Hydrogen Bonding in Water by Poly(vinyldiaminotriazine) for the Molecular Recognition of Nucleic Acid Bases and Their Derivatives[1]

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ABSTRACT: Poly(2-vinyl-4,6-diamino-1,3,5-triazine) (PVDAT) binds efficiently pyrimidine derivatives from water solutions by hydrogen bonding. The order in the adsorbing activity (thymine, uridine, uracil, thymidine ≫ cytidine, cytosine > pyrimidine = 0) coincides with the number of hydrogen bonds formed between the guests and the 4,6-diamino-1,3,5-triazine residues in PVDAT. Purines and their derivatives are also adsorbed from their aqueous solutions by PVDAT. Hydrogen bonding is confirmed by the shift of the carbonyl stretching band of uracil toward a smaller wavenumber upon adduct formation with PVDAT. The association constant of the PVDAT−thymine adduct in water (150 M^{-1}) is comparable with the corresponding value for the 4,6-diaminotriazine−thymine adduct in chloroform (90 M^{-1}). Neither a monomer model of PVDAT nor its dimer model shows guest-binding activity in water. The hydrogen bonding of PVDAT is strengthened by benzene moieties attached to the diaminotriazine residues. The polymer microenvironment is essential for this molecular recognition through hydrogen bonding in water.

Introduction

Mimicking the molecular recognition by biological systems is one of the most attractive themes for chemists. To date, a variety of artificial receptors, which selectively bind the target guest molecules through hydrogen-bond formation, have been reported.[2−6] In order to accomplish high selectivities and great binding activities, the hydrogen-bonding sites in the receptors should be complementary with those in the guests. The importance of appropriate flexibility of the receptor molecules has also been documented.

However, most of the artificial receptors previously reported are effective only in aprotic organic solvents, such as chloroform and carbon tetrachloride. Otherwise, the hydrogen bonds with the guests are destroyed by competition with the solvent molecules.[7] Therefore, artificial receptors, which form hydrogen bonds in water (as natural receptors do), are of growing interest. Hamilton *et al*. demonstrated that the bis(alkylguanidium) receptor recognizes glutaric acid, even in mixtures of dimethyl sulfoxide (DMSO) and water,[3b] and Rebek reported a synthetic receptor which bound 3′,5′-cyclic adenosine monophosphate in water.[2c] These authors as well as Constant *et al*.[8] proposed that cooperation with electrostatic and/or stacking interactions is necessary to form hydrogen bonds efficiently in protic solvents.

An alternative way to facilitate hydrogen bonding is to adjust the physicochemical properties of the environment where the bonds are formed. According to Kurihara *et al*.,[9] a Langmuir−Blodgett film of an amphiphile, which bears a 4,6-diaminotriazine residue, binds thymidine at the air/water interface through hydrogen bonding. An adenine−thymine base pair was formed in an aqueous micellar system.[10] These results strongly suggest that polymeric receptors are promising candidates for the molecular recognition of guests through hydrogen bonding in water.

In the present paper, we report that poly(2-vinyl-4,6-diamino-1,3,5-triazine) (PVDAT) recognizes efficiently nucleic acid bases and their derivatives in water solution. Hydrogen-bond formation between guests and PVDAT has been confirmed by infrared (IR) spectroscopy. The thermodynamic parameters for the hydrogen bonding were determined. The effect of a chemical modification of PVDAT on the guest binding activity has also been demonstrated. The polymer microenvironment has been found to be essential for molecular recognition of nucleic acid bases in water solution.

Experimental Section

Materials. 2-Vinyl-4,6-diamino-1,3,5-triazine (VDAT; Figure 1d), nucleic acid bases, nucleosides, and other reagents were purchased from Tokyo Kasei Kogyo Co. or other commercial sources. 2,2′-Azobisisobutyronitrile (AIBN) was recrystallized from methanol. Water was purified by a Millipore Milli-XQ purification system, and its specific resistance was greater than 18.3 MΩ·cm.

Poly(2-vinyl-4,6-diamino-1,3,5-triazine) (PVDAT; Figure 1a). PVDAT was prepared by a radical polymerization of VDAT (0.29 M) in DMSO at 70 °C with AIBN (3.9 mM) as the initiator. The mixture was at first homogeneous, but the polymer gradually precipitated as the polymerization proceeded. The polymer was washed successively with DMSO and with methanol and dried in vacuo. The reduced specific viscosity ([η]) in acetic acid was 0.30 dL/g at 30 °C.[11] Scanning electron microscopy on a JSM-5400LV spectrometer showed that the PVDAT was composed of particles with a mean diameter of about 1 μm. The PVDAT powder was directly used for the guest adsorption experiments.

