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Short Communication

Molecular Recognition of Nucleic Acid Bases in Water by Hydrogen Bonding of Poly(vinyldiaminotriazine)

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Abstract. Poly(2-vinyl-4,6-diamino-1,3,5-triazine) efficiently binds nucleic acid bases and nucleosides in water by using complementary hydrogen bonding. The binding activity decreases in the order: U, T > A ≫ C, G. The corresponding monomer shows virtually no activity, indicating a predominant role of polymer effect for the molecular recognition in water.

Key words: Poly(2-vinyl-4,6-diamino-1,3,5-triazine), molecular recognition, hydrogen bonding, nucleic acid base.

1. Introduction

The mimicking of molecular recognition in biological systems has been attracting a great deal of interest. A variety of elegant organic receptors, which selectively bind the target guest molecules through hydrogen bonding, have been designed [1–3]. In most cases, aprotic organic solvents such as chloroform and carbon tetrachloride were used as the media, since otherwise the hydrogen bonds are destroyed by competition with the solvents [4]. Novel receptors are required to achieve precise recognition of guests in water, as is done in nature.

In 1991, Kunitake *et al*. showed that the Langmuir-Blodgett film of an amphiphile bearing 4,6-diaminotriazine binds uridine and thymidine at the air/water interface through hydrogen bonding [5, 6]. In the present communication we report that poly(2-vinyl-4,6-diamino-1,3,5-triazine) (PVDT) efficiently recognizes nucleic acid bases and nucleosides in bulk water. The complementary hydrogen bonds are formed even in water, since an appropriate reaction field is provided by the polymer.

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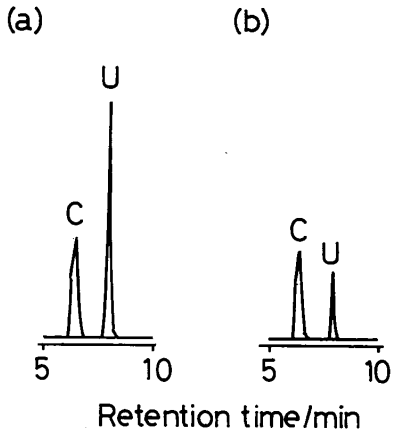

Figure 1. HPLC patterns for aqueous solution of a uracil/cytosine mixture (a) before and (b) after the contact with PVDT. One milliliter of the solution ([uracil] = [cytosine] = 1 mM) was treated with 100 mg of PVDT at 20 °C (see Experimental section for detail).

2. Experimental

PVDT was prepared by a radical polymerization of 2-vinyl-4,6-diamino-1,3,5-triazine (VDT, 0.29 mol dm^{-3}) in DMSO at 70 °C with AIBN (3.9 mmol dm^{-3}) as the initiator. The adsorption experiments were carried out by incubating PVDT in 1 mL of aqueous solution of guest with intermittent shaking. The pH was maintained at 6.5 $\pm$ 0.5 without the use of buffer. The mixture was then centrifuged, and the liquid phase was analyzed by reversed-phase HPLC. As internal standard, 4-hydroxypyridine, which shows no measurable interaction with PVDT and is detectable by a UV detector, was added to the incubation mixture.

3. Results and Discussion

When an aqueous solution of uracil and cytosine was incubated at 20 °C with PVDT (which is insoluble in water), the concentration of uracil in the liquid phase decreased (compare (b) with (a) in Figure 1). Equilibrium was attained within 15 min. In contrast, cytosine was adsorbed only faintly. The eminent and selective uracil-binding activity of PVDT in water is conclusive. On elevating the temperature to 70 °C, the adsorbed uracil was released to the aqueous phase, confirming the reversibility of the adsorption [7].

Uridine, thymine, and thymidine were also significantly adsorbed by PVDT, while the adsorption of cytidine was much smaller (Table I). Apparently, the complementary hydrogen bonding between the guests and the 4,6-diaminotriazine (DAT) residues in the polymer is responsible for the adsorption: U and T have three hydrogen bonding sites toward DAT (Figure 2(a)), whereas C has only two (b). Three hydrogen bonds are required to bind pyrimidines effectively in water

Table I. Adsorption of nucleic acid bases and nucleosides by PVDT at 25 °C

Nucleic acid base	Adsorbing activity[a]	
	Base itself	Nucleoside
Uracil	0.20	0.22
Thymine	0.26	0.18
Cytosine	0.04	0.05
Adenine	0.13	0.12
Guanine	–[b]	0.04

[a] The ratio of the decrease in the guest concentration, observed on contact with 10 mg of PVDT containing 73 μmol of DAT residue, with respect to the initial guest concentration (1 mM). The amount of the solution is 1 mL (see Experimental).
[b] Water solubility of guanine is too low for the experiment.

