Molecular Recognition of Sugars by Monolayers of Resorcinol-Dodecanal Cyclotetramer[1]

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Abstract: Resorcinol-dodecanal cyclotetramer (**1**) forms a stable monolayer at the air-water interface. Molecular interactions of this monolayer with sugars and related water-soluble substances have been studied by combinations of potentiometric responses of monolayer-modified SnO_2 electrodes, UV-visible, FT-IR, and XPS spectroscopies of LB films, and surface pressure-area isotherms. Sugars and water-soluble polymers bearing hydrogen-bonding groups bind to this monolayer selectively, resulting in anodic potentiometric responses of the **1**-modified electrode. The affinity of sugars to monolayer **1** increases in the order: glucose < fucose ~ galactose ~ arabinose < xylose < ribose. This selectivity is different from that of sugar extraction into CCl_4 from the aqueous phase: ribose is bound to **1** effectively in both systems, while fucose, which is easily extracted into CCl_4, is less effective in the monolayer system. Galactose, which is complexed weakly in CCl_4, shows significant binding to the monolayer. Formation of sugar complexes which expose more hydroxyl groups and less lipophilic parts to water is preferred at the interface. Binding to the monolayer host is efficient owing to the high densities of host molecules aligned at the interface. Spectroscopic techniques are applied to study binding of riboflavin and poly(vinylpyrrolidone) to the monolayer of **1**. Binding of sugars to monolayers of octadecyl alcohol, *N,N*-didodecyl(gluconoamido)hexamide, and octadecanoic acid have also been studied. The present results strongly indicate that the specificity of sugar binding is brought out by hydrogen bonding; the presence of multiple hydroxyl groups and their fixed mutual arrangements in resorcinol cyclotetramer is required to obtain efficient sugar binding.

Introduction

"Molecular recognition" has become a prominent field in chemistry; it serves as a basic concept to understand biological functions and offers a key in developing new types of functionalized materials.[2] Organized molecular monolayers and bilayers provide unique environments for molecular interactions and consequently for molecular recognition.[3] New molecular recognition systems can be developed by using monolayer assemblies, which may possess characteristics uniquely different from those in homogeneous media. These supramolecular systems should be important (1) in applications such as chemical sensors, (2) in understanding molecular interactions on biological cell surfaces, and (3) in developing novel two-dimensional molecular assemblies composed of multiple chemical species. In spite of these interesting features, only a few investigations have been reported concerning molecular recognition by well-defined monolayer systems. Monolayers of nucleolipids[4] and monolayers of amphiphiles functionalized by cyclodextrin[5], crown ether,[6,7] and calixarene[8] have been studied in this regard.

Specific pairing of hydrogen bonding is useful for precise recognition of organic molecules, as demonstrated amply in biological macromolecules. Therefore, molecular recognition employing hydrogen bonding has been a major focus in current host-guest chemistry.[9-12] Aoyama et al. recently provided the first example of stereoselective complexation of sugars in apolar organic media (CCl_4) using a synthetic polyhydroxy macrocycle **1** as a lipophilic polar host.[12] Multiple hydrogen bonding involving adjacent hydroxy groups of the macrocycle and the stability of the resulting complexes in apolar media were considered to be responsible for this selectivity. These investigations examine host-guest complexations only in homogeneous apolar media[9a,10-12] or in host-guest crystal lattices.[9b] These interactions, if realized in aqueous media, should be highly relevant to what occurs in biological systems.

We plan in a series of forthcoming papers[13] to discuss the effectiveness of specific hydrogen bonding in molecular recognition of organic compounds which remain exposed to aqueous media. Molecular monolayers on water are advantageous in tackling this problem. They provide well-defined molecular interfaces between the aqueous phase and the organic (monolayer) phase, and a variety of physical techniques are available for studying the structure and property of the interface. Secondly, the hydrogen-bonding function can be anisotropically aligned at the interface by using appropriate monolayer compounds. The anisotropic organization of host molecules should have an important bearing on the specificity of molecular recognition.

In the present study, we prepared monolayers from compound **1** and studied binding of sugars and other related substances from the bulk aqueous phase. Sugars are polyhydroxy aldehydes (aldoses) or polyhydroxy ketones (ketoses). They exist as cyclic hemiacetals or hemiketals and are composed of groups of closely

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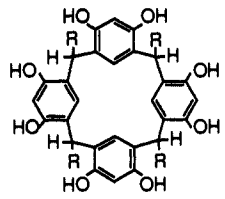
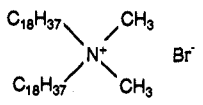
Chart I



R: $(CH_2)_{10}CH_3$

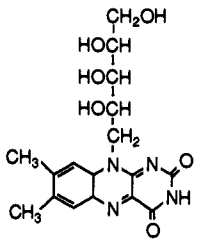
1



5

$CH_3(CH_2)_{17}OH$

2



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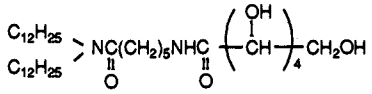

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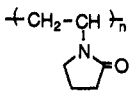
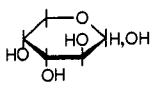


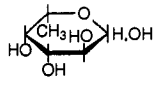
7

$CH_3(CH_2)_{16}COOH$

4



8 arabinose



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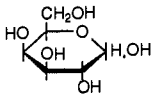

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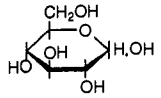
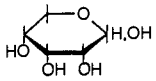
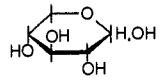


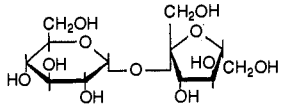
11 glucose



12 ribose



13 xylose



14 sucrose

related stereoisomers. Compound **1** has eight hydroxyl groups inwardly facing into a cavity, which served as a binding site in CCl_4. To our great surprise, the monolayer obtained from **1** did bind highly water-soluble sugars at low concentrations. The observed specificity was different from that in CCl_4.

