Structure and Electrochemistry of Self-Organized Fullerene – Lipid Bilayer Films

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Abstract: The unique properties of C_{60}-bearing artificial lipids with three C_{16} (lipid **1**), C_{14} (lipid **2**), or C_{12} (lipid **3**) alkyl chains have been characterized by a variety of techniques, including ^{13}C NMR, UV/Vis, and FT-IR spectroscopies, differential scanning calorimetry (DSC), X-ray diffraction, and electrochemistry. The ^{13}C NMR and UV/Vis spectra show that the lipids **1** – **3** have a closed aziridine structure at a 6/6-ring junction of C_{60}. The DSC data reveal that cast films of **1** exhibit two endothermic peaks at temperature ranges of 35 – 40 °C (main transition) and 47 – 49 °C (subtransition) in air, water, and 0.5 M aqueous tetraethylammonium chloride solution, while cast films of **2** and **3** each display one endothermic peak at 50 – 57 °C. The results of temperature-dependent FT-IR and UV/Vis studies of cast films of **1** – **3**, together with the above data, reveal that the main peak in the DSC thermogram of a film of **1** can be attributed to a typical phase transition as seen in lipid bilayer membranes, while the sub-endothermic peak seen with **1** and the peaks for **2** and **3** stem from a change in the orientation of the C_{60} moieties. X-ray diffraction patterns of each of the cast films of **1** – **3** show a diffraction peak corresponding to the (001) plane, suggesting the formation of molecular bilayer membrane structures. Cyclic voltammograms and Osteryoung square-wave voltammograms obtained from cast films of **1** – **3** on basal plane pyrolytic graphite (BPG) electrodes show strong temperature dependences. Finally, the thermodynamics of the binding of nine different alkylammonium ions and two alkylphosphonium ions to the electrogenerated radical monoanions and dianions of **1** – **3** cast on electrodes is described.

Keywords: electron transfer · fullerenes · lipids · self-assembly · supramolecular chemistry

Introduction

The chemistry and physics of fullerenes have received increasing attention from many research groups.[1] The preparation, characterization, and functionalization of fullerene thin films are also of interest from both a fundamental and a practical point of view.[2] The solution electrochemistry of fullerenes and the electrochemistry of fullerene films have been widely studied and remain the subject of much research effort from both theoretical and experimental approaches.[3] We[4] and others[5] have been interested in combining fullerene chemistry with the chemistry of lipid bilayer membranes. Synthetic lipid bilayer membranes exhibit fundamental physicochemical properties similar to those of biomembranes and, through a variety of means, can be immobilized on various substrates as multibilayer films. We have previously reported that electrodes modified with synthetic lipid bilayer membranes exhibit unique electrochemical behavior arising from the properties of the lipid bilayer membranes themselves.[4, 6] A combination of fullerene chemistry with the chemistry of synthetic lipid bilayer membranes can be expected to open up an exciting area in science.[4]

Several groups, including our own, have described the synthesis of water-soluble, fullerene-bearing amphiphiles and their aggregation structures in water.[7] We report herein, in greater detail, our results concerning the structure of water-insoluble, self-organized thin films of fullerene lipids and the electrochemistry of immobilized thin films of these lipids on electrodes. In a preliminary account of this study,[4a] we reported the synthesis and characterization of a self-assembled triple-chain fullerene lipid **1**, in which the cross-sectional area of the triple alkyl chain is close to that of the C_{60} moiety (Figure 1).[4a] We have previously described the formation of stable monolayers of **1** and the phase-transition-dependent electronic properties of Langmuir – Blodgett films of **1**,[4e] and have presented a theoretical thermodynamic treatment to describe the binding of electrolyte cations to the radical anion and the dianion of the fullerene moieties in a cast film of **1** on

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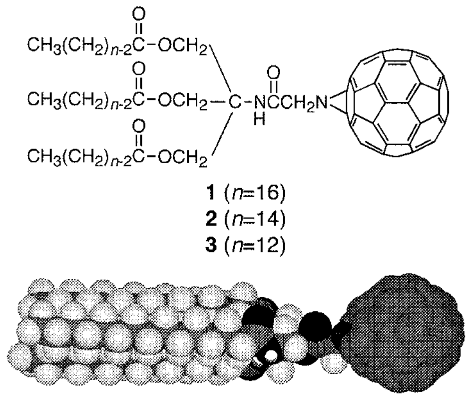


Figure 1. Chemical structure and the CPK model of **1**.

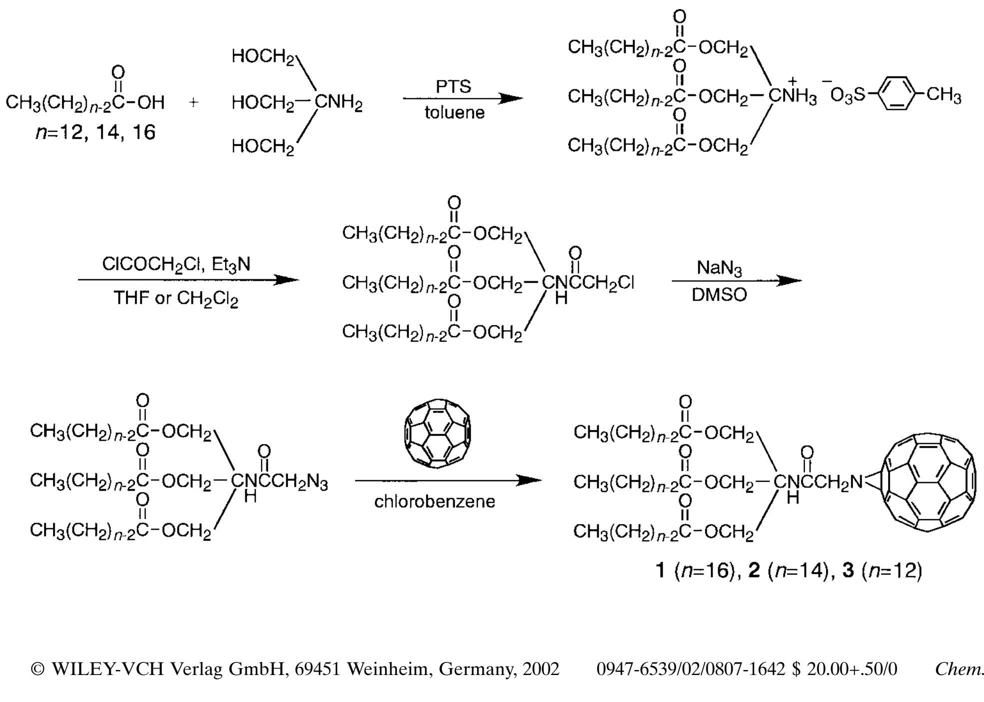
a basal plane graphite (BPG) electrode in aqueous solution.[4f] In the present study, we describe: 1) the structural and spectral characteristics of **1**–**3**, where **2** and **3** are fullerene lipids with three C_{14} or C_{12} alkyl chains, respectively, 2) the electrochemical behavior of cast films of **1**–**3** on electrodes in aqueous solution, and 3) the thermodynamics of the binding between the fullerene radical monoanions and dianions of **1**–**3** and electrolyte cations.