Poly[4-(4,6-diamino-1,3,5-triazinyl)styrene] (PDATS; Figure 1b). The monomer 4-(4,6-diamino-1,3,5-triazinyl)-styrene was synthesized via methyl (4-vinylphenyl)acetate as an intermediate. First, (4-vinylphenyl)acetic acid (1.5 g, 10 mmol) and thionyl chloride (8 mL, 10 mmol) were reacted in 50 mL of dichloromethane. After being refluxed for 2 h, the reaction mixture was added to an ice-cooled methanol/triethylamine mixture (10 mL/4 mL) and was extracted with dichloromethane. The organic layer was successively washed with aqueous solutions of Na_2CO_3 and of NaCl and then concentrated by evaporation. Silica gel column chromatography (hexane/ethyl acetate = 10:1) afforded methyl (4-vinylphenyl)-acetate in 90 mol % yield. ^{1}H-NMR (270 MHz, DMSO-d_6): δ 7.66 (d, J = 7.6 Hz, 2H), 7.13 (d, J = 7.6 Hz, 2H), 6.43 (m, 1H), 5.49−5.56 (m, 2H), 3.58 (s, 3H).

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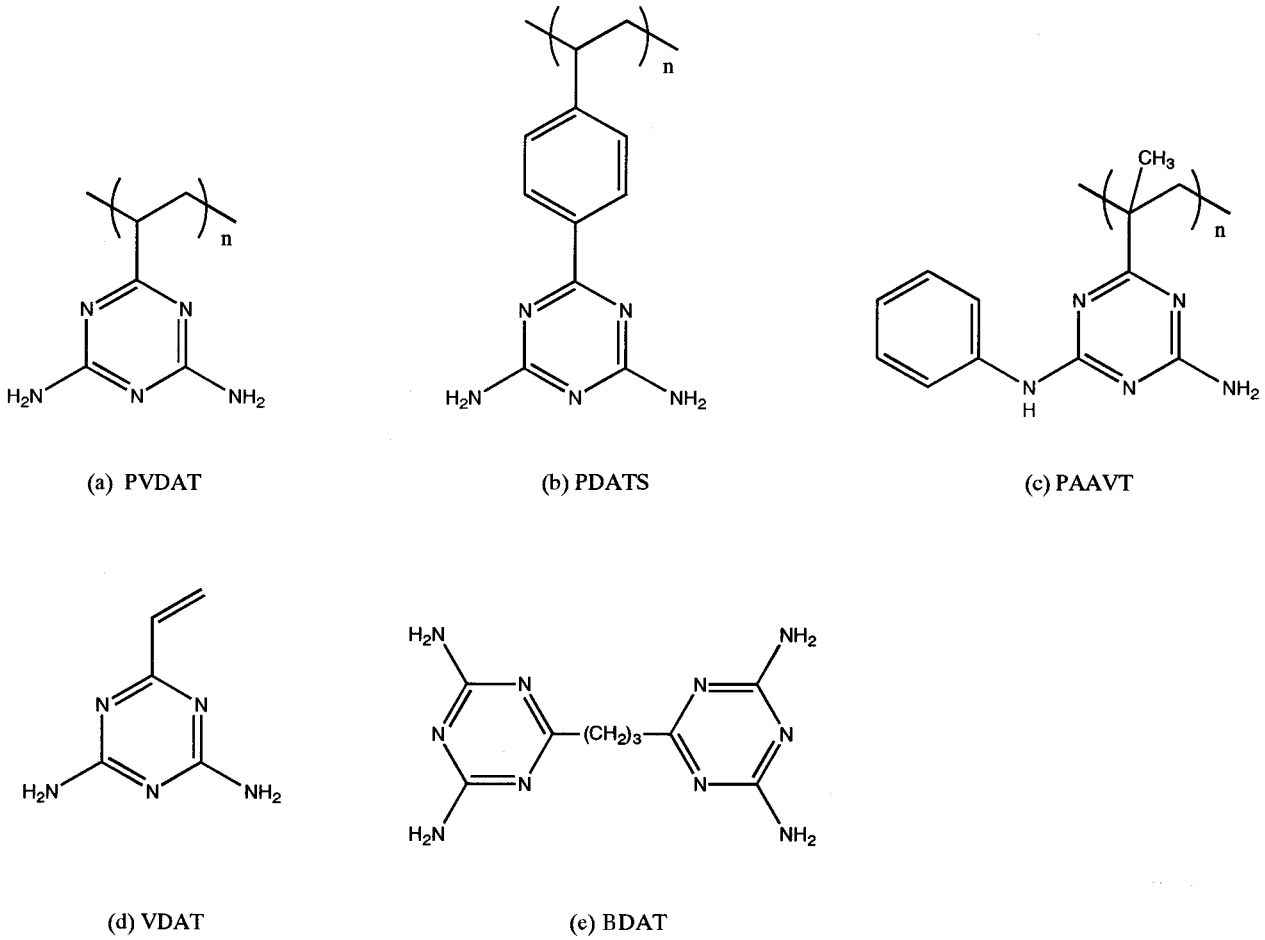

Figure 1. Chemical structures of the artificial receptor molecules. (a): Poly(2-vinyl-4,6-diamino-1,3,5-triazine) (PVDAT) (b): Poly[4-(4,6-diamino-1,3,5-triazinyl)styrene] (PDATS) (c): Poly[2-amino-4-anilino-6-(2-methylvinyl)-1,3,5-triazine] (PAAVT) (d): 2-Vinyl-4,6-diamino-1,3,5-triazine (VDAT) (e): 1,3-Bis(4,6-diamino-1,3,5-triazinyl)propane (BDAT).