Experimental Section

Materials. The synthesis of resorcinol–dodecanal cyclotetramer (**1**) has been reported.[12] Octadecyl alcohol (**2**, Gasukuro Kogyo), *N,N*-didodecyl(gluconoamido)hexamide (**3**, Sogo Pharmaceutical Co.), octadecanoic acid (**4**, Gasukuro Kogyo), and *N,N*-dioctadecyldimethylammonium bromide (**5**, Sogo Pharmaceutical Co.) were used as supplied. Sugars (Kishida Chemicals, except for L-rhamnose and D-arabinose from Wako Pure Chemicals, and L-fucose from Nacarai Tesque), riboflavin (**6**, Kishida Chemicals), and Na_2SO_4 were of reagent grade and used as supplied unless stated otherwise. Poly(vinylpyrrolidone) (**7**, average molecular weight (MW) 40 000), poly(acrylic acid) (MW 8000–12 000), poly(vinyl alcohol) (MW 22 000), and poly(ethylene glycol) (MW 200) were supplied from Wako Pure Chemicals and used without further purification. The spreading solvents for monolayers were of spectra grade (Kishida Chemicals). The subphase water was deionized and doubly distilled by Nanopure II and Fi-streem 48D glass still system (Barnstead).

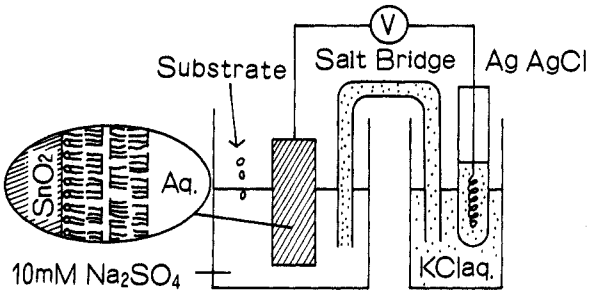


Figure 1. The setup for the potential measurement of monolayer modified electrodes.

Pressure–Area (π–A) Isotherms. A computer-controlled film balance system FSD 20 (San-esu Keisoku) was used for measuring surface pressure as a function of molecular area (trough size was 150 × 600 mm). Isotherms were taken at a compression rate of 24 mm/min, and the temperature of the aqueous subphase was maintained at 20.0 ± 0.1 °C. The solvents used for spreading monolayers were benzene for **1**–**4**, and a 7:2:1 (by volume) mixture of benzene/ethanol/chloroform for **5**. Monolayers were spread over aqueous guest molecules and compression was started after incubation for appropriate periods of time (10 min to 2 h). In order to equilibrate the interaction of monolayers with substrates, the π–A curves were compared for an incubation time of 2 h. The Langmuir–Blodgett (LB) deposition of the monolayer from a guest subphase was performed after the same incubation period.

Transfer of Monolayers onto Solid Substrates. The (LB) deposition of monolayers was performed in the vertical mode by using a computer-controlled film balance (FSD 50) and lifter (FSD 21) system (San-esu Keisoku). The monolayer of **1** was transferred to solid substrates at a surface pressure of 25.0 ± 0.2 mN/m and a deposition rate of 20 mm/min; that of **2** at 30.0 ± 0.5 mN/m and 20 mm/min; **3** at 35.0 ± 0.2 mN/m and 20 mm/min; **4** at 25.0 ± 0.2 mN/m and 50 mm/min; **5** at 25.0 ± 0.2 mN/m and 20 mm/min, respectively. The transfer ratios were unity (1.0 ± 0.1) for **1**–**5** unless otherwise stated.

Electrode Modification and Potential Measurement. SnO_2 glass electrodes (10 × 50 mm, Matsuzaki Shinku) were immersed in fuming nitric acid and rinsed with distilled water. The surface of the SnO_2 plates was made hydrophobic by deposition of one layer of monolayer **5**. The electrodes were further modified by depositing three layers of **1**[14] or one layer of **2**, **3**, or **4**. The last layers were deposited in the down-stroke mode. The modified electrodes were kept under water in small glass vessels and taken out from the trough without exposing the hydrophilic part of the monolayer to the air. The potential of modified electrodes against the Ag/AgCl reference electrode was monitored by using a Keithley 617 electrometer at room temperature (22 ± 1 °C). Aqueous Na_2SO_4 (10 mM) was used as a supporting electrolyte. Figure 1 illustrates the setup for the electrochemical measurement. Typically, the response of five identically modified electrodes, which were prepared separately, was measured for each substrate in order to establish the reproducibility of the data.

Spectroscopic Measurements. Absorption spectra were recorded on a Hitachi 330 spectrophotometer. FT-IR measurements were carried out on a Nicolet 710 FT-IR spectrometer by both the transmission method and the RAS (reflection absorption spectroscopy) method. Monolayers were transferred onto CaF_2 plates (for the transmission method) or onto Ag-coated (1000 Å, vapor deposition) slide glasses (for the RAS method). In order to minimize the influences of water vapor and CO_2 on the spectra, the system was purged by dry air for 3–4 h before measurements. X-ray photoelectron spectra were measured by a Perkin-Elmer PHI 5300 ESCA system with a Mg Kα X-ray source. LB films deposited onto solid substrates were mounted directly on the sample stage. The stage was cooled to below −100 °C by an internal thermal conductor connected to an external cryogenic Dewar. An X-ray power of 12 kV, 300 W, and a data acquisition time of 0.5 to 1 min were used for the measurements. Repeat scans over the same region of surface gave the identical spectra.

(14) A transfer ratio of the first layer of **1** was low (0.5 ~ 0.6); thus three layers were deposited on SnO_2 in the case of **1**.

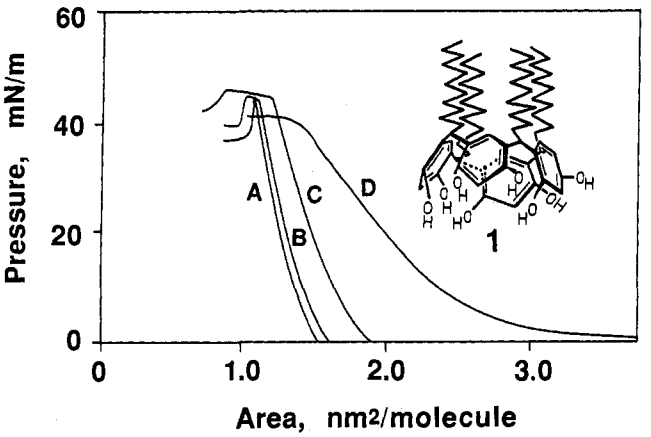


Figure 2. Surface pressure–area isotherms of resorcinol–dodecanal cyclotetramer at 20.0 ± 0.1 °C: (A) on pure water, incubation time 10 min; (B) on pure water, incubation time 2 h; (C) on 1.0×10^{-4} M aqueous riboflavin, incubation time 2 h; (D) on 2×10^{-2} unit M aqueous poly(vinylpyrrolidone), incubation time 2 h.

