Precise recognition of nucleic acid bases by polymeric receptors in methanol. Predominance of hydrogen bonding over apolar interactions †

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In methanol, poly(2-vinyl-4,6-diamino-1,3,5-triazine) (PVDAT) precisely recognizes nucleic acid bases and their derivatives through hydrogen-bond formation. The binding activity (uracil, thymine ⩾ cytosine, adenine > pyrimidine, purine ≒ 0) exactly coincides with increasing number in the complementary hydrogen-bonding sites of the guest towards the diaminotriazine residue. The guest-selectivity is higher than that achieved previously in water (H. Asanuma *et al.*, *Macromolecules*, 1998, 31, 371), mainly because the guest-binding occurs mostly *via* the hydrogen bonding at aprotic sites provided by the polymeric receptor. Stacking and apolar interactions are ineffective in methanol. The copolymers of 2-vinyl-4,6-diamino-1,3,5-triazine with hydrophobic monomers (styrene and 4-vinylbiphenyl) show still higher guest-selectivities, due to the increased aprotic environment around the diaminotriazine residues.

Introduction

Recognition of target molecules with high affinity is a fundamental step in biological systems. Natural receptors show exceedingly high selectivity and affinity by forming complementary hydrogen bonds with the target molecules. Based on this strategy, a variety of artificial receptors have been designed.[2–5] The technique of molecular imprinting has also been developed.[6] However, most of these artificial receptors are effective only in aprotic media (chloroform, carbon tetrachloride, and so on), since otherwise the hydrogen bonds are competitively destroyed by solvent molecules.[7] Thus, current interests are focused on guest-recognition in protic solvents, which is done by natural receptors.[2,3c,4a–4c]

In a previous paper,[8] we reported that poly(2-vinyl-4,6-diamino-1,3,5-triazine) (PVDAT) satisfactorily recognizes uracil and thymine in water through the formation of three complementary hydrogen bonds with the diaminotriazine (DAT) residue. The hydrogen bonding was confirmed by the shift of the carbonyl stretching band of uracil upon the adduct formation with PVDAT. The importance of polymeric microenvironment for hydrogen-bond formation was demonstrated. In these systems, however, stacking and apolar interactions between the guest and the polymeric receptor were concurrently taking place. Thus, even adenine (which has two complementary hydrogen-bonding sites towards DAT) and purine (one hydrogen-bonding site) were rather strongly adsorbed by PVDAT. In order to attain more strict recognition, stacking and apolar interactions must be suppressed.

The present paper reports on the binding of nucleic acid bases by PVDAT in methanol, in which stacking and apolar interactions are minimized. Both purine and pyrimidine bases are strictly recognized by the number of complementary hydrogen-bonding sites toward DAT residue. Furthermore, still higher guest-selectivity is achieved by the copolymers of vinyldiaminotriazine and apolar monomers such as styrene and 4-vinylbiphenyl (see Fig. 1).

† See reference 1.

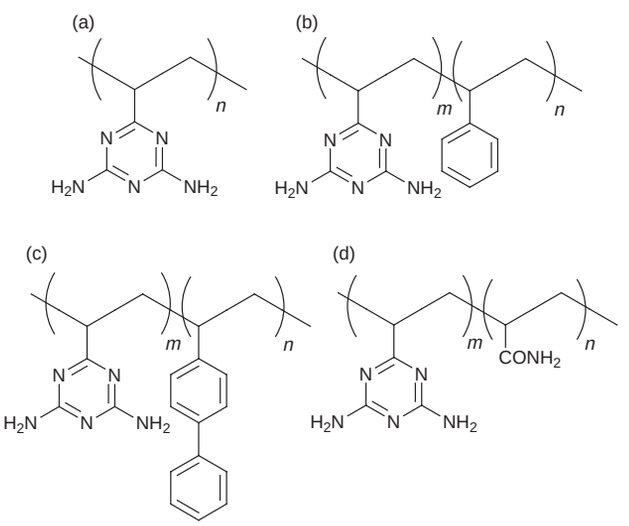

Fig. 1 Chemical structures of artificial polymeric receptors used in this study. (a) Poly(2-vinyl-4,6-diamino-1,3,5-triazine) (PVDAT), (b) Poly[styrene-*co*-(2-vinyl-4,6-diamino-1,3,5-triazine)] (PST-VDAT; $m:n = 15:85$), (c) Poly[(4-vinylbiphenyl)-*co*-(2-vinyl-4,6-diamino-1,3,5-triazine)] (PVB-VDAT; $m:n = 9:91$), (d) Poly[(acrylamide)-*co*-(2-vinyl-4,6-diamino-1,3,5-triazine)] (PAAm-VDAT; $m:n = 43:57$).

