Molecular Recognition of Nucleotides by the Guanidinium Unit at the Surface of Aqueous Micelles and Bilayers. A Comparison of Microscopic and Macroscopic Interfaces

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Received March 26, 1996[⊗]

Abstract: Molecular recognition of the guanidinium/phosphate pair was investigated at microscopic interfaces of aqueous micelles and bilayers. Monoalkyl and dialkyl amphiphiles with guanidinium head groups were synthesized and dispersed in water to form micelles and bilayers having guanidinium groups at the aggregate surface. Binding of nucleotides such as AMP to these functionalized aggregates was evaluated by using an equilibrium dialysis (ultrafiltration) method. The observed binding constants of 10^2-10^4 M^{-1} are much larger than the corresponding binding constant reported for a monomerically dispersed pair in the aqueous phase (1.4 M^{-1}) but are smaller than those found at the macroscopic air−water interface (10^6-10^7 M^{-1}). Therefore, the macroscopic interface promotes guanidinium−phosphate interaction more effectively than the microscopic interface. The present finding indicates that the microscopic interface can strengthen hydrogen bonding and/or electrostatic interaction even in the presence of water. Saturation binding phenomena were different between micelles and bilayers. All of the guanidinium groups in fluid micelles are effective for phosphate binding, but part of the guanidinium group in bilayers are not effective probably because of steric restriction.

Introduction

Molecular recognition in biological systems is achieved through combined noncovalent interactions such as hydrogen bonding, electrostatic interaction, and hydrophobic interaction. These noncovalent interactions are also useful for designing artificial host molecules. The hydrogen bonding interaction has been frequently used for preparing specific host molecules.[1−4] However, the hydrogen-bond-mediated interactions are not effective in aqueous systems, because bulk water forms strong hydrogen bonds with host molecules. Therefore, these host systems are not directly relevant to biological phenomena. Nowick et al.[5] investigated specific binding between thymine and adenine in aqueous micelles by the NMR titration method. But the thymine functional group is buried in the hydrophobic core of micelles, and effective hydrogen bonding is realized by avoiding direct contact of water with the host/guest system. Bonar−Law also investigated hydrogen bonded association of a hydrophobic porphyrin-based receptor inside SDS micelle and found the binding was energetically similar to binding in methanol.[6]

Molecular recognition in biological systems usually proceeds at microscopic interfaces such as cell surface and protein surface. We have found that complementary hydrogen bonding acts efficiently for molecular recognition at the air−water interface. For example, effective binding was observed for the complementary pairs of diaminotriazine monolayer and barbituric acid or nucleic acid bases (thymine, etc.),[7] orotate monolayer and nucleic acid bases (adenine etc.),[8] and guanidinium monolayer and phosphate (ATP, etc.).[9] It appears that the presence of the macroscopic interface is a key to accomplish effective molecular recognition in contact with the aqueous phase.

It is important to know to what extent the interface must be macroscopic in order to realize the effective hydrogen bonding. Aqueous micelles and bilayers provide microscopic interfaces appropriate for this purpose. We selected the guanidinium/phosphate pair for molecular recognition. Guanidinium groups are found at the arginine side chain in proteins. They form specific bonds with carboxylates and phosphates[10] and play important roles in keeping tertiary structures of proteins[11] and in providing anion recognition sites in some enzymes,[12,13]

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⊗ Abstract published in *Advance ACS Abstracts,* August 1, 1996.

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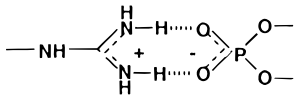
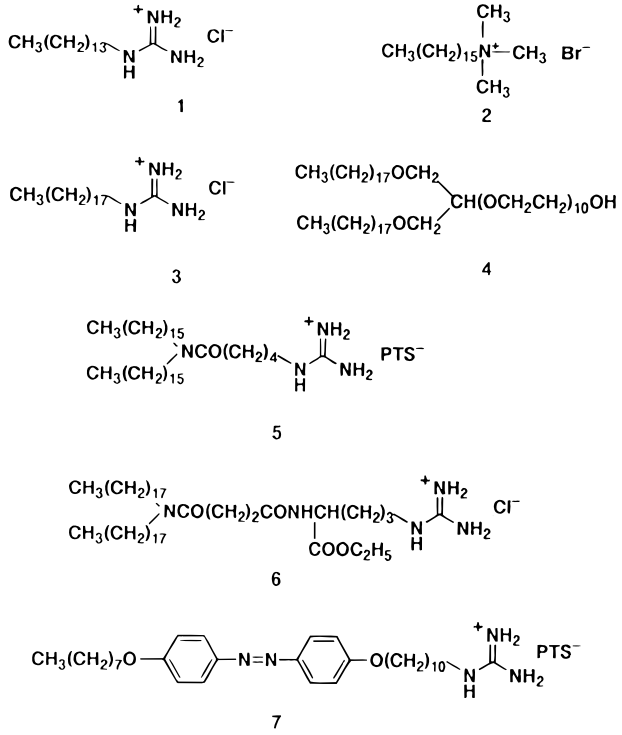
S0002-7863(96)00991-2 CCC: $12.00

because they are protonated in the wide pH range and can form hydrogen bonded ion pairs.[14]



The use of the guanidinium group as recognition sites has been investigated in artificial systems. For example, Lehn and co-workers reported a bicyclic guanidinium compound as a selective anion receptor,[15] and Anslyn et al. described a bis-guanidinium compound that increased the rate of imidazole-catalyzed mRNA hydrolysis by 20-fold in water.[16] Hamilton et al. synthesized an artificial enzyme that possessed two guanidinium moieties and increased the lutidine-catalyzed transterification of a *p*-nitrophenyl-activated RNA analog by nearly 1000-fold in acetonitrile,[17] and Schmidtchen investigated the phosphate binding ability of a linear ditopic anion host with a guanidinium moiety. Its binding constant in water, 10.6 M^{-1}, was ca. 7.5 times larger than that of the monomeric system.[18] We reported extremely effective binding of nucleotides with guanidinium monolayers.[9] In this study, we synthesized monoalkyl and dialkyl amphiphiles with guanidinium groups as hydrophilic head groups and examined their binding behavior toward adenine nucleotides in aqueous micelles and bilayers.