(the stacking interaction is not so important here: *vide infra*). Formation of the hydrogen bonding adducts is further corroborated by the fact that maleimide was notably adsorbed by PVDT but *N*-methylmaleimide was not.

The adsorption of uracil by PVDT in water fitted the Langmuir plot fairly well, as shown in Figure 3: the formation constant, *K*, of the complex between the DAT residue and uracil was calculated to be 93 M^{-1} at 20 °C from the intercept and the slope of the straight line. This value is overwhelmingly greater than the corresponding value for the monomer, 2-vinyl-4,6-diamino-1,3,5-triazine (VDT). According to an NMR titration, the K value for the VDT–uracil complex in DMSO is only 0.3 M^{-1}, and is 0.2 M^{-1} in 7/3 DMSO/H_2O mixture (the value in water should be much smaller, although it could not be directly determined because of the poor solubility of VDT) [8]. Thus the binding activity of DAT residue in PVDT is enormously promoted by a polymer effect. It is noteworthy that the K value for uracil-binding by PVDT in water is almost identical with that (90 M^{-1}) for the complex formation between 2,6-dibutyramidopyridine (used as a model compound of DAT) and 1-butylthymine in non-competitive chloroform [2a].

It is assumed that the hydrogen bonding between the guest and the DAT residue in PVDT takes place in apolar microspheres formed by several DAT residues. Because of the segmental restraints in the polymer, these residues, which readily stack with each other, are arranged less regularly and constitute the microspheres. The diaminotriazine residues therein are relatively free from the solvation with water, and thus possess strong hydrogen bonding activities [9]. Consistently, the DAT residue in a 1 : 5.5 copolymer of VDT and styrene showed almost the same activity as did the DAT in PVDT, since the benzene rings of the styrene and the

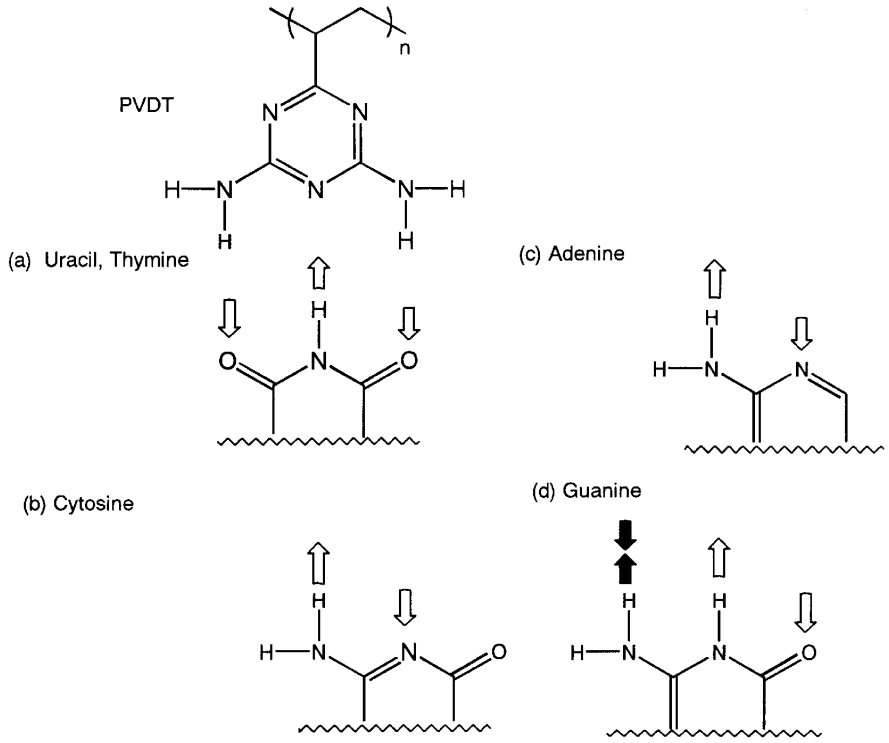

Figure 2. Hydrogen bonding of PVDT with nucleic acid bases and nucleosides. The open arrows show the complementary hydrogen bonds, whereas the closed ones refer to potential steric repulsion.

DAT residues can stack with each other [10]. When VDT was copolymerized with methyl acrylate (in a molar ratio 1 : 5), however, the guest-binding activity totally disappeared because of the absence of stacking of the DAT residues.