Results and Discussion

Design and synthesis of fullerene lipids and their solubility: Fullerene lipids **1**, **2**, and **3** were prepared by azide addition reactions to C_{60} (Scheme 1), in yields of 26.4, 9.3, and 9.2%, respectively. The ^{13}C NMR spectrum of **1** featured 12 peaks (including four unresolved signals),[4a] while the spectra of **2** and **3** featured 13 peaks (including three unresolved signals) in the region $\delta = 140-150$, thus indicating C_{2v} symmetry of the fullerene moiety.[8] In each of the spectra of **1**–**3**, a peak was seen near $\delta = 83$, corresponding to the two sp^3 hybridized carbons. The UV/Vis spectra of **1**–**3** in hexane each showed a strong absorption at 209 nm and a weak absorption at 420 nm. The absorption at 420 nm has been reported to be specific for closed 6/6-ring-bridged fullerene derivatives.[9] All of these data are consistent with the fact that **1**–**3** have closed aziridine structures at a 6/6-ring junction of C_{60}.

Compounds **1**–**3** are not soluble in water or methanol because of the lack of a highly hydrophilic moiety in their chemical structures. The solubility of the lipids in acetonitrile is also rather limited. In contrast, **1**–**3** are soluble in a variety of organic solvents such as DMF, DMSO, chloroform, benzene, and hexane. The compounds are also soluble, albeit to a limited extent, in ethanol and isopropanol.

Phase transition: The phase transition of lipid bilayers between crystalline and liquid-crystalline phases is one of the most fundamental characteristics of lipid bilayer membranes, and the lipid bilayer properties depend on the change in fluidity of the bilayers.[10] Figure 2 (traces a–c) shows typical differential scanning calorimetry (DSC) thermograms for cast films of **1** in various media. As has been described elsewhere,[4a] cast films of **1** exhibit two endothermic peaks at 35.2 °C (main peak) and 47.0 °C (subpeak) in air. Phase transitions for cast films of **1** appear at 39.7 °C (main peak) and 47.6 °C (subpeak) in the presence of water, at 33.8 °C (main peak) and 47.9 °C (subpeak) in the presence of 0.5 M aqueous tetraethylammonium chloride (TEAC), and at 31.5 °C (main peak) and 43.8 °C (subpeak) in the presence of acetonitrile. It is interesting to note that the phase-transition behavior of **1** does not differ greatly in the various media, that is, the lipid retains a similar molecular orientation in these media. We have previously reported that cast films of artificial lipids can retain an organized lipid bilayer structure and a fundamental bilayer characteristic (phase transition) in organic solvents.[11] Phase-transition temperatures (T_c), transition enthalpies (ΔH), and transition entropies (ΔS) of **1** are summarized in Table 1. Transition entropies (ΔS) indicate the degree of randomness in the



Scheme 1.

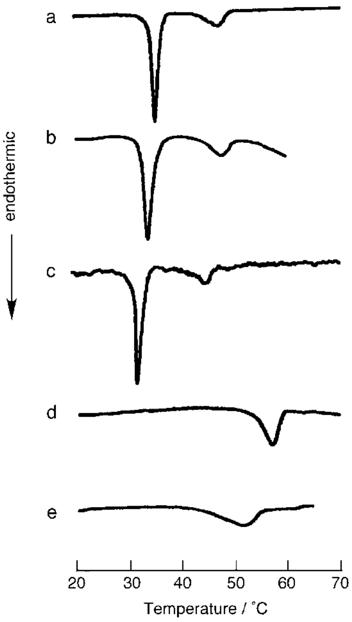


Figure 2. DSC thermograms of cast films of **1** (a–c), **2** (d), and **3** (e) in air (a,d,e), in 0.5 M aqueous TEAC solution (b) and in acetonitrile (c).

Table 1. Phase-transition temperature (T_c) for cast films of **1**–**3**.

Lipid	Medium	T_c [°C]	ΔH [kJ mol^{-1}]	ΔS [J mol^{-1}K^{-1}]
1	air	35.2, 47.0	26.8, 8.9	86.9, 27.8
	H_2O	39.9, 48.6	31.8, 2.5	101.6
	TEAC (0.5 M in H_2O)	33.8, 47.9	30.8, 8.9	100.2, 27.8
	CH_3CN	31.5, 43.8	39.7, 8.3	130.2, 26.2
2	air	57.0	11.9	40.0
3	air	51.5	10.5	32.4

hydrocarbon chain.[10] In this study, the ΔS values per methylene unit for the main peaks of **1** in air, water, and 0.5 M TEAC were evaluated as 1.93, 2.26, and 2.23 J mol^{-1} K^{-1}, respectively. These values are comparable with those of liposomal membranes[10] and synthetic bilayer membranes,[12] thus suggesting that the observed endothermic peaks arise from a transition between crystalline and liquid-crystalline states similar to that observed for aqueous bilayer membranes.[10, 12]

Multiscan DSC measurements on the films of **1** revealed that the main transition soon recovered when the temperature was cycled back after heating up to 40 °C, while ageing at temperatures below 20 °C was required to recover the original two endothermic peaks when the temperature was cycled back from temperatures above the subpeak temperature. This is probably due to the formation of a less ordered structure at such high temperatures.

