Recognition of aqueous flavin mononucleotide on the surface of binary monolayers of guanidinium and melamine amphiphiles

Katsuhiko Ariga, Ayumi Kamino, Hiroshi Koyano and Toyoki Kunitake*†

Supermolecules Project, JST(Former JRDC), Kurume Research Park, 2432 Aikawa, Kurume 839, Japan

Recognition of aqueous flavin mononucleotide (FMN) on the surface of binary monolayers of guanidinium amphiphiles (monoalkyl derivative, C_{18}Gua, or dialkyl derivative, $2C_{18}$Gua) and the melamine amphiphile ($2C_{18}$mela-NN) has been investigated by p–*A* isotherms, FTIR spectroscopy, and XPS measurements. p–*A* Isotherms and FTIR spectra of C_{18}Gua–$2C_{18}$mela-NN (1 : 1) monolayers show that there is no direct hydrogen bonding and/or coulombic interactions between C_{18}Gua and $2C_{18}$mela-NN on pure water and that the C_{18}Gua component is dissolved into the subphase upon compression. In contrast, the presence of aqueous FMN prevented C_{18}Gua molecules from dissolving into the subphase. Maximum condensation was observed at a 1 : 1 ratio in C_{18}Gua–$2C_{18}$mela-NN mixed monolayers on aqueous FMN. XPS analyses revealed that one FMN molecule was bound to one C_{18}Gua–$2C_{18}$mela-NN (1 : 1) unit and binding was saturated at 5×10^{-6} mol dm^{-3} FMN. Peak shifts observed in FTIR spectra indicated that the isoalloxiazine ring in FMN formed hydrogen bonds with $2C_{18}$mela-NN. These results support a model that the isoalloxazine and phosphate functions in FMN are bound *via* hydrogen bonding to melamine in $2C_{18}$mela-NN and guanidinium in C_{18}Gua, respectively. Similar binding behaviour of FMN was observed for mixed monolayers of $2C_{18}$Gua–$2C_{18}$mela-NN.

Hydrogen bonding is highly directive unlike other secondary valence forces and plays an important role in molecular design of receptor–guest systems with high specificity.[1–4] It has been believed that molecular recognition *via* hydrogen bonding is difficult in polar media such as water due to competition from the latter, and most effective designs have been carried out in non-aqueous media. Thermodynamic analyses by Williams *et al.*[5] and Adrian and Wilcox[6] suggested that a molecular design to induce entropic gain upon releasing bound water would compensate an enthalpic disadvantage in polar media. This disadvantage may be avoided by creation of a local non-aqueous environment in water. Nowick *et al.*[7] and Bonar-Law[8] incorporated recognition sites in the hydrophobic core of micelles, while Komiyama *et al.*[9] immobilized hydrogen bonding sites in a water-insoluble polymer.

Unlike these approaches, we have studied the aqueous phase near a hydrophobic phase, *i.e.*, interfaces with water. Our quantum chemical calculation based on a multidielectric model revealed that hydrogen bonding was enhanced at the air/water interface, since electronic properties of molecules located in water close to the hydrophobic phase are affected by the low dielectric medium, thus the molecules behave as if they are in a less polar medium.[10] We have also demonstrated experimentally that molecular recognition through hydrogen bonding is effective at the air/water interface. Nucleotides,[11] nucleic acid bases,[12] sugars,[13] amino acids[14] and peptides[15] dissolved in the aqueous subphase are effectively bound by receptor monolayers. Molecular recognition at the air/water interface has been recently reported by other groups as well.[16–20] This new finding is also applicable to microscopic interfaces formed by micelles and bilayers dispersed in bulk water.[21]

These monolayer interfaces can be composed of a variety of amphiphile molecules, thus creating varied recognition sites. We already reported that a ternary monolayer of guanidinium (C_{18}Gua), diaminotriazine (C_{10}AzoAT) and orotate ($2C_{18}$Oro) recognizes flavin adenine dinucleotide (FAD) [Fig. 1(A)].[22] In this system, guanidinium, diaminotriazine and orotate functions in monolayers are bound to phosphate, isoalloxazine and adenosine units in FAD, respectively. It was also confirmed from AFM observation that a regular pattern of monolayer components was formed upon binding of FAD to a C_{18}Gua–$2C_{18}$Oro mixed monolayer.[23] It is expected that one can make various patterns through combinations of different amphiphiles and aqueous guests.

However, difficulties in preparing desirable recognition systems are still sometimes encountered. One of the major difficulties is undesirable interactions among component amphiphiles. For example, recognition of aqueous AMP by a mixed monolayer of C_{18}Gua and $2C_{18}$Oro [Fig. 1(A)] is not efficient,[22] because ion pairing between guanidinium and orotate competes with their binding to the guest.[24] Two-dimensional crystallization of an azobenzene-type monolayer of C_{10}AzoAT sometimes disturbs the formation of a desirable recognition site.[25] In order to develop mixed monolayer systems which can recognize various kinds of aqueous guests, we have to establish a strategy to avoid unfavourable interactions within monolayers.

Here, we demonstrate recognition of flavin mononucleotide (FMN) by mixed monolayers of guanidinium amphiphiles (monoalkyl derivative, C_{18}Gua, and dialkyl derivative, $2C_{18}$Gua) and a melamine amphiphile ($2C_{18}$mela-NN) [Fig. 1(B), (C)]. The following aspects were considered to achieve effective recognition: (1) a guanidinium component was selected, as the strong interaction between guanidinium and phosphate[11a] would enhance the recognition efficiency; (2) the *N*,*N*-disubstituted $2C_{18}$mela-NN molecule has only one face of the three-point recognition site of the melamine ring. We thus expect a 1 : 1 recognition in contrast to network formation observed for *N*,*N*′-disubstituted melamines;[26] (3) ion pairing can be avoided between guanidinium and melamine under normal conditions.

Results and Discussion

Monolayer behaviour of mixtures of guanidinium and melamine amphiphiles on water

The behaviour of mixed monolayers of C_{18}Gua and $2C_{18}$mela-NN was examined on pure water. The monoalkyl guanidinium amphiphile C_{18}Gua is relatively hydrophilic forming only an unstable monolayer which is readily dissolved into water upon

† Permanent address: Faculty of Engineering, Kyushu University, 1 Hakozaki, Higashiku, Fukuoka 812, Japan.

