immediately after its preparation, but after allowing the sample to stand overnight the maximum was shifted to 235 nm (Figure 3 A, curves a and b). Apparently, the initially formed aggregate (designated as the K-aggregate) changed into the thermodynamically more stable aggregate (designated as the T^1-aggregate). The difference in the UV absorption maximum indicated that the T^1-aggregate displays a higher degree of intermolecular electronic interaction between the tetraene chromophores than the K-aggregate, thereby suggesting that the T^1-aggregate exhibits a tighter packing than the K-aggregate. Interestingly, the third aggregate state (designated as the T^2-aggregate) was also detected; when the buffer solution was allowed to stand at room temperature for one week, the UV spectrum changed to the spectrum shown as curve c in Figure 3 A. The observed broad absorptions indicate that this aggregate is not well ordered.[13]

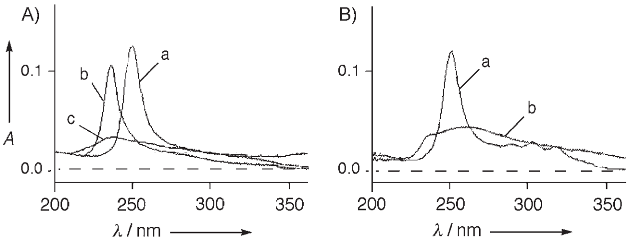


Figure 3. UV spectral behavior of TE-FAs in aqueous solution (0.05 M sodium phosphate buffer, pH 7.0) at (23 ± 2) °C. A) C_{24}-TE-FA (**1e**): a) freshly prepared solution; b) after 3 h; c) after one week. B) C_{20}-TE-FA (**1c**): a) freshly prepared solution; b) after 3 h.

Interestingly, the UV spectral behavior of C_{20}-*t,t,t,t*-TE-FA (**1c**) is different to that of C_{24}-*t,t,t,t*-TE-FA (**1e**). The freshly prepared sample of **1c** displayed a blue-shifted absorption at 250 nm (curve a, Figure 3 B) corresponding to the K-aggregate of **1e**. However, on allowing the sample to stand at room temperature for several hours or overnight, the UV spectrum changed to a broad absorption (curve b, Figure 3 B) corresponding to the T^2-aggregate of **1e**.[13] Although no direct experimental support is available, we assume that, like in the C_{24}-TE-FA (**1e**) series, the T^2-aggregate was formed via the T^1-aggregate but it did not accumulate in the **1c** TE-FA series.

With these results, we then studied the properties of three different types of aggregates formed from *t,t,t,t*-TE-FAs. First, we conducted the doping experiments of K-, T^1-, and T^2-aggregates with saturated (sat-) FAs. As discussed, the unique blue-shifted UV absorption of K- and T^1-aggregates can be attributed to the intermolecular electronic interactions of the tetraene chromophore within the head-to-head H-aggregates. Then, on doping these aggregates with a sat-FA, one expects that the blue-shifted UV absorption should be affected if the sat-FA penetrates into these aggregates. On addition of sat-FA, the H-aggregate of **1c** was conserved, but its composition changed from a homogeneous to a heterogeneous aggregate in which the intermolecular electronic interaction between the tetraene chromophores was prevented. Upon addition of sat-C_{20}-FA, the K-aggregate that formed from C_{20}-*t,t,t,t*-TE-FA (**1c**) exhibited the UV behavior fully consistent with this expectation (Figure 4 A). This property of K-aggregates is referred to as "dynamic". Interestingly, a clear trend was observed that the efficiency of penetration is dependent on the size-matching of sat-FA with C_{20}-*t,t,t,t*-TE-FA (**1c**): C_{20}-sat-FA was far more effective than C_{16}-sat-FA, and C_{12}-sat-FA had virtually no effect.[6]

Upon addition of C_{20}-sat-FA, the broad UV absorption of the T^2-aggregate of **1c** showed virtually no change (Figure 4 B). This UV spectral behavior is indicative that the T^2-aggregate is not penetrated by C_{20}-sat-FA, and this property is referred to as "static".