DSC thermograms of cast films of **2** and **3** exhibit endothermic peaks at 57.0 and 51.5 °C, respectively (Figure 2, traces d and e), which may be attributed to the phase transitions of the fullerene moieties, as discussed in the following section. For aqueous liposomal[10] and synthetic lipid bilayer[12] membranes and for lipid cast films,[6b] the phase-transition temperature decreases with a decrease in the alkyl chain length by about 13–18 °C for every two methylene groups. The phase-transition behavior of **2** and **3** differs from that of liposomal[10] and synthetic lipid bilayer[12] membranes, since we could not detect a main transition peak like that seen for a film of **1**, even when DSC measurements were made over the range −20 to +70 °C.

Spectral properties: In order to assign the endothermic peaks of the DSC thermograms described in the previous section, we examined the temperature dependence of the FT-IR and UV/Vis spectra of cast films of **1**–**3** in air. The wavenumbers of the asymmetric and symmetric methylene stretching vibrations in the FT-IR spectra of the film of **1** changed drastically near 35 °C (Figure 3a). The shifts of $\nu_{as}(CH_2)$ from 2917.9 to 2922.0 cm^{-1} and of $\nu_s(CH_2)$ from 2849.2 to 2851.0 cm^{-1} can be attributed to a *trans–gauche* conformational change of the long alkyl chain,[13] which leads to the phase transition of the thin film. It is evident that the main peak in the DSC thermogram of the film of **1** is attributable to the bilayer phase transition typically observed for liposomal and synthetic lipid bilayer membranes. In contrast, no temperature dependence was observed for cast films of **2** and **3** (Figure 3b,c). The $\nu_{as}(CH_2)$ and $\nu_s(CH_2)$ vibrations appear at 2922.7 and 2851.3–2851.8 cm^{-1} for **2** and at 2921.7 and 2851.3–2851.8 cm^{-1} for **3**. These data suggest that the alkyl chains in both **2** and **3** adopt a *gauche* conformation at the temperatures of the measurements.

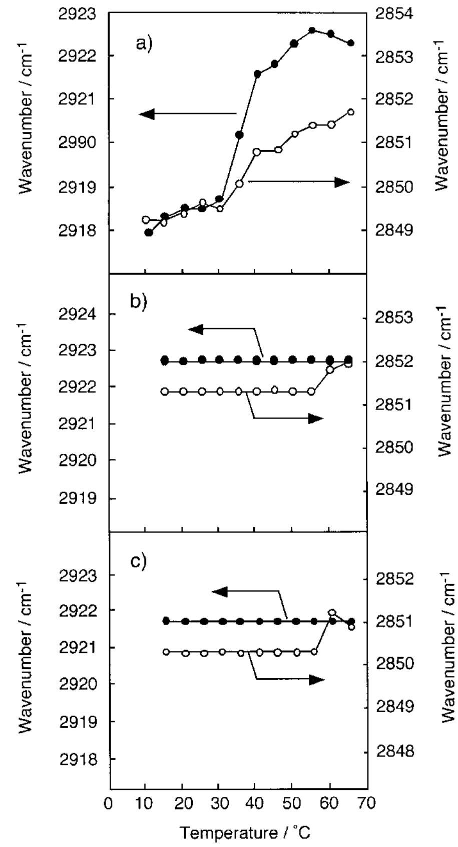


Figure 3. Plots of peak wavenumbers of the antisymmetric (closed circles) and symmetric (open circles) methylene stretching bands in the FT-IR spectra of cast films of **1** (a), **2** (b), and **3** (c) as a function of temperature.

As described previously, the UV/Vis spectrum of a film of **1** in air at temperatures below 35 °C shows three bands (labeled as A, B, and C) with absorption maxima at 215.5, 262.5, and 329.5 nm, respectively; the peak maxima for bands A, B, and C are shifted to longer wavelengths upon increasing the temperature above the subphase transition (Figure 4a), thus indicating the existence of an electronic interaction between the C_{60} moieties in the film of **1**. Similar temperature-dependent spectra were observed for films of both **2** and **3** (Figure 4b,c). The breaks near 55 °C in Figure 4b and 50 °C in Figure 4c are close to the phase-transition temperatures of the corresponding films determined by DSC. Thus, it is evident that the phase transition of the film regulates an electronic interaction between the C_{60} moieties for lipids **1**–**3**.

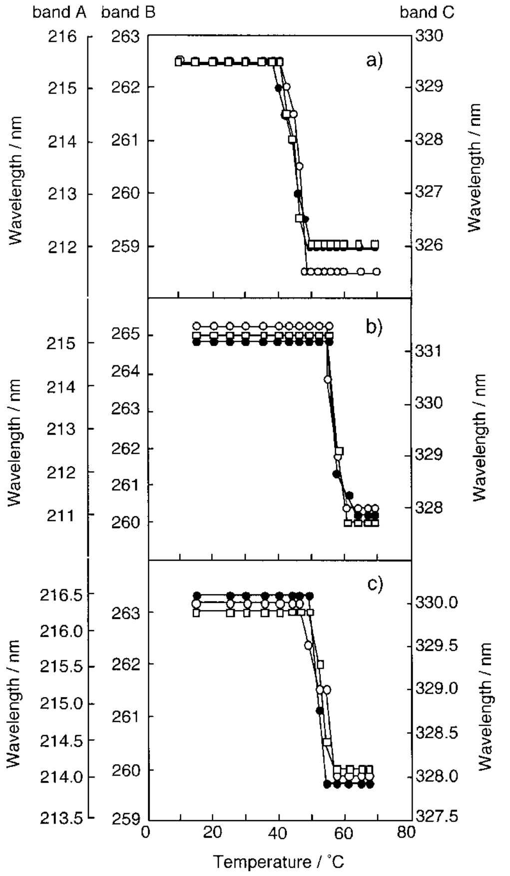


Figure 4. Plots of peak wavelength for bands A (closed circles), B (open squares), and C (open circles) in the UV/Vis spectra of cast films of **1** (a), **2** (b), and **3** (c).