Chart 1. Structures of the Amphiphiles Used in This Study



Experimental Section

Materials. Adenosine-5′-monophosphate disodium salt (AMP), adenosine-5′-diphosphate disodium salt (ADP), and adenosine-5′-triphosphate disodium salt (ATP) were purchased from Oriental Yeast Co. Bis(2-hydroxyethyl)iminotris(hydroxymethyl)methane (Bis-Tris) and 2,6-dichlorophenolindophenol (DCPIP) were purchased from Wako Pure Chemical Industries. Ultrafilter Molcut II LC (fractionating molecular weight, 5000) was purchased from Nihon Millipore Kogyo. Water used in this study was ion-exchanged by Millipore WQ 500 (Yamato), and its specific resistance was around 18 MΩ·cm. Molecular structures of amphiphiles used in this study are shown in Chart 1. Hexadecyltrimethylammonium bromide (**2**) was purchased from Tokyo Chemical Industries, Ltd. Dialkyl nonionic amphiphile **4** was provided by a research group at Kyushu University.[19] Guanidinium amphiphiles **1**, **3**, **5**, and **6** were synthesized as described below. L-Argenine ethyl ester dihydrochloride (Kokusan Chemical Works) for the preparation of **6** was used as supplied.

1-Tetradecylguanidinium Chloride (1). Methylisothiourea sulfate (1.65 g, 11.9 mmol) was dispersed in methanol (30 mL). Sodium (0.303 g, 10.2 mmol) dissolved in methanol (30 mL) was added slowly at 0 °C, and the resulting mixture was stirred for 1 h at 0 °C. Tetradecylamine (2.10 g, 9.85 mmol) dissolved in methanol (20 mL) was added slowly to the dispersion at room temperature. The whole mixture was stirred at room temperature for 2 h and then at 40 °C for 42.5 h. *p*-Toluenesulfonic acid monohydrate (*p*-TsOH·H_2O) (3.80 g, 20.0 mmol) was added to the solution. The solution was heated to 60−70 °C, and the precipitate was filtered off. The filtrate was kept at −20 °C to give colorless crystals (1.09 g, mp, 98−101 °C). Recrystallization from acetonitrile (100 mL) gave 1-tetradecylguanidinium *p*-toluenesulfonate salt as fine colorless crystals (0.887 g, 21%): mp 106.9−108.2 °C; ^{1}H NMR (DMSO-d_6) δ 0.88 (t, 3H, J = 6.7 Hz, CH_3), 1.25 (m, 2H, 11 CH_2), 1.51 (m, 2H, $NHCH_2CH_2$), 2.29 (s, 3H, $ArCH_3$), 3.02 (m, 2H, $NHCH_2$), 7.19 (d, 2H, J = 8.0 Hz, Ar), 7.48 (d, 2H, J = 8.2 Hz, Ar), 6.94 and 7.39 (br and m, respectively, 5H, guanidinium). Anal. Calcd for $C_{22}H_{41}N_3O_3S$: C, 61.79; H, 9.66; N, 9.83. Found: C, 61.71; H, 9.52; N, 9.85. The *p*-toluenesulfonate salt was converted to chloride by an ion exchange on IRA-400 resin (Cl^-). Chloride (**1**): ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 6.6 Hz, CH_3), 1.25 (m, 22H, 11 CH_2), 1.60 (m, 2H, $NHCH_2CH_2$), 3.11 (m, 2H, $NHCH_2$), 6.46 and 7.87 (br and m, respectively, 5H, guanidinium).

1-Octadecylguanidinium Chloride (3) was obtained by ion-exchange of 1-octadecylguanidinium *p*-toluenesulfonate salt to improve water dispersibility. 1-Octadecylguanidinium *p*-toluenesulfonate salt was synthesized by operations similar to those for **1**. 1-Octadecylguanidinium *p*-toluenesulfonate salt (19% yield): mp 117−118 °C; ^{1}H NMR (DMSO-d_6) δ 0.85 (t, 3H, J = 6.6 Hz, CH_3), 1.24 (m, 30H, 15 CH_2), 1.44 (m, 2H, $NHCH_2CH_2$), 2.29 (s, 3H, $ArCH_3$), 3.07 (m, 2H, $NHCH_2$), 7.11 (d, 2H, J = 8.0 Hz, Ar), 7.47 (d, 2H, J = 8.0 Hz, Ar), 6.80 and 7.37 (br and m, respectively, 5H, guanidinium). Anal. Calcd for $C_{26}H_{49}N_3O_3S$: C, 64.55; H, 10.21; N, 8.69. Found: C, 64.53; H, 10.19; N, 8.52. Chloride (**3**): ^{1}H NMR (DMSO-d_6) δ 0.84 (t, 3H, J = 6.4 Hz, CH_3), 1.23 (m, 30H, 15 CH_2), 1.43 (m, 2H, $NHCH_2CH_2$), 3.06 (m, 2H, $NHCH_2$), 7.21 and 7.43 (br and m, respectively, 5H, guanidinium).

Dihexadecylamine. A mixture of hexadecylamine (61.9 g, 256 mmol), hexadecyl bromide (65.0 g, 213 mmol), Na_2CO_3 (56.6 g, 534 mmol), and ethanol (400 mL) was refluxed for 116 h. The solvent was removed by evaporation to dryness, and the residual solid was suspended in $CHCl_3$. This was washed with aqueous Na_2CO_3 and water (×2) followed by drying over Na_2SO_4. The solvent was removed by evaporation, and the residue was recrystallized three times from hexane to give dihexadecylamine (27.0 g, 27%) as a colorless powder: mp 65.7−65.9 °C; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6H, J = 6.7 Hz, 2 CH_3), 1.2−1.4 (m, 52H, 26 CH_2), 1.4−1.6 (m, 4H, 2 CH_2CH_2N), 2.64 (t, 4H, J = 7.5 Hz, 2 CH_2N); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 14.07, 22.67, 27.41, 29.34, 29.58, 29.60, 29.68 (br), 30.16, 31.91, 50.13; HRMS (EI) m/z calcd for $C_{32}H_{67}N$ 465.5273, found 465.5272. Anal. Calcd for $C_{32}H_{67}N\cdot{}^1/_2H_2O$: C, 80.93; H, 14.43; N, 2.95. Found: C, 81.02; H, 14.26; N, 2.97.

