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Molecular Recognition of Barbiturates by Diaminotriazine Functionalized Monolayers

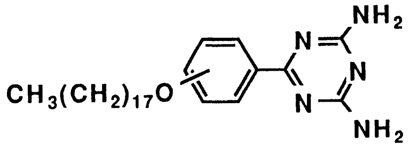
Yoshihiro HONDA, Kazue KURIHARA, and Toyoki KUNITAKE*,†
Molecular Architecture Project, JRDC, Kurume Research Park, Kurume 830

Diaminotriazine functionalized amphiphiles were synthesized to form stable monolayers at the air-water interface. Combined examination of FT-IR, ESCA, and reflection absorption spectroscopies confirmed specific binding of barbituric acid and thiobarbituric acid to the monolayer, due to hydrogen bonding between the diaminotriazine head of amphiphiles and the imide moiety of barbiturates.

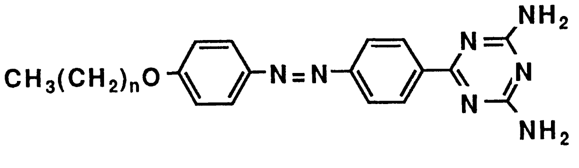
Molecular recognition by way of multiple hydrogen bonding is one of the most active fields in organic chemistry, due to its implications in the biochemical process. Most of the recent examples include recognition of nucleic acid bases and related hetero cycles[1-5] and sugars.[6] These recognition experiments are conducted in organic solvents, thus lead to exaggerated roles of hydrogen bonding. Since the molecular recognition process in the biological system is usually exposed to the aqueous environment, it is desirable to examine the hydrogen bonding interaction in the presence of the aqueous phase. We have been using surface monolayers as a means to study the molecular recognition, because of their technical advantages[7-10] For instance, monolayers of a polyhydroxyl macrocycle and of Kemp's triacid derivatives could bind aqueous sugars,[8] and nucleic acid bases,[10] respectively, at very low concentrations.

In the present study, we synthesized long-chained derivatives of diaminotriazine, **1**-**4**, and examined their molecular recognition capabilities at the air-water interface.

Octadecyl bromide was condensed with p- and m-cyanophenols and the products were allowed to react with dicyanodiamide in methoxyethanol to give **1** and **2**. The reaction of 4-alkoxyl-4'-cyano azobenzene with dicyanediamide gave **3** and **4**.[11]



m-: **1**
p-: **2**



n=9: **3**
n=17: **4**

Hamilton and coworkers showed that macrocyclic derivatives of 2,6-diaminopyridine bound barbiturates and nucleic acid bases[1,2] effectively. It is also known that adenine derivatives form specific hydrogen bonds with barbiturates.[12-14] The mode of hydrogen bonding in the diaminotriazine ring is identical to those of 2,6-diaminopyridine and adenine. There-

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† Permanent address: Department of Organic Synthesis, Faculty of Engineering, Kyushu University, Hakozaki, Fukuoka 812.

fore, diaminotriazine monolayers are expected to recognize barbiturates.

Surface pressure-area relations (π-A curve)[15)] of these triazine compounds are shown in Fig. 1. Compound **1** did not show any pressure increase, but **2** and **3** gave almost identical monolayer behavior. The collapse pressure of **4** was lower than those of **2** and **3**. They all displayed expanded phases.