Structure of fullerene lipid bilayer films: Cast films of **1** have a multi-bilayer structure with the molecular layer tilted by 42.8° from the basal plane.[4a] An X-ray diffraction study revealed that cast films of **2** and **3** gave diffraction peaks at $2\theta = 1.97°$ and $2\theta = 2.12°$, respectively (data not shown), which can be assigned to the (001) plane of these films. By assuming molecular lengths of 3.28 nm for **2** and 3.04 nm for **3**, as estimated from CPK space-filling models, it is clear that the cast films of both **2** and **3** form a biomembrane-mimetic multi-bilayer structure like that seen with **1**. Since the cast films of both **2** and **3** have a *gauche* conformation of the alkyl chains (see previous section), the tilting angles from the basal plane of the molecular layers are expected to be smaller than the calculated values,[14] provided that the alkyl chains are in an all-*trans* conformation.

The X-ray diffraction patterns of the films were found to be temperature dependent. The diffraction peak of the film of **1** was maintained at temperatures below 35 °C (which corresponds to the main transition of the film), but on increasing the temperature to 50 °C, a temperature higher than the subphase-transition temperature, the diffraction peak almost disappeared. These results suggest that at higher temperatures the structure of the film of **1** becomes rather disordered. The observed temperature dependence was reversible, that is, when the temperature was cycled back to 25 °C, the X-ray intensity of the films was recovered, although ageing was required (data not shown).

The DSC results, together with the spectral and X-ray data, suggest a possible schematic model for the structure and phase-transition behavior for cast films of **1**–**3** (Figure 5). Phase 1 consists of the lipids in a rigid crystalline state, in which all the alkyl chains have a *trans* conformation. Lipid **1** only forms this state. Phase 2 is a fluid state in which the alkyl chains have a *gauche* conformation, while the orientation of the fullerene moieties remains constant. Phase 3 is a fluid state with a less ordered structure than those in phases 1 and 2.

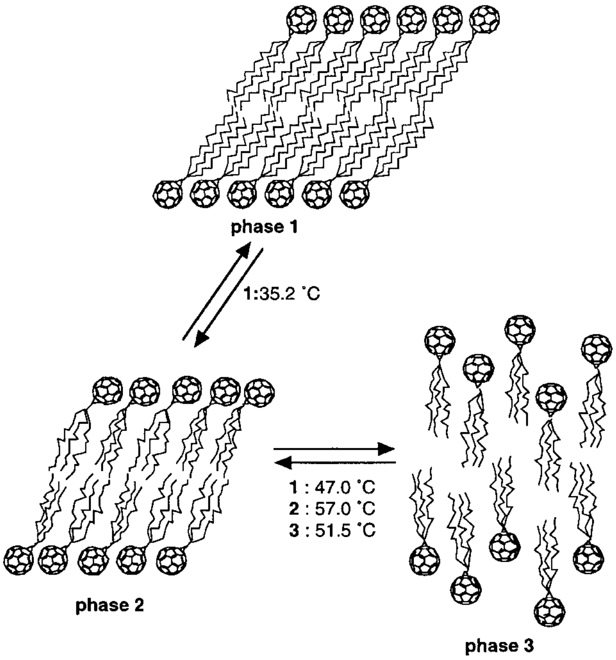


Figure 5. A possible model for the phase transitions of cast films of **1**, **2**, and **3**.

Electrochemical behavior: Typical cyclic voltammograms of a cast film of **1** on a BPG electrode in 0.5 M aqueous tetra-*n*-butylammonium chloride (TBAC) as electrolyte are shown in Figure 6. Well-defined redox waves leading to the generation of the fullerene dianion of **1** can be seen. The first reduction/oxidation process was found to be stable on potential cycling, but this was not case on cycling up to – 1.0 V, at which point

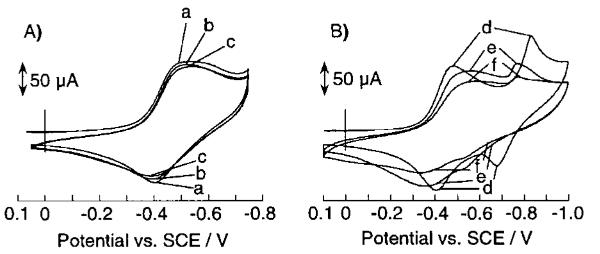


Figure 6. Cyclic voltammograms (a and d: 1st scan, b and e: 5th scan, c and f: 10th scan) at a scan rate of 100 mV s^{-1} for a cast film of **1** on a BPG electrode in 0.5 M aqueous TBAC solution at 55 °C. Scan ranges for A (left) and B (right) are 0.05 to −0.85 V and 0.1 to −1.0 V, respectively.

the fullerene dianion is produced. This results in a decrease in the redox charge, and thus the second reduction current almost disappeared after ten scans. The decrease in the reduction charge suggests the formation of an electroinactive film of the lipid at the electrode surface. Similar cyclic voltammetric behavior was observed for the **2**- and **3**-modified electrodes. Numerical data from the cyclic voltammograms (CVs) on the first scan for the modified electrodes are summarized in Table 2. Despite the differences in the chain lengths, the cathodic and anodic peak potentials, and the formal potential ($E^{0\prime}$) do not differ greatly. In contrast, electroactive amounts of the fullerene moiety on **1**–**3** were found to increase with decreasing alkyl chain length. If one assumes a uniform thickness of the films, more than 140 monolayers of **3** on the BPG electrode are electroactive at 60 °C (see Table 2). In other words, about 50 % of the C_{60} moieties in the film on the electrode are electroactive. Evidently, the fullerene moieties in the films are arranged in such a way as to allow facile electrochemical communication both between each other and with the underlying electrode surface.