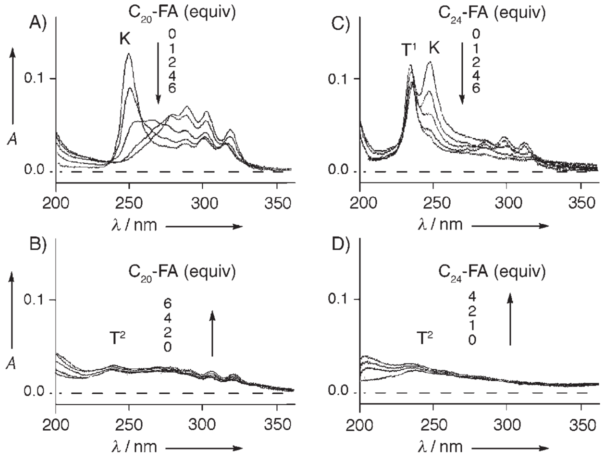
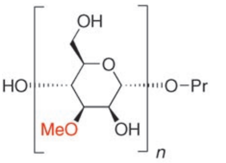


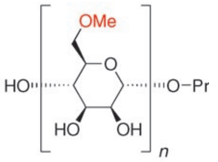
Figure 4. Doping experiments of TE-FA aggregates with sat-FA in aqueous solution (0.05 M sodium phosphate buffer, pH 7.0) at (23 ± 2) °C. A) Doping the K-aggregate of C_{20}-*t,t,t,t*-TE-FA (**1c**) with C_{20}-sat-FA. B) Doping the T^2-aggregate of **1c** with C_{20}-sat-FA. C) Doping the mixture of K- and T^1-aggregates of C_{24}-*t,t,t,t*-TE-FA (**1e**) with C_{24}-sat-FA. D) Doping the T^2-aggregate of **1e** with C_{24}-sat-FA.

Figure 4C illustrates the UV spectral behavior of the aggregates of C_{24}-TE-FA (**1e**). Before addition of C_{24}-sat-FA, the UV spectrum showed that **1e** existed as a mixture of the K-aggregate ($\lambda_{max} = 248$ nm) and the T^1-aggregate ($\lambda_{max} = 235$ nm). Upon addition of C_{24}-sat-FA, only the K-aggregate exhibited the same behavior as observed for the K-aggregate of C_{20}-TE-FA (**1c**), whereas the T^1-aggregate remained virtually unchanged. Interestingly, as with the C_{20}-TE-FA series, a clear trend in the size-matching preference was again noticed.[6] Lastly, the UV experiment summarized in Figure 4D showed that the T^2-aggregate is static. Overall, these doping experiments demonstrated that the K-aggregate is dynamic, whereas the T^1- and T^2-aggregates are almost and practically static, respectively.[14] This conclusion is further supported by titration experiments of the K-, T^1-, and T^2-aggregates with sMGP and sMMP.

sMMP and sMGP are the structural analogues of natural MMP and MGP.[4] Note that, unlike natural MMP and MGP, these synthetic analogues are available as chemically well-defined and homogeneous materials. Also relevant to the present work, we should mention that, like natural MMP and MGP, these synthetic analogues form a 1:1 complex with at least monomeric TE-FAs. This unique property was used to study the dynamic and static natures of K-, T^1-, and T^2-aggregates.



sMMP
n=6–20



sMGP
n=6–20

Figure 5A summarizes the titration experiment of the K-aggregate of C_{20}-TE-FA (**1c**) with sMGP 20-mer ($n = 20$). On addition of sMGP, the characteristic blue-shifted UV absorption changed to that normally found for tetraenes. Owing to the complexation with sMGP, the K-aggregate is disaggregated which gives rise to the normal absorption for tetraenes. Figure 5B presents the titration result of the T^2-aggregate of C_{20}-TE-FA (**1c**) with sMGP 20-mer. On addition of sMGP, the characteristic broad UV absorption of **1c** was almost unaffected. These results support the dynamic and static natures as concluded from the doping experiments with sat-FAs. Likewise, the titration experiments of C_{24}-TE-FA (**1e**) with sMGP 20-mer (Figure 5C,D) gave the results consistent with the previous conclusion; that is, the K-aggregate is dynamic, whereas T^1- and T^2-aggregates are almost and practically static, respectively.[14]