Methyl (4-vinylphenyl)acetate (1.4 g, 8.7 mmol) was reacted with biguanide (1.0 g, 7.3 mmol) in dry methanol at room temperature for 4 h. The resultant white precipitate was washed with ethanol and recrystallized from methanol. The yield of the desired product (4-(4,6-diamino-1,3,5-triazinyl)-styrene) was 65 mol %. ^{1}H-NMR (DMSO-d_6): δ 8.21 (d, J = 8.4 Hz, 2H), 7.55 (d, J = 8.4 Hz, 2H), 6.74 (m, 5H), 5.92 (d, J = 17.8 Hz, 1H), 5.34 (d, J = 10.8 Hz, 1H).

Radical polymerization of the monomer was achieved in DMSO using AIBN as the initiator ([monomer]$_0$/[AIBN]$_0$ = 74). The polymer was purified by repeated reprecipitation from DMSO into methanol.

Poly[2-amino-4-anilino-6-(2-methylvinyl)-1,3,5-triazine] (PAAVT; Figure 1c). Methyl methacrylate (0.84 g, 8.4 mmol) and phenylbiguanide (1.24 g, 7.0 mmol) were treated in dry methanol at room temperature for 4 h. The white precipitate was collected and washed with ethanol. Recrystallization from methanol gave 2-amino-4-anilino-6-(2-methylvinyl)-1,3,5-triazine in 32 mol % yield. ^{1}H-NMR (DMSO-d_6): δ 7.91 (d, J = 8.4 Hz, 2H), 7.35 (t, J = 8.4 Hz, 3H), 6.95 (m, 3H), 6.35 (s, 1H), 5.48 (s, 1H), 2.08 (s, 3H). The monomer was polymerized by AIBN in DMSO, and the polymer was reprecipitated by methanol and dried in vacuo.

1,3-Bis(4,6-diamino-1,3,5-triazinyl)propane (BDAT; Figure 1e). This dimer model of PVDAT was prepared according to the literature.[12] Dicyandiamide (4.7 g, 56 mmol), glutaronitrile (2.3 g, 24 mmol), and KOH (1.3 g, 23 mmol) were treated in dimethylformamide (30 mL) at 135 °C for 1.5 h. The precipitate was thoroughly washed with hot DMSO. The yield was 63 mol % (3.8 g).

Adsorption of Guests in Water by the Polymeric Receptors. Finely powdered PVDAT was added to 1 mL of an aqueous solution of guest(s), and the mixture was magnetically stirred at a predetermined temperature for 1 h (PVDAT was virtually insoluble in water). The pH was maintained at 6.5 ± 0.5, although no buffer was used.

After incubation, the mixture was centrifuged (1400 rpm), and the liquid phase was analyzed by reversed-phase HPLC (Merck LiChrosphere RP-18(e) ODS column). As an internal standard, 4-hydroxypyridine, which showed no measurable interaction with PVDAT (as confirmed by careful control experiments), was added to the incubation mixture together with the guests. Guest adsorption by other receptors was achieved in virtually the same way.

Infrared Spectroscopy on the Adduct Formation by PVDAT. A thin film of PVDAT was prepared by casting an acetic acid solution of the polymer on CaF_2 crystal plates, followed by desiccation. The infrared spectrum of the film was measured by a JASCO FT/IR-230 spectrometer. The film was then dipped into a 20 mM aqueous solution of a guest at 25 °C for 1 h. The solution on the film was wiped off, and the film was again subjected to IR spectroscopy. The spectrum of the film before the guest adsorption was subtracted from the spectrum after the adsorption.

Evaluation of Binding Constants and Thermodynamic Parameters for Adduct Formation between the Polymeric Receptors and Guests. The binding constants (K) for guest−PVDAT adducts were determined by the Langmuir adsorption isotherm,[13]

$$c/(\theta A) = c/A + 1/(KA) \quad (1)$$

Here, c is the equilibrium concentration of free guest in the liquid phase, whereas A and θ are the maximal amount of the adsorbed guest and the surface coverage, respectively. The thermodynamic parameters for adduct formation were evalu-

ated by a van't Hoff plot of the K values, obtained at different temperatures.