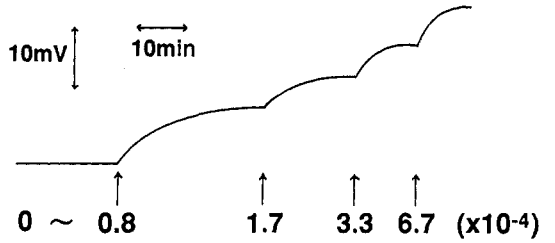


Figure 3. The time course of the potential shift observed on the **1**-modified electrode after the successive addition of ribose to the aqueous phase.

Results and Discussion

Monolayer of Resorcinol Cyclotetramer 1 at the Air–Water Interface. The π–A isotherm of Figure 2 shows that resorcinol-dodecanal cyclotetramer (**1**) forms a stable monolayer. The collapse area of 1.1 nm^2/molecule corresponds to the minimum molecular area, 1.1 nm^2, of the cyclic head group of **1** estimated from the CPK molecular model. The monolayer expands only slightly with increasing incubation times from 10 min to 2 h.

In the initial stage of experiment, we noticed that the π–A curve becomes expanded by the presence of sugars in the subphase.[1] The extent of the expansion showed a close correlation with the response of the monolayer-modified electrode; ribose gave the largest expansion. Johnston et al.[15] indicated some years ago that film expansion occurred as a result of the interaction of carbohydrates with phospholipid monolayers. Later careful work by Arnett and others[16] demonstrated, however, that the expansion disappeared when the sugars were recrystallized and/or treated with activated charcoal. The latter authors thus concluded that the phospholipid isotherm was sensitive to unknown surface-active impurities in sugars. Since this danger was conceivable in our case, we treated commercial ribose with activated charcoal.[17] The treated ribose (0.05 M in the subphase) did not cause any expansion, unlike the untreated ribose. It is strange that untreated sugars cause monolayer expansion reproducibly, even when we use different lots of sugars. Our efforts to detect those impurities by spectroscopic and thin layer chromatographic techniques failed.

Potentiometric Response of Monolayer-Modified Electrode to Sugars. The surface potential of monolayers is affected by adsorption of a second species which causes changes in the surface charge and dipole densities.[18] We decided to use this principle to detect possible sugar binding to the monolayer. SnO_2 glass electrodes were modified by deposition of monolayer **1** so that the polar end of the amphiphilic molecule remains exposed to the aqueous phase. The potential of the modified electrode was monitored against the Ag/AgCl electrode. It changes in the anodic direction upon addition of sugars to the aqueous phase beyond certain critical concentrations. Figure 3 illustrates a time course of the potential change observed after addition of ribose to the aqueous phase at concentrations of $(0.8–6.7) \times 10^{-4}$ M. This response occurs slowly; the potential reaches its equilibrium value in 10–15 min. In contrast, pH changes of the aqueous phase by 0.1 to 1 pH unit cause a quick change in the potential by 3 to 30 mV within 1 min. The critical concentration of ribose, beyond which the response appears, varied from 2.3×10^{-5} M to 7.2×10^{-5} M with an average value of $(4.0 \pm 1.5) \times 10^{-5}$ M, when we measured responses of seven independently prepared electrodes. The bare electrode without monolayer modification also shows an anodic response to ribose, but the response is seen at much higher concentrations: above 1×10^{-3} M. The electrode, which is modified with a monolayer of dialkylammonium salt **5** and has a hydrophobic surface, does not register any stable potential. Ribose treated with activated charcoal or with an anion exchange resin gave analogous responses, unlike the case of the π–A isotherm. Therefore, neither surface-active impurities, which supposedly caused expansion of monolayer **1** nor anionic impurities, which could induce anodic responses, influenced the electrode response. It is clear from these results that adsorption of ribose to the hydrophilic moiety of monolayer **1** is responsible for the observed potential change. Ribose, galactose, and glucose provide similar responses, as shown in Figure 4. The magnitude of the potential shift increases in proportion to the logarithmic concentration of sugars beyond certain critical concentrations with

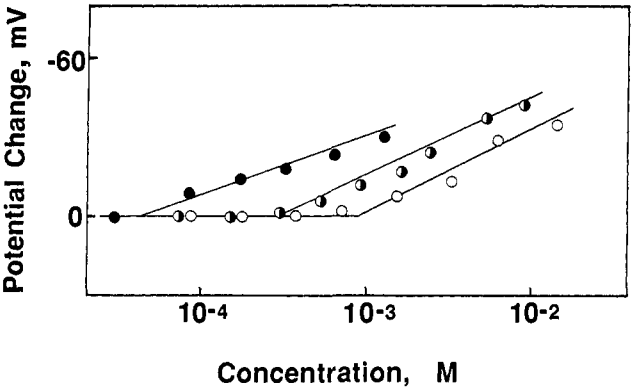


Figure 4. The potential changes of the **1**-modified electrode plotted against the logarithmic concentration of sugars: (●) ribose, (◑) galactose, (○) glucose.

Table I. Critical Concentrations beyond Which Monolayer Modified Electrodes Provide Anodic Responses

	$10^4 \times$ critical concentration (M)			
substrate	monolayer **1**	**2**	**3**	**4**
ribose	0.42 ± 0.22	1.7 ± 0.3	4.8	1.0 ± 0.6
xylose	2.23 ± 0.36	1.8 ± 0.6	4.6	0.6
arabinose	4.4 ± 1.1			
fucose	4.1 ± 1.3			
galactose	4.2 ± 0.7	14 ± 6	5.0	
glucose	6.0 ± 0.5	8.4 ± 0.8	17	0.4
sucrose	8.7 ± 1.8	14 ± 6	25	3.6 ± 1.0
poly(vinyl-pyrrolidone)	$(25.0 \pm 0.5) \times 10^{-3}$ (unit M)			
riboflavin	0.013			

(15) Johnston, D. S.; Coppard, E.; Parera, G. V.; Chapman, D. *Biochemistry* **1984**, *23*, 6912–6919.