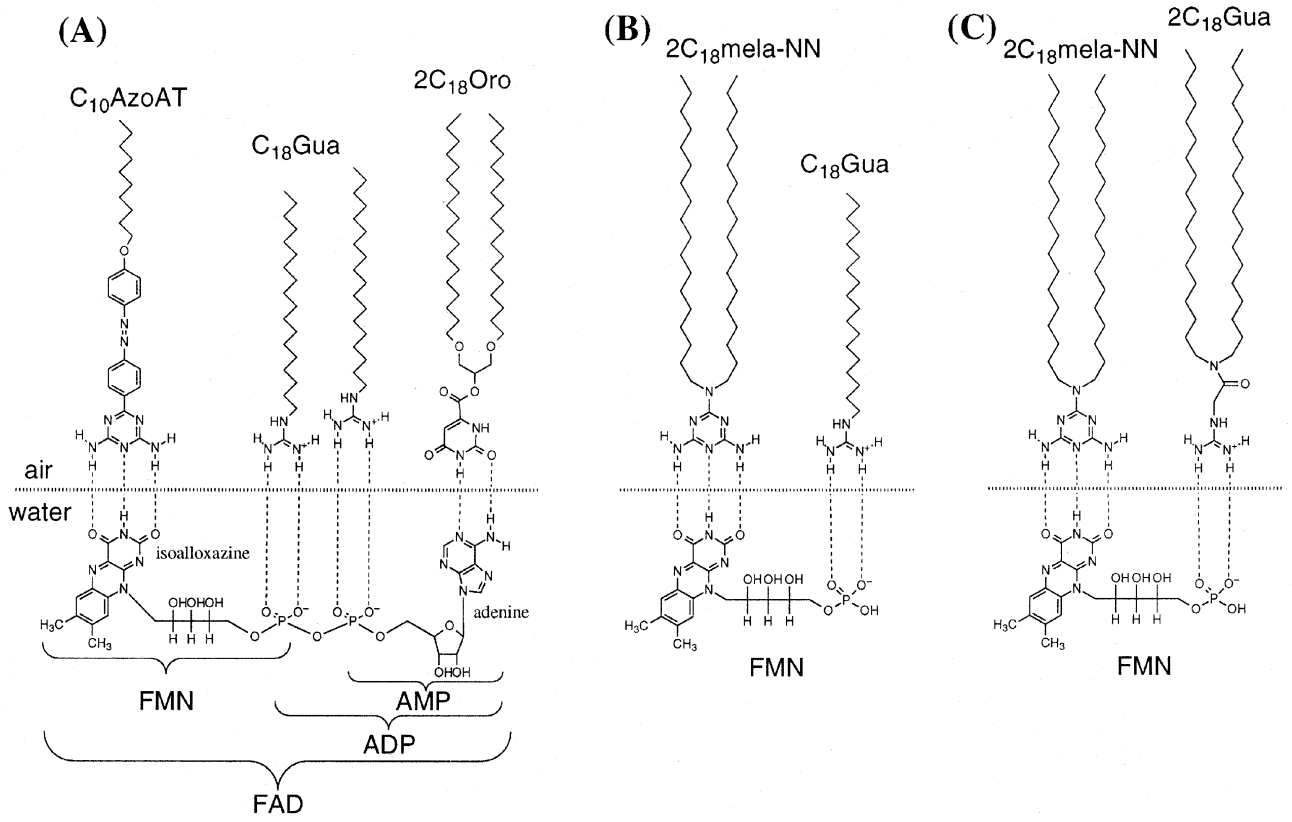

Fig. 1 Multisite binding of mixed monolayers with complementary aqueous guests: A, mixed monolayer of C_{10}AzoAT, C_{18}Gua and $2C_{18}$Oro on FAD; B, mixed monolayer of $2C_{18}$mela-NN and C_{18}Gua on FMN; C, mixed monolayer of $2C_{18}$M and $2C_{18}$Gua monolayers on FMN

compression.[24] Therefore, p–*A* isotherms of the C_{18}Gua monolayer have a poor reproducibility on pure water and the molecular area is smaller than the cross-sectional area of the monoalkyl chain.

p–*A* Isotherms of $2C_{18}$mela-NN and C_{18}Gua–$2C_{18}$mela-NN (1 : 1) monolayers on pure water are shown in Fig. 2(A). Since the molecular areas are normalized by the number of $2C_{18}$mela-NN molecules, the difference in molecular area between the two isotherms represents the area occupied by the C_{18}Gua component in the mixed monolayer. The isotherm of the $2C_{18}$mela-NN monolayer displays only a condensed phase with a limiting area of 0.44 nm^2, indicating formation of a well packed monolayer. The isotherm of C_{18}Gua–$2C_{18}$mela-NN (1 : 1) monolayer is similar in shape to that of the $2C_{18}$mela-NN single-component monolayer. The difference in the molecular area between the two isotherms is only 0.05 nm^2, and is much smaller than the cross-section of one monoalkyl chain. The area of a mixed monolayer compressed at 30 mN m^{-1} was observed to decrease with time. These results strongly suggest that C_{18}Gua is dissolved in water upon compression in spite of the presence of the $2C_{18}$mela-NN component.

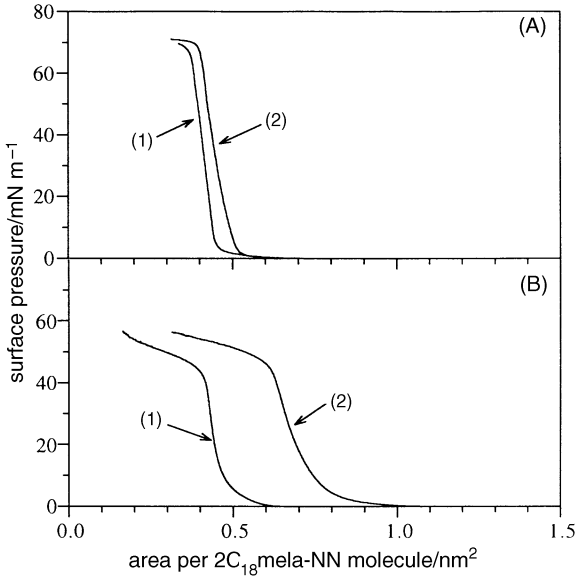

Fig. 2 p–*A* Isotherms of (1) $2C_{18}$mela-NN and (2) C_{18}Gua–$2C_{18}$mela-NN (1 : 1) monolayers at 20 °C: A, on pure water; B, on 1×10^{-5} mol dm^{-3} of aqueous FMN. Molecular area was calculated on the basis of the number of $2C_{18}$mela-NN molecules.