Table 2. Electrochemical data for CVs of the modified electrodes in 0.5 M aqueous TBAC solution at 60 °C. E_{pc1} and E_{pa1} are the first cathodic and anodic peak potentials, respectively.

	1-modified electrode	**2**-modified electrode	**3**-modified electrode
E_{pc1} [mV]	−458	−463	−484
E_{pa1} [mV]	−404	−407	−396
$E^{0\prime}$ [mV]	−431	−435	−440
charge[a] [µC]	129	411	926
electroactive fullerene in films[a] [nmol]	1.37	4.26	9.60
electroactive amounts[a] [%] (monolayers)	6.7 (20)	21.3 (62)	48.0 (140)

[a] Data were obtained from the first reduction in the CVs measured at the scan rate of 10 mV s^{-1}.

The scan rate dependence for the first reduction process was also examined (data not shown). For the **1**-, **2**-, and **3**-modified electrodes, both the cathodic and anodic peak currents were found to be proportional to the potential sweep rates (v) in the range 10–30 mV s^{-1}, as expected for thin-layer electrochemical behavior. In contrast, the currents were proportional to the square root of v at 100–3000 mV s^{-1}, as expected for a diffusion-controlled process.

We have previously reported that the electrochemistry of C_{60} embedded in an artificial lipid film is tunable by the temperature-controlled phase transition of the lipid bilayer films on electrodes.[4c] In this study, Osteryoung square-wave voltammetry was used to evaluate the effect of the phase transition of the fullerene films. Typical Osteryoung square-wave voltammograms (OSWVs) for a **1**-modified electrode in water containing TBAC as an electrolyte are shown in Figure 7. Plots of the reduction current for a **1**-modified electrode in water containing TBAC or TEAC as a function of temperature are shown in Figure 8. For both aqueous systems, we see shoulders near 35 and 48 °C, which are close to the main and subphase transitions, respectively, of films of **1**, thus suggesting that the phase transition of the films affects the electrochemistry of the modified electrodes.

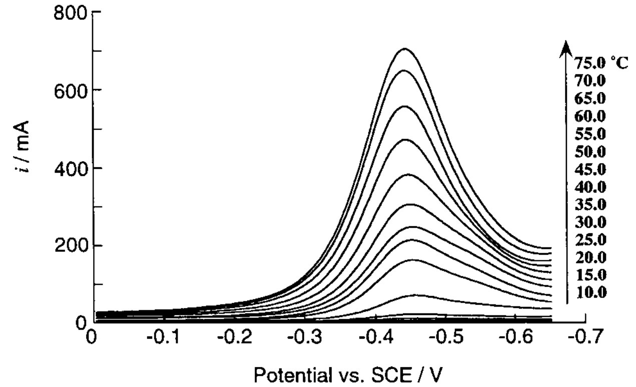


Figure 7. Temperature dependence of the OSWVs (pulse amplitude 25 mV, frequency 15 Hz) for a cast film of **1** on a BPG electrode in 0.5 M aqueous TBAC solution.

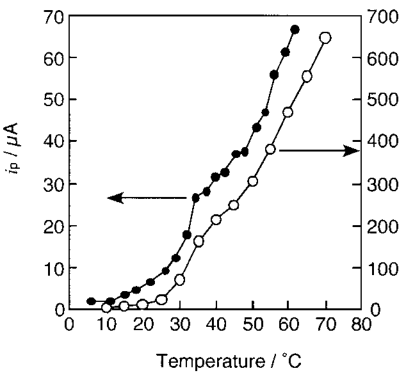


Figure 8. Plots of cathodic peak current as a function of temperature for the OSWVs (pulse amplitude 25 mV, frequency 15 Hz) of a cast film of **1** on a BPG electrode in 0.5 M aqueous TBAC solution (open circles) or 0.5 M aqueous TEAC solution (closed circles).

An unexpected temperature dependence was observed for the OSWVs of both **2**- and **3**-modified electrodes (Figure 9); that is, at temperatures below 25 °C, the modified electrodes showed almost no faradaic current, as is the case for the **1**-modified electrode. The shoulders seen near 30 °C for **2**- and **3**-modified electrodes are not consistent with the transition temperatures obtained by DSC (see Figure 2, traces d and e). It is evident that the phase transition of the fullerene lipids **2** and **3** does not regulate the electrochemistry of the modified electrodes. Similar temperature dependences were observed in the CVs of **1**-, **2**-, and **3**-modified electrodes measured at a scan rate of 1.0 V s^{-1} and for OSWVs at a frequency of 200 Hz

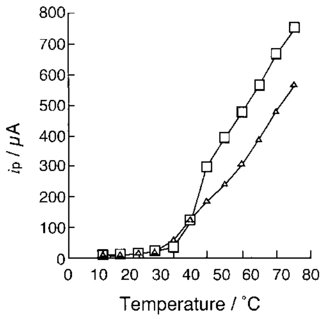


Figure 9. Plots of cathodic peak current as a function of temperature for the OSWVs (pulse amplitude 25 mV, frequency 15 Hz) of cast films of **2** (squares) and of **3** (triangles) on BPG electrodes in 0.5 M aqueous TBAC solution.

and a pulse amplitude of 25 mV. The observed strong temperature dependences might stem from some structural changes of the films that are not detectable by DSC; however, a clear explanation of this phenomenon at the molecular level is difficult based on our present knowledge.

The temperature dependences of the modified electrodes proved to be reversible. Construction of redox-switchable molecular devices[15] based on fullerene films would be of interest with regard to the application and utilization of fullerenes.