4-(Dihexadecylcarbamoyl)butylammonium Chloride. A solution of dihexadecylamine (7.99 g, 17.1 mmol), 5-bromovaleryl chloride (3.50 mL, 24.7 mmol), and triethylamine (3.60 mL, 25.9 mmol) in CH_2Cl_2

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(300 mL) was stirred at room temperature for 2.5 h followed by washing with aqueous Na_2CO_3 (×2) and water (×5). After being dried over Na_2SO_4, the solvent was removed by evaporation to give a yellow oil. Hexane (300 mL) was added, and the insoluble material was removed by filtration. The filtrate was evaporated to give a yellow oil, which was chromatographed on SiO_2 (3:1 hexane/ethyl acetate) to give crude 5-bromo-*N*,*N*-di(hexadecyl)pentanoylamide (7.68 g, 71%) as an oil: TLC R_f 0.73 (2:1 hexane/ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6H, J = 6.6 Hz, 2 CH_3), 1.2−1.4 (m, 52H, 26 CH_2), 1.4−1.6 (m, 4H, 2 NCH_2CH_2), 1.7−2.0 (m, 4H, 2 CH_2), 2.31 (t, 2H, J = 7.1 Hz, CH_2C(O)), 3.19 (t, 2H, J = 7.7 Hz, NCH_2), 3.28 (t, 2H, J = 7.6 Hz, NCH_2), 3.42 (t, 2H, J = 6.6 Hz, CH_2Br). A mixture of this product and potassium phthalimide (4.56 g, 24.6 mmol) and *N*,*N*-dimethylformamide (DMF) (150 mL) was stirred at 80−90 °C for 21 h. DMF was removed under vacuum, and the residue was dissolved in CH_2Cl_2 (500 mL). This solution was washed with aqueous Na_2CO_3 (×2) and water (×2) and dried over Na_2SO_4. The solvent was removed by evaporation, and the residue was chromatographed on SiO_2 (3:1 hexane/ethyl acetate) to give crude 5-phthalimido-*N*,*N*-di(hexadecyl)pentanoylamide (6.04 g, 71%) as a colorless wax: TLC R_f 0.57 (2:1 hexane/ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6H, J = 6.6 Hz, 2 CH_3), 1.2−1.4 (m, 52H, 26 CH_2), 1.4−1.6 (m, 4H, 2 NCH_2CH_2), 1.6−1.8 (m, 4H, 2 CH_2), 2.34 (t, 2H, J = 7.0 Hz, CH_2C(O)), 3.19 (t, 2H, J = 7.7 Hz, NCH_2), 3.27 (t, 2H, J = 7.6 Hz, NCH_2), 3.71 (t, 2H, J = 6.8 Hz, CH_2N), 7.6−7.9 (m, 4H, Ar). A portion of this product (1.0 g) was refluxed with hydrazine monohydrate (288 mg, 5.69 mmol) and 95% aqueous ethanol (30 mL) for 16 h. Concentrated hydrochloric acid was added, and the resulting insoluble material was removed by filtration through a Celite pad. The filtrate was concentrated, and the residue was recrystallized twice from ethyl acetate to give the title compound (690 mg, 80%) as a colorless powder: mp 71.7−72.7 °C; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6H, J = 6.7 Hz, 2 CH_3), 1.1−1.4 (m, 56H, 28 CH_2), 1.4−1.6 (m, 4H, 2 CH_2), 1.7−1.9 (m, 4H, 2 CH_2), 2.37 (br s, 2H, CH_2C(O)), 3.06 (br s, 2H, CH_2NH_3), 3.1−3.3 (m, 4H, 2 CH_2N), 8.50 (br s, 3H, NH_3). Anal. Calcd for $C_{37}H_{77}N_2OCl$: C, 73.89; H, 12.90; N, 4.66. Found: C, 73.95; H, 12.93; N, 4.63.

1-(4-(Dihexadecylcarbamoyl)butyl)guanidinium *p*-Toluenesulfonate (5). Methylisothiourea sulfate (417 mg, 3.0 mmol) was dissolved in 0.44 N NaOH (8 mL) at room temperature. The resulting clear solution was stirred for 10 min followed by addition of 4-(dihexadecylcarbamoyl)butylammonium chloride (300 mg, 0.50 mmol) in ethanol (8 mL). The whole mixture was stirred at 70 °C for 16 h. *p*-TsOH·H_2O (1.34 g, 7.0 mmol) was added to the mixture followed by further stirring at room temperature for 6 h. This mixture was extracted with $CHCl_3$. The combined extracts were dried over Na_2SO_4 and concentrated under reduced pressure. To the residue was added CH_3CN/THF (1:1) with heating, and the insoluble material was removed by filtration. The filtrate was concentrated, and the residue was subjected to column chromatography on alumina (2:1 CH_3CN/CH_3OH) to give **5** as a colorless oil (152 mg, 39%): ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6H, J = 6.7 Hz, 2 CH_3), 1.1−1.4 (m, 52H, 26 CH_2), 1.4−1.7 (m, 8H, 4 CH_2), 2.31 (br s, 2H, CH_2C(O)), 2.35 (s, 3H, $ArCH_3$), 3.1−3.3 (m, 6H, 2 CH_2N and CH_2NH), 7.00 (br s, 4H, 2 NH_2), 7.17 (d, 2H, J = 8.0 Hz, Ar), 7.62 (br s, 1H, NH), 7.72 (d, 2H, J = 8.0 Hz, Ar). Anal. Calcd for $C_{45}H_{86}N_4O_4S$: C, 69.36; H, 11.12; N, 7.19. Found: C, 69.18; H, 11.08; N, 7.10.

Dioctadecylamine was prepared by the procedures similar to those of dihexadecylamine in 37% yield: mp 72.0−72.5 °C; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6H, J = 6.7 Hz, 2 CH_3), 1.2−1.4 (m, 60H, 30 CH_2), 1.4−1.6 (m, 4H, 2 CH_2CH_2N), 2.61 (t, 4H, J = 7.4 Hz, 2 CH_2N). Anal. Calcd for $C_{36}H_{75}N$: C, 72.83; H, 14.48; N, 2.68. Found: C, 82.70; H, 14.41; N, 2.67.