(16) Arnett, E. M.; Harvey, N.; Johnson, E. A.; Johnston, D. S.; Chapman, D. *Biochemistry* **1986**, *25*, 5239–5242.

(17) Following the procedure by Arnett et al.,[16] activated charcoal (7.5 g/100 mL) was added to the hot sugar aqueous solutions (0.1 M) and removed by filtration. The concentration was adjusted by monitoring the optical activity.

(18) Davies, J. T.; Rideal, E. K. *Interfacial Phenomena*; Academic Press: New York, 1961.

slopes of 27 ± 3 mV/decade for ribose; 26 ± 4 mV/decade for galactose; and 25 ± 3 mV/decade for glucose. These slopes are identical for all the sugars we studied within experimental errors. The critical concentrations for the potentiometric response are summarized in Table I. The affinity of sugars to monolayer **1** can be evaluated from these values; they increase in the order: glucose < fucose ~ galactose ~ arabinose < xylose < ribose.

In order to confirm binding of sugars to the monolayer, attempts were made to detect sugars in the LB films by FT-IR and XPS measurements. However, it was difficult to detect small amounts of bound sugars because of structural similarities between sugars and **1**. The sugars lack strong IR peaks distinguishable from those of **1**. Sugars and **1** contain only oxygen and carbon as detectable elements by XPS. When higher sugar concentrations in the subphase were used to achieve saturation in binding, it was difficult to eliminate incorporation of free aqueous sugars which could be transferred along with the monolayer during the transfer process.[19]

Responses of Electrodes Modified by Monolayers of 2–4. The preceding results establish that the electrode response is a sensitive means to detect the binding of sugars to a hydroxylated monolayer. In a subsequent experiment, we examined other hydroxylated monolayers of **2**, **3**, and **4** to determine if they can interact with water-soluble sugars. Octadecyl alcohol (**2**) and octadecanoic acid (**4**) form condensed monolayers, exposing the hydroxyl and carboxylic acid groups to water in relatively well-aligned states. Their π–A isotherms show transitions from the liquid condensed phase to the solid condensed phase at areas of 0.2 mm^2/molecule (for both **2** and **4**), and at pressures of 13 and 24 mN/m, respectively. In contrast, a monolayer of *N*,*N*-didodecyl(gluconoamido)hexamide (**3**), which contains a noncyclic sugar head group, exhibits only the expanded phase with pressures appearing at an area of 1.2 nm^2/molecule. The monolayers of **2** and **4** were transferred onto SnO_2 electrodes at surface pressures of 30 mN/m (for **2**) and 25 mN/m (for **4**), where the surface densities of functional groups (OH in **2**, and COOH in **4**) are 5 groups/nm^2. The deposition of monolayer **3** was performed at 25 mN/m and at a surface density of 1.8 gluconoamide groups/nm^2.

All of these modified electrodes exhibit anodic potentiometric responses similar to that of the electrode modified by **1**, but the response is clearly different among the monolayers. The electrode modified with a monolayer of octadecyl alcohol shows responses to ribose, glucose, and galactose that are rather similar to those by the electrode modified with **1** (Figure 5A). However, the sensitivity as evaluated by the critical concentrations is smaller: (1.7 ± 0.3) × 10^{-4} M for ribose, (14 ± 6) × 10^{-4} M for galactose, and (8.4 ± 0.8) × 10^{-4} M for glucose. The selectivity of binding can be evaluated from the ratios of the critical concentration for different sugars. It is also lower in this case compared with those of monolayer **1**. The electrode modified by *N*,*N*-dioctadecyl-(gluconoamido)hexamide (**3**) produces potential shifts at concentrations three to ten times higher than those for **1**, showing its smaller sensitivity to sugars (Figure 5B). On the other hand, in case of the octadecanoic acid (**4**) electrode, the responses appear at low concentrations; some are even lower than those for **1** (Figure 5C). Both of the **3** and **4** electrode give lower sugar selectivities than the **1** electrode. The critical concentrations of the potential response for various electrodes are summarized in Table I. The selectivity indexes expressed as the ratio of the critical concentrations for ribose, the most sensitive sugar, to those for glucose, the least sensitive among monosaccharides, are 22 (**1**), 4.9 (**2**), 3.5 (**3**), and 0.4 (**4**). The potential change is proportional to the logarithmic concentrations of sugars, providing the slope of about 30 mV/decade for **2** and **4**, and 10 ± 1 mV/decade for **3**.

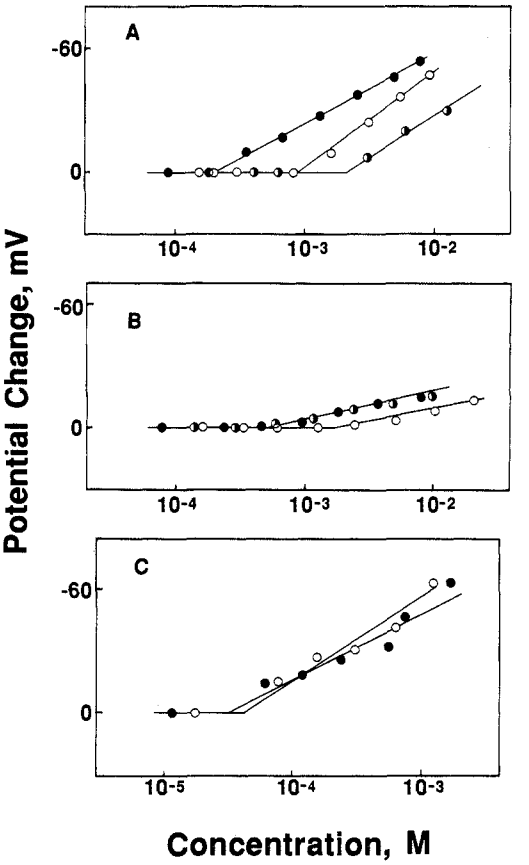


Figure 5. The potential changes of modified electrodes plotted against the logarithmic concentration of sugars: (●) ribose, (◑) galactose, (○) glucose. Electrodes are modified with different monolayers: (A) octadecyl alcohol, (B) *N*,*N*′-didodecyl(gluconoamide)hexamide, (C) octadecanoic acid.