The monolayers on pure water were transferred onto gold-deposited glass plates and their FTIR spectra were measured in the reflection–absorption mode (RAIRS). Fig. 3 shows spectra of a cast film of C_{18}Gua and LB films of $2C_{18}$mela-NN and C_{18}Gua–$2C_{18}$mela-NN (1 : 1). The cast film is a substitute for an LB film of the C_{18}Gua component, since LB transfer of the C_{18}Gua monolayer from pure water was difficult. In the spectrum of C_{18}Gua [Fig. 3(A)], ν(C=N) and δ(NH) peaks of the guanidinium moiety are seen at 1680 and 1628 cm^{-1}, respectively.[27] The triazine ν(C=N) peak at 1579 cm^{-1} and broad δ(NH) peak at 1600–1700 cm^{-1} are observed in the spectrum of $2C_{18}$M LB film [Fig. 3(B)].[28] The spectrum of the C_{18}Gua–$2C_{18}$mela-NN (1 : 1) LB film [Fig. 3(C)] is essentially identical to that of the single-component $2C_{18}$mela-NN LB film. The former spectrum does not contain any feature of the C_{18}Gua component. These spectral characteristics clearly reveal dissolution of the C_{18}Gua component into subphase during compression.

These results also indicate the absence of specific (hydrogen bonding and/or coulombic) interaction between C_{18}Gua and $2C_{18}$mela-NN. In the case of a mixed monolayer of C_{18}Gua and $2C_{18}$Oro, guanidinium and orotate functions form a stable 1 : 1 ion pair with specific peak shifts in the FTIR spectrum.[24]

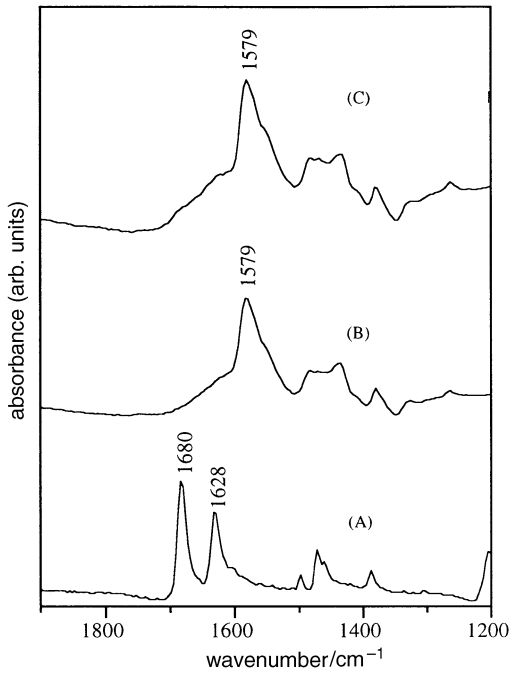

Fig. 3 FTIR spectra of multilayer films (5 Y-type films) on gold-coated glass plates: A, C_{18}Gua cast film; B, $2C_{18}$mela-NN LB film transferred from pure water; C, C_{18}Gua–$2C_{18}$mela-NN (1:1) LB film transferred from pure water

Thus, guanidinium and melamine functions should act on the FMN guest without mutual interference.

p–*A* Isotherms of mixed monolayers of C_{18}Gua–$2C_{18}$mela-NN on aqueous FMN

We subsequently investigated the interaction of aqueous FMN with the mixed monolayer. In remarkable contrast with the preceding results, a monolayer of C_{18}Gua becomes stable on 1×10^{-5} mol dm^{-3} FMN and gives satisfactory reproducibility in its p–*A* isotherm. Apparently, binding of FMN to the monolayer prevents the guanidinium component from dissolution into subphase, as also observed with aqueous FAD.[24] Fig. 2(B) shows isotherms of $2C_{18}$mela-NN and C_{18}Gua–$2C_{18}$mela-NN (1:1) monolayers on 1×10^{-5} mol dm^{-3} FMN. Again, molecular areas are normalized by the number of $2C_{18}$mela-NN molecules. The difference in the molecular area between two isotherms is 0.20–0.25 nm^2, in reasonable agreement with the cross-sectional area of monoalkyl C_{18}Gua.

In order to investigate the stoichiometry of interacting components, p–*A* isotherms of the mixed monolayer were measured in varying mixing ratios on 1×10^{-5} mol dm^{-3} FMN. The data were normalised by the total number of amphiphile molecules used and are shown in Fig. 4(A). Deviations of the observed molecular area from that of an ideal mixture were calculated according to the following equations,[29]

$$A_{\text{ideal}} = xA_{\text{a}} + (1-x)A_{\text{b}} \quad (1)$$

$$\text{Normalized deviation} = (A_{\text{obs}} - A_{\text{ideal}})/A_{\text{ideal}} \quad (2)$$

where A_{a}, A_{b}, A_{ideal}, A_{obs}, and x represent the molecular area of component a, molecular area of component b, mean molecular area for an ideal mixture, observed mean molecular area, and mole fraction of component a, respectively. Normalized deviations obtained with eqn. (2) are plotted in Fig. 4(B). At low surface pressures, the negative deviation (condensation effect) is maximized at an equimolar mixing ratio. This result might be considered to originate from an entropic contribution

