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Cooperative Binding of Adenine via Complementary Hydrogen Bonding to an Imide Functionalized Monolayer at the Air-Water Interface

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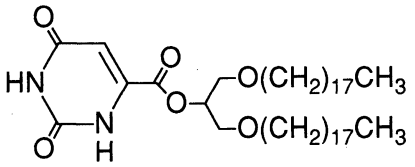
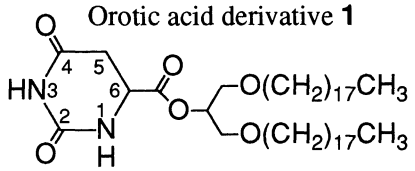
Double-chain derivatives of orotic acid (**1**) and hydroorotic acid (**2**) formed stable monolayers at the air-water interface. FT-IR and ESCA spectroscopies revealed specific cooperative binding of adenine from the aqueous subphase to monolayer **1** in preference to thymine. Monolayer **2** did not bind these nucleic acid bases.

We have shown in a series of recent publications that hydrogen bonding interactions are an effective means of molecular recognition by molecular monolayers on water.[1-5] The interfacial aqueous environments do not interfere with the hydrogen bonding, in spite of the fact that artificial hydrogen bonding hosts have been mostly studied in aprotic media in order to avoid detrimental effects of aqueous media.[6,7] These monolayers, therefore, provide useful models for molecular recognition via hydrogen bonding that occurs at the surface of biological macromolecules in exposure to the aqueous environment.

We report herein cooperative substrate binding via complementary base pairing at the air-water interface. Amphiphiles we employed are double- chain derivatives of orotic acid (**1**) and hydroorotic acid (**2**).[8] These functional units commonly contain cyclic imide (uracil-like and hydrouracil-like) structures that are complementary to adenine, but their electronic and steric structures are different. Amphiphiles **1** and **2** form stable monolayers on pure water with collapse pressures of 40-50 mN/m and limiting molecular areas of 0.4 nm^2 $molecule^{-1}$ as shown in Fig. 1. The latter values are two times the molecular cross section of the alkyl chain[9] and imply the presence of well-packed alkyl chains perpendicular to the water surface. The collapse pressure of monolayer **1** decreased at adenine concentrations greater than 1 mM (M = mol dm^{-3}) in the subphase; whereas the surface pressure-area (π-A) behavior of monolayer **2** was not affected by adenine. Addition of thymine in the subphase did not cause any change in π-A isotherms of the two monolayers. This monolayer behavior strongly suggests that only adenine is selectively bound to monolayer **1**.



Orotic acid derivative **1**



Hydroorotic acid derivative **2**

Direct confirmation of substrate binding was subsequently conducted by FT-IR and X-ray photoelectron (XPS) spectroscopies. As shown in Fig. 2, an FT-IR reflection spectrum (Nicolet, Model 710) of a Langmuir-Blodgett (LB) film of **1** transferred from pure water[10] gives ν_{NH} peaks at 3086 cm^{-1} (for the N1 position) and at 3200 cm^{-1} (for the

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N3 position) that are characteristic of the dimeric imide structure.[11-13] The carbonyl peaks at the C2 and C4 positions are located at 1693 and 1681 cm^{-1}, respectively, and are also indicative of the dimer structure, as has been reported for barbiturates.[13] The corresponding transmission spectrum does not possess the $\nu_{N3\text{-}H}$ peak at 3200 cm^{-1}. Thus, the N-H bond at the N3 position must be placed perpendicular to the film surface, and hydrogen-bonded imide dimers are formed between the opposing LB layers.

For an LB film of **1** transferred from 3 mM aqueous adenine, the dimer ν_{NH} peak at 3068 and 3200 cm^{-1} are lessened and new peaks assignable to adenine appeared at 3445 (ν_{NH}), 3380 (ν_{NH}), 3336 (ν_{NH}), 1677 (δ_{NH}) and 1607 (δ_{NH}) cm^{-1}. The ν_{NH} peaks are shifted to shorter wavenumbers due to hydrogen bonding with the orotate unit, as already noted in similar systems.[14] The peaks at 3336 and 3445 cm^{-1} are attributable to hydrogen-bonded adenine N-H and the 3380 cm^{-1} peak is assigned to the monomeric (non-hydrogen-bonded) orotate structure. These IR data strongly suggest that the adenine-orotate pair replaces the orotate-orotate (dimer) pair in the LB film.

An LB film of **1** did not display any IR change when thymine was added to the subphase in place of adenine. Furthermore, both of adenine and thymine in the subphase did not produce any change in IR spectra of monolayer **2** which contains the hydroorotate unit. It is clear, therefore, that only the orotate-adenine pair leads to binding at the interface.