Strong dependence of the electrode response on the modifying reagent is obvious. Adsorption of neutral molecules per se does not lead to large potential shifts. The anodic shift of the potential implies that the monolayer surface becomes more negatively charged against the Ag/AgCl electrode. Two mechanisms are conceivable for this change: one is charge separation upon hydrogen bond formation between sugars and monolayers, and the other is dipole density change induced by the adsorption of polar neutral molecules (sugars) at the monolayer–water interface. In either case, the two-dimensional molecular organization of monolayers and specific hydrogen bonding will produce well-defined orientations of sugar molecules relative to the host monolayer, and large potential changes ensue. The smaller slope (10 mV/decade) observed by the electrode modified with compound **3** supports this interpretation, because a flexible chain of five hydroxyl groups is involved in sugar orientation in this case. The carboxylic acid monolayer binds sugars efficiently, but the binding appears to be too strong to obtain significant selectivity.

Binding of Riboflavin and Electrode Response. As is clear from the unsuccessful spectroscopic attempts, sugar binding is difficult to detect by physicochemical means other than the electrode response. In order to circumvent this difficulty, we subsequently used a chromophoric sugar derivative, riboflavin, as guest molecule. Riboflavin has been reported to form a complex with **1** in CCl_4.[12a] The π–A isotherm of **1** becomes expanded upon introduction of riboflavin in the subphase; see Figure 2.

The potentiometric response of the **1**-modified electrode was examined in the presence of riboflavin. The potential of the modified electrode changes also in the anodic direction upon addition of riboflavin to the aqueous phase beyond the critical concentration of 1.3 × 10^{-6} M. The magnitudes of the potential

(19) The amount of water withdrawn along with monolayers can be determined by using a quartz crystal microbalance.[1] The maximum amount of co-transferred water thus determined is 10 nL/cm^2 for the monolayer in the first deposition cycle onto Ag layer from pure water. It decreases gradually in the subsequent cycles. The amount of co-transferred substrates which is contained in 10 nL of the aqueous phase can be calculated to be 0.006 molecule/nm^2 for 0.001 M solution, 0.12 molecule/nm^2 for 0.02 M, and 3 molecules/nm^2 for 0.5 M. The LB film of **1** on Ag prepared from 0.5 M aqueous xylose (in six deposition cycles) reveals that molar ratio of **1** to xylose to be 1:2.4 (XPS analysis), which corresponds to the density of two xylose molecule/nm^2. This density is small when compared with the above estimated value. Xylose may redissolve in the aqueous phase during repeated deposition cycles.

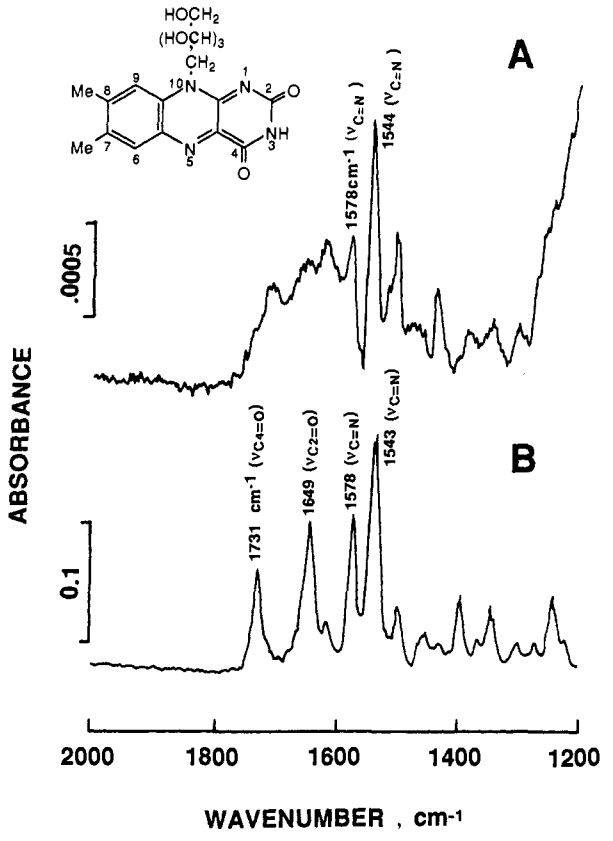


Figure 6. Comparison of FT-IR spectra of riboflavin: (A) difference spectrum obtained by substracting a spectrum of a LB film of **1** alone from that of a LB film of **1** transferred onto a CaF_2 plate (by six deposition cycles) from 1.0×10^{-4} M aqueous riboflavin (the 2924-cm^{-1} peak was used as reference in the substraction); (B) riboflavin spectrum in a KBr disk.

shift are proportional to the logarithmic concentration of riboflavin with the slope being (24 ± 4) mV/decade.

The binding of riboflavin to the monolayer can be monitored by UV spectroscopy. The monolayer of **1** was transferred onto quartz plates in the absence and the presence of riboflavin. Co-transfer of riboflavin in the one-layer LB film was proved by the presence of absorption spectral maxima at 267 and 227 nm. The molar ratio of riboflavin guest and host molecule of **1** is determined to be 1:5 from absorption intensity, as described before.[1] This result supports the premise that the potential shift is indeed ascribed to adsorption of riboflavin to monolayers.