Binding of fullerene anions and electrolyte cations: We have previously described the thermodynamics of the binding of electrolyte cations (tetraethylammonium and tetra-*n*-butylammonium ions) to the electrogenerated radical mono- and dianions of fullerene moieties in a molecular bilayer membrane film of **1** cast on an electrode surface.[4f] A theoretical treatment of the binding was based on Equations (1) and (2):

$$E_{1/2,1} = E_1^{0'} + \frac{RT}{F}\ln K_1 + \frac{pRT}{F}\ln C \tag{1}$$

$$E_{1/2,2} = E_2^{0'} + \frac{RT}{F}\ln\frac{K_2}{K_1} + \frac{(q-p)RT}{F}\ln C \tag{2}$$

Here, $E_{1/2,1}$ and $E_{1/2,2}$ are the half-wave potentials for the first and second redox processes,respectively, $E_1^{0'}$ and $E_2^{0'}$ are the standard redox potentials for the two elementary redox processes, p and q are numbers of cations bound to fullerene anions in the first and second reduction processes, respectively, K_1 and K_2 are the binding constants between the fullerene radical monoanions and the electrolyte cations, respectively, and c is the concentration of the electrolyte (for further details, see ref. [4f]).

We have extended the initial study by using nine different alkylammonium ions and two alkylphosphonium ions. The electrolytes used were: tetramethylammonium chloride (TMAC), trimethylbenzylammonium chloride (TMBAC), TEAC, tetraethylphosphonium chloride (TEPC), triethylbenzylammonium chloride (TEBAC), tetra-*n*-propylammonium chloride (TPAC), tri-*n*-butylbenzylammonium chloride (TBBAC), TBAC, and tetra-*n*-butylphosphonium chloride (TBPC). Details of the analytical treatment of the binding are described in the literature.[4f] The electrochemistry of the modified electrodes was examined using differential pulse voltammetry in the presence of different concentrations of electrolytes. As the electrolytes, aqueous KCl solutions of varying concentrations as well as mixed aqueous solutions of a given electrolyte + KCl were used. For the mixed solutions, the total concentration of the two 1:1 electrolytes was adjusted to 0.5 M in order to maintain approximately constant ionic strength. The results are summarized in Table 3. The interesting features are as follows.

1) For all cations used, p is almost 1 and q is almost 2.
2) K_1 shows a strong alkyl chain length dependence. This is in sharp contrast to the binding of electrochemically generated fullerene anions in organic solvents with tetraalkylammonium cations, for which no alkyl chain length dependence was reported.[16]
3) K_1 values for tetraalkylphosphonium cations are about one order of magnitude greater than those for tetraalkylammonium cations having the same alkyl chain length. The "softness" of tetraalkylphosphonium cations would explain this stronger complexation, since fullerene anions are "soft" anions.
4) For the binding with fullerene radical anions, the introduction of a benzyl moiety in the electrolyte shows a stronger effect than that seen with methyl or ethyl groups, but a weaker effect than that seen with a butyl group.

Table 3. Parameters for the binding of the reduced forms of **1** and alkylammonium ions and phosphonium ions at the reduction processes of **1** films on BPG at 328 K.

Electrolyte	p	q	K_1 [M^{-1}]	$\Delta G_{1,328\,\mathrm{K}}$ [kJ mol^{-1}]
TMAC	1.03	–	3.80×10	−9.9
TMBAC	1.08	–	5.24×10^3	−23.4
TEAC	1.05	–	7.93×10^3	−25.7
TEPC	1.16	–	5.00×10^4	−29.5
TEBAC	1.04	–	1.79×10^5	−34.6
TPAC	1.10	2.15	0.96×10^6	−37.6
TBBAC	0.97	1.99	1.01×10^8	−50.3
TBAC	0.97	2.11	1.71×10^8	−50.6
TBPC	1.06	1.98	1.70×10^9	−58.0

The Gibbs free energy change will involve contributions from 1) the displacement of water molecules from the fullerene surface, 2) the interaction between fullerene and tetraalkylammonium ion, and 3) the relaxation of the polarization of the medium by the formation of an ion pair.[4f] Interactions leading to binding would include attractive electrostatic interactions, and hydrophobic interactions between the fullerene anion moieties and the alkyl chains of the cation. A contribution from the $\pi-\sigma$ interaction between the fullerene anion moieties and the alkyl chains might also be involved. We conclude that the binding due to the electrostatic interaction is enhanced by increasing the alkyl chain length, since the value of ΔG_1 increases significantly with increasing chain length.

A similar electrolyte dependence was observed for the **2**- and **3**-modified electrodes, for which, with all the cations used, the p and q values were evaluated as 0.93 – 1.00 and 1.95 – 2.05, respectively. The K_1 values for TEAC, TEPC, and TBAC are 0.96×10^2, 1.67×10^4, and 1.18×10^6 M^{-1}, respectively, at **2**-modified electrodes, while those for TEPC and TBAC at **3**-modified electrodes are 1.90×10^3 and 1.08×10^5 M^{-1}, respectively. The smaller binding constants obtained for **2**- and **3**-modified electrodes compared to **1**-modified electrodes stem from the positive shift[17] of the half-wave potentials of **2**- and **3**-modified electrodes in aqueous KCl, in which the pseudo standard redox potential for the first redox process of the C_{60} moiety on the fullerene lipids was determined (for details, see ref. [4f].

Conclusion

We have described herein the structure and electrochemical behavior of cast films of the fullerene lipids **1**–**3**. DSC, FT-IR and UV/Vis spectroscopies, and X-ray diffraction measurements have revealed that **1**–**3** form multibilayer structure films that exhibit phase transitions attributable to the lipid bilayer phase transition typically observed for liposomal and synthetic lipid bilayer membranes and/or to a change in orientation of the C_{60} moieties. We have been able to represent the phase transitions of the lipid films schematically (see Figure 5). Regulated electrochemistry at the fullerene lipid-modified electrode devices has been possible. The electrogenerated radical mono- and dianions of the fullerene lipids have been found to bind strongly with "soft" electrolyte cations, namely large tetra-*n*-butylammonium and tetra-*n*-butylphosphonium ions. We have pointed out secondary factors that play important roles with regard to complex formation in aqueous systems.

Finally, we would like to emphasize that the present study represents a foundation en route to the construction of fullerene lipid bilayer devices, which might be useful in the field of nanoscience and nanotechnology of fullerenes.