***N*,*N*-Dioctadecylsuccinamic Acid.** A solution of dioctadecylamine (2.47 g, 4.74 mmol) and succinic anhydride (956 mg, 9.56 mmol) in THF (100 mL) was stirred at room temperature for 17 h. Solvent was removed by evaporation, and the residue was dissolved in CH_2Cl_2. This was washed with 1 N aqueous HCl (×2) and dried over Na_2SO_4. Evaporation of the solvent and recrystallization of the residue from CH_3CN gave *N*,*N*-dioctadecylsuccinamic acid (2.26 g, 77%): mp 65.8−66.5 °C; ^{1}H NMR ($CDCl_3$) δ 0.87 (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), 2.68 (s, 4H, $C(O)CH_2CH_2C(O)$), 3.22 (t, 2H, J = 7.8 Hz, CH_2N), 3.31 (t, 2H, J = 7.7 Hz, CH_2N). Anal. Calcd for $C_{40}H_{79}NO_3$: C, 77.23; H, 12.80; N, 2.25. Found: C, 77.20; H, 12.74; N, 2.27.

***N*,*N*-Dioctadecyl-*N*′-ethylarginylsuccinamide HCl Salt (6).** *N*,*N*-Dioctadecylsuccinamic acid (0.50 g, 0.80 mmol) was dissolved in CH_2Cl_2 (150 mL), and diethyl phosphorocyanidate (DEPC) (0.150 mL, 0.99 mmol) was added to the solution at 0 °C. After 15 min arginine ethyl ester dihydrochloride (0.265 g, 0.97 mmol) and triethylamine (0.60 mL, 4.30 mmol) dissolved in DMF (50 mL) was added slowly. The whole mixture was stirred at room temperature for 120 h. Solvent was removed in vacuo, and water was added to the residue. The supernatant was removed by decantation. The solid residue was dispersed in acetonitrile (100 mL). *p*-TsOH·H_2O (3.03 g, 15.9 mmol) was added to the dispersion, and the solid was dissolved with sonication. Water (50 mL) was slowly added to the resulting clear solution. The precipitates (the crude *p*-toluenesulfonate) were collected and dissolved in methanol. Concentrated HCl was added to the solution and the resulting precipitates were collected by filtration and recrystallized from acetonitrile/ethanol (5:1) (7 mL) to give **6** as colorless crystals (0.156 g, 23%): mp 168−169 °C; ^{1}H NMR ($CDCl_3$), δ 0.88 (t, 6H, J = 6.7 Hz, 2 CH_3), 1.25 (m, 63H, 30 CH_2 and $COOCH_2CH_3$), 1.4−1.7 (m, 8H, 2 NCH_2CH_2 and $CHCH_2CH_2CH_2NH$), 2.59 (br, 2H, CH_2CO), 2.70 (br, 2H, CH_2CO), 3.23 (br, 6H, 2 NCH_2 and $CH_2NHC(NH_2)NH_2$), 4.17 (q, 2H, J = 7.1 Hz, $COOCH_2CH_3$), 4.42 (br, 1H, $NHCHCO$), 7.26 (br, 1H, CONH), 6.91 and 7.89 (br each, 5H, guanidinium). Anal. Calcd for $C_{48}H_{96}N_5O_4Cl$: C, 68.41; H, 11.48; N, 8.31. Found: C, 68.36; H, 11.38; N, 8.06.

Characterization of the Aggregates. The critical micellar concentration (CMC) of monoalkyl amphiphiles **1** and **2** were determined by a dye method using DCPIP.[20] DCPIP shows partial deprotonation in water at neutral pH. The presence of cationic micelles shifts its equilibrium to the deprotonated species (blue) because of incorporation of the dye in micelles and subsequent stabilization of the anionic species. The CMC values of the amphiphiles were measured as follows. Mixed solutions of amphiphile **1** or **2** and DCPIP were prepared (**1**/DCPIP = 0−1/0.02 mM and **2**/DCPIP = 0−5/0.02 mM), and the observed λ_{max} value was monitored as a function of amphiphile concentration. The λ_{max} value of DCPIP was ca. 520 nm below CMC and shifted to ca. 600 nm at sufficiently high concentrations of the amphiphile. The CMC values were determined from the minimum amphiphile concentration where the λ_{max} showed a shift to a long wavelength. UV absorption measurements were performed in water or in 0.5 mM Bis-Tris buffer (pH 7) at 25 °C with a JASCO UV/VIS/NIR spectrometer equipped with a JASCO EHC-441 temperature controller. The measurements were carried out.

Differential scanning calorimetry (DSC) measurements were performed with a Seiko SSC/5200H calorimeter equipped with DSC 120 module. Aqueous dispersions of **3/4** (0.5/1.0 mM), **5** (1.0 mM), and **6** (1.0 mM) were prepared, subjected to ultrasonic treatment with a probe-type sonicator (Bransonic Sonifier Model 250), and sealed (60 μL) in a Ag sample pan, and scaning was carried out at a rate of 1 °C·min^{-1} from 5 to 70 °C.

Transmission electron microscopy (TEM) observation of aqueous dispersions of the amphiphiles was performed as follows. Aqueous dispersion of the amphiphile (0.5−1.0 mM) were negatively stained by mixing with equal volumes of saturated aqueous uranyl acetate. The mixture was kept at room temperature for 1 h. Aliquots of the solution were placed on a carbon-coated copper mesh and dried in vacuo. The sample was observed with a HITACHI H-600S electron microscope at 12 000−60 000 magnification.