Results

Precise recognition of nucleic acid bases by PVDAT in methanol through hydrogen-bond formation

As shown in Table 1, PVDAT notably binds uracil and thymine in methanol. Both of the guests possess three complementary hydrogen-bonding sites toward DAT (see Fig. 2). The binding of adenine and cytosine, which have two complementary hydrogen-bonding sites, is less efficient. Pyrimidine and purine (one hydrogen-bonding site) are not bound to a measurable extent. Thus, nucleic acid bases are strictly recognized by PVDAT in methanol by the number of complementary hydrogen-bonding sites towards DAT.[9] Maleimide having three complementary hydrogen-bonding sites is also adsorbed by

Table 1 Adsorption of nucleic acid derivatives by PVDAT at 25 °C

Guest molecules	No. of hydrogen bonding sites toward DAT	Adsorption activity[a]	
		In methanol	In water
Uracil	3	0.30	0.20
Thymine	3	0.24	0.26
Adenine	2	0.03	0.13
Cytosine	2	0.02	0.04
Pyrimidine	1	0.01>	0.01>
Purine	1	0.01>	0.05

[a] Molar ratio of the adsorbed guest to the initially fed guest (1 μmol in 1 ml) on the contact with 10 mg (73 μmol) of PVDAT at 25 °C.

Table 2 Langmuir analysis of the thymine adsorption by PVDAT in protic media at 25 °C[a]

Protic solvent	K/M^{-1}	A/mmol (g PVDAT)$^{-1}$
Methanol	110	4.4
Water	150	2.6

[a] The K and A values were determined by using eqn. (1).

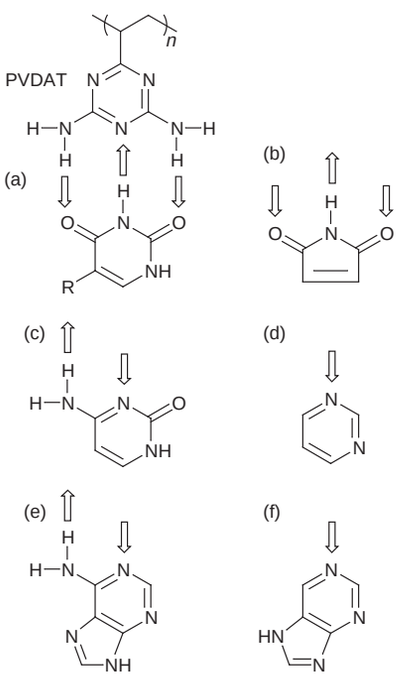

Fig. 2 Hydrogen-bond formation of 4,6-diaminotriazine (DAT) residue with various guest molecules. The open arrows indicate the complementary hydrogen bonds. (a) R = H: uracil; R = Me: thymine; R = Et: 5-ethyluracil; R = Pr: 5-propyluracil; (b) maleimide; (c) cytosine; (d) pyrimidine; (e) adenine; (f) purine.

PVDAT. These guest-bindings are reversible. When the thymine–PVDAT mixture is kept at equilibrium at 25 °C and then heated to 50 °C, 24% of the adsorbed thymine is released to the liquid phase. The binding constant (K) for the thymine–PVDAT adduct in methanol, determined by using eqn. (1), is 110 M^{-1} at 25 °C (see Table 2). This value is close to that (150 M^{-1}) for the adduct formation in water, which was evaluated previously.[10]

Comparison of the guest-recognizing activity of PVDAT in methanol with that in water

