Evaluation of Binding and Origins of Specificity of 9-Ethyladenine Imprinted Polymers

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Abstract: An investigation of 9-ethyladenine (9-EA) imprinted polymers has provided insight into the origins of binding and recognition by imprinted polymers. Porogens and polymerization temperature are important variables during the polymerization. Factors that influence rebinding of the 9-EA imprinted polymer include pH and utilization of the porogen as mobile phase. Finally, quantitative structure−binding relationship studies elicited the substructures of 9-EA primarily responsible for creating the binding sites within the polymers.

The development of synthetic receptors that recognize nucleotide bases and their derivatives is an important area in chemistry today.[1] Apart from a more detailed understanding of the interactions and mechanisms in DNA−DNA or protein−DNA recognition, applications are envisioned in the fields of biosensors, drug therapy, separation science, and genetic engineering. Rationally designed host molecules have been prepared that bind nucleotides and their analogues with especially strong binding found for adenine.[1] Binding sites for nucleotide bases by macromolecular structures have also been successful using antibodies,[2] synthetic macromolecules,[3] and monolayers.[4]

The interest in DNA specific binding molecules prompted us to apply the molecular imprinting technique to this important area.[5] The concept of molecular imprinting is illustrated in Scheme 1.

The process begins with the desired target molecule denoted T, or template, which serves two functions. The first is as a space-filling three-dimensional object around which a complementary polymer cavity can be formed. The second is to organize complementary interactions between groups on the template and functional monomers during polymerization. Organization of polymerizable functional monomers by the template may be achieved by either covalent bonds and/or noncovalent forces such as hydrogen-bonding, electrostatic, and hydrophobic interactions. The polymerization reaction mixture consists of the template along with functional monomers and a large excess of cross-linking monomer. An equal volume of inert solvent (porogen) and free-radical initiator make up the remainder of the polymerization solution. Thermal or photochemical initiated polymerization results in a highly cross-linked insoluble network polymer. The template may be reversibly removed from the polymer network, while the functional monomers remain covalently bound to the polymer itself. Left in the polymer matrix are three-dimensional cavities that are complementary in shape to the template with desired functionality in a specific arrangement.

Application of molecular imprinting to form an adenine receptor was inspired by proton NMR studies[6] of the interaction between butyric acid and 9-ethyladenine (9-EA) in chloroform. An association constant of $K_a = 160\ M^{-1}$ was determined. This example of binding between adenine and a carboxylic acid was a good indicator of its potential to preorganize methacrylic acid (MAA) in a pre-polymerization complex for molecular imprinting. This is illustrated in Scheme 2. The interaction of methacrylic acid (MAA) with 9-EA prior to and during copolymerization with ethylene glycol dimethacrylate (EGDMA) was designed to produce an array of carboxylic acids complementary to 9-EA.

Polymerization mixtures consist of MAA (12.0 mol %) and 9-EA (1.0 mol %) in $CHCl_3$ containing EGDMA (86.0 mol %) and AIBN (1.0 mol %). Photopolymerization gave a highly cross-linked network polymer.[7] Control polymers containing the same number of carboxylate groups but incorporating a "generic" template, benzylamine [P(BA)], and materials prepared without any template [P(BL)] were also prepared. The template, 9-EA, was removed by Soxhlet extraction to afford a polymer containing putative adenine recognition sites. The binding and specificity of the imprinted polymer can be