Competitive Adsorption between Thymine and Various Guests toward PVDAT. Various guests were adsorbed to PVDAT in the presence of thymine, in order to obtain information concerning their binding sites. A 10 mg amount of PVDAT was added to 1 mL of an aqueous solution containing both a guest (1 mM) and thymine (20 mM), and the mixture was incubated at 25 °C with stirring for 1 h to attain equilibrium. Then, the amounts of the guest and thymine in the liquid phase were determined by HPLC.

If the guest molecule and thymine occupy the same binding sites in PVDAT, the amount of the adsorbed guest (n_g) in the presence of thymine should be given by

$$n_g = [g]_0 n_t / \{K_t[t]_0/K_g + (1 - K_t/K_g)(10^3 n_t)\} \qquad (2)$$

Here, n_t is the amount of the thymine adsorbed by PVDAT. The terms $[g]_0$ and $[t]_0$ refer to the initial concentrations of the guest and thymine. The binding constants K_g (of the guest−PVDAT adduct) and K_t (of the thymine−PVDAT adduct) were determined by independent adsorption experiments.

Determination of the Binding Constant of the Uracil−VDAT Adduct in Solutions. The binding constants of the adduct (K) in DMSO and in DMSO/H_2O mixtures were determined by ^{1}H-NMR titration at 20 °C. The downfield shifts (Δ_{obs}) of the amino protons of the 4,6-diaminotriazine (DAT) residue of VDAT (due to hydrogen-bond formation with uracil) were monitored as a function of the uracil concentration. The concentration of VDAT ($[VDAT]_0$) was kept constant at 50 mM. The ^{1}H-NMR spectra were measured on a JEOL 270 MHz NMR spectrometer with DMSO as an internal standard. The data were analyzed in terms of the following equation, which is based on 1:1 complex formation between VDAT and uracil:

$$\Delta_{obs} = {}^1/_2\Delta_{max}\{(X + 1 + 1/(K[VDAT]_0) - [(X^2 + 1 + 1/(K[VDAT]_0)^2 - 2X + 2X/(K[VDAT]_0) + 2/(K[VDAT]_0)]^{1/2}\} \qquad (3)$$

Here X is the ratio of the initial concentration of uracil to that of VDAT. The Δ_{max} value is the maximal change in the chemical shift with respect to the value in free VDAT.

RESULTS

Recognition of Pyrimidine Bases by PVDAT through Hydrogen-Bond Formation in Water. When an aqueous solution containing uracil, thymine, cytosine, and pyrimidine was incubated at 25 °C with PVDAT (which is insoluble in water), the concentrations of uracil and thymine in the liquid phase rapidly decreased (compare the HPLC pattern b with a in Figure 2). Equilibrium was attained within 15 min. By contrast, cytosine was adsorbed only faintly, and pyrimidine was not adsorbed at all. A remarkably selective binding of uracil and thymine by PVDAT in water was demonstrated. When the mixture was heated to 70 °C, about half of the adsorbed uracil was released to the aqueous phase. The amount of the uracil released was identical to the value estimated from an independent adsorption experiment at 70 °C. Thus, the present adsorption of uracil and thymine by PVDAT is totally reversible.

The adsorbing activities of PVDAT toward various nucleic acid bases and nucleosides are in the following order: thymine, uridine, uracil, thymidine ≫ cytidine, cytosine > pyrimidine = 0 (Table 1). This order coincides fairly well with that in the number of hydrogen-bonding sites in the guest, which are complementary

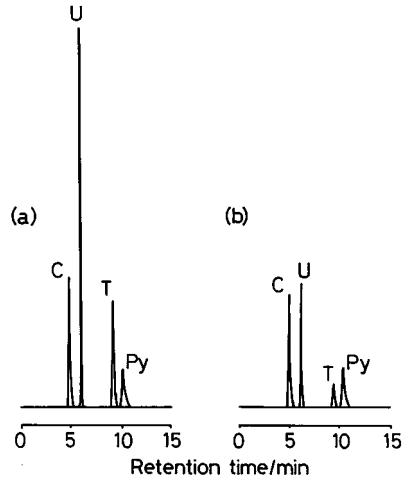

Figure 2. HPLC patterns for an aqueous solution containing uracil, thymine, cytosine, and pyrimidine before (a) and after (b) contact with PVDAT. One milliliter of the solution ([each of the guest]$_0$ = 1 mM) was treated with 100 mg of PVDAT at 25 °C.