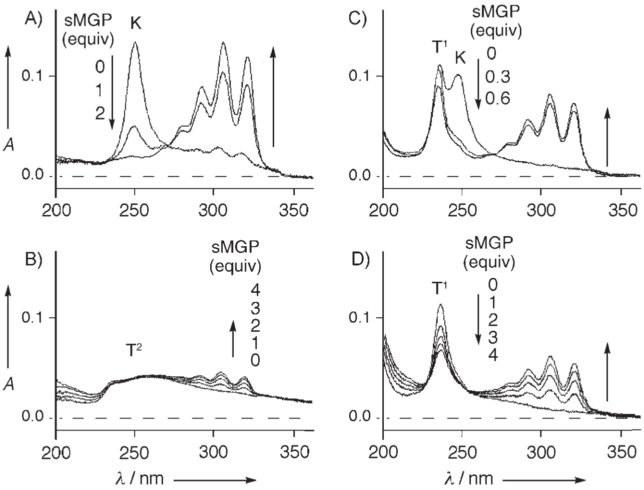


Figure 5. Titration of TE-FAs (2×10^{-6} M) with sMGP 20-mer in aqueous solution (0.05 M sodium phosphate buffer, pH 7.0) at (23 ± 2) °C. A) Titration of the K-aggregate of C_{20}-*t,t,t,t*-TE-FA (**1c**) with sMGP 20-mer. B) Titration of the T^2-aggregate of C_{20}-*t,t,t,t*-TE-FA (**1c**) with sMGP 20-mer. C) Titration of the mixture of K- and T^1-aggregates of **1e** with sMGP 20-mer. D) Titration of the T^1-aggregate of **1e** with sMGP 20-mer. See Supporting Information for the titration of the T^2-aggregate of **1e** with sMGP 20-mer.

Overall, three K-, T^1-, and T^2-aggregates were detected by UV spectroscopy. The reported results demonstrate that 1) both K- and T^1-aggregates of TE-FAs adopt head-to-head H-aggregates, 2) the T^1-aggregate is more tightly packed than the K-aggregate, 3) the K-aggregate is dynamic, but the T^1-aggregate is almost static, and 4) the T^2-aggregate, characterized by its broad UV absorption, is thermodynamically most preferred and static. At present, we have no data to suggest a global structure or even an average molecular size for an aggregate. However, we would speculate that all of the K-, T^1-, and T^2-aggregates are lamellar-type in a local sense but different in the packing-mode of the FA backbone: the K-aggregate is a melted type, the T^1-aggregate is a crystalline type, and the T^2-aggregate is a disordered type with no defined mode of intermolecular electronic interaction between the tetraene chromophores (Figure 6). The experiments on C_{24}-TE-FA (**1e**) uncovered the aggregate-forming processes; that is, an initial formation of the dynamic K-aggregate, which is transformed first into the static T^1-aggregate and then into the static T^2-aggregate.[15] The step of dynamic K-aggregate formation is reversible, whereas the following steps (dynamic K-aggregate→static T^1-aggregate and static T^1-aggregate→static T^2-aggregate) are (almost) irreversible.[14]

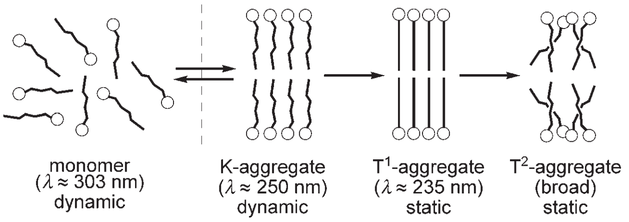


Figure 6. Schematic representation of the formation process and characteristics of the aggregates.