Experimental Section

Materials: DMSO was dried over CaH_2 and distilled twice prior to use; other solvents used in the syntheses were distilled prior to use. Chloroform and benzene used for analytical measurements were of spectrophotometric grade. Water was purified by passage through a Milli-Q Plus ultra-pure water system (Millipore Co.). Its resistivity thereafter was over 18 MΩ cm. C_{60} (>99%) was purchased from Matsubo (Tokyo). All other chemicals used were of reagent grade. *O,O′,O″*-Trihexadecanoyl-*N*-(chloroacetyl)-tris(hydroxymethyl)aminomethane (**4**) was synthesized according to a literature procedure.[18]

***O,O′,O″*-Trihexadecanoyl-*N*-(azidoacetyl)tris(hydroxymethyl)aminomethane (5)**: A solution of **4** (1.9 g, 2.0 mmol) and sodium azide (0.26 g, 4.0 mmol) in dry DMSO (50 mL) was stirred for 19 h at 60 °C under N_2 atmosphere. The reaction mixture was then poured into water and extracted three times with dichloromethane. The combined organic layers were washed three times with brine and dried over anhydrous $MgSO_4$. After evaporation of the solvent in vacuo, the residue was reprecipitated from methanol to afford **5** as a white solid. Yield 1.1 g (59.8%); m.p. 61.0–62.0 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 6.72$ (s, 1H; NH), 4.44 (s, 6H; OCH_2), 4.35 (s, 2H; $COCH_2N_3$), 2.34 (t, $^3J(H,H) = 7.69$ Hz, 6H; CCH_2CO), 1.58 (m, 6H; CH_2CCO), 1.24 (m, 72H; $(CH_2)_{12}$), 0.88 (t, $^3J(H,H) = 6.96$ Hz, 9H; CH_3); IR (KBr): $\tilde{\nu} = 2120, 1740, 1665$ cm^{-1}; elemental analysis calcd (%) for $C_{54}H_{102}N_4O_7$ (919.6): C 70.52, H 11.18, N 6.09; found C 70.80, H 11.20, N 5.79.

Hexadecanoic acid, 2-[(1′*H*-[5,6]fullereno-C60-*I*$_h$-[1,9-*b*]azirin-1′-ylacetyl)-amino]-2-{[(1-oxohexadecyl)oxy]methyl}-1,3-propanediyl ester (fullerene lipid 1): The synthetic procedure has been described in brief in ref. [4a]. A solution of **5** (147 mg, 0.16 mmol) in chlorobenzene (10 mL) was added dropwise to a gently refluxing solution of C_{60} (115 mg, 0.16 mmol) in chlorobenzene (70 mL) over a period of 10 min under N_2 atmosphere, and then the mixture was vigorously refluxed for 24 h. The solvent was subsequently evaporated in vacuo, and the residue was purified by chromatography on SiO_2 (eluent: ethyl acetate/hexane, 5:1) to give compound **1** as a dark brown solid. Yield: 68 mg (26.4%); m.p. 50.0–51.0 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.88$ (s, 1H; NHCO), 4.61 (s, 6H; OCH_2), 3.91 (s, 2H; $COCH_2N$), 2.33 (t, $^3J(H,H) = 7.51$ Hz, 6H; CH_2CO), 1.60 (m, 6H; CH_2CCO), 1.26 (m, 72H; $(CH_2)_{12}$), 0.88 (t, $^3J(H,H) = 6.78$ Hz, 9H; CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 173.24$ (COO), 168.10 (NHCO), 141.1, 141.6, 142.1, 143.0, 143.2, 143.8, 144.2, 144.4, 144.7, 145.0, 145.4, 145.5 (sp^2 of C_{60}), 83.5 (sp^3 of C_{60}), 62.7 (OCH_2), 58.3 (CNH), 54.1 ($\mathrm{CO}\mathit{C}\mathrm{H_2N}$), 34.2 ($\mathit{C}\mathrm{H_2COO}$), 32.0 ($\mathit{C}\mathrm{H_2CH_2COO}$), 29.6 ($(CH_2)_{10}$), 25.0 ($\mathrm{CH_3CH_2}\mathit{C}\mathrm{H_2}$), 22.7 ($\mathrm{CH_3}\mathit{C}\mathrm{H_2}$), 14.1 ($CH_3$); IR (KBr): $\tilde{\nu} = 1740, 1660$ cm^{-1}; UV/Vis (*n*-hexane): λ_{max} (ε) = 420.5 (4450), 322.0 (37100), 255.0 (105700), 209.5 nm (115400 mol^{-1} dm^3 cm^{-1}); FAB-MS (*m*-NBA): *m*/*z* (%): 1611 (1.1) [M^++1], 720 (18.1) [C_{60}^+]; elemental analysis calcd (%) for $C_{114}H_{102}N_2O_7 \cdot H_2O$ (1630.2): C 84.00, H 6.43, N 1.72; found C 84.31, H 6.61, N 1.97.

Tetradecanoic acid, 2-[(1′*H*-[5,6]fullereno-C60-*I*$_h$-[1,9-*b*]azirin-1′-ylacetyl)-amino]-2-{[(1-oxotetradecyl)oxy]methyl}-1,3-propanediyl ester (fullerene lipid 2) and dodecanoic acid, 2-[(1′*H*-[5,6]fullereno-C60-*I*$_h$-[1,9-*b*]azirin-1′-ylacetyl)amino]-2-{[(1-oxododecyl)oxy]methyl}-1,3-propanediyl ester (fullerene lipid 3): Compounds **2** and **3** were synthesized in a similar manner as that described for **1**. After our preliminary report of this study had been published,[4a] Jie and co-workers[19] described the synthesis of fullerene lipids with similar chemical structures.