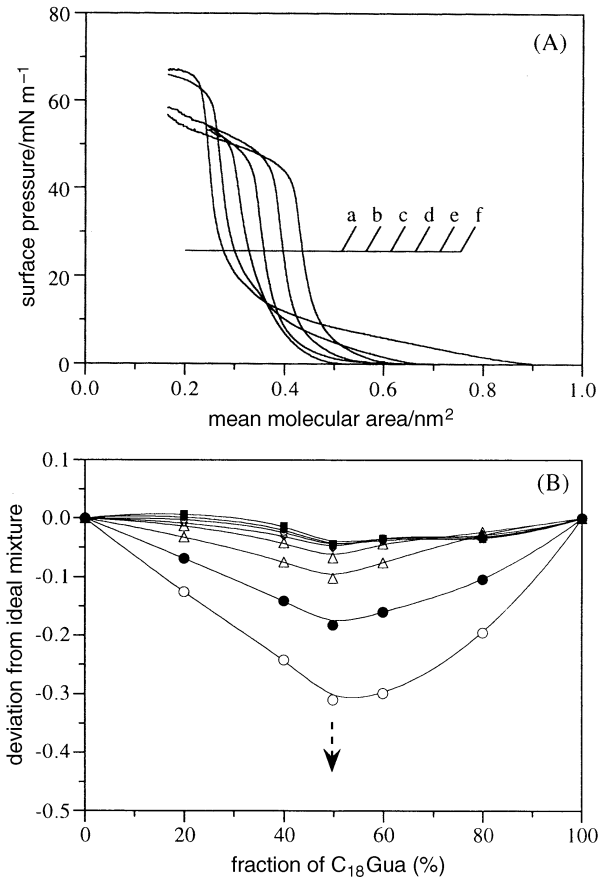

Fig. 4 (A) p–*A* Isotherm of mixed monolayers of C_{18}Gua and $2C_{18}$mela-NN at 20 °C: a, C_{18}Gua 100.0%; b, 79.9%; c, 59.9%; d, 39.9%; e, 19.9%; f, 0.0%. (B) Plots of normalized deviation against the fraction of C_{18}Gua at 5, 10, 15, 20, 25, 30, 35 and 40 mN m^{-1} (from the bottom).

upon mixing. However, it is more likely that condensation of the mixed monolayer *via* FMN binding is most pronounced for the equimolar monolayer. As the surface pressure increases, the condensation effect is suppressed because of increased molecular packing at all the mixing ratios. However, binding of FMN to the monolayer proceeds even at high surface pressures, as confirmed by XPS and FTIR results as discussed below.

Binding analysis of aqueous FMN to mixed monolayers of C_{18}Gua–$2C_{18}$mela-NN by XPS measurements

Quantitative analysis of FMN binding was conducted by XPS measurements. The amount of FMN bound to monolayers was determined from the elemental ratio of phosphorus and nitrogen in XPS measurement of the transferred monolayer (Table 1). The FMN/C_{18}Gua ratio is close to unity for a C_{18}Gua monolayer, indicating that one FMN molecule is bound to each C_{18}Gua molecule; the guanidinium group is stoichiometrically bound to a phosphate unit. Although weak interactions to carbonyl and/or the lone pair on nitrogen in the isoalloxazine unit of FMN are also conceivable,[11b,17b] the equimolar binding ratio observed strongly suggests that the binding occurs between guanidinium and phosphate and that the other possibilities are unlikely.

The ratio of FMN bound to single-component $2C_{18}$mela-NN monolayer is 0.44 under the same conditions. The binding constant reported for aqueous cyclic imide (thymine) and a diaminotriazine monolayer is only 2×10^2 dm^3 mol^{-1},[12a] while the constant between aqueous phosphate (AMP) and a guanidinium monolayer is 3×10^6 dm^3 mol^{-1}.[11a] The hydrogen bond-

Table 1 Binding of FMN to monolayers as determined by XPS[a]

amphiphile unit	[FMN]/mmol dm^{-3}	P (%)	N (%)	R^b
C_{18}Gua	0.01	1.52	9.92	1.07
$2C_{18}$mela-NN	0.01	0.50	11.28	0.44
C_{18}Gua–$2C_{18}$mela-NN (1:1)	0.00	0.00	11.05	0.00
C_{18}Gua–$2C_{18}$mela-NN (1:1)	0.0001	0.55	11.55	0.62
C_{18}Gua–$2C_{18}$mela-NN (1:1)	0.005	0.90	11.04	1.06
C_{18}Gua–$2C_{18}$mela-NN (1:1)	0.01	0.93	10.96	1.10
C_{18}Gua–$2C_{18}$mela-NN (1:1)	0.10	0.94	10.93	1.12
$2C_{18}$Gua–$2C_{18}$mela-NN (1:1)	0.01	0.76	10.10	1.06

[a]LB films (9 or 10 layers) were used for measurement. [b]R = Bound FMN/amphiphile.

ing interactions between a neutral receptor and guest, *e.g.*, $2C_{18}$mela-NN–isoalloxazine (FMN), is less efficient.

The XPS results reveal that the binding ratio of FMN to C_{18}Gua–$2C_{18}$mela-NN (1:1) is close to unity at FMN concentrations $>5\times10^{-6}$ mol dm^{-3}, consistent with the binding motif of Fig. 1(B) where one FMN molecule simultaneously binds to one guanidinium and one melamine. Since the binding efficiency is *ca.* 50% at 10^{-7} mol dm^{-3} FMN and is saturated at 5×10^{-6} mol dm^{-3} FMN, the binding constant is estimated to be in the range of 10^7 dm^3 mol^{-1}.

FTIR examination of the receptor–guest interaction

The mode of the receptor–guest interaction was studied by FT RAIRS spectroscopy of monolayer receptors transferred onto a gold-deposited plate. IR spectral changes caused by FMN binding were characterized separately for the two functional components of the receptor monolayer (Fig. 5 and 6). Fig. 5 shows IR spectral characteristics of C_{18}Gua and FMN in the region 1200–1900 cm^{-1}. According to the literature,[30,31] the peaks observed for an FMN cast film [Fig. 5(C)] are assigned as follows: $\nu(C_4{=}O)$ at 1729, $\nu(C_2{=}O)$ at 1681; $\nu(C{=}N)$ at 1579 and $\nu(C{=}N)$ at 1550 cm^{-1}. The spectrum of a C_{18}Gua LB film transferred from 1×10^{-5} mmol dm^{-3} of aqueous FMN [Fig. 5(B)] is basically a superimposition of the two components, although some peak broadening by overlapping is seen in the 1600–1700 cm^{-1} region. The presence of FMN