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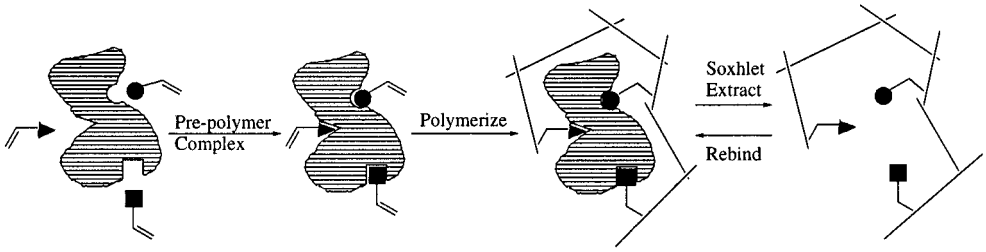
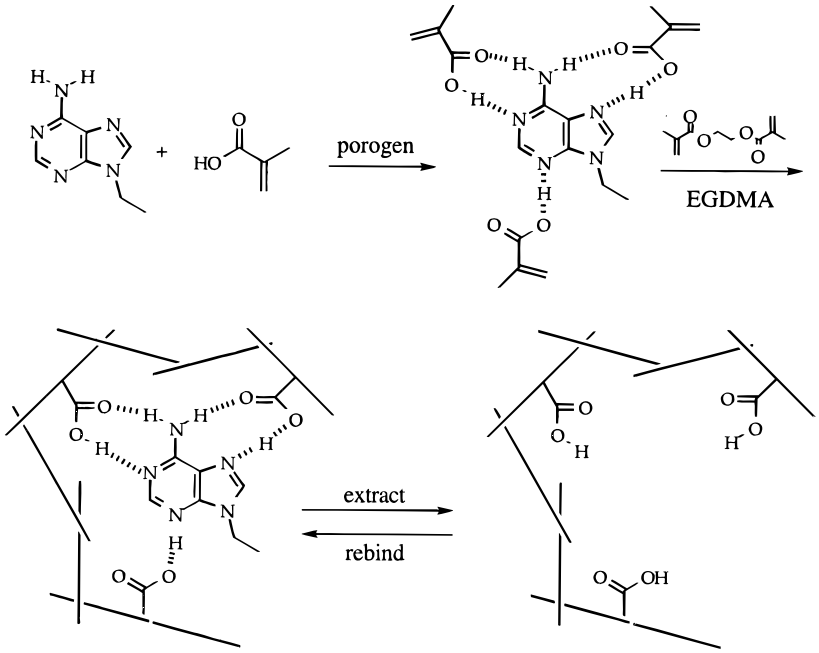
Scheme 1. Molecular Imprinting Strategy for Creating Selective Sites in Macroporous Polymers



Scheme 2. Template Strategy for Creating Selective Sites to 9-EA within Network Polymers[a]



[a] Dashed lines show potential H-bonding interactions between 9-EA and MAA.

evaluated either in the batch or chromatographic mode. The binding of 9-EA to its own imprinted polymer was found to be 76 000 M^{-1} in chloroform, a value approaching the binding power of antibodies.[5] In contrast, the generic polymers show little affinity for 9-EA. In addition to accentuated binding affinity by the polymer, specificity is clearly seen in chromatographic data presented for a column packed with 9-EA imprinted polymer which showed little retention of any nucleotide derivatives except adenine (*vide infra*). The evidence suggests that the imprinting method creates a microenvironment based on both shape selection and position of functional groups that recognizes best the 9-EA template molecule.

To explore the factors that influence binding by polymers imprinted with 9-EA, three studies were undertaken. First, the effect of temperature on the free-radical polymerization was examined. Next, the influence of porogen (polymerization solvent) on creating the binding site during polymerization was investigated, as well as the effect of solvent on post polymerization recognition. Finally, the dependence of chromatographic retention on pH of the mobile phase was studied. In addition to the above studies, quantitative structure−binding studies were carried out to learn what elements of the structure of 9-EA contribute to the binding and specificity of the polymer complement. Small molecules containing substructural elements of 9-EA were imprinted and evaluated chromatographically.

Results

Photoinitiated versus Thermal Polymerization. Initial chromatographic studies of photoinitiated 9-EA imprinted polymer revealed a selective retention of adenine derivatives over the other purine or pyrimidine bases.[5] The polymers were prepared at approximately 5 °C by photoinitiation using AIBN as initiator. Photoinitiation was chosen on the basis of the hypothesis that the pre-polymer complex equilibrium lies further toward the complex at low temperature.[8,9] It was of interest to see if polymers prepared by thermal initiation would in fact show decreased binding and selectivity for 9-EA. A polymer mixture of composition identical to that of the photoinitiated polymer was polymerized at 80 °C for 6 h. A comparison of the performance of photoinitiated versus thermal-initiated polymer is shown in Table 1. The results clearly show that photoinitiated polymerization provides substantially better binding.

Investigation of Solvent Effects on Binding Site Fidelity. An analysis of capacity factors for 9-EA imprinted polymers made in different porogens reveals that the porogen can have an influence on the binding behavior. For example, in Table 2, the retention of 9-EA is almost 3 times greater for a polymer made using acetonitrile as porogen than for polymer made using chloroform, in an acetonitrile-containing mobile phase.