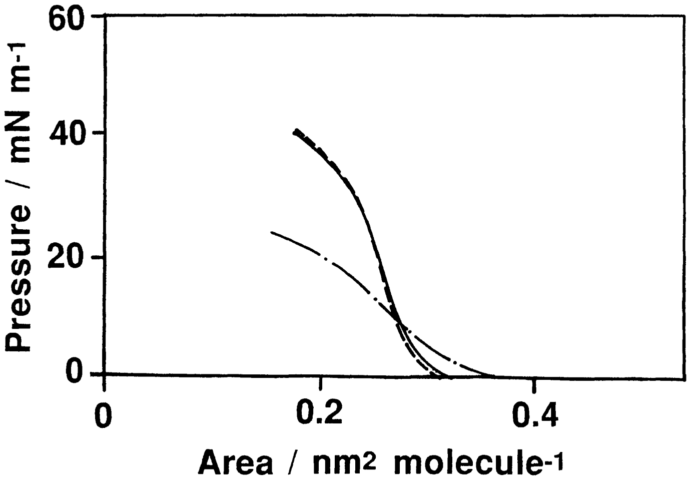

Fig. 1. The π-A isotherm of **2** (-----), **3** (——),and **4** (—·—) on pure water at 20.0 ± 0.2 °C.

Subsequently, influences of guest molecules dissolved in the subphase were examined. The following guest molecules did not alter the π-A isotherms: uracil, alloxane, sodium barbital, barbital **5**, dimethylbarbituric acid **6**, riboflavin, glucose, and NaN_3.[16)] In contrast, some barbital derivatives, particularly barbituric acid **7** and thiobarbituric acid **8**, caused expansions of the π-A curve, as shown in Fig. 2 for monolayer **3**. Similar results were obtained with other stable monolayers **2** and **4**. The expansion suggested specific interaction of these barbiturates with the monolayer.

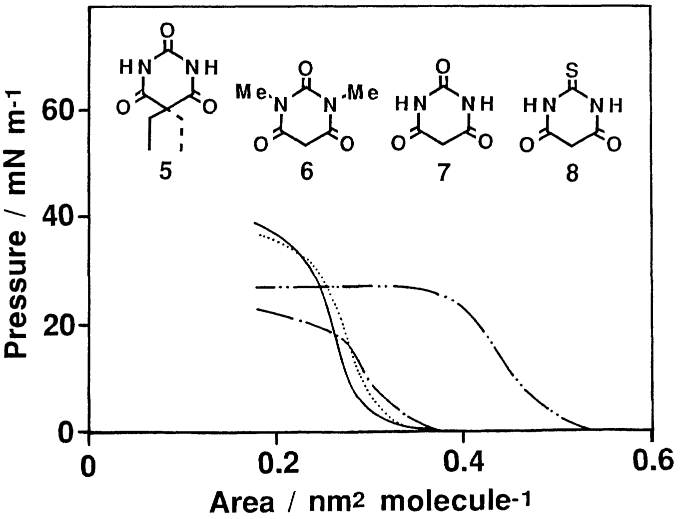

Fig. 2. The π-A isotherm of **3** (——) on pure water, and on 0.01 M aqueous solutions of **5** (-----), **7** (—·—), **8** (—··—) at 20.0 ± 0.2 °C.

FT-IR spectra (Nicolet, Model 710) of LB films were then used to evaluate the barbiturate binding. LB films (4-10 layers) of **3** were transferred onto CaF_2 plates (for the transmission method) or Au (100 nm thickness) -deposited glass slides (for the reflection method) from various subphases.[17)] A FT-IR transmission spectrum of the LB film transferred from pure water shows peaks at 3396, 1605, and 1529 cm^{-1} (Fig. 3a). The first peak is attributed to the NH stretching and the latter two are to the phenyl breathing mode. On the other hand, LB films from aqueous barbituric acid exhibit shift and broadening of the NH peak of **3** at ca.3300 cm^{-1}, and additional peaks, which are characteristics of barbituric acid,[18)] at around 3200 ($\nu_{NH, imide}$), 1700 ($\nu_{C=O, imide}$), 1400 cm^{-1} (Fig. 3b, 3c).[19)] Increasing concentration of barbituric acid from 0.001 M (M = mol dm^{-3}) to 0.01 M enhances the spectrum change, indicating better binding of barbituric acid. Addition of barbituric acid decreases the pH of the subphase.[20)] However, an IR spectrum of the film obtained from 0.005 M aqueous H_2SO_4 (pH 3.0) is identical to that obtained from pure water. The majority of the diaminotriazine groups should not be protonated at these pH's. Thus, ionic binding of the barbiturate anion pH's. Thus, ionic binding of the barbiturate anion with the protonated diaminotriazine group appears not significant. The binding is attributed to complementary hydrogen bonding between the diaminotriazine group and the imide moiety of barbituric acid. Thiobarbituric acid (0.01 M) also causes a similar spectrum change (Fig.3d), whereas barbital (0.01 M) does not cause any change. Inferior binding of barbital to that of barbituric acid may be explained by lessened hydrogen donor ability of the former (pKa 7.4 and 4.0, respectively),[21)] and by steric[18)] and hydrophobic[8)] destabilization of bound barbital.