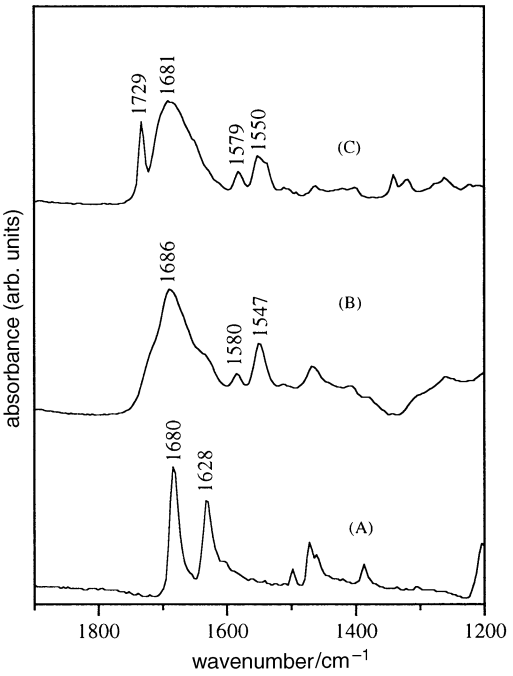

Fig. 5 FTIR spectra of multilayer films (5 Y-type films) on gold-coated glass plates: A, C_{18}Gua cast film; B, C_{18}Gua LB film transferred from aqueous 1×10^{-5} mol dm^{-3} FMN; C, FMN cast film

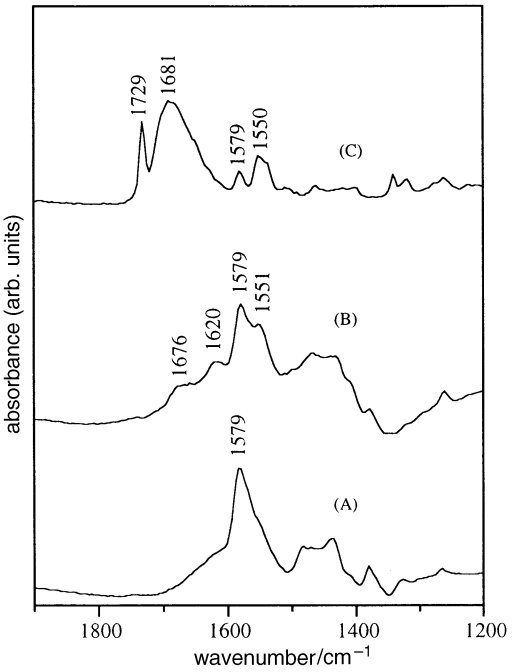

Fig. 6 FTIR spectra of multilayer films (5 Y-type films) on gold-coated glass plates: A, $2C_{18}$mela-NN LB film transferred from pure water; B, $2C_{18}$mela-NN LB film transferred from aqueous 1×10^{-5} mol dm^{-3} FMN; C, FMN cast film

peaks indicates the binding of FMN to the C_{18}Gua monolayer. The absence of significant shifts of C=O peaks implies that the guanidinium unit in the monolayer is not bound to the isoalloxiazine ring of FMN.

Fig. 6 summarizes similar FTIR data for the $2C_{18}$mela-NN monolayer. Comparison of IR spectra of $2C_{18}$mela-NN LB films transferred from pure water [Fig. 6(A)] and from 1×10^{-5} mol dm^{-3} aqueous FMN [Fig. 6(B)] reveals that the latter spectrum shows new peaks at 1676, 1620 and 1551 cm^{-1} which can be assigned to $\nu(C_4{=}O)$, $\nu(C_2{=}O)$ and a $\nu(C{=}N)$ stretch, respectively. The former two peaks show shifts to lower wavenumbers relative to the corresponding peaks of the FMN cast film. This suggests that $2C_{18}$mela-NN binds to isoalloxazine of FMN *via* hydrogen bonding. Similar spectral shifts were reported by Kyogoku *et al.*[32] for hydrogen bonding between FMN and adenine derivatives.

Fig. 7 shows spectra of a C_{18}Gua–$2C_{18}$mela-NN (1:1) LB film transferred from aqueous FMN at different concentrations. The $\nu(C{=}O)$ peaks of isoalloxazine are shifted to 1676 and 1626 cm^{-1}, indicating that FMN is bound to the mixed monolayer through hydrogen bonding. The peak at 1580 cm^{-1} is an overlapped peak of $2C_{18}$mela-NN and FMN, while that at 1550 cm^{-1} arises from bound FMN only. Therefore, the intensity of the latter peak is relatively weak at 1×10^{-7} mol dm^{-3} FMN where the ratio of bound FMN to amphiphile is low.

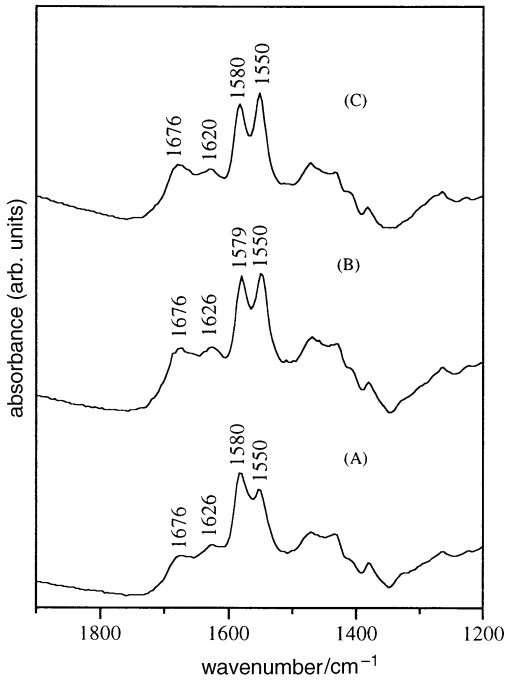

Fig. 7 FTIR spectra of the C_{18}Gua–$2C_{18}$mela-NN (1:1) LB films (5 Y-type films) transferred from aqueous FMN: A, 1×10^{-7} mol dm^{-3}; B, 1×10^{-5} mol dm^{-3}; C, 1×10^{-4} mol dm^{-3}

The IR data are again consistent with the binding motif of Fig. 1(B), *i.e.*, one FMN molecule is bound to one guanidinium and one melamine with formation of a guanidinium–phosphate pair and isoalloxazine–melamine hydrogen bonding.

Binding of aqueous FMN with $2C_{18}$Gua–$2C_{18}$mela-NN monolayers

We conducted a similar investigation by using a dialkyl guanidinium amphiphile, $2C_{18}$Gua. p–*A* Isotherms of $2C_{18}$Gua–$2C_{18}$mela-NN monolayers on 1×10^{-5} mol dm^{-3} aqueous FMN are shown in Fig. 8(A). Normalized deviations of molecular area calculated using eqn. (1) and (2) are plotted in Fig. 8(B) as a function of the fraction of $2C_{18}$Gua. p–*A* Isotherms show a condensed phase alone, and condensation effects are not significant at any mixing ratio. Collapse pressures are minimized at a monolayer composition close to equimolar mixing [curve (c) for 40.3% $2C_{18}$Gua and curve (d) for 60.3% of $2C_{18}$Gua].