Table 1. Adsorption of Pyrimidine Derivatives by PVDAT at 25 °C

nucleic acid base	no. of hydrogen-bonding sites toward DAT	adsorbing activity[a]	
		base	nucleoside
uracil	3	0.20	0.22
thymine	3	0.26	0.18
cytosine	2	0.04	0.05
pyrimidine	1	0.01>	

[a] Molar ratio of adsorbed guest to the initially charged guest, observed on the contact of PVDAT (10 mg) with 1 mL of aqueous guest solution (initial guest concentration is 1 mM).

with those of the 4,6-diaminotriazine (DAT) residue. Uracil (U) and thymine (T) have three hydrogen-bonding sites, cytosine (C) has two, and pyrimidine (Py) has only one (see Figure 3). Apparently, the complementary hydrogen bonding between the guests and the DAT residues in the polymer is responsible for the guest adsorption. Three hydrogen bonds are required to bind the pyrimidine derivatives effectively in water under physiological conditions. The adsorption of a nucleic acid base is almost the same as that of the corresponding nucleoside, indicating that the ribose residues do not make any significant contribution to the adsorption. The formation of the hydrogen-bonding adducts is further supported by the fact that 3-methyluracil, in which the imido residue in uracil is N-methylated (Figure 3c), was only slightly adsorbed by PVDAT.

Spectroscopic Evidence for Hydrogen Bonding between Uracil and PVDAT in Water. Hydrogen-bond formation between uracil and PVDAT in water was further confirmed by FT-IR analysis. When a film of PVDAT was contacted with an aqueous solution of uracil, shoulder bands appeared around 1700−1740 cm^{-1} (compare Figure 4b with Figure 4a). In the difference spectrum c (=b − a), two new peaks were seen at 1712 and 1681 cm^{-1}. They were assignable to the C=O stretching bands of the uracil bound to PVDAT (in the absence of PVDAT, the C=O stretching bands are located at 1737 and 1716 cm^{-1}, Figure 4d).[14] The IR spectrum of a PVDAT film remained unchanged when it was dipped into aqueous solutions of cytosine, which is only slightly adsorbed by PVDAT (spectrum f is almost superimposed with e).

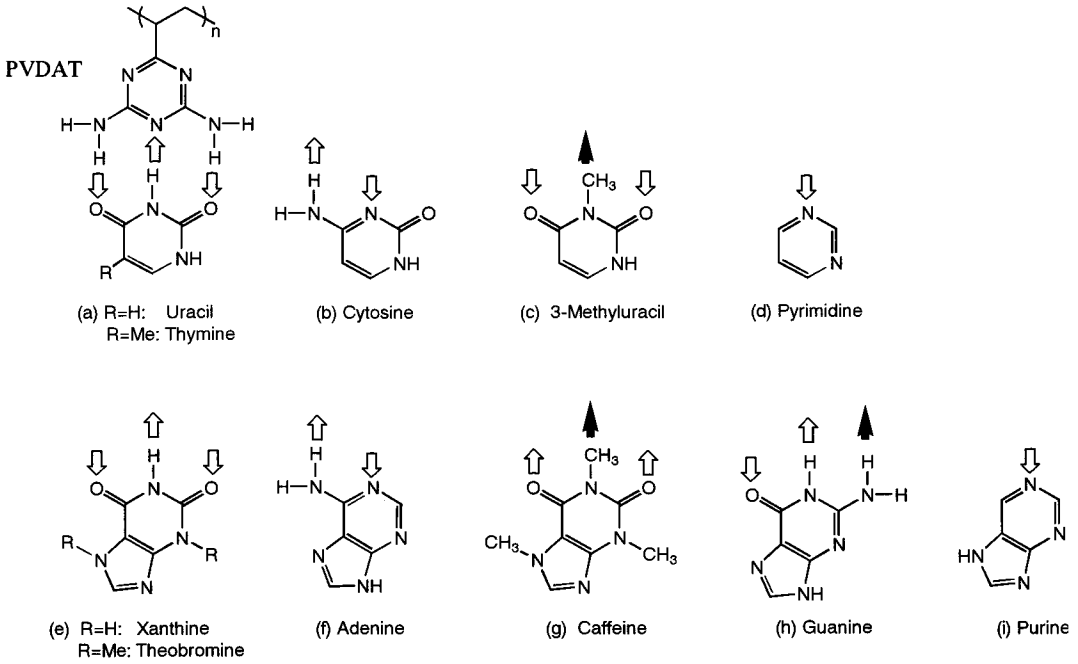

Figure 3. Hydrogen-bond formation of 4,6-diaminotriazine (DAT) residue with various guest molecules. The open arrows indicate the complementary hydrogen bonds and the closed ones refer to potential repulsion.