The adenine binding can be quantitatively estimated by the XPS method (Perkin-Elmer PHI 5300 ESCA system). The observed elemental ratio of an LB film of **1** (C : O : N = 87.8 ± 0.6 : 9.0 ± 0.5 : 3.3 ± 0.1 at a take off angle of 90°) showed a satisfactory agreement with the theoretical ratio (88.21 : 8.47 : 3.32) which was obtained upon correction of the depth of each atom from the very surface of the Y-type LB film.[3,10] The amount of adenine bound at the orotate unit was estimated by a similar procedure. The bound adenine increases with increasing adenine concentrations in the subphase toward the stoichiometric ratio, as shown in Fig. 3. These data do not obey the simple Langmuir adsorption isotherm which assumes independent binding sites, unlike previous examples of guest binding at the interface.[3-5] The data show a better fit to the cooperative adsorption scheme, although the data point that corresponds to saturated binding cannot be obtained because of the low solubility of adenine. A tentative estimate indicates that, on average, 3.5 molecules of adenine cooperatively binds to the monolayer. This

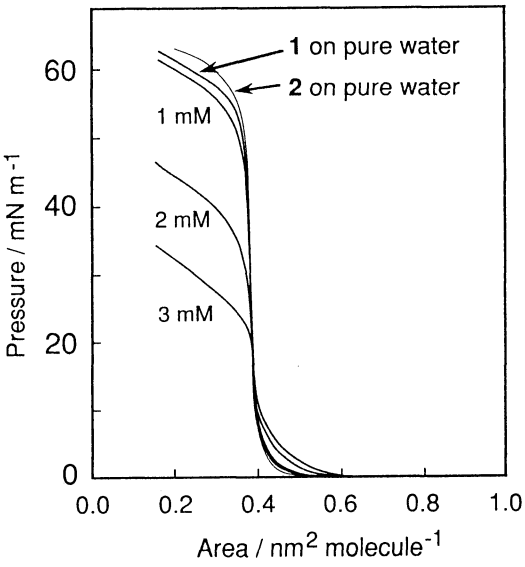

Fig. 1. Surface pressure-area (π-A) isotherms of monolayers of **1** on pure water and aqueous adenine and of **2** on pure water at 20.0 °C.

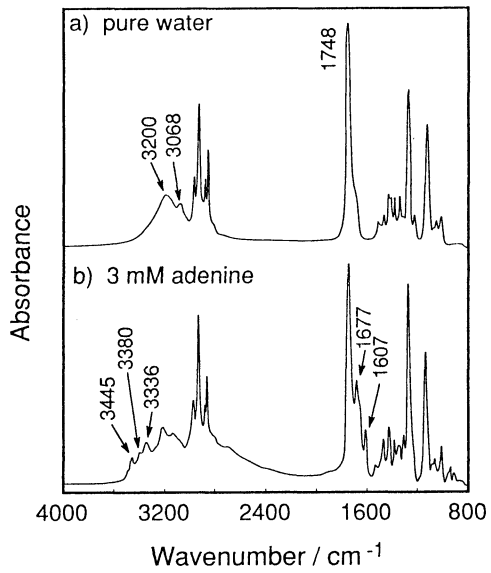

Fig. 2. IR refrection spectra of LB films (6 layers) of **1** prepard from pure water (a) and from 3 mM aqueous adenine (b).

cooperativity will come from the stacking effect of bound adenine, as illustrated in Fig. 4.

The selective binding of monolayer **1** with adenine but not with thymine is readily explained by assuming the Watson-Crick hydrogen bonding mechanism. We have shown that a diaminotriazine monolayer binds nucleic acid bases bearing the imide moiety (e.g., thymine) selectively. The reversed host-guest relationship in the identical hydrogen bonding mode is presented in this study. However, binding of thymine to a diaminotriazine monolayer did obey a simple adsorption isotherm.[3)]

Another interesting feature is that monolayer **1**, but not **2**, binds adenine. The uracil ring in **1** is planar, but **2** assumes a non-planar lactam (dihydrouracil) structure in preference to four kinds of less stable lactim tautomers.[15)] Rohrer and Sundaralingam reported the crystal structure of dihydrouracil in which the direction of the carbonyl bond at the C2 and C4 positions are deviated from the C2-N3-C4 plane by 11.1° and 3.6°, respectively.[16)] The lack of coplanarity in the interacting imide moiety would exert serious detrimental effects on complementary hydrogen bonding with adenine.

An additional, related factor which differentiates **1** and **2** is their acidities. The pK_a values of orotic acid and hydroorotic acid are estimated to be 9.7 and ca.12, respectively.[17)] Kyogoku et al. compared pK_a values of uracil derivatives and their binding constants with adenine in $CHCl_3$, and found that the binding constant decreased with increasing pK_a values.[14)] According to their data, the binding constants of adenine by 1-cyclohexyluracil and 1-cyclohexylhydrouracil are 100 M^{-1} and 30 M^{-1}, respectively. The enhanced proton donating ability of the orotate (uracil) N-H unit relative to that of the hydroorotate (hydrouracil) unit (as reflected in their pK_a values) must be certainly effective in promoting adenine binding, in addition to the coplanarity of the hydrogen bonding unit.