To establish whether optimum binding occurs when polymer rebinding employs the same solvent used as porogen, two polymers imprinted with 9-EA utilizing two different porogens

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Table 1. Comparison of Capacity Factors (k')[10] of Nucleotide Analogues on HPLC Columns Packed with Identical Amounts of 9-EA Imprinted Polymers Initiated Either Thermally or Photochemically (Benzoic Acid Imprinted Polymer Was Used as a Control)[a]

	P(9-EA)		P(BzA)
substrates	photoinitiated	thermal-initiated	photoinitiated
9-EA	54.8	3.8	1.7
1-cyclohexyluracil	3.9	0.2	0.3
1-propylcytosine	0.3	0.9	2.4
1-propylthymine	0.4	0.1	0.2
benzoic acid	0.0	0.0	0.0

[a] The polymers (25−38 μM particle size) were slurry packed in stainless steel chromatographic columns (length, 100 mm; i.d., 4.6 mm). Mobile phase = 92.5:5:2.5 MeCN/AcOH/H_2O; λ = 260; flow rate = 1.0 mL/min at room temperature. 10.0 μL of 1.0 mM solutions were injected onto the column.

Table 2. Comparison of Capacity Factors (k') for Nucleotide Analogues on 9-EA Imprinted Polymers Employing Chloroform versus Acetonitrile as Porogen[a]

substrates	P(9-EA), $CHCl_3$ as porogen	P(9-EA), MeCN as porogen
9-EA	4.83	11.32
1-cyclohexyluracil	1.07	8.26
1-propylcytosine	0.62	0.14
1-propylthymine	0.76	0.17
adenine	1.90	2.81
guanine	0.30	0.24
uracil	0.37	0.01
cytosine	0.44	0.08
thymine	0.44	0.26
adenosine	0.48	0.36
benzylamine	0.56	0.02

[a] Columns prepared as in Table 1. Mobile phase = 3/7 KH_2PO_4−K_2HPO_4 (0.1 M (aq), pH = 4.5)/MeCN; UV detection at λ = 260; flow rate = 1.0 mL/min at room temperature. 10.0 μL of 1.0 mM solutions were injected onto the column.

Table 3. Capacity Factors of 9-EA Polymers Employing Chloroform and Acetonitrile as Porogen and Mobile Phase[a]

polymer	$CHCl_3$/AcOH (85/15)	MeCN/AcOH (85/15)
P(9-EA/$CHCl_3$ as porogen)	16.58	2.70
P(9-EA/MeCN as porogen)	1.96	7.45

[a] Columns prepared as in Table 1. Mobile phase indicated in the Table; UV detection at λ = 260; flow rate = 1.0 mL/min at room temperature. 10.0 μL of 1.0 mM solutions were injected onto the column.

were evaluated chromatographically using solvent systems incorporating each porogen. The solvent systems chosen were 85/15 acetonitrile/acetic acid and 85/15 chloroform/acetic acid to recreate pre-polymerization conditions as well as obtain suitable chromatograms. Table 3 shows retention times of 9-EA for all four cases.

These results indicate that solvent does affect the microenvironment of the binding sites created in the polymer. It also appears that there is enhanced binding in polymers immersed in the solvent in which they were polymerized. This suggests that ideal rebinding conditions for a given template should include the solvent used as porogen.

Chromatographic Retention as a Function of pH. Retention of the 9-EA template on its imprinted polymer is a function of pH. Using an aqueous buffer solvent system, KH_2PO_4−K_2HPO_4 (aq)/acetonitrile (3/7), a correlation between retention and mobile phase pH is seen in Figure 1 for the 9-EA imprinted polymer. The pH was altered by adjusting the balance of mono- and dibasic phosphate salts (or adding HCl to KH_2HPO_4 solution for pH values lower than 4.2), while the total concentration of phosphate salts was held constant at 0.01 M.

(10) Capacity factor (k') is used as a retention parameter; $k' = [V(t) - V(o)\}/V(o)]$, where $V(t)$ is the retention volume and $V(o)$ is the dead volume or the retention volume of a nonbinding substrate. This parameter is superior to retention time because it is independent of column size or amount of stationary phase in the column.

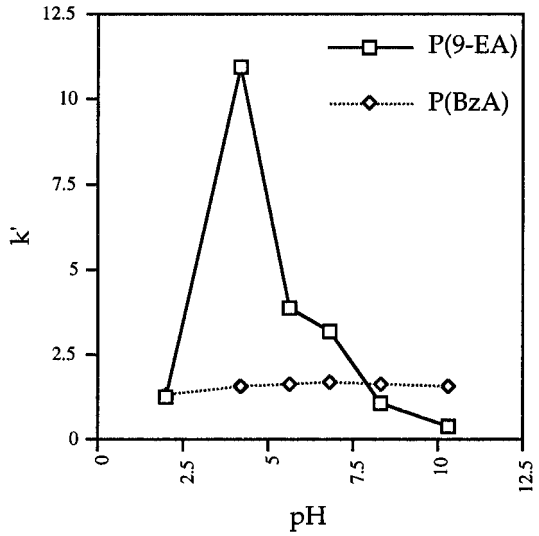


Figure 1. Retention of 9-EA on P(9-EA) and P(BzA) as a function of pH.