Fig. 9 shows FTIR spectra of a $2C_{18}$Gua LB film transferred from pure water [Fig. 9(A)], a $2C_{18}$Gua LB film transferred from 1×10^{-5} mol dm^{-3} aqueous FMN [Fig. 9(B)], and $2C_{18}$Gua–$2C_{18}$mela-NN (1:1) LB film transferred from 1×10^{-5} mol dm^{-3} aqueous FMN [Fig. 9(C)]. The latter two spectra show evidence of FMN binding, *i.e.*, ν(C=N) peaks of FMN at 1579 and 1549 cm^{-1} and peak broadening in the 1600–1700 cm^{-1} region due to overlapped ν(C=O) peaks of FMN. Therefore, FMN is bound to both the $2C_{18}$Gua monolayer and the $2C_{18}$Gua–$2C_{18}$mela-NN (1:1) monolayer. Binding of FMN is also confirmed by XPS (bottom row in Table 1). The observed elemental ratio indicates the presence of one FMN molecule per $2C_{18}$Gua–$2C_{18}$mela-NN (1:1) unit and the spectroscopic data are in accord with the binding motif shown in Fig. 1(C).

Conclusion

Binding of aqueous FMN to guanidinium–melamine binary monolayers has been investigated. The monolayer behaviour on pure water shows the absence of specific functional inter-

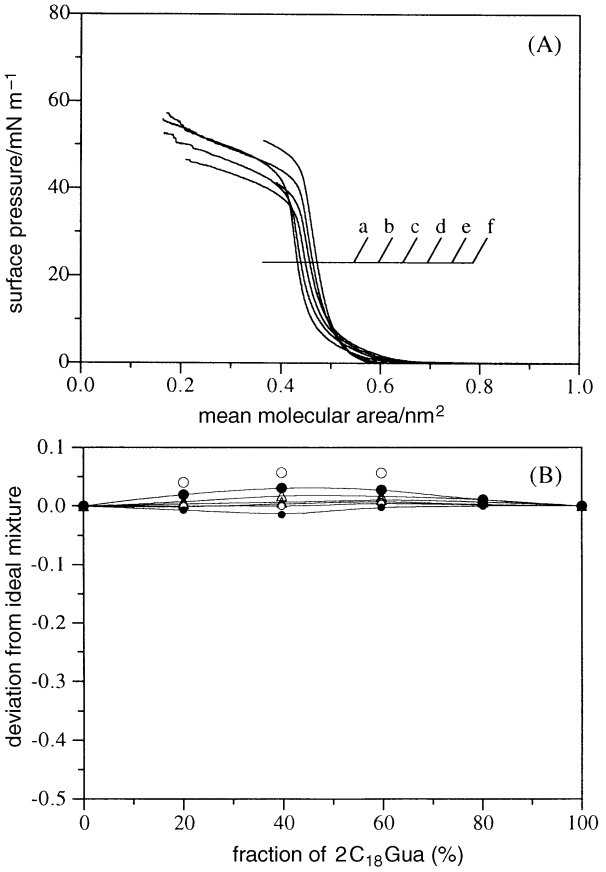

Fig. 8 (A) p–*A* Isotherms of mixed monolayers of $2C_{18}$Gua and $2C_{18}$mela-NN at 20 °C: a, $2C_{18}$Gua 0.0%; b, 19.9%; c, 40.3%; d, 60.3%; e, 79.9%; f, 100.0%. (B) Plots of normalized deviation against the fraction of C_{18}Gua at 5 (#), 10 ($), 15 ('), 20 (+), 25 (1), 30 (#), and 35 ($) mN m^{-1}.

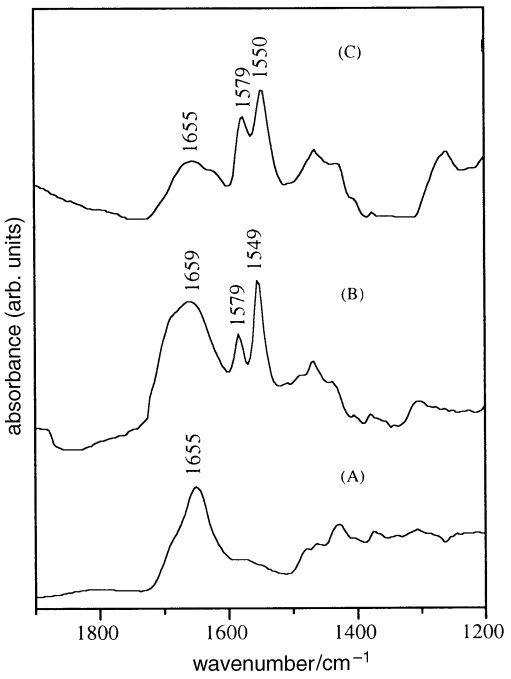

Fig. 9 FTIR spectra of multilayer films (5 Y-type films) on gold-coated glass plates: A, $2C_{18}$Gua LB film transferred from pure water; B, $2C_{18}$Gua LB film transferred from 1×10^{-5} mol dm^{-3} of aqueous FMN; C, $2C_{18}$Gua–$2C_{18}$mela-NN (1:1) LB film transferred from 1×10^{-5} mol dm^{-3} of aqueous FMN

actions between the two kinds of amphiphiles. Both amphiphiles are basic and cannot form a strongly interacting complex between themselves. FTIR data suggests the presence of hydrogen bonding between the melamine amphiphile and the isoalloxazine unit of FMN. Quantitative analysis by XPS measurements reveals that one FMN molecule binds to one guanidinium molecule and one melamine molecule with a binding constant of *ca.* 10^7 dm^3 mol^{-1}. This effective binding originates owing to the absence of competitive amphiphile–amphiphile interactions.