When the adsorption by PVDAT is conducted in water, pyrimidine derivatives are also satisfactorily recognized in terms of the number of complementary hydrogen-bonding sites towards DAT, as reported previously (see Table 1).[8] However, the selectivity for the binding of purine bases is much less strict than that in methanol. For example, adenine (two complementary hydrogen-bonding sites) is strongly adsorbed by PVDAT, which is in contrast with its poor binding in methanol. Its binding activity in water is considerably greater than the corresponding value for cytosine, which has also two hydrogen-bonding sites towards DAT. Even purine (one hydrogen-bonding site) is notably adsorbed in water, and its binding activity is close to that on cytosine. Apparently, stacking and apolar interactions are concurrently taking place here, and making the difference in the magnitude of hydrogen bonding less explicit. These arguments are supported by the fact that the adsorbing activity on 5-substituted uracil derivatives in water monotonically increases with increasing length of the alkyl substituent [H < methyl < ethyl < propyl: Fig. 3(b)]. In methanol, however, the order is entirely reversed,[11] since stacking and apolar interactions are minimized [Fig. 3(a)].[12]

Table 3 Adsorption of thymine and adenine by VDAT-copolymer in methanol at 25 °C

Polymeric receptor	Adsorption activity[a]	
	Thymine	Adenine
PVDAT	0.5 (0.26)[b]	0.1> (0.13)
PAAm-VDAT	— (0.24)	— (0.12)
PST-VDAT	0.10	0.01>
PVB-VDAT	0.17 (0.60)	0.02 (0.30)

[a] Molar ratio of the adsorbed thymine to the initially fed thymine (1 μmol in 1 ml) on the contact with polymeric host containing 15 μmol of DAT residue at 25 °C. [b] Parentheses indicate the adsorption activities in water on the contact with the hosts containing 73 μmol of DAT residue at 25 °C.

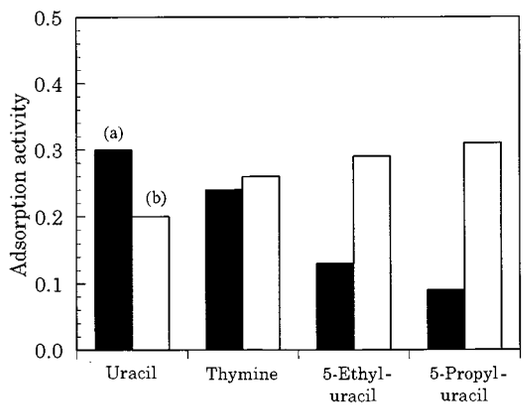

Fig. 3 Adsorption of uracil derivatives by PVDAT at 25 °C in methanol (a) and in water (b). Adsorption conditions are as in footnote *a* of Table 1. The ordinate (adsorption activity) indicates the molar ratio of the adsorbed guest to the initially fed guest.

Promoted guest-recognition by the copolymers of VDAT and styrene or 4-vinylbiphenyl

The guest-binding activity of PVDAT in methanol is greatly increased by copolymerization with hydrophobic co-monomers (Table 3). The activity of the copolymer of VDAT and styrene (PST-VDAT) for thymine-binding in methanol is two times as great as that of PVDAT. The binding activity of the copolymer with 4-vinylbiphenyl (PVB-VDAT) is still greater. In contrast, the binding activity toward adenine is little affected by the copolymerization. Thus, the selectivity for thymine-binding is enhanced by the copolymerization with these hydrophobic monomers.[13] When VDAT is copolymerized with hydrophilic acrylamide (PAAm-VDAT), however, the binding activity is virtually unchanged. In water, the binding activity towards thymine is also promoted by the copolymerization with hydrophobic monomers.[14]

Discussion

In methanol, nucleic acid bases are strictly recognized by PVDAT through hydrogen-bonding (Table 1). Quite a high guest-selectivity is achieved, since stacking and apolar interactions are minimized there.[12] As previously shown,[8] PVDAT adsorbs nucleic acid bases at two types of binding sites. The 'type I binding site' is rather small and binds pyrimidine bases preferentially to purine bases by complementary hydrogen-bonding. On the other hand, the 'type II binding site' is responsible for the binding of purine bases, where stacking and apolar interactions function cooperatively with hydrogen bonding. Both sites are rather aprotic due to the polymer effects, and thus hydrogen bonds are efficiently formed even in protic solvents. Probably the aprotic binding sites consist of several DAT residues which are arranged less regularly due to the segmental restraints in the polymer. By using methanol as the medium in place of water, hydrophobic interactions between the guest and the polymeric receptors, which are unfavorable for the strict recognition of nucleic acid bases (especially of purine bases), are suppressed.[12]

The guest-binding selectivity is still more increased by copolymerization with hydrophobic monomers (styrene and 4-vinylbiphenyl). The hydrophobic residues, introduced in the vicinity of DAT residues, enhance the aprotic nature at both the 'type I binding site' and the 'type II binding site', and facilitate the hydrogen-bonding interactions. Consistently, copolymerization with hydrophilic acrylamide does not affect the guest-selectivity at all.