Ultrafiltration and Determination of the Component Concentration. Binding of substrate molecules to aqueous aggregates was evaluated by the equilibrium dialysis method (ultrafiltration method)[21] which can separate unbound substrates from bound substrates (Figure 1). All of the solutions used here were prepared with 0.5 mM Bis-Tris buffer (pH 7). Amphiphiles and nucleotides were mixed at the following concentrations: **1**/AMP = 0.5/0−1.0 mM, **1**/ADP = 0.5/0−1.0 mM, **1**/ATP = 0.5/0−1.0 mM, **2**/AMP = 10.0/0−10.0 mM, **3/4**/AMP = 0.5/1.0/0−1.0 mM, **5**/AMP = 0.5/0−1.0 mM, and **6**/AMP = 0.5/0−1.0 mM. The mixture was put into the upper cup of ultrafilter

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Molcut II LC (low adsorption type filter with fractionating molecular weight of 5000). It was kept in a thermostated bath at 25 °C for 1 h until the set up was thermally equilibrated. Pressure was then applied to the upper cup to accelerate filtration. The nucleotide concentrations in the filtrates were determined from absorbance of the adenine ring ($\epsilon = 13 \times 10^3$ at 260 nm). Concentrations of guanidinium amphiphiles in the filtrates were determined from the absorption (535 nm) of the dye mixture that was formed from their reaction with diacetyl and 1-naphthol (the V−P reaction).[22] An aqueous sample (2 mL) and 2 N NaOH (1 mL) were mixed. 1-Propanol solution of 0.025 wt% diacetyl and 5 wt% 1-naphthol (1 mL) was added, and the absorbance at 535 nm was measured after 30-min reaction.

The concentration of the bound substrates $[S]_{bound}$ was estimated by subtracting the concentrations of unbound substrate $[S]_{free}$ from initial substrate concentration $[S]_{initial}$.

$$[S]_{bound} = [S]_{initial} - [S]_{free} \quad (1)$$

The ratio of the bound substrate to the guanidinium group, *y*, was estimated from eq 2

$$y = \frac{[S]_{bound}}{[H]} = \frac{[S]_{initial} - [S]_{free}}{[H]} \quad (2)$$

where [H] is the concentration of guanidinium group in aggregates that is corrected with the amount of the molecularly dispersed amphiphile. Binding of the substrates to guanidinium amphiphiles is represented as eq 3 by assuming Langmuir type adsorption (eqs 3 and 4)[23]

$$y = \frac{\alpha \times [S]_{free}}{1/K + [S]_{free}} \quad (3)$$

$$\frac{[S]_{free}}{y} = \frac{1}{\alpha \times K} + \frac{1}{\alpha} \times [S]_{free} \quad (4)$$

where α and *K* represent saturation binding ratio of substrates and binding constant, respectively. The α and *K* values can be obtained from the intercept and the slope, respectively, of eq 4.

Results and Discussion

Recognition of Nucleotides by Guanidinium Micelles. Critical micellar concentrations (CMC) of monoalkyl amphiphiles **1** and **2** were determined as described in the Experimental Section. The CMC value of amphiphile **2** was 9.0×10^{-4} M in water and 4.3×10^{-4} M in Bis-Tris buffer (pH 7 and 25 °C). The former value is in good agreement with the reported values.[24,25] The CMC value of amphiphile **1** was 1.3×10^{-4} M in water and 5.7×10^{-5} M in BisTris buffer (pH 7 and 25 °C). Monoalkyl guanidinium amphiphile **1** showed a lower CMC value than monoalkyl quarternary ammonium amphiphile **2**. All the binding experiments described as below were carried out at concentrations above these CMC.

The ultrafiltration experiments for aqueous substrates in the absence of the amphiphiles were performed prior to binding study as a control. The substrates contained in the initial solutions were detected in the filtrate within ±0.5% error in all cases. As the experimental error is estimated to be approximately 1.0%, nonspecific adsorption of the substrate on the filter is negligible. Control experiment for filtration of the guanidinium **1** was also carried out in the absence of the substrate. The concentration of **1** detected in the filtrate by V−P reaction was 5.0×10^{-5} M and was almost the same as its CMC value. Therefore, only molecularly dispersed amphiphile molecules passed through the filter, and the micellar **1** was trapped on the filter.

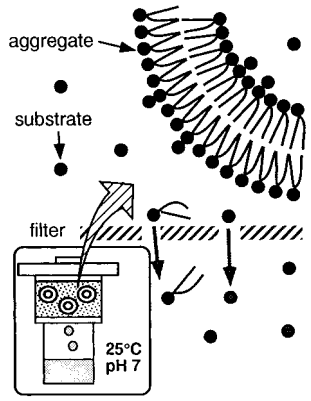

Figure 1. Scheme of equilibrium dialysis (ultrafiltration).

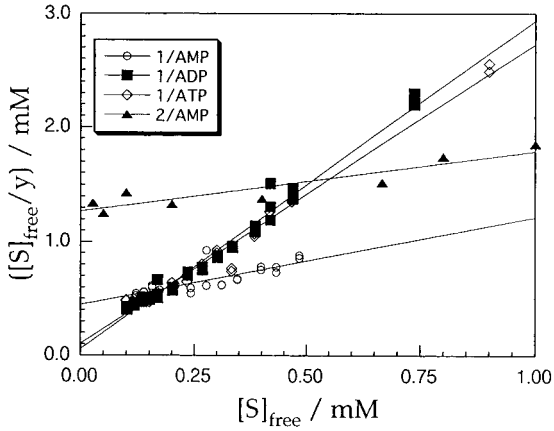

Figure 2. Reciprocal plots of the Langmuir binding isotherm for guanidinium micelles and nucleotides; 25 °C and pH 7.

In order to investigate the binding ability of amphiphile **1** toward nucleotides, ultrafiltration was applied to aqueous mixtures of micelle **1** and substrates, AMP, ADP, and ATP. As the adsorption of the substrates on the filter is negligible, the substrate concentration in the filtrate is equal to the concentration of unbound substrates in the original micelle/substrate mixtures. These experimental data are plotted according to eq 4 in Figure 2. All of the plots show linear relationships indicating the occurrence of the Langmuir type adsorption. Saturation binding ratio α and binding constant *K* were obtained from intercept and slope, respectively, and summarized in Table 1, together with determination coefficient R^2.

The combination of micellar **1** and AMP substrate gives $K = 1.8 \times 10^3$ M^{-1} and $\alpha = 1.3 \pm 0.3$. This α value suggests formation of an equimolar complex from guanidinium and phosphate. The obtained binding constant is larger than the value reported for the corresponding molecularly dispersed system in aqueous media (1.4 M^{-1})[10] by a factor of 10^2.