In accordance with the proposed structure of the binding sites, adenine and adenosine are bound 2–3 fold more strongly than cytosine and cytidine (see Table I). All of these guests have two hydrogen bonding sites to DAT (Figure 2). Undoubtedly, the two-hydrogen bonding adducts of A are stabilized by the stacking interaction between the purine and the DAT residue(s) at the binding site [11]. A small binding activity toward guanosine is ascribed to a steric repulsion on complex formation, depicted by the closed arrows in Figure 2(d). The present finding indicates that synthetic polymers are useful for the design of artificial receptors which can bind guest molecules in an aqueous phase.

Acknowledgments

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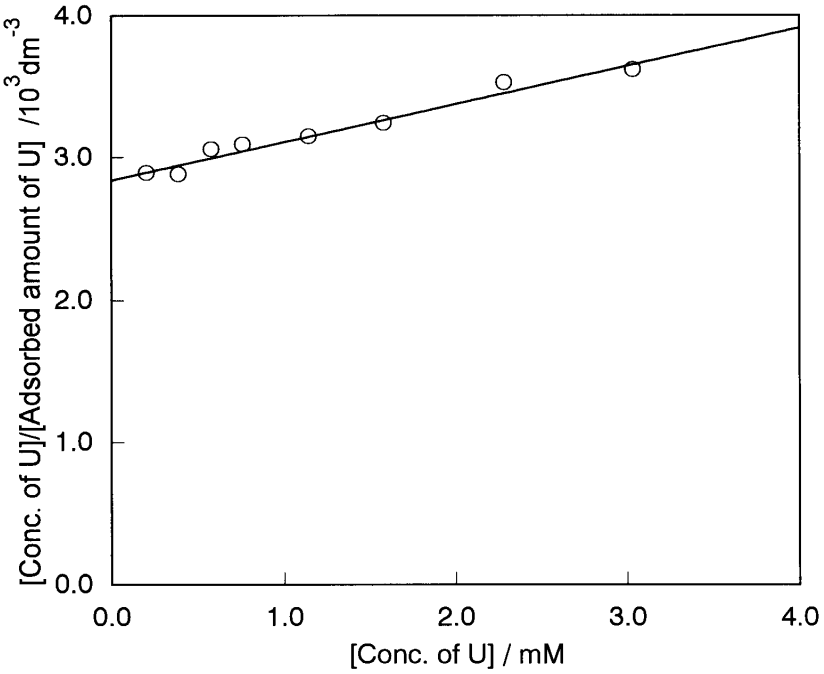

Figure 3. Langmuir plot for the adsorption of uracil by PVDT. Ten milligrams of the polymer was contacted with 1 mL of uracil solutions of various concentrations at 20 °C. The horizontal axis refers to the concentration of uracil in the liquid phase in equilibrium, and the vertical axis shows its ratio to the adsorbed amount of uracil.

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6. Synthetic receptors, which take advantage of electrostatic interactions and can bind guests even in polar solvents, are reported in Ref. 2b.
7. After uracil was adsorbed at 20 °C, the mixture was heated to 70 °C. About half of the adsorbed uracil was released to the aqueous phase, exactly as expected from an independent adsorption experiment at 70 °C.
8. Solid VDT did not adsorb uracil in water.
9. The maximum uracil-binding capacity of PVDT (3.8 μmol for 10 mg polymer), estimated from the Langmuir plot, corresponds to one binding site per 19 DAT residues.

10. When 51.2 mg of styrene-VDT copolymer (containing 73 μmol of DAT residue as does 10 mg of PVDT) was contacted with uracil solution, 22% of initially charged uracil was adsorbed at 25 °C. This value was almost the same as that of PVDT (see Table I). Moreover, the isotherm for the adsorption by the copolymer almost superimposed that for the adsorption by PVDT.
11. Some contribution of stacking interaction in the guest binding was evidenced by the fact that 80% of xanthine, with respect to the initial concentration (0.1 mM), was adsorbed by PVDT at 25 °C, whereas only 14% of adenine was adsorbed under identical conditions. A similar cooperation of hydrogen bonding and stacking interaction was indicated previously: (a) J.F. Constant, J. Fahy, and J. Lhomme: *Tetrahedron Lett.* **28**, 1777 (1987). (b) V.M. Rotello, E.A. Viani, G. Deslongchamps, B.A. Murray, and J. Rebek, Jr.: *J. Am. Chem. Soc.* **115**, 797 (1993).