FT-IR spectroscopy was subsequently employed to examine the interaction of riboflavin with the monolayer of **1**. FT-IR spectra of monolayer **1** transferred onto CaF_2 plates (by six deposition cycles)[20] from pure water and from aqueous riboflavin were compared. The former spectrum is identical with that of compound **1** in a KBr disk. A difference spectrum (Figure 6A), where the former spectrum is subtracted from the latter by using the 2924-cm^{-1} peak (ν_{CH_2} of **1**) as reference, agrees with a spectrum of riboflavin in KBr except for the shape of imide peaks (CO–NH–CO) around 1700 cm^{-1}. An IR spectrum of riboflavin in KBr (Figure 6B) exhibits peaks at 1731 cm^{-1} ($\nu_{C_4=O}$), 1649 cm^{-1} ($\nu_{C_2=O}$), 1578 cm^{-1} ($\nu_{C=N}$), and 1543 cm^{-1} ($\nu_{C=N}$), in which state the C_2=O group is considered to interact with both the N_3–H group and the ribityl moiety by forming intramolecular and/or

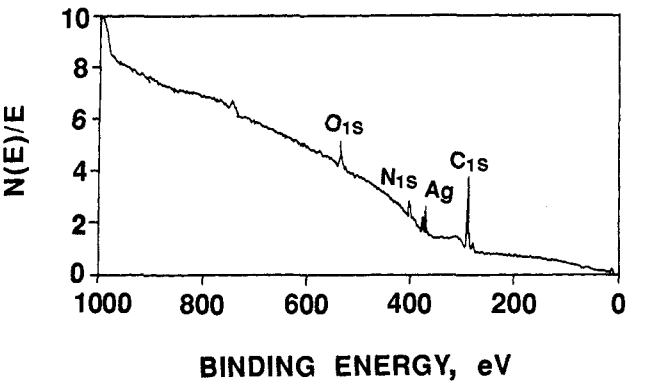


Figure 7. Wide-range XPS spectrum of an LB film of **1** obtained at a take-off angle of 45°. The LB film (6 layers) was transferred on a Ag-coated slide glass from 2×10^{-2} unit M aqueous poly(vinylpyrrolidone).

intermolecular hydrogen bonding.[21] When riboflavin is bound to the monolayer of **1**, the two peaks assigned to $\nu_{C_4=O}$ and $\nu_{C_2=O}$ become one broad peak, while the peaks of $\nu_{C=N}$ (1578 cm^{-1}) and $\nu_{C=N}$ (1544 cm^{-1}) are separatedly seen. Anisotropic samples are known to give IR absorption intensities that depend on the orientation of each bond; the transmission method emphasizes vibrations parallel to the surface, while the RAS method emphasizes those perpendicular to the surface.[22] However, the peak broadening at 1650–1700 cm^{-1} cannot be produced by the sample anisotropy, since it is also found in the RAS spectrum. Instead it is probably caused by frequency shift and broadening of the original two peaks upon complex formation of riboflavin with **1**. The hydroxyl groups of **1** would hydrogen bond with the imide moiety of riboflavin, and hydrogen bonds between the ribityl unit and the imide group in free fiboflavin would be replaced by those with **1**. The previous work on the complexation of riboflavin and **1** in CCl_4[12a] assumed that the imide moiety was primarily responsible for the binding. The FT-IR spectrum changes in Figure 6 suggest that the imide moiety and possibly the ribityl group are also responsible for hydrogen bonding between riboflavin and monolayer **1**.

Binding of Poly(vinylpyrrolidone) and Electrode Response. The hydrogen-bonding interaction as observed between the monolayer of **1** and sugars should be enhanced if multisite hydrogen-bonding compounds are used as guest molecule. We therefore examined the π–A behavior of **1** in the presence of several water-soluble polymers. A marked expansion of the π–A curve was observed when the subphase contains 2×10^{-2} unit M of poly(vinylpyrrolidone), as shown in Figure 2. Other polymers [poly(acrylic acid), poly(vinyl alcohol), and poly(ethylene oxide)] showed smaller effects. In fact, poly(vinylpyrrolidone) has been known as an efficient hydrogen-bond acceptor. It binds aqueous dye molecules effectively[23] and forms stable complexes with polymeric hydrogen-bond donors such as poly(acrylic acid).[24]

The electrode response to poly(vinylpyrrolidone) was also investigated. Addition of the polymer to the aqueous phase produced anodic shifts at concentrations above 7×10^{-9} M (2.5×10^{-6} unit M). Plots of the shift as a function of the logarithmic polymer concentration are linear with a slope of 18 mV/decade. The critical concentration is much lower than that (1.3×10^{-6} M) riboflavin.

Binding of poly(vinylpyrrolidone) to the monolayer was confirmed by X-ray photoelectron spectroscopy (XPS) and IR

(20) The transfer ratio of monolayer **1** on pure water onto CaF_2 in the down-stroke mode decreases from 0.6 (1st cycle) to 0.2 (3rd cycle), while those in the up-stroke mode and from aqueous riboflavin (except for the value of 0.5 in the first down-stroke mode) were always unity. These differences in the deposition characteristics also support the existence of interactions between monolayers and substrates. The transfer ratio of monolayer **1** from aqueous riboflavin onto Ag layer is lower: 0.2 in the down-stroke mode (except for the value of 0.6 in the first cycle) and unity in the up-stroke mode.

(21) Assignment of IR peaks of riboflavin: (a) Abe, M.; Kyogoku, Y.; Kitagawa, T.; Kawano, K.; Ohishi, N.; Takai-Suzuki, A.; Yagi, K. *Spectrochim. Acta* **1986**, *42A*, 1059–1068. (b) Schopfer, L. M.; Morris, M. D. *Biochemistry* **1980**, *19*, 4932–4935.

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(23) Sholtan, W. *Macromol. Chem.* **1953**, *11*, 131–230.

(24) Okhrimenko, I. S.; Efremov, I. F.; D'yakonova, E. B.; Miroshenko, G. V. *Vysokomol. Soedin.* **1966**, *8*, 1707–1711.

spectroscopy. Monolayer **1** was transferred onto Ag-coated (1000 Å) slide glasses.[25] A wide-scan XPS spectrum of an LB film of **1** deposited from aqueous poly(vinylpyrrolidone) (2×10^{-2} unit M) is shown in Figure 7. The relative peak intensity is affected by take-off angles; however, it was constant at angles greater than 45°. The peak areas give atomic compositions of the sample after correction of relative sensitivity factors.[26] The oxygen/carbon ratio of an LB film **1** transferred from pure water was determined to be 10.1:89.9 (%, ±0.4), in close agreement with the theoretical ratio (1:9) for the molecular structure of **1**. Here, the average values of data obtained at 45°, 75°, and 90° were used for calculation. The XPS spectrum displays an N_{1s} peak at 403.5 eV, which is absent in the spectrum of an LB film of **1** alone. The N_{1s} peak was also not detected for a Ag-coated plate which was dipped in aqueous poly(vinylpyrrolidone) (2×10^{-2} unit M) through the monolayer-free surface under otherwise the same conditions. The atomic composition of the LB film of **1** transferred from aqueous poly(vinylpyrrolidone) is 81.0:11.5:7.5 (%, ±0.4) for C, N, and O. This corresponds to a molar ratio of 15 ± 1 for pyrrolidone unit/**1**. The ratio is ca. two times as large as that expected from complete pairing of the hydroxyl group in the resorcinol and the amide group in the monomer unit. The complete pairing appears difficult owing to steric crowding. Not all the pyrrolidone units are involved in the binding.