We suggest that these steps are universally involved in the aggregation of TE-FAs and likely sat-FAs as well. To test this notion, we studied the UV spectral behavior of a series of TE-FAs at a concentration of 2 μM in aqueous solution (sodium phosphate buffer, pH 7.0) at (23 ± 2) °C (Figure 7). Under this condition, C_{16}-TE-FA (**1a**) exists as a monomer, C_{18}-TE-FA (**1b**) exists as a mixture of monomer and K-aggregate, and

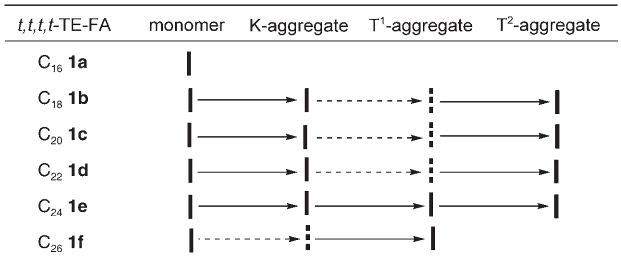


Figure 7. Summary of the steps involved in the aggregation of TE-FAs **1a–f** in aqueous solution (0.05 M sodium phosphate buffer, pH 7.0) at 2×10^{-6} M at (23 ± 2) °C. A solid vertical line represents an aggregate that is detectable by UV spectroscopy, whereas a dashed vertical line represents an aggregate that is not detectable by UV spectroscopy.

C_{20}- (or longer) TE-FAs exist as aggregates. The behavior/properties of C_{20}-and C_{22}-TE-FAs compared well. However, in the C_{26}-TE-FA series the K-aggregate was not detected by UV spectroscopy, and the T^1-aggregate formed was found to be stable at room temperature at least for one week.[13] Overall, on the balance of enthalpy and entropy, each TE-FA exhibited a unique behavior.

Last, we note that the insights gained through this study have led us to 1) uncover intriguing behavior of the chiral aggregates formed from TE-FA derivatives[16] and 2) develop a simple method to follow the association/dissociation events between TE-FAs and sat-FAs with sMMP and sMGP.[7]

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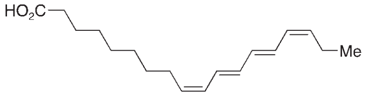
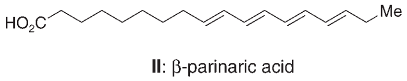
[1] See Supporting Information for the syntheses of TE-FAs and TriE-FAs.

[2] C_{18}-*c,t,t,c*- and C_{18}-*t,t,t,t*-TE-FAs **I** and **II** are known as α- and β-parinaric acids, respectively. Both **I** and **II** were isolated from the plant *Parinarium glaberrimum* [a) J. P. Riley, *J. Chem. Soc.* **1950**, 12; b) L. A. Sklar, B. S. Hudson, R. D. Simoni, *Proc. Natl. Acad. Sci. USA* **1975**, *72*, 1649] and were used as a fluorescent probe for monitoring the complexation between methylated polysaccharides and fatty acids: c) K. K. Yabusaki, C. E. Ballou, *Proc. Natl. Acad. Sci. USA* **1978**, *75*, 691; d) T. Kiho, C. E. Ballou, *Biochemistry* **1988**, *27*, 5824.



I: α-parinaric acid



II: β-parinaric acid

[3] For the isolation and characterization of MMP, see: a) G. R. Gray, C. E. Ballou, *J. Biol. Chem.* **1971**, *246*, 6835; b) M. Ilton, A. W. Jevans, H. B. White III, D. Vance, K. Bloch, *Proc. Natl. Acad. Sci. USA* **1971**, *68*, 87; c) S. K. Maitra, C. E. Ballou, *J. Biol. Chem.* **1977**, *252*, 2459, and references therein.

[4] The design and synthesis of sMMP and sMGP will be reported elsewhere. a) M. C. Hsu, J. Lee, Y. Kishi, unpublished results; b) M. Meppen, Y. Wang, H.-S. Cheon, Y. Kishi, unpublished results.