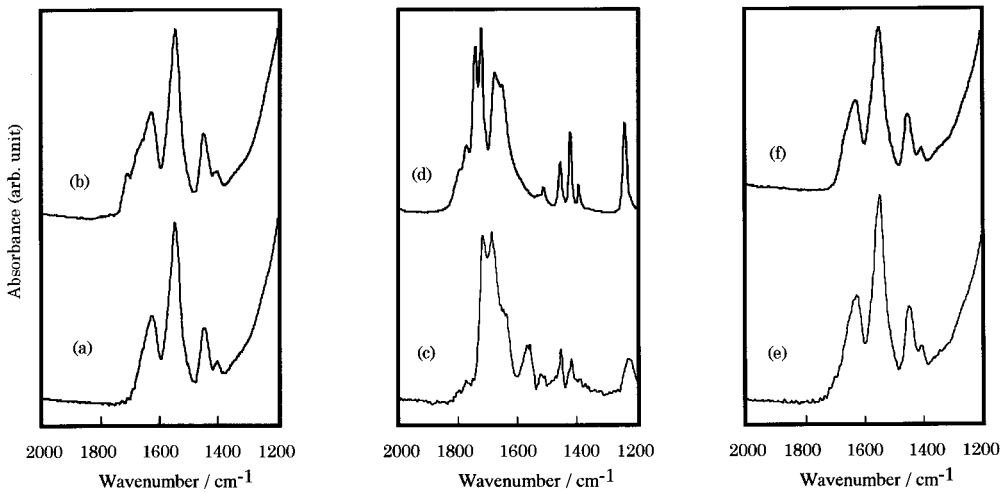

Figure 4. FT-IR spectra of PVDAT film before (a) and after (b) contact with 20 mM aqueous solution of uracil at 25 °C for 1 h. Spectrum (c) was obtained by subtracting (a) from (b), whereas (d) shows the spectrum of solid uracil, measured by the conventional KBr method. Spectra (e) and (f) refer to the PVDAT film before and after contact with 20 mM of cytosine solution.

Adsorption of Purine Bases and Their Derivatives by PVDAT in Water. Purine derivatives are also efficiently adsorbed from water by PVDAT through hydrogen-bond formation. As expected, the adsorption increases monotonically with an increasing number of complementary hydrogen-bonding sites toward the DAT residue (Table 2). Adenine, which has two hydrogen-bonding sites, is adsorbed much more strongly than purine, which has only one site (Figure 3). Guanosine is adsorbed less efficiently than adenine, since a steric and/or electrostatic repulsion also occurs upon complex formation with PVDAT (see the closed arrow in Figure 3h). Theobromine, as well as xanthine (Figure 3e), is strongly adsorbed by forming three complementary hydrogen bonds. Caffeine (the N-methylated derivative of theobromine) is adsorbed by PVDAT, but only to a much smaller extent.

Guest-Adsorbing Activities of the Monomer Unit of PVDAT (VDAT) and Its Dimer Model (BDAT). Evidence for the Dominant Role of a Polymer Effect. Results of a ^{1}H-NMR analysis on complex formation between VDAT and uracil in homogeneous solutions are represented in Figure 5. The binding constants (K) for the VDAT−uracil complex in DMSO, determined from the plot, is only 0.3 M^{-1}. In a 7:3 DMSO/H_2O mixture, the K value is smaller (0.2 M^{-1}).[15] Thus, the K value in pure water is expected to be even smaller (it cannot be directly determined because of the poor solubility of VDAT). It is concluded that the

Table 2. Adsorption of Purine Derivatives by PVDAT at 25 °C

guest molecules	no. of hydrogen-bonding sites toward DAT	adsorbing activity[a]
adenine	2	0.13
guanine	1	0.04[b]
purine	1	0.05
theobromine	3	0.52
caffeine	1	0.10

[a] The adsorption conditions are as in footnote *a* of Table 1. [b] Guanosine was used in place of guanine, since the solubility of guanine is too small for the experiment.

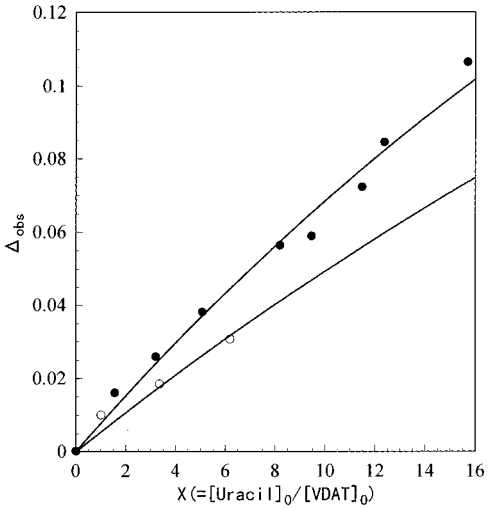

Figure 5. Plots of the NMR shift (Δ_{obs}) of the amino protons of VDAT as a function of the $[uracil]_0/[VDAT]_0$ ratio in DMSO (●) and in a 7/3 DMSO/H_2O mixture (○) at 20 °C. The solid lines are theoretically obtained by using eq 3.