FT-IR reflection absorption spectroscopy (RAS) was applied to the LB film of **1** (6 layers)[25] deposited from 2×10^{-2} unit M aqueous poly(vinylpyrrolidone). The spectrum shows a $\nu_{C=O}$ peak at 1694 cm^{-1} with a shoulder at 1653 cm^{-1}, revealing the presence of poly(vinylpyrrolidone). A film of poly(vinylpyrrolidone) cast from its aqueous solution shows a $\nu_{C=O}$ peak at 1694 cm^{-1}, which is reported to be characteristic of the free pyrrolidone group.[27] The peak at 1653 cm^{-1} corresponds to that bound to the hydroxyl group.[27] The frequency shift between two peaks can be ascribed to hydrogen bonding of poly(vinylpyrrolidone) with monolayer **1**. The presence of both free and hydrogen-bonded pyrrolidone groups agrees well with the result of XPS analysis.

Molecular Recognition by Monolayers. The results described above clearly demonstrate that sugars and other substrates are specifically bound to the monolayer of **1** from dilute subphases at the air–water interface. These specific interactions are most likely brought out by hydrogen bonding. The affinity and selectivity of substrate binding should be determined by the orientation and densities of the hydroxyl group in the monolayer. The arrangement of the hydroxyl group in the monolayer of resorcinol–dodecanal cyclotetramer (**1**), which serves as a 1:1 host for sugars in CCl_4,[12] offers high affinity and selectivity in sugar binding. Lower affinities and selectivities observed for monolayers **2** and **3** indicate the importance of multiple hydroxyl groups and their fixed mutual arrangement in the resorcinol cyclotetramer.

Interestingly, the binding selectivity is different between the air–water interface and the bulk phase (CCl_4). The electrode modified with monolayer **1** responds to sugars with a sensitivity order of glucose < fucose ~ galactose ~ arabinose < xylose < ribose. In contrast, extraction of these sugars with **1** into the CCl_4 phase was facilitated in the order: xylose ~ galactose ~ glucose (the ratio of sugar to **1**:very small) < arabinose (0.1) < ribose (0.5) < fucose (1.0).[12b] Ribose shows high affinities in both systems, whereas fucose, which is readily extracted into CCl_4, works moderately in the monolayer system. Xylose and galactose, which are complexed weakly in CCl_4, provide significant affinities at the interface.

(25) Deposition of monolayer **1** onto Ag layer was incomplete: The transfer ratio from pure water was 0.8 in the up-stroke mode and decreased from 0.7 (1st cycle) to 0.1 (6th cycle) in the down-stroke mode; that from 2×10^{-2} unit M aqueous poly(vinylpyrrolidone) was 0.9 in the up-stroke mode, and −0.3 in the down-stroke mode.

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(27) IR study of the pyrrolidone group in complexes with poly(vinyl alcohol), and in water and CCl_4: Pozdnyakov, V. M.; Saviskaya, A. N.; Klimenko, I. B.; Vol'f L. A.; Meos, A. I. *Vysokomol. Soedin.* **1969**, *11*, 649–652. Afanasenko, L. D.; Yarym-Agaev, N. L.; Bilobrov, V. M. *Ukr. Khim. Zh.* **1987**, *53*, 153–155.

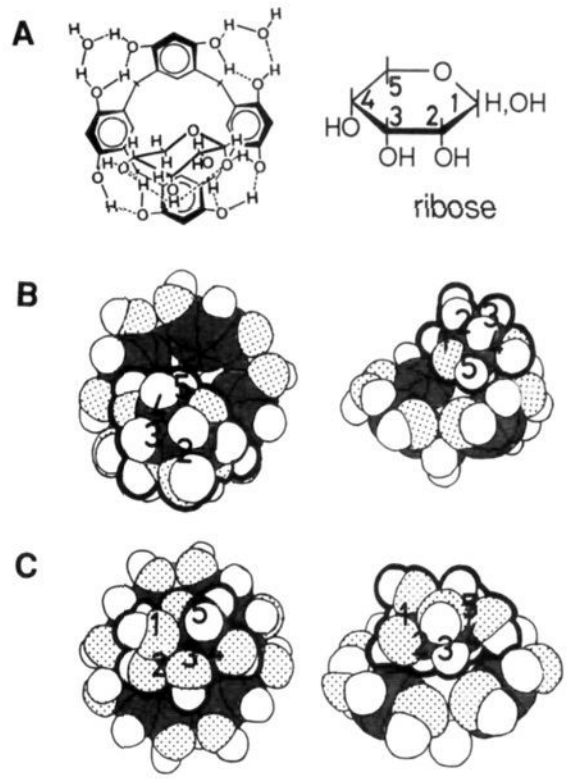

Figure 8. Schematic representations of 1:1 complex of **1** and ribose: (A, B) hydrophobic complex, (C) hydrophilic complex; carbon (dark), hydrogen (white), oxygen (dotted). Ribose molecules are encircled by thick lines. The left-hand sides of B and C illustrate top views of complexes, and the right-sides, side views. Ring carbons of ribose are numbered. It is seen that the hydrophilic face attaches to the cavity in B, and the hydrophobic face attaches to the cavity in C.

A detailed study of Aoyama[12b] on the complexation of **1** and sugars in CCl_4 concludes that sugars are doubly bound to two binding sites (phenolic binding sites) of **1** that are separated by a *m*-phenylene bridge. Stability of **1**-sugar complexes is attained through maximization of favorable hydrogen-bonding interaction and minimization of unfavorable exposure of the sugar OH groups to bulk solvent. A proposed structure of such a complex in CCl_4 is shown in Figure 8. Fucose possesses a methyl group on 5-C of the pyranose ring, instead of hydrogen in arabinose and the hydroxymethyl group in galactose. The highest extractability of fucose is governed conceivably by the lipophilicity of the resulting complex. Similar factors should determine the affinity of sugars to the monolayer of **1**, although, at present, it is difficult to specify which hydroxyl groups are involved in binding.