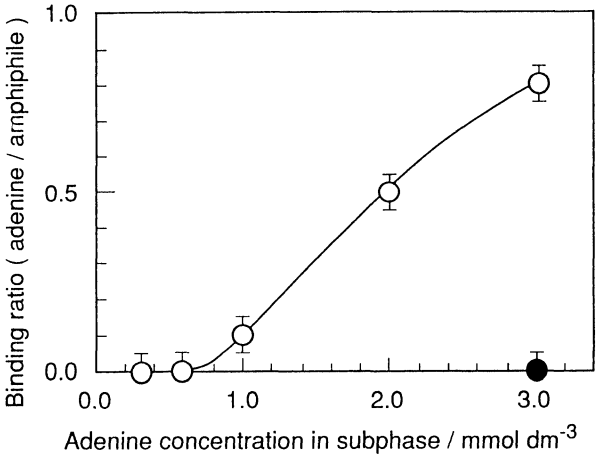

Fig. 3. Binding of adenine to imide functionalized monolayers **1** (○) and **2** (●) at 20 °C.

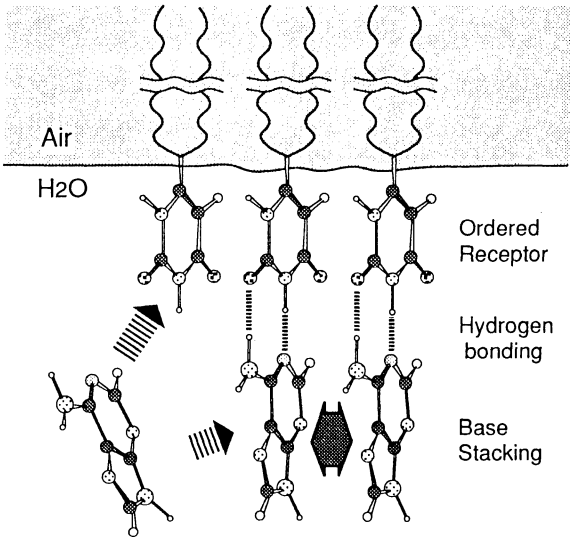

Fig. 4. Cooperative binding of adenine to orotate monolayer **1**.

Implications of the present findings on biological molecular recognition are as follows. First, it is confirmed again that complementary hydrogen bonding acts as an effective means of molecular recognition at the air-water interface. Second, the contrasting recognition behavior of the orotate and hydroorotate monolayers is noteworthy, in view of the fact that in t-RNA molecules the uracil base is present in the paired base region, whereas the hydrouracil unit is found in loop regions where intramolecular base pairs are not formed.[18)] Third, guest binding is promoted cooperatively by base stacking. Apart from its unquestionable importance in nucleic acid base pairing, interplay of hydrogen bonding and aromatic stacking has been noticed in complex formation

between daunomycin and an oligonucleotide[19] and in specific recognition of 2'-guanylic acid by ribonuclease T1(protein).[20]

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8) Syntheses of **1** and **2** will appear in a future publication. Crystalline **1**: colorless powder; mp 102.4-103.4 °C. ^{1}H NMR (JEOL JNM-GSX 400 spectrometer, $CHCl_3$): δ 8.56 (br s, 1H, 3-NH), 8.43 (br s, 1H, 1-NH), 6.41 (s, 1H, CH), 5.33 (tt, J=3.7 Hz, 1H, OC<u>H</u>CH_2), 3.62 (d, J=4, 4H, $CHC\underline{H}_2O$), 3.43 (m, 4H, OCH_2), 1.59, 1.53 and 1.24 (m, 64H, alkyl tail), 0.87 (t, J=5, 6H CH_3). IR (KBr): 3200, 3080, 2914, 2846, 1673, 1492, 1465, 1261, 1125 cm^{-1}. Anal. Found : C, 71.98; H, 11.26; N, 3.67%. Calcd for $C_{44}H_{82}N_2O_6$: C, 71.89; H, 11.24; N, 3.81%. Crystalline **2**: colorless powder; mp 107.2-108.4 °C. ^{1}H NMR ($CHCl_3$): δ 7.33 (br s, 1H, 3-NH), 5.81 (br s, 1H, 1-NH), 5.23 (tt, J=3.8 Hz, 1H, OC<u>H</u>CH_2), 4.31 (m, 1H, 6-CH), 3.56 (dd, 4H $CHC\underline{H}_2O$), 3.41 (m, 4H, OCH_2), 2.99 and 2.82 (dd, 1H, 5-CH_2), 1.55 and 1.25 (m, 64H, alkyl tail), 0.88 (t, J=5, 6H, CH_3). IR (KBr): 3210, 3090, 2914, 2846, 1741, 1704, 1476, 1217, 1134 cm^{-1}. Anal. Found : C, 71.67; H, 11.43; N, 3.79%. Calcd for $C_{44}H_{84}N_2O_6$: C, 71.69; H, 11.49; N, 3.80%.
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