Direct Evidences for the Hydrogen Bonding in Water by Polymeric Receptors Carrying Diaminotriazine

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In homogeneous aqueous solutions, copolymers of 2-vinyl-4,6-diamino-1,3,5-triazine with acrylamide selectively bind thymine and theobromine through complementary hydrogen bonding, as confirmed by ^{1}H-NMR and ultrafiltration experiments. The corresponding monomer is inactive, indicating the predominance of polymer effect for the recognition in water.

Hydrogen bonding is one of the fundamental interactions used in natural systems for the molecular recognition. Elegant artificial receptors were synthesized by placing hydrogen-bonding sites complementarily towards target molecules.[1-3] However, most of these receptors do not effectively function in water, because the hydrogen bondings are competitively destroyed by the solvent molecules. Recent interests are focusing on the artificial receptors which successfully operate in water as naturally occurring ones do.[4]

In a previous paper,[5] water-insoluble homopolymers of poly(2-vinyl-4,6-diamino-1,3,5-triazine) adsorbed nucleic acid bases through hydrogen bonding in water. The order in binding activity precisely coincided with the number of hydrogen-bonding sites of the guests towards 4,6-diamino-1,3,5-triazine (DAT) residue. The hydrogen bonding was substantiated by the decrease in the carbonyl-stretching frequency of thymine on the adduct formation. Furthermore, Langmuir-Blodgett films of an amphiphile carrying a DAT residue bound thymidine at the water/air interface through hydrogen bonding.[6] Guanine nucleosides were also bound to L-B films of an amphiphile carrying cytosine.[7] The hydrogen-bond formation was directly observed by quartz-crystal microbalance system.[8] However, all of these systems involve the boundary of different phases (solid/water or air/water), and it is still controversial whether the boundary is essential for the hydrogen bonding or not. In the present paper, water-soluble copolymers carrying DAT residues are prepared, and the guest binding by them in water is investigated by ^{1}H-NMR spectroscopy and ultrafiltration experiments. The evidences for hydrogen-bond formation in homogeneous aqueous phase are presented.

Water-soluble polymeric receptors were prepared by the copolymerization of 2-vinyl-4,6-diamino-1,3,5-triazine (VDAT) and acrylamide (Figure 1).[9] All the aqueous solutions containing the copolymer (P(VDAT-*co*-AAm)) and thymine were homogeneous. When the polymer was added to aqueous solutions of thymine, ^{1}H-NMR signal of the imido N-H proton (H^1) of thymine notably shifted towards the lower magnetic field (Figure 2).[10] The magnitude of the chemical-shift change monotonically increased with increasing amount of the polymer. Hydrogen bonding between thymine and the DAT residue in water is clearly shown (note that the H^1 proton is directly associated with the hydrogen bonding to DAT: see Figure 1). Consistently, the chemical shifts of both the amide N-H proton (H^2) and the methine proton (H^3) of thymine, which are far away from the hydrogen-bonding site, remained virtually unchanged. A similar result was obtained for the adduct formation with theobromine, which also has three complementary hydrogen-bonding sites towards DAT. The imido N-H proton of

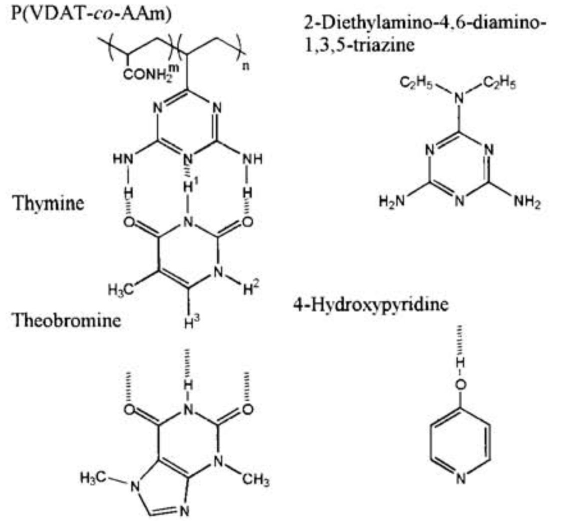

Figure 1. Chemical structures of the polymeric receptors and the substrates used in this study.

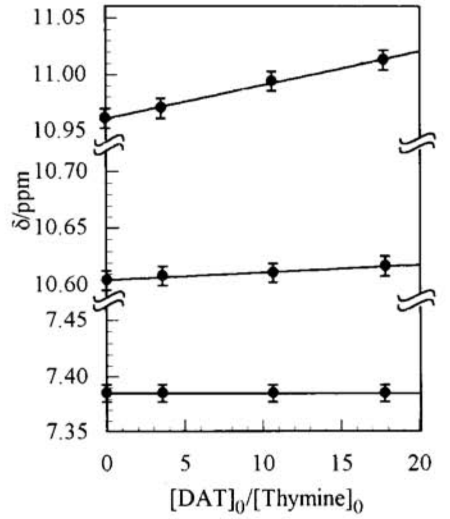

Figure 2. Plots of the ^{1}H-NMR shifts (δ in ppm) of the imido proton (upper), the amide proton (middle), and the methine proton (lower) of thymine as a function of the [DAT residue in P(VDAT-*co*-AAm)]$_0$/[thymine]$_0$ ratio. In the absence of the polymeric receptor, the ^{1}H-NMR signals for the imido N-H, the amido N-H, and the C-H protons are at δ10.961, 10.604 and 7.385 ppm, respectively.

theobromine showed a specific downfield shift (from δ10.870 to 10.904 ppm), when the polymer (45 mmol dm^{-3} with respect to DAT residue) was added ([theobromine]$_0$ = 2 mmol dm^{-3}). In contrast, chemical shifts of the other protons of theobromine did not change to measurable extents.