Both of complementary hydrogen bonding and electrostatic attraction are believed to act as binding forces for this system. In order to estimate the contribution of these interactions, we conducted a control experiment by using micellar **2** that is not capable of hydrogen bonding with AMP. The binding constant in this case was 8.1×10^2 M^{-1} and was half the value obtained for **1**/AMP. Thus, both of complementary hydrogen bonding and electrostatic attraction clearly contributed to the guanidinium/phosphate recognition on the micellar surface.

Subsequently, binding of guanidinium micelle **1** with ADP, ATP, and adenosine that have two, three, and zero phosphate

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(24) Klevens, H. B. *J. Am. Oil. Chem. Soc.* **1953**, *30*, 74.
(25) Ralston, A. W.; Eggenberger, D. N. *J. Am. Chem. Soc.* **1947**, *70*, 977.

Table 1. Binding Parameters between Guanidinium Aggregates and Substrates

amphiphile	substrate	K^a/M^{-1}	α^a	R^2
		Micelle		
1	AMP	$1.8 \pm 0.1 \times 10^3$	1.3 ± 0.3	0.95
1	ADP	$5.4 \pm 0.3 \times 10^4$	0.4 ± 0.1	0.99
1	ATP	$2.5 \pm 0.7 \times 10^4$	0.4 ± 0.1	0.98
1	adenosine	no binding[b]		
2	AMP	$8.1 \pm 0.1 \times 10^2$	1.1 ± 0.2	0.97
		Bilayer		
3/4 (1:2)	AMP	$1.8 \pm 0.1 \times 10^2$	0.6 ± 0.2	0.98
3/4 (1:2)	AMP	$1.1 \pm 0.1 \times 10^2$	1.2 ± 0.2	0.98 (sonicated)
5	AMP	$1.0 \pm 0.1 \times 10^3$	0.7 ± 0.1	0.99
5	AMP	$1.0 \pm 0.1 \times 10^3$	0.5 ± 0.1	0.99 (sonicated)
6	AMP	$3.3 \pm 0.1 \times 10^2$	0.9 ± 0.2	0.98
		Monolayer[c]		
7	AMP	3.2×10^6	1.0	
7	ATP	1.7×10^7	0.3	
		Bulk Water[d]		
guanidinium	$H_2PO_4^-$	1.37		

[a] The ± values are standard deviations from the least squares analysis. [b] Detectable binding was not observed. [c] Data from ref 9a. [d] Data from ref 10.

groups, respectively, were performed (Table 1). In the case of adenosine substrate, binding was not detectably observed. This result endorses that the observed binding of nucleotides to guanidinium micelles is based on the specific interaction between guanidinium and phosphate groups. The adenine moiety, by itself, is not effective for the binding. Results for ADP and ATP summarized in Table 1 are different from those of AMP in both of the binding constant and the saturation binding ratio. The saturation binding ratio α is 0.4 ± 0.1 for **1**/ADP and **1**/ATP, being much smaller than that of **1**/AMP. Clearly, the two phosphate units in ADP are used in the equimolar complexation. It is known that two of the three phosphate groups in ATP are deprotonated at neutral pH.[26] Therefore, ATP molecule may bind to two guanidinium groups, unless additional dissociation of the phosphate group proceeds due to a pK_a shift on the cationic micelle. Or, if all three phosphate units are dissociated, the third phosphate may not be used for binding due to the particular structural feature of the micellar surface. Unfortunately, the observed α value of 0.4 ± 0.1 is not consistent with either of the 1:2 or 1:3 guanidinium/phosphate complexation. In contrast, a guanidinium monolayer of **7** clearly formed a 1:3 complex with ATP at the air−water interface.[9a] Globular micelles are known to have fluid hydrophobic cores in the dynamic equilibrium with dispersed monomeric surfactant species, whereas surface monolayers are composed of static molecular organizations when compressed. This structural difference may be related to different modes of binding at the surface of globular micelles and surface monolayers.

Both ADP and ATP showed larger binding constants to the guanidinium micelle than AMP. Apparently the multiple interaction in the former cases enhances the *K* value. However, the increment is not proportional to the number of binding sites. The binding constant for **1**/ATP (2.5×10^4 M^{-1}) is significantly smaller than that for **1**/ADP (5.4×10^4 M^{-1}), in spite of the fact that ATP has a larger number of the possible binding sites. The free energy gain upon binding appears to be affected by factors other than the number of binding sites. Structural matching of micelle surface and nucleotide conformation, lipophile/hydrophile balance of host and guest, and steric crowding may be involved as additional factors. These factors might represent unique characteristics of molecular recognition at the surface of aggregates.

Table 2. Characterization of Aqueous Bilayer

	DSC			
bilayer	T_c/°C	ΔH/kJ·mol^{-1}	ΔS/J·mol^{-1}·K^{-1}	TEM observation
3/4 (1:2)	46 and 53[a]	28	87[b]	fragment (multilayer)
4	55	55	168	multilamellae vesicle
5[c]				string (multilayer)
6	40	11	35	string

[a] A broad peak with two tops was observed. [b] T_c = 50 °C was assumed for the calculation. [c] Endothermic peak was not detected from 5 to 70 °C.

Characterization of Aqueous Bilayers of Guanidinium Amphiphiles. The preceding binding study reveals that the interaction between guanidinium and phosphate is strengthened at an aqueous microscopic interface of micelles. In order to extend this finding to other aqueous aggregates, we investigated binding behavior of nucleotides onto the surface of bilayer membranes. Bilayers provide large and stable microscopic interfaces where exchange of amphiphile molecules is much slower than that in micelles.

Double-chain amphiphiles **5** and **6** and single-chain amphiphile **7** are expected to produce stable bilayer membranes when dispersed in water, on the basis of the past molecular design of bilayer-forming compounds. Monoalkyl amphiphile **3** forms a fluid micelle by itself, but it can be incorporated in a typical bilayer membrane of **4** without disrupting the bilayer structure.

The formation of bilayer structures was confirmed by differential scanning calorimetry (DSC) and by transmission electron microscopy (TEM), prior to binding experiments. Results of DSC measurements are summarized in Table 2. Aqueous dispersion of **5** did not show any endothermic peak in the temperature range examined (5−70 °C), while aqueous dispersions of **3/4** (1:2) and **6** gave endothermic peaks due to crystal−liquid crystal phase transition. The phase transition of bilayer **5** exists probably below 5 °C, as judged from DSC data of other related amphiphiles.[19a] In fact, the melting point of solid **5** was lower than room temperature (see synthesis of **5**).