Multisite binding in a mixed monolayer would produce a regular molecular arrangement in a two-dimensional plane as shown in Fig. 1. In fact, AFM observations revealed that a mixed monolayer of C_{18}Gua and $2C_{18}$Oro on aqueous FMN formed a regular two-dimensional molecular pattern.[23] A large variety of molecular patterns can be created by appropriate combinations of receptor and guest molecules. For example, system A, B, and C of Fig. 1 would form different patterns. However, mutual interactions in monolayers sometimes disturb guest binding. A mixed monolayer of C_{18}Gua–$2C_{18}$Oro (1:1) does not bind AMP efficiently because of ion pairing between the two components and shows that mutual interaction between receptor components can seriously limit the patterning.[24] In order to obtain designed molecular patterns, we have to avoid such interactions and the present system of guanidinium, melamine and FMN meets these conditions. Further development of suitable recognition systems will lead to an increased variety of two-dimensional molecular patterns.

Experimental

Materials

Flavine mononucleotide monosodium salt (FMN) was commercially supplied (Wako Pure Chem.). The water used for the subphase was deionized and doubly distilled using a Nanopure II-4P and Glass Still D44 System (Barnstead). Spectroscopic grade benzene and ethanol (Wako Pure Chem.) were used as spreading solvents. Gold (99.999%) and chromium (99.99%) used for the surface modification of solid substrates were purchased from Soekawa Chemicals. Synthetic methods for $2C_{18}$Gua and C_{18}Gua are described elsewhere.[33] The melamine amphiphile, $2C_{18}$mela-NN, was synthesized as follows.

2,4-Diamino-6-(dioctadecylamino)triazine ($2C_{18}$mela-NN)

A mixture of 2,4-diamino-6-chlorotriazine (290 mg, 1.99 mmol), dioctadecylamine[21] (1.04 mg, 1.99 mmol) and $KHCO_3$ (200 mg, 1.99 mmol) in 1,4-dioxane (20 cm^3) was refluxed for 6 h. Water (50 cm^3) was added to the mixture and the insoluble material was collected by filtration. The solid collected on the filter was washed with water and dried to give a slightly yellow powder. This was chromatographed on SiO_2 (200 g; CH_2Cl_2–MeOH, 10:1). The product fractions were collected and concentrated to give a solid. This was recrystallized from EtOH–MeOH to give $2C_{18}$mela-NN (458 mg, 36%) as a colourless solid. Mp, 49.7–55.8 °C; TLC R_f 0.49 (CH_2Cl_2–methanol, 10:1); ^{1}H NMR ($CDCl_3$, 300 MHz) d 0.88 (t, 6H, $J=6.6$ Hz, 2 CH_3), 1.2–1.4 (m, 60H, 30 CH_2), 1.4–1.6 (m, 4H, 2 CH_2CH_2N), 3.44 (t, 4H, $J=7.6$ Hz, 2 CH_2N), 5.23 (br s, 4H, 2 NH_2). Anal. Calc. for $C_{39}H_{78}N_6 \cdot 0.5H_2O$: C, 73.18; H, 12.44; N, 13.13. Found: C, 73.28; H, 12.41; N, 12.83%.

p–*A* Isotherm measurement and LB transfer

p–*A* isotherms were measured with a computer-controlled film balance system FSD-50 (USI System, Fukuoka). A mixture of benzene–ethanol (80:20, v/v) was used as a spreading solvent. Compression was started about 10 min after spreading at a rate of 0.2 mm s^{-1} (or 20 mm^2 s^{-1} based on area). The subphase temperature was kept at 20 ± 0.2 °C. The surface pressures were measured by a Wilhelmy plate, which was calibrated with the transition pressure of an octadecanoic acid monolayer.

LB films were transferred onto gold-deposited glass plates for reflection–absorption FTIR spectroscopy. The substrate was prepared as follows. A slide glass (pre-cleaned, $176\times26\times1$ mm, Iwaki Glass) was immersed in a detergent solution overnight (Dsn 90, Bokusui Brown). The glass was washed with a large excess of ion-exchanged water to remove the detergent completely, and subjected to sonication in fresh ion-exchanged water several times. After the glass was dried *in vacuo* for over 1 h, thin layers of chromium and gold were consecutively formed by the vapour-deposition method (1000 Å Au/50 Å Cr/slide glass) with a vapour-deposition apparatus VPC-260 (ULVAC Kyushu).

LB transfer was carried out with a FSD-21 instrument (USI System, Fukuoka) by the vertical dipping method. Monolayers were transferred on Au-coated glass plates at 30 mN m^{-1} with dipping speeds of 20 mm min^{-1} (downstroke) and 5 mm min^{-1} (upstroke). Transfer of $2C_{18}$Gua–$2C_{18}$mela-NN (1:1) monolayer from aqueous FMN was conducted at 20 mN m^{-1} because of its low collapse pressure. Transfer ratios were almost unity and Y-type transfer was used.

Characterization of LB films

FTIR spectra (reflection–absorption mode) were measured with LB films (5 Y-type films) transferred onto gold-deposited glass plates with a Nicolet 710 FTIR spectrometer.

X-Ray photoelectron spectra (XPS) were measured for the LB films (5 Y-type films) on Au/Cr/glass with a Perkin-Elmer PHI 5300 ESCA instrument using an Mg-Ka X-ray source (300 W). Repeated scans over the same surface region at a take-off angle of 45° gave reproducible spectra. The elemental composition was obtained by dividing the observed peak area by the intrinsic sensitivity factor of each element.