The polymeric structure of P(VDAT-*co*-AAm) is essential for the hydrogen bonding in water. When 2-diethylamino-4,6-diamino-1,3,5-triazine (a water-soluble monomeric analog of the DAT residue in the P(VDAT-*co*-AAm)) was used in place of the polymer, the imido proton of thymine showed an upfield shift, rather than a downfield shift assignable to hydrogen bonding.[11] Homopolymers of acrylamide caused no change in the chemical shifts of the protons of thymine. Assumedly, P(VDAT-*co*-AAm) provides rather apolar micro-domain around the DAT residues, in which the hydrogen bonds with the guests are formed. Furthermore, the polymer chain can impose a steric restraint on the DAT residues and suppress their stacking with the guest molecules.[12]

The proposed hydrogen bondings in water by the water-soluble polymeric receptors were further confirmed by the following ultrafiltration experiments.[13] Thymine freely permeates through the membrane used (the cut-off molecular weight 5000), but P(VDAT-*co*-AAm) cannot. When aqueous solutions containing both the polymeric receptor and thymine were treated with this membrane, the concentrations of thymine in the filtrates were considerably smaller than those in the feed solutions. Apparently, some of the thymine formed adducts with the polymer in water and thus could not permeate through the membrane. On the other hand, 4-hydroxylpyridine, which has only one hydrogen-bonding site towards DAT (Figure 1) and is hardly bound by P(VDAT-*co*-AAm), completely passed through the membrane, even in the presence of the polymer. Thus, the number of hydrogen-bonding sites governs the strength of guest binding by the polymeric receptors.

In conclusion, water-soluble copolymers carrying 4,6-diamino-1,3,5-triazine residues satisfactorily bind thymine and theobromine in homogeneous aqueous solutions through hydrogen-bond formation. Synthetic polymers are promising candidates for artificial receptors which are effective in water.

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9 Copolymerization of VDAT and acrylamide was achieved in DMSO at 65 °C with AIBN as the initiator. After the reaction, the polymer was dialyzed overnight against pure water to remove free monomers. The copolymer used for Figure 2 has a molecular weight of 5900 as determined by MALDI-TOFMS and the VDAT/AAm ratio in this polymer is 11/89 from elemental analysis.

10 The NMR measurements were made in 9/1 H_2O/D_2O mixture at pH 5.0 (50 mmol dm^{-3} acetate buffer) and 25 °C on a Brucker AXM-500 spectrometer by a Jump-and-Return sequence to eliminate the signal of water: G. Kawai, T. Yokoyama, K. Nishikawa, T. Ueda, T. Hashizume, J. A. McCloskey, S. Yokoyama, and K. Watanabe, *Nucleosides Nucleotides*, **13**, 1189 (1994). The signal for HDO was used as the internal standard.

11 The signals of thymine at δ10.961 (for the imido proton) and 7.385 ppm (for the methine proton) shifted to δ10.900 and 7.380 ppm, respectively, on the addition of three times as much amount of the monomeric analog. These upfield shifts are probably due to stacking of the thymine with the monomeric analog.

12 Direct contribution of the amide moieties in the acrylamide residues in the copolymer to the recognition of thymine is ruled out, since the copolymers of VDAT with N,N-dimethylacrylamide induced similar downfield shift of the imido proton of thymine. The shift was 0.028 ppm for the former polymer (the composition = 12/88), whereas the shift for the P(VDAT-*co*-AAm) was 0.033 ppm. Recognition of thymine with plural DAT residues in the polymer (e.g. stacked DATs) is also unlikely because P(VDAT-*co*-AAm)s of different compositions gave almost the same downfield shifts. When the composition was 7/93, the shift for the imido proton was 0.039 ppm. The [DAT]$_0$ in the polymer was kept at 50 *mM* in each case.

13 The mixtures of P(VDAT-*co*-AAm) and thymine were incubated at 25 °C for 1 h and then charged to a Millipore Ultrafree-MC centrifugal filter. The filtrates, obtained by centrifuging the mixtures, were analyzed by HPLC. For example, the concentration of the thymine in the filtrate was about 13% smaller than the value in the feed solution, under the following conditions: [DAT residue in the polymer]$_0$ = 30 and [thymine]$_0$ = 0.30 mmol dm^{-3} at pH 6.5. In the absence of the polymer, both thymine and 4-hydroxypyridine freely passed through the membrane, and their concentrations in the filtrates were identical with the values in the feed solutions.