Aqueous dispersion of **3/4** (1:2) showed overlapping two DSC peaks at 46 and 53 °C. As this peak pattern was observed in repeated scans, it cannot be ascribed to metastable phases. As one of the peak temperature, 53 °C is almost the same as that observed in aqueous dispersion of single component **4** (T_c = 55 °C with ΔH = 55 kJ·mol^{-1}), the mixed bilayer of **3/4** (1:2) is presumed to possess a phase-separated domain of pure **4** (T_c = 53 °C). The total ΔH value for the **3/4** mixture, 28 kJ·mol^{-1}, is significantly smaller than that (55 kJ·mol^{-1}) of pure **4**. When we recalculated ΔH based on the mole of **4** alone, ΔH is still 42 kJ·mol^{-1} and is lower than that of single component **4**. Therefore, mixing of **3** causes considerable disorder in bilayer **4**. Aqueous dispersion of **6** showed its phase transition at 40 °C with ΔH = 11 kJ·mol^{-1}. This enthalpy change is smaller than those reported for typical bilayer membranes such as phospholipids[27,28] **4** and other synthetic amphiphiles[19a] and may indicate inferior molecular ordering. DSC data show that **3/4** (1:2) and **6** bilayers are in less-ordered crystalline states and **5** is in the liquid-crystalline state at 25 °C where the binding study was performed.

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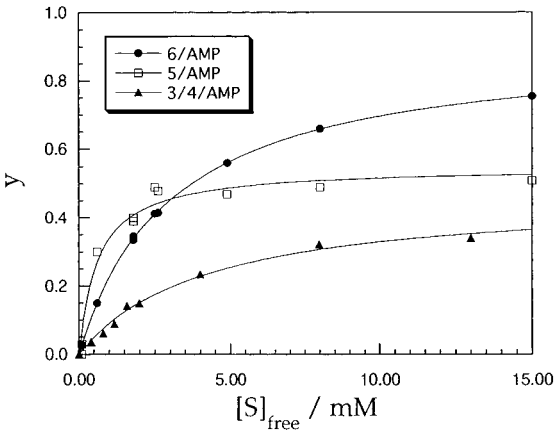

Figure 3. Saturation binding of guanidinium bilayers and nucleotides at 25 °C and pH 7.

In the TEM observation, the **3/4** (1:2) mixture showed the presence of fragments of multibilayer structures and the absence of the inner aqueous core, although **4** itself is known to form clear multilamellar vesicles.[19b] Mixing with monoalkyl **3** appears to deteriorate the bilayer structure of **4** and hinder vesicle formation. Amphiphile **5** showed a string-like multilayer structure. Amphiphile **6** also showed a string-like structure, but the multibilayer structure was not obvious. All the amphiphiles used in this study did not possess the inner water core unlike vesicles, and the substrate trapping in the inner core need not be considered in the following binding study.

Nucleotide Recognition on Guanidinium Bilayers. Ultrafiltration of aqueous bilayer dispersions without substrates was first conducted as a control experiment. Guanidinium amphiphiles **3** in **3/4** (1:2), **5**, and **6** were not detected in the corresponding filtrates, when their aqueous dispersions were subjected to ultrafiltration. The detection limit of guanidinium moiety by V−P reaction is approximately 1×10^{-6} M. As these amphiphiles form stable bilayer assemblies, the amount of molecularly dispersed guanidinium amphiphiles (at CMC) is less than 1×10^{-6} M, and bilayer aggregates remain on the filter completely. Therefore, only the unbound substrates are detectable in the filtrate.

Binding of AMP on the bilayer of **3/4** (1:2), **5**, and **6** was investigated by equilibrium dialysis (ultrafiltration) similar to that employed in the micelle system. Ultrafiltration was carried out just after mixing of bilayer dispersion and AMP. The plots of bound substrate, y, against free (unbound) substrate $[S]_{free}$ (eq 3) gave saturation curves as shown in Figure 3. It was found that the reliability of the Langmuir plot of these data was seriously affected by large errors at low substrate concentrations. Therefore, we used, instead, nonlinear curve fitting to estimate K and α. The results are included in Table 1. The binding constants are in the range of 10^2-10^3 M^{-1} and are comparable to the value observed in the micelle system. However, the binding constant for bilayers **3**, **4**, and **6** are smaller than those for bilayer **5** and micelle **1** by factors of 5−10. This difference may be related to the microenvironment at the aggregate surface, since the chemical structure of the receptor guanidinium is identical. We could not detect the phase transition for aqueous bilayer **5** at 5 to 80 °C and assumed that the bilayer is in the liquid crystalline state. Therefore, it is probable that the rate difference is derived from difference in the physical state of the aggregates. The guanidinium function at the surface of fluid aggregate (micelle and bilayer) must be more efficient than that on the crystalline aggregate surface of **3/4** and **6**. As another probable factor, long oligo(ethyleneglycol) chains in the matrix bilayer of **4** may suppress the interaction with the guanidinium moiety of **3** by steric hindrance.

The saturation binding, α, is essentially unity for the guanidinium function on bilayer **6**. However, this is not the case with bilayer **3/4** and **5**. This lack of saturation may come from the guanidinium function that is not exposed to the other bilayer surface. Thus, we carried out additional binding experiments for bilayer/AMP mixtures upon sonication. Unfortunately, TEM observation did not indicate the presence of the inner (closed) bilayer surface. The binding experiment for the sonicated samples showed contrasting results: the binding ratio essentially reached unity in the case of the bilayer **3/4**, whereas it appeared to remain unchanged for bilayer **5**. The stoichiometric binding of AMP toward single-component bilayer **5** may be suppressed by steric crowding. In contrast, the guanidinium function on bilayer **3/4** become fully exposed to AMP upon sonication.