X-Ray photoelectron spectroscopy (Perkin-Elmer PHI 5300 ESCA system) further supports the substrate binding. The carbon / nitrogen / oxygen ratio of an LB film of **3** transferred from pure water onto a Au-coated slide glass was determined to be 81.4:14.7:3.9 (%, ± 0.3, at a take-off angle of 45 °), in close agreement with the theoretical ratio (81.7 : 15.0 : 3.3).[22)] The C/N/O ratio of an LB film from 0.001 M barbituric acid is 79.5 ± 1.3 : 14.6 ± 1.4 : 5.8 ± 0.2 (%). From the increase in the oxygen content, we can estimate the molar ratio of **3** to barbituric acid in the LB film to be 1 : 0.4.[24)]

Binding of substrates affects molecular orientation of amphiphiles in monolayers. The azobenzene chromophore has been successfully used to evaluate the molecular orientation in surface monolayers.[25)] A reflection spectrum (Otsuka Electronics, Multi Channel Transient Spectrophotometer MCPD-110) of monolayer **3** on pure water shows an absorption peak (λ_{max}) at 323 nm that is ascribed to parallel orientation of the chromophores (H-aggregates).[26)] Reflection spectra of **3** on 0.01 M aqueous barbital (λ_{max}, 330 nm), dimethylbarbituric acid (λ_{max}, 324 nm) and 0.025 M H_2SO_4 (λ_{max}, 334 nm) are similar to that on pure water. In contrast, 0.01 M barbituric acid and thiobarbituric acid shift the reflection peak to 345 nm and 375 nm, respectively, which correspond to the tilted or the random orientation of chromophores.[25)] These observation also supports specific interaction of barbituric acid and thiobarbituric acid with monolayer **3**.