Analysis for **2**: Yield 66 mg (9.3%); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.86$ (s, 1H; NHCO), 4.61 (s, 6H; OCH_2), 4.34 (s, 2H; OCH_2NC_{60}), 2.34 (t, $^3J(H,H) = 7.70$ Hz, 6H; CH_2COO), 1.63 (m, 6H; CH_2CH_2COO), 1.24 (m, 60H; $(CH_2)_{10}$), 0.88 (t, $^3J(H,H) = 6.78$ Hz, 9H; CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 173.2$ (COO), 168.1 (NHCO), 140.0, 141.1, 141.5, 142.2, 143.0, 143.2, 143.9, 144.7, 144.8, 144.9, 145.4, 146.2, 147.3 (sp^2 for C_{60}), 83.4 (sp^3 for C_{60}), 62.7 (OCH_2), 58.3 (CNH), 54.1 ($\mathrm{CO}\mathit{C}\mathrm{H_2N}$), 34.1 ($\mathit{C}\mathrm{H_2COO}$), 31.9 ($\mathit{C}\mathrm{H_2CH_2COO}$), 29.5 ($(CH_2)_8$), 24.9 ($\mathrm{CH_3CH_2}\mathit{C}\mathrm{H_2}$), 22.7 ($\mathrm{CH_3}\mathit{C}\mathrm{H_2}$), 14.1 ($CH_3$); IR (neat): $\tilde{\nu} = 2923, 2851, 1743, 1685$ cm^{-1}; UV/Vis (*n*-hexane): λ_{max} (ε) = 421.5 (2900), 323.0 (42000), 255.5 (140000), 209.5 nm (130000 mol^{-1} dm^3 cm^{-1}); TOF-MS (α-CHCA): *m*/*z* (%): 1550 (28.6) [M^++Na], 721 (11.9) [C_{60}^++1]; elemental analysis calcd (%) for $C_{108}H_{90}N_2O_7 \cdot 0.5\,H_2O$ (1537.0): C 84.40, H 5.97, N 1.82; found C 84.33, H 6.06, N 1.80.

Analysis for **3**: Yield 71 mg (9.2%); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.86$ (s, 1H; NHCO), 4.61 (s, 6H; OCH_2), 4.35 (s, 2H; $COCH_2NC_{60}$), 2.34 (t, $^3J(H,H) = 7.70$ Hz, 6H; CH_2COO), 1.63 (m, 6H; CH_2CH_2COO), 1.25 (m, 48H; $(CH_2)_8$), 0.87 (t, $^3J(H,H) = 6.78$ Hz, 9H; CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 173.2$ (COO), 168.1 (NHCO), 140.0, 141.1, 141.6, 142.3, 143.0, 143.3, 143.9, 144.7, 144.8, 144.9, 145.4, 146.1, 147.2 (sp^2 of C_{60}), 83.4 (sp^3 of C_{60}), 62.7 (OCH_2), 58.3 (CNH), 54.1 ($\mathrm{CO}\mathit{C}\mathrm{H_2N}$), 34.2 ($\mathit{C}\mathrm{H_2COO}$), 31.9 ($\mathit{C}\mathrm{H_2CH_2COO}$), 29.5 ($(CH_2)_6$), 24.7 ($\mathrm{HC_3CH_2}\mathit{C}\mathrm{H_2}$), 22.7 ($\mathrm{CH_3}\mathit{C}\mathrm{H_2}$), 14.1 ($CH_3$); IR (neat): $\tilde{\nu} = 2922, 2850, 1742, 1686$ cm^{-1}; UV/Vis (*n*-hexane): λ_{max} (ε) = 421.5 (2600), 322.5 (41000), 255.5 (130000), 209.0 nm (120000 mol^{-1} dm^3 cm^{-1}); TOF-MS (α-CHCA): *m*/*z* (%): 1466 (32.1) [M^++Na], 721 (16.5) [C_{60}^++1]; elemental analysis calcd (%) for $C_{102}H_{78}N_2O_7 \cdot 0.5\,H_2O$ (1452.8): C 84.33, H 5.48, N 1.93; found C 84.30, H 5.64, N 1.79.

DSC of fullerene lipid films: A 10 μL portion of a solution of **1** (or **2** or **3**) (0.5 mg) in chloroform was placed in a DSC sample vessel and the solvent was evaporated by air-drying overnight. DSC measurements were made on a Shimadzu DSC-60 apparatus in air or in the presence of 20 μL of water, water containing 0.5 M tetraethylammonium chloride, or acetonitrile at a heating rate of 2.0 °C min^{-1}.[6b]

FT-IR measurements: A 20 μL portion of a solution of **1** (or **2** or **3**) (1.0 mM) in chloroform was spread on a CaF_2 plate, and the solvent was evaporated by air-drying overnight. The modified plate thus obtained was placed in a temperature-controlled flow-through cell (Harrick Scientific Corporation).[20] Temperatures were kept constant to within ±0.1 °C (Neslab Instruments, Circulator RTE-100). FT-IR spectra were measured on a Nicolet Protégé-460 with 64 scans accumulation for each spectrum.

UV/Vis absorption spectral measurements: A 50 μL portion of a solution of **1** (or **2** or **3**) (1.0 mM) in chloroform was spread on the exterior or interior wall of a quartz cell in order to allow measurement of the spectra of films of **1**–**3**. An Hitachi U-3000 spectrophotometer was used for the measurements.

X-ray diffraction measurements: A cast film of **1** (or **2** or **3**) was prepared on a slide glass. The X-ray diffraction patterns of the films were measured in air on a Rigaku RINT-2000 diffractometer.

Electrochemical measurements: The typical procedure for the preparation of a modified electrode was as follows. A 20 µL portion of a solution of **1** (1.0 mM) in benzene was placed on a BPG electrode (geometric area 0.36 cm^2), and the modified electrode was left to dry in air for a day. It was then annealed in 0.5 M electrolyte solution at 50 °C for 30 min. A temperature-controlled electrochemical cell was used. A saturated calomel electrode (SCE) and a Pt plate electrode were used as the reference and the counterelectrodes, respectively. A BAS-100BW electrochemical analyzer was used for all of the cyclic voltammetry, differential pulse voltammetry, and Osteryoung square-wave voltammetry.

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