Guanidinium/Phosphate Binding at Microscopic and Macroscopic Interfaces. We investigated in this study binding of nucleotides, AMP, ADP, and ATP, toward the guanidinium function at the surface of micelles and bilayers. Their binding constants were in the range of 10^2-10^4 M^{-1}. The guanidinium function must be located at the aggregate surface due to its hydrophilic nature and the particular molecular structure of the component. These values are much greater than that (1.4 M^{-1}) found for guanidinium chloride and simple phosphate in bulk water.[10] Therefore, the binding interaction is strengthened by factors of 10^2-10^4 at the microscopic surface of micelles and bilayers, and the water molecules near the aggregate surface provide much less interference for the binding capability of the guanidinium unit. We investigated previously the binding of AMP and ATP to the guanidinium unit at the air−water interface.[9] Binding of these nucleotides toward monolayer **7** was stoichiometric, and the binding constants were 10^6-10^7 M^{-1}. These values are greater than those observed for micelles and bilayers by factor of 10^2-10^4. Thus, the binding efficiency is enhanced by factors of 10^2-10^4 each, as the binding environment is converted from bulk water to microscopic interface (micelle and bilayer), and to macroscopic interface. Interpretation of these environmental effect is not straightforward.

Figure 4 illustrates binding patterns in bulk water, at a microscopic surface, and at a macroscopic surface, together with the corresponding binding constants and ΔG values for binding. The host−guest interaction in bulk water may be defined to occur at a molecular surface. The free energy of binding is enhanced by ca. 20 kJ·mol^{-1} in each step. The enhanced stabilization of the guanidinium/phosphate pair is not yet fully explicable. A theoretical approach was recently applied to this interaction at the interface by Sakurai et al.[29] As illustrated in Figure 5, ethyl guanidinium cation and phosphate anion were placed near/at the interface of two different dielectric media that correspond to hydrocarbon ($\epsilon = 2.0$) and water ($\epsilon = 80$). The binding energy profile was obtained by calculating by reaction field theory the whole system as a function of the guanidinium phosphate distance, **d**, and the position of a guanidinium N relative to the interface, **R**. Potential minima were found when the binding pair was exposed to water ($d =$ 0 to −3.4 in Figure 5) indicating favored pairing of the two functions under these interfacial arrangements. The free energy gained by the pairing can be comparable to the experimentally observed binding energy.

Recently, we observed submicron range attraction between hydrophobic surfaces of monolayer-modified mica in water by

(29) Sakurai, M.; Tamagawa, H.; Furuki, T.; Inoue, Y.; Ariga, K.; Kunitake, T. *Chem. Lett.* **1995**, 1001.

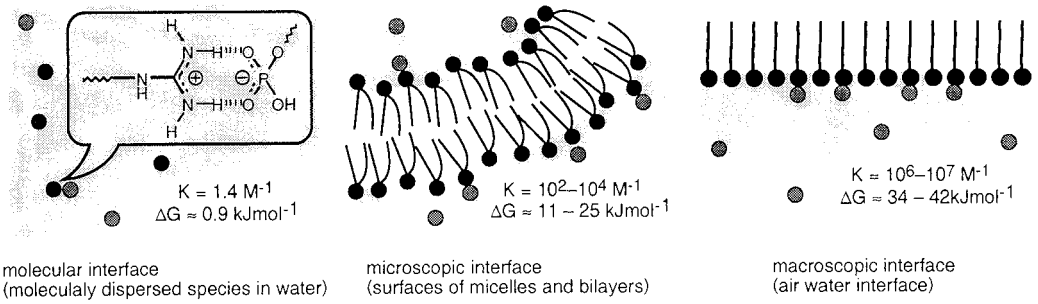

Figure 4. Typical binding constant (K), and binding energy (ΔG) of guanidinium and phosphate system at varying interfaces.

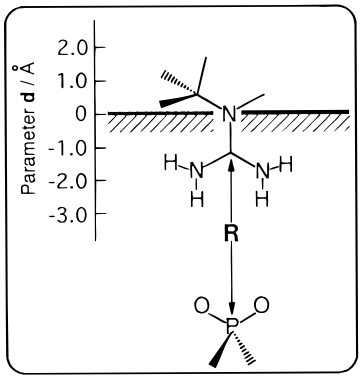

Figure 5. A continuum model of the lipid−water interface for the interaction of guanidinium and phosphate.[29] **d**: position of guanidinium nitrogen with respect to the interface (dielectric boundary). Here, the parameter **d** is taken to be zero. **R**: distance between the guanidinium carbon and the phosphorus atom.

the surface forces measurement.[30] We proposed on the basis of these surprising results that the attraction between opposing surfaces is much enhanced and becomes long-ranged if the surfaces are sufficiently large, molecularly smooth, and strongly hydrophobic. This unique feature of the interface between water and the hydrophobic surface may be related to the enhanced guanidinium/phosphate interaction, as the theoretical approach suggests. Larger binding energies are gained if the interacting pair is buried deeper in the aqueous phase. The observed difference of the binding energy among molecular interface, microscopic interface, and macroscopic interface is consistent with this presumption. The air−water interface is much larger and smoother than surfaces of aqueous micelles and bilayers, that, in turn, provides supramolecular surfaces larger than that of simple molecular pairs. Bonar−Law reported binding behavior of porphyrin−based receptor in SDS micelle where the binding was energetically similar to that in methanol (ϵ = 32.6).[8] Interior of micelle might provide lower dielectric media than hydrocarbon. As for the hydrogen bonding interaction at interfaces, Nowick and co-workers studied the thymine/adenine pairing in anionic SDS micelles.[5] The effective binding of aqueous thymine with alkylated adenine was observed in the hydrophobic core of the micelle, and the binding constant was comparable to that observed in $CHCl_3$ medium. These binding sites in the micellar interior, however, cannot be conceived as existing at the microscopic interface and cannot be directly compared with our system.

In conclusion, we demonstrated that nucleotides were bound to the guanidinium-functionalized micelles and bilayers (microscopic interface) via specific hydrogen bonding and electrostatic attraction. It is revealed that the microscopic interface strengthens hydrogen bonding and/or electrostatic interaction even in the presence of water.

Acknowledgment. We are grateful to Dr. Izumi Ichinose, Kyushu University, for assistance in TEM measurements. We also appreciate Shinkai Chemirecognics Project, JRDC for permission to use their DSC instrument.

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