References

1 (*a*) J. Rebek Jr. and D. Nemeth, *J. Am. Chem. Soc*., 1986, **108**, 5637; (*b*) J. Rebek Jr., *Acc. Chem. Res*., 1990, **23**, 399.
2 (*a*) S-K. Chang, D. Van Engen, E. Fan and A. D. Hamilton, *J. Am. Chem. Soc*., 1991, **113**, 7640; (*b*) A. D. Hamilton and D. Van Engen, *J. Am. Chem. Soc*., 1987, **109**, 5035.
3 (*a*) Y. Aoyama, Y. Tanaka, H. Toi and H. Ogoshi, *J. Am. Chem. Soc*., 1988, **110**, 634; (*b*) Y. Aoyama, Y. Tanaka and S. Sugahara, *J. Am. Chem. Soc*., 1989, **111**, 5397.
4 (*a*) J-M. Lehn, *Pure Appl. Chem*., 1994, **66**, 1961; (*b*) K. C. Russell, E. Leize, A. Van Dorsselaer and J-M. Lehn, *Angew. Chem., Int. Ed. Engl*., 1995, **34**, 209; (*c*) A. Marsh, E. G. Nolen, K. M. Gardinier and J-M. Lehn, *Tetrahedron Lett*., 1994, **35**, 397.
5 (*a*) A. J. Doig and D. H. Williams, *J. Am. Chem. Soc*., 1992, **14**, 338; (*b*) D. H. Williams, J. P. L. Cox, A. J. Doig, M. Gardner, U. Gerhard, P. T. Kaye, A. R. Lal, I. A. Nicholls, C. J. Salter and R. C. Mitchell, *J. Am. Chem. Soc*., 1991, **113**, 7020.
6 (*a*) J. C. Adrian and C. S. Wilcox, *J. Am. Chem. Soc*., 1991, **113**, 678; (*b*) 1992, **114**, 1398.
7 (*a*) J. S. Nowick and J. S. Chen, *J. Am. Chem. Soc*., 1992, **114**, 1107; (*b*) J. S. Nowick, J. S. Chen and G. Noronha, *J. Am. Chem. Soc*., 1993, **115**, 7636; (*c*) J. S. Nowick, T. Cao and G. Noronha, *J. Am. Chem. Soc*., 1994, **116**, 3285.
8 R. P. Bonar-Law, *J. Am. Chem. Soc*., 1995, **117**, 12397.
9 H. Asanuma, S. Gotoh, T. Ban and M. Komiyama, *Chem. Lett*., 1996, 681.
10 M. Sakurai, H. Tamagawa, T. Furuki, Y. Inoue, K. Ariga and T. Kunitake, *Chem. Lett*., 1995, 1001.
11 (*a*) D. Y. Sasaki, K. Kurihara and T. Kunitake, *J. Am. Chem. Soc*., 1991, **113**, 9685; (*b*) 1992, **114**, 10994; (*c*) D. Y. Sasaki, M. Yanagi, K. Kurihara and T. Kunitake, *Thin Solid Films*, 1992, **210/211**, 776.
12 (*a*) K. Kurihara, K. Ohto, Y. Honda and T. Kunitake, *J. Am. Chem. Soc*., 1991, **113**, 5077; (*b*) T. Kawahara, K. Kurihara and T. Kunitake, *Chem. Lett*., 1992, 1839.
13 (*a*) K. Kurihara, K. Ohto, Y. Tanaka, Y. Aoyama and T. Kunitake,

J. Am. Chem. Soc., 1991, **113**, 444; (*b*) K. Kurihara, K. Ohto, Y. Tanaka, Y. Aoyama and T. Kunitake, *Thin Solid Films*, 1989, **179**, 21.
14 Y. Ikeura, K. Kurihara and T. Kunitake, *J. Am. Chem. Soc*., 1991, **113**, 7342.
15 (*a*) X. Cha, K. Ariga, M. Onda and T. Kunitake, *J. Am. Chem. Soc*., 1995, **117**, 11833; (*b*) X. Cha, K. Ariga and T. Kunitake, *J. Am. Chem. Soc*., 1996, **118**, 73; (*c*) 9545.
16 H. Kitano and H. Ringsdorf, *Bull. Chem. Soc. Jpn*., 1985, **58**, 2826.
17 (*a*) T. M. Bohanon, S. Dezinger, R. Fink, W. Paulus, H. Ringsdorf and M. Weck, *Angew. Chem., Int. Ed. Engl*., 1995, **34**, 58; (*b*) R. Ahuja, P-L. Caruso, D. Möbius, W. Paulus, H. Ringsdorf and G. Wildburg, *Angew. Chem., Int. Ed. Engl*., 1993, **32**, 1033.
18 X.-C. Chai, S.-G. Chen, Y.-L. Zhou, Y.-Y. Zhao, T.-J. Li and J.-M. Lehn, *Chin. J. Chem*., 1995, **13**, 385.
19 M. Shimomura, O. Karthaus and K. Ijiro, *Supramol. Sci*., 1996, **3**, 61.
20 Y. Ebara, K. Itakura and Y. Okahata, *Langmuir*, 1996, **12**, 5165.
21 M. Onda, K. Yoshihara, H. Koyano, K. Ariga and T. Kunitake, *J. Am. Chem. Soc*., 1996, **118**, 8524.
22 K. Taguchi, K. Ariga and T. Kunitake, *Chem. Lett*., 1995, 701.
23 (*a*) Y. Oishi, Y. Torii, M. Kuramori, K. Suehiro, K. Ariga, K. Taguchi, A. Kamino and T. Kunitake, *Chem. Lett*., 1996, 411; (*b*) Y. Oishi, Y. Torii, T. Kato, M. Kuramori, K. Suehiro, K. Ariga, K. Taguchi, A. Kamino, H. Koyano and T. Kunitake, *Langmuir*, 1997, **13**, 519.
24 A. Kamino, K. Taguchi, H. Koyano, K. Ariga and T. Kunitake, manuscript in preparation.
25 K. Taguchi, A. Kamino, H. Koyano, K. Ariga and T. Kunitake, manuscript in preparation.
26 H. Koyano, K. Yoshihara, K. Ariga, T. Kunitake, Y. Oishi, O. Kawano, M. Kuramori and K. Suehiro, *Chem. Commun*., 1996, 1769.
27 L. J. Bellamy, *The Infrared Spectra of Complex Molecules*, Chapman and Hall, London, 3rd edn., vol. 1, 1975.
28 M. Scoponi, E. Polo, F. Pradella, V. Bertolasi, V. Carassiti and P. Goberti, *J. Chem. Soc., Perkin Trans. 2*, 1992, 1127.
29 A. Kamino, K. Ariga, T. Kunitake, V. Birault, G. Pozzi, Y. Nakatani and G. Ourisson, *Colloids Surf*., 1995, **103**, 183.
30 S. Suzuki, S. Isoda and M. Maeda, *Jpn. J. Appl. Phys*., 1989, **28**, 1673.
31 V. I. Birss, A. S. Hinman, C. E. McGarvey and J. Segal, *Electrochim. Acta*, 1994, **39**, 2449.
32 Y. Kyogoku and B. S. Yu, *Bull. Chem. Soc. Jpn*., 1969, **42**, 1387.
33 A. Kamino, H. Koyano, K. Ariga and T. Kunitake, *Bull. Chem. Soc. Jpn*., 1996, **69**, 3619.

Paper 7/00081B; *Received* 3*rd January*, 1997