[5] For the isolation and characterization of MGLP/MGP, see: a) Y. C. Lee, C. E. Ballou, *J. Biol. Chem.* **1964**, *239*, PC3602; b) L. S. Forsberg, A. Dell, D. J. Walton, C. E. Ballou, *J. Biol. Chem.* **1982**, *257*, 3555, and references therein. On the basis of the structure of the polysaccharide isolated from *Mycobacterium bovis* BCG, Rivière suggested a revision on the structure proposed by Ballou; see: G. Tuffal, R. Albigot, M. Rivière, G. Puzo, *Glycobiology* **1998**, *8*, 675. Regarding the structural heterogeneity of MGP, Ballou commented that at least two forms of MGP containing 21 hexoses exist; see: K. Kamisango, A. Dell, C. E. Ballou, *J. Biol. Chem.* **1987**, *262*, 4580; see also: G. Tuffal, R. Albigot, B. Monsarrat, C. Ponthus, C. Picard, M. Rivière, G. Puzo, *J. Carbohydr. Chem.* **1995**, *14*, 631.

[6] See Supporting Information for details.

[7] UV spectroscopy studies showed that **1c** forms a 1:1 complex with sMMP 16-mer and sMGP 16-mer: H.-S. Cheon, Y. Wang, J. Ma, Y. Kishi, *ChemBioChem* **2007**, DOI: 10.1002/cbic.200600499.

[8] a) R. M. Hochstrasser, M. Kasha, *Photochem. Photobiol.* **1964**, *3*, 317; b) M. Kasha, H. R. Rawls, M. A. El-Bayoumi, *Pure Appl. Chem.* **1965**, *11*, 371; c) for a review on this subject, see: P. W. Bohn, *Annu. Rev. Phys. Chem.* **1993**, *44*, 37.

[9] a) D. G. Whitten, *Acc. Chem. Res.* **1993**, *26*, 502; b) N. C. Maiti, S. Mazumdar, N. Periasamy, *J. Phys. Chem. B* **1998**, *102*, 1528.

[10] For the aggregation of fatty acids containing an aromatic system, see: a) *trans*-stilbene: X. Song, C. Geiger, M. Farahat, J. Perlstein, D. G. Whitten, *J. Am. Chem. Soc.* **1997**, *119*, 12481; b) *trans*-azobenzene: X. Song, J. Perlstein, D. G. Whitten, *J. Am. Chem. Soc.* **1997**, *119*, 9144; c) diphenylpolyene: S. P. Spooner, D. G. Whitten, *J. Am. Chem. Soc.* **1994**, *116*, 1240; d) phenyl/biphenyl/terphenyl: H. C. Geiger, J. Perlstein, R. J. Lachicotte, K. Wyrozebski, D. G. Whitten, *Langmuir* **1999**, *15*, 5606.

[11] In preliminary studies, we have noticed that 1) the position of the chromophore in the backbone, 2) the cation of phosphate buffer (i.e. Na^+ vs Mg^{2+}), and 3) the pH (pH 3.0, 7.0, and 10.0) significantly affect the formation and properties of the aggregates.

[12] The aggregate formed from a 1:1 mixture of **3a** and **3c** did not give a characteristic blue-shifted UV peak, but rather gave a broad UV absorption. See Supporting Information for details.

[13] On dilution with methanol, the sample with a broad UV absorption gave the normal UV absorption for tetraenes, thereby verifying that the broad UV absorption does not result from decomposition of the TE-FA.

[14] On the basis of the doping experiment with sat-FAs, we suggest that the process of K- to T^1-aggregates is virtually irreversible. From the titration experiment with sMMPs/sMGPs, however, we noticed that the T^1-aggregate probably disaggregates slowly. Interestingly, in the C_{26}-TE-FA series, both experiments demonstrated that the T^1-aggregate is irreversible. For details, see Figure 10S in the Supporting Information.

[15] In the aggregation of C_{20}-TriE-FAs **4a** and **4c**, all of the K-, T^1-, and T^2-states were detected by UV spectroscopy. See Figure 7S in the Supporting Information.

[16] The aggregation behavior of chiral tetraenoic fatty acids will be reported elsewhere: J. Ma, H.-S. Cheon, Y. Kishi, *Org. Lett.* **2007**, in press.