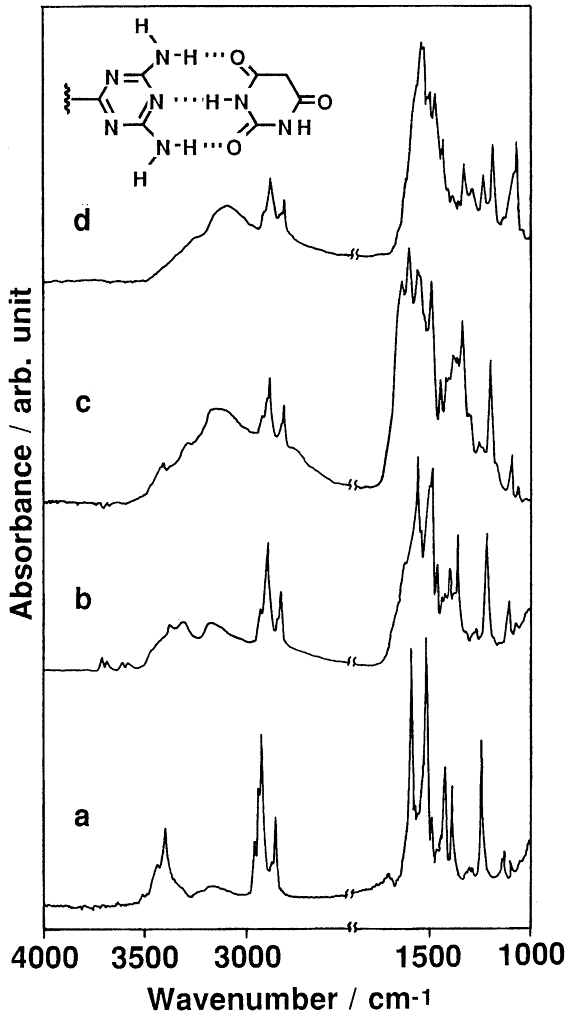

Fig. 3. FT-IR transmission spectra of LB films of **3** transferred from pure water (**a**), 0.001 M barbituric acid (**b**), 0.01 M barbituric acid (**c**) and 0.01 M thiobarbituric acid (**d**). The films consist of five LB layers (**a**, **c**, **d**) and 12 layers (**b**).

In conclusion, we demonstrated specific binding of some barbital derivatives with diaminotriazine monolayers. Complementary hydrogen bonding between the diaminotriazine and the imide group of substrates must be responsible for the binding. High efficiency and specificity of the binding were observed even when the receptor was exposed to the aqueous subphase. The corresponding binding by the synthetic host has been observed only in aprotic organic solvents.[1,2)]

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11) Compound **1**: colorless crystals, mp 125 - 126 °C; Anal.Found: C, 71.21; H, 9.96; N, 15.37 %. Calcd for $C_{27}H_{45}N_5O$: C, 71.17; H, 9.95; N, 15.37 %. Compound **2**: colorless crystals, mp 152 - 153 °C; Anal.Found: C, 71.28; H, 9.93; N, 15.34 %. Calcd for $C_{27}H_{45}N_5O$: C, 71.17; H, 9.95; N, 15.37 %. Compound **3**: orange crystals, mp 214 - 216 °C (liquid crystal); Anal.Found: C, 67.08; H, 7.42; N, 21.93 %. Calcd for $C_{25}H_{33}N_7O$: C, 67.09; H, 7.43; N, 21.91 %. Compound **4**: orange crystals, mp 198 - 203 °C (liquid crystal); Anal.Found: C, 70.74; H, 8.85; N, 17.42 %. Calcd for $C_{33}H_{49}N_7O$: C, 70.80; H, 8.82; N, 17.51 %.
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15) The π-A curves were measured with a computer-controlled film balance (San-esu Keisoku, FSD 20) at 20 °C. The spreading solvent was $CHCl_3$ for **1** and **2** (0.5 - 1.0 mM), and a 9:1 mixture of $CHCl_3$ and ethanol for **3** and **4** (1.0 mM).
16) Substrate concentrations were 0.005 M for uracil, 0.05 M for alloxane, sodium barbital and glucose, 0.01 M for barbital and dimethylbarbituric acid, 0.00001 M for riboflavin and 0.008 M for NaN_3.
17) LB films of **3** were deposited in the vertical mode at a surface pressure of 25 mN m^{-1} and at a transfer rate of 20 mm min^{-1}. The transfer ratios were unity in the up-stroke mode and zero in the down-stroke mode.
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19) The transmission and the reflection method emphasize different vibration modes, though changes described here are common in both spectra.
20) The pH of 0.01 M aqueous barbituric acid is 3.1, and that of 0.01 M thiobarbituric acid is 2.3.
21) *The Merck Index (Ninth Edition)*, Merck & Co, Rahway (N. J.), 967 (1976).
22) The elemental composition of compound **3** is C : N : O = 76 : 21 : 3 (%). A theoretical value was calculated for an ideal z-type LB film (4 layers) by using an optimized mean free path of electron of 40 Å[23] for all C, N and O. A slightly larger oxygen content was observed owing to adsorption of an unknown species to the LB film surface which broadened the oxygen peak.
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24) The computed atomic ratio for the substrate binding of 1 : 0.4 (**3** to barbituric acid) is C : N : O = 79.4 : 15.5 : 5.1. The increase in the oxygen content by unknown surface species is assumed to be the same as that of the film from pure water (0.6 %), and corrected.
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26) Compression of monolayers does not change the location of the reflection peak.

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