In conclusion, PVDAT reversibly and selectively binds nucleic acid bases in methanol through hydrogen-bond formation. The binding activity and selectivity are further promoted by copolymerization with hydrophobic monomers. The present findings strongly indicate that a variety of guests can be bound in protic solvents through hydrogen-bonding by designing the polymeric field around the recognition sites.

Experimental

Materials

2-Vinyl-4,6-diamino-1,3,5-triazine (VDAT), nucleic acid bases, and their derivatives were purchased from Tokyo Kasei Kogyo Co. (or other commercial sources) and used without further purification. Styrene was distilled before use. 2,2′-Azoisobutyronitrile (AIBN) was recrystallized from methanol. 4-Vinylbiphenyl was purified also by recrystallization. Water was treated with a Millipore Milli-XQ purification system, and its specific resistance was greater than 18.3 MΩ cm^{-1}.

Poly(2-vinyl-4,6-diamino-1,3,5-triazine) [PVDAT: Fig. 1 (a)]

PVDAT was prepared by radical polymerization of VDAT (0.29 M) in DMSO at 70 °C with AIBN (3.9 mM) as the initiator, as described previously.[8] The averaged molecular weight (M_v) was around 10 000, as estimated from the reduced specific viscosity ($[\eta]$ = 0.30 dl g^{-1} at 30 °C in acetic acid).

Copolymerization of VDAT with various co-monomers [Fig. 1 (b)–(d)]

The copolymerization was carried out in DMSO at 70 °C by using AIBN (8.1 mM) as the initiator. The total monomer concentration was 0.56 M, and the VDAT/co-monomer ratio in the polymerization mixtures was 1/5 (for styrene and 4-vinylbiphenyl) or 1/1 (for acrylamide).[15] After the reaction, the mixture was poured into methanol. The white precipitate was recollected and dried *in vacuo* after being washed with methanol and ethanol. The compositions of copolymers, determined by elemental analysis, are shown in parentheses in Fig. 1.

Adsorption of guests by polymeric receptors

A fine powder of the polymeric receptors containing 73 μmol of DAT residues was added to a guest solution (1 ml), and the mixture was magnetically stirred at 25 °C. The equilibrium for the adsorption by PVDAT in methanol was attained within 15 min.[16] The amount of the guest bound to the polymers was evaluated from the guest concentration in the liquid phase, which was determined by HPLC. The polymeric receptors used in this study were virtually insoluble in either methanol or water. The details of experimental procedures have been described previously.[8]

Evaluation of binding constants for adduct formation between polymeric receptors and guests

The binding constants (K) for guest–receptor adducts were determined by Langmuir's adsorption isotherm, eqn. (1).[17]

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

Here, c is the equilibrium concentration of free guest in the liquid phase, whereas A and θ are the maximal amounts of the adsorbed guest and the surface coverage, respectively.

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9 Direct evidence of hydrogen bond formation in methanol was obtained by FTIR measurement: the carbonyl stretching band of uracil shifted to lower frequency on the adduct formation with PVDAT. A similar IR shift was also observed on the hydrogen bond formation between PVDAT and uracil in water, as previously reported in reference 8. This argument was further confirmed by the fact that 3-methyluracil, whose hydrogen bonding site is blocked by a methyl group, was hardly adsorbed by PVDAT.
10 These values are slightly larger than the corresponding value for the complex formation between 2,6-dibutyramidopyridine (a model compound of DAT) and 1-butylthymine (90 M^{-1}) in non-competitive chloroform.[3b]. In contrast, VDAT (monomer of PVDAT) hardly bound uracil in homogeneous DMSO–water mixture. See ref. 8.
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