Examination of the CPK molecular model of **1**–sugar complexes and the chemical structure of sugars reveals interesting features which explain the observed selectivity well. We built the molecular models which maximize the hydrogen-bonding interaction as well as the exposure of sugar hydroxyl groups to bulk water. Ribose molecule (**12**, all-cis) consists of a lipophilic face and a hydrophilic face, thus enabling formation of both lipophilic and hydrophilic complexes (Figure 8). This explains its high affinity to **1** observed both in an apolar solvent and in a monolayer. On the contrary, glucose (**11**, all-trans) cannot form a well-fit complex with **1**, because equal numbers of the hydroxyl group exist in both of the two faces. Xylose (**13**) –**1** complex is hydrophilic owing to exposure of two hydroxyl groups to the bulk phase. The formation of this complex is favorable at the monolayer surface, but is suppressed in CCl_4. Fucose (**9**), which forms a lipophilic complex, does not bind to monolayer **1** efficiently.

A condensed monolayer of octadecyl alcohol **2**, where hydroxyl groups align at the monolayer surface at a density of 5 groups/nm^2, presents binding behavior similar to that of **1**. The alignment of the hydroxyl groups may produce a convergent binding site similar

to that of **1**. Rather effective binding of sugars by octadecyl alcohol (**2**) monolayer is consistent with this mechanism.

The high sensitivity of the electrode response (at 10^{-5} M sugars) is surprising. The extraction experiment of **1**-CCl_4-water system required much higher guest concentrations, typically 3-5 M for sugars.[12] These high concentrations were not necessary in the present work. The much enhanced sugar binding at the interface may be produced by either or both of the following factors. First, host molecules are aligned at the interface in a high density. This is expected to shift the equilibrium in favor of complex formation and to help sugar binding entropically. The advantage of multisite guest molecules like poly(vinylpyrrolidone) is obvious. Secondly, the structure of water at the interface is different from that of bulk water.[28] Host compound **1** in the bulk phase is strongly hydrogen-bonded to water, forming a stable tetrahydrate. The pattern and strength of hydrogen bonding with water may change extensively when this compound is in contact with the interfacial water.[12] The water of hydration may become more exchangeable relative to that in the bulk. The anisotropic alignment of the host functional group could also modify the pattern of hydration, enhancing sugar binding at low concentrations.

(28) Israelachivili, J. N. *Intermolecular and Surface Forces*; Academic Press: London, 1985.

Previous examples of well-defined guest monolayers are limited, especially with regard to organic guest molecules. Inclusion of azobenzene derivatives in cyclodextrin monolayers has been monitored by visible absorption spectroscopy and by circular dichroism.[5b] In this case, the host-guest monolayers were formed by spreading organic solutions of host and guest molecules (complexes) over the aqueous subphase; thus the host-guest interaction is not induced at the interface. Specific interactions of nucleolipid monolayers with water-soluble organic molecules have been studied only by the π-A isotherm data.[4] The present investigation represents, to the best of our knowledge, the first example of direct observation of guest binding to a monolayer.

Conclusions

It is demonstrated for the first time that molecular recognition is effectively accomplished by a hydrogen-bonding host monolayer that is exposed to aqueous subphases. The unexpected effectiveness is attributable to the high density of host molecules and peculiar properties of water at the interface. The mode of sugar binding is apparently different between the bulk phase and the interface. These features may be related to unique biological processes occurring at the cell membrane surface. An important implication of the present result is that novel chemical sensors can be designed on the basis of two-dimensional alignment of specifically interacting functional groups.

Formation of Organic Thin Films by Electrolysis of Surfactants with the Ferrocenyl Moiety

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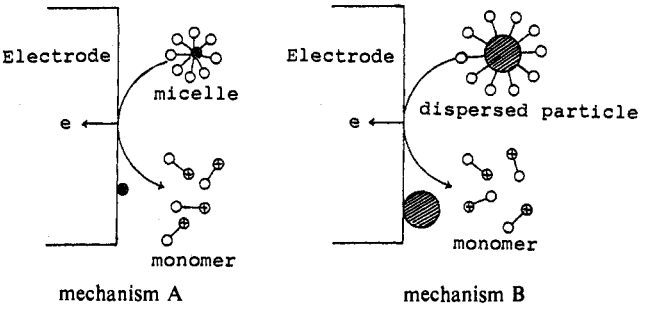
Abstract: The formation of organic thin films by controlled-potential electrolysis (oxidation) of an aqueous solution containing surfactants with a ferrocenyl moiety and an organic compound incorporated in micelles or in a dispersed organic pigment with use of the surfactants is examined. Opaque films of five azo dyes, vinylcarbazole, cetyl alcohol, dioctadecyldimethylammonium chloride, and 4,4′-didodecylviologen are formed through the former mechanism. Transparent films of phthalocyanine compounds (MPc (M = H_2, Cu)), four halogen derivatives of CuPc, three perylene derivatives, and two quinones are formed through the latter mechanism. The scanning electron micrograph studies show that crystal size of the film increases with electrolysis time in the case of the former mechanism and is the same for the added particles in the case of the latter mechanism. The adsorption isotherm of the surfactant on the pigment particle surface shows that they form monolayers at saturation and their desorption starts at slightly above the critical micelle concentration (cmc). These results suggest that the films formed through the former and the latter mechanisms are prepared by disruption of micelles and desorption of surfactant from the pigment surface, respectively.

Introduction

Although electrochemistry of organic compounds solubilized in micellar solutions has been extensively studied by several researchers,[1] no electrochemistry of micelles formed by redox-active surfactants has been reported. Recently, we demonstrated that a micelle formed by surfactants with the ferrocenyl moiety can be broken up into monomers when the surfactants are oxidized chemically or electrochemically.[2,3] Reversibility of such control

Scheme I



of the formation-disruption of a micelle was also demonstrated by a spectroscopic observation that a dye is solubilized or released accordingly as the micelles are formed or broken up. This procedure was extended to preparation of organic thin films.[4] This

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