Table 3. Competitive Adsorption to PVDAT of Various Guests in the Presence of Thymine toward PVDAT at 25 °C

	competitive suppression of guest adsorption by thymine[a]	
guest	obsd	theor[b]
uracil	0.29	0.30
adenine	0.00	0.43
theobromine	0.14	0.29

[a] The ratio of the decrease in the amount of adsorbed guest, caused by the presence of 20 mM thymine, to the amount of the guest adsorbed in the absence of thymine. [b] As estimated from eq 2.

thymine−PVDAT adduct (K = 150 M^{-1} at 25 °C) is far more stable than the thymine−VDAT adduct.

A dimer model of PVDAT (BDAT, Figure 1e), in which two DAT residues are connected by a trimethylene chain (exactly as in PVDAT), did not adsorb either uracil or thymine to a measurable extent.[16] The dominant role of a "polymer effect" in the adsorption by PVDAT in water has thus been demonstrated.

Competitive Adsorption of Various Guests by PVDAT in the Presence of Thymine. The adsorption of uracil by PVDAT is considerably suppressed by the presence of thymine (Table 3). The suppression is as predicted from eq 2 (note that this equation is derived under the assumption that the binding of these two guests takes place competitively). Thus, the adsorption of uracil and thymine occurs at identical binding sites ("type-I binding sites") in PVDAT.

In contrast, the adsorption of adenine is not affected by thymine. If adenine adsorption takes place at the type-I binding sites, the adsorption of adenine would be competitively suppressed by the thymine around 2-fold. Thus, adenine is adsorbed at "type-II binding sites", which are different from the type-I binding sites. The magnitude of the suppression by thymine on the adsorption of theobromine is intermediate between that for competitive binding (estimated from eq 2) and that for independent binding. Theobromine can be bound to both the type-I binding sites and the type-II binding sites (*vide infra*).

Thermodynamic Analysis on the Guest Adsorption by PVDAT. The adsorption of the pyrimidine and purine derivatives by PVDAT fits fairly well Langmuir-type plots (a typical one is presented in Figure 6). The binding constants (K) and the thermodynamic parameters, determined by these analyses, are listed in Table 4. The binding constant of the thymine−PVDAT adduct (150 M^{-1}) is 25 times that for the cytosine−PVDAT adduct (6 M^{-1}). The formation of the thymine adduct ($\Delta H = -5.6$ kcal mol^{-1}) is more exothermic than for the cytosine adduct ($\Delta H = -4.2$ kcal mol^{-1}). On the other hand, the entropy changes (ΔS) are similar. Thus, thymine is adsorbed by PVDAT more strongly than cytosine, mainly because of a more favorable enthalpy change. The adsorption of pyrimidine derivatives by PVDAT is strictly governed by the number of complementary hydrogen bonds which are formed between the guest and the DAT residue (three for thymine and two for cytosine).

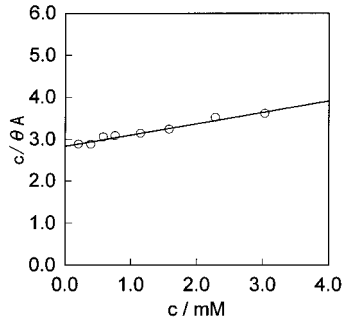

Figure 6. Langmuir-type plot for the adsorption of uracil by PVDAT. Ten milligrams of the polymer were contacted with 1 mL of uracil solutions of various concentrations at 20 °C. The horizontal axis refers to the equilibrium concentration of uracil in the liquid phase, and the vertical axis shows its ratio to the amount of adsorbed uracil.

Table 4. Thermodynamic Parameters for the Guest Adsorption by PVDAT

guest	maximal binding capacity,[a] mmol/(g of PVDAT)	binding constant at 25 °C, M^{-1}	ΔH, kcal mol^{-1}	ΔS, eu
thymine	0.26	150	−5.6 ± 0.6	−8.9 ± 2.0
cytosine	0.30	6	−4.2 ± 0.4	−10.6 ± 1.3
adenine	0.19	100	−7.0 ± 0.9	−14.3 ± 3.1
theobromine	0.24	490	−7.4 ± 0.9	−12.5 ± 3.0
uracil	0.38	90[b]		

[a] These values correspond to A in eq 1. [b] At 20 °C.

Adsorption of Guests in Water by the Derivatives of PVDAT. When benzene moieties were introduced between the DAT residues of PVDAT and its main chain, the resultant polymer (PDATS; Figure 1b) adsorbed 58 mol % of thymine under the adsorption conditions described in footnote *a* of Table 1. This value is about twice as great as the corresponding value (26%) for PVDAT. However, the adsorption of cytosine was almost unchanged.

Similar results were obtained for PAAVT, in which one of the amino residues in the DAT is replaced by an anilino residue (Figure 1c). About half (52 mol %) of the thymine was adsorbed by PAAVT, whereas the adsorption of cytosine was virtually unchanged by the modification. Thus, the selectivity for thymine adsorption vs cytosine adsorption is significantly improved by the introduction of benzene rings next to the DAT residues.

DISCUSSION

Hydrogen Bonding in Water by Polymeric Receptors. The adsorptions of pyrimidine and purine derivatives in water solution on PVDAT depend on the number of complementary hydrogen bonds (Tables 1 and 2). *Hydrogen bonding* is, or is mostly, responsible for adduct formation. Neither a monomeric receptor VDAT nor a dimer model of PVDAT (BDAT) is active. Only the polymeric receptors (PVDAT, PDATS, and PAAVT) efficiently form hydrogen bonds in water. A remarkable polymer effect has been demonstrated.

According to competitive-adsorption experiments, PVDAT has two types of hydrogen-bonding sites (Table 3). The type-I binding sites adsorb pyrimidine derivatives preferentially to purine derivatives. In order to be efficiently adsorbed to these sites from a water solution, the guests must form three complementary hydrogen bonds with the DAT residues. On the other hand, type-II binding sites are effective only for the adsorption of purine derivatives. As expected, the adsorption of adenine, which forms only two hydrogen bonds with DAT (and is hardly adsorbed at type-I sites), is independent of thymine adsorption. However, the adsorption of theobromine is partially competitive with thymine adsorption, since this guest forms three hydrogen bonds with the DAT residue and is bound at the type-I as well as type-II binding sites.

In a proposed mechanism, PVDAT and its derivatives provide a rather aprotic microenvironment at both the type-I and type-II binding sites for hydrogen-bond formation in water. This microenvironment, constituted by a number of DAT residues, is apolar, and the DAT residues therein are rather free from solvation by water molecules. Thus, hydrogen bonds with the guests are formed even in water solution. The K value for the thymine−PVDAT adduct (150 M^{-1}) in water is comparable with the value (90 M^{-1}) for complex formation between 2,6-dibutyramidopyridine (a model compound of DAT) and 1-butylthymine in chloroform.[3a]

The argument is strengthened by the fact that the hydrogen bonding of PVDAT with the guests is improved when benzene moieties are introduced near to the DAT residues (the thymine-adsorbing activities of PDATS and PAAVT, defined as in footnote *a* in Table 1, are twice as great as that of PVDAT). The benzene residues increase the apolar character around the DAT residues, increasing their hydrogen bonding of the guests in water solution. However, the adsorbing activities of PVDAT toward cytosine, which forms only two complementary hydrogen bonds with DAT, are little affected by the introduction of benzene groups.

Type-I and Type-II Binding Sites. In the guest binding at the type-I sites, hydrogen bonding plays a dominant role. Thus, the exothermicity as well as the binding constant increases significantly with an increasing number of hydrogen-bonding sites.[17] At the type-II binding sites, however, a stacking interaction with DAT residues favors hydrogen bonding. The difference in the number of hydrogen-bonding sites affects to a lesser extent the strength of guest binding than for the type-I sites (compare Table 2 with Table 1).[18] Accordingly, adenine is strongly adsorbed with a $\Delta H = -7.0$ kcal mol^{-1} compared to the corresponding value for cytosine adsorption ($\Delta H = -4.2$ kcal mol^{-1}): note that both of the guests have two hydrogen-bonding sites for DAT and that the latter guest is bound to the type-I binding sites. Thymine interferes little with the adsorption of adenine.

Thermodynamic Analysis for the Guest Binding by PVDAT in Water. The exothermicity for the hydrogen bonding in water with PVDAT (especially at the type I binding site) is greater than for the non-polymeric receptors (see Table 4). According to Rebek and his co-workers,[2c] hydrogen bonds can be formed even in water, when supported by stacking interactions between the guest and the receptor. The addition of two hydrogen bonds between the guest and the receptor increases the exothermicity by 1.6 kcal mol^{-1} (thus the exothermicity for one hydrogen bond is 0.8 kcal mol^{-1}). The formation of one hydrogen bond with PVDAT has $\Delta H = -1.4$ kcal mol^{-1} at the type-I binding sites, as estimated from the difference between the ΔH for the thymine−PVDAT adduct ($\Delta H = -5.6$ kcal mol^{-1}, three hydrogen bonds) and that for the cytosine adduct ($\Delta H = -4.2$ kcal mol^{-1}, two hydrogen bonds). The apolar character of the microenvironment, provided by the polymer, facilitates the hydrogen bonding.

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References and Notes

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(16) The dimer model (73 μmol), which was insoluble in water, was incubated with 1 mL of aqueous solutions of the pyrimidine derivatives (1 mM) at 25 °C for 24 h.
(17) The size of the type-I binding sites would fit the pyrimidine derivatives but would be too small for the purine derivatives.
(18) The structure of type-II binding sites would be sterically less favorable for hydrogen bonding.

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