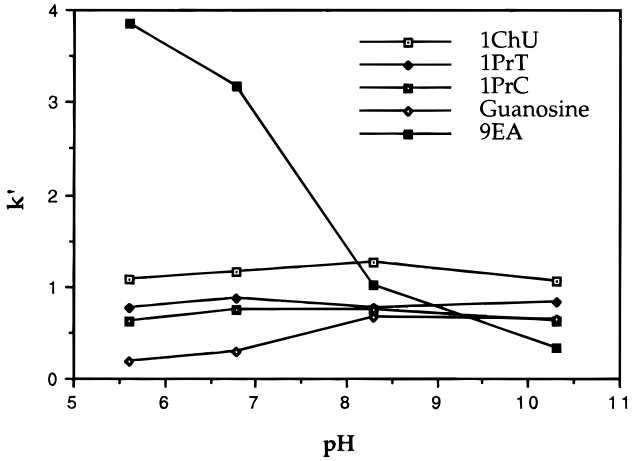


Figure 2. Comparison of capacity factors for various nucleotide base derivatives on the P(9-EA) column at different pHs. 1ChU = 1-cyclohexyluracil, 1PrT = 1-propylthymine, and 1PrC = 1-propylcytosine.

In addition, Figure 2 reveals that non-adenine nucleotide bases show little retention on the 9-EA column as pH is changed. Thus, it appears that the pH selectivity of the polymer is specific only for the template molecule.

Quantitative Structure−Binding Studies. The good binding characteristics of the 9-EA imprinted polymer prompted us to investigate the origins of the template interactions. Of interest was which elements of 9-EA's structure and topography contribute to the binding and specificity of the polymer. To probe these factors, a series of simple molecules that contain substructural features of 9-EA (Figure 3) were imprinted under the same conditions used to imprint 9-EA.

Each of the imprinted polymers were evaluated chromatographically using each of the different templates as substrate. Table 4 summarizes the capacity factors found for each combination of polymer and substrate (note that 9-EA did not elute from the P(9-EA) column even after 6 h, thus there is no entry).

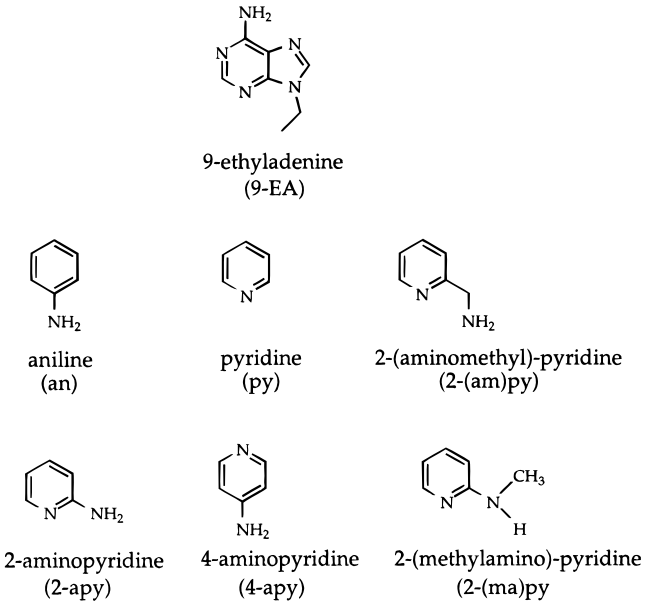


Figure 3. Simple analogues of 9-EA imprinted and compared to P(9-EA).

Table 4. Capacity Factor (k') Values of Small Molecule Analogues of 9-EA[a]

polymers[c]	an[b]	py	2-apy	2-(am)py	4-apy	2-(ma)py	9-EA
P(an)	**1.2**	1.6	0.1	0.4	0.5	1.5	0.2
P(py)	1.5	**1.9**	0.9	0.7	3.8	2.3	0.6
P(2-apy)	3.4	4.7	**6.9**	2.0	13.7	10.1	1.7
P(2-(am)py)	4.7	4.2	2.8	**10.4**	3.1	2.4	1.7
P(4-apy)	2.0	3.0	1.0	0.6	**6.0**	2.4	0.9
P(2-(ma)py)	2.0	3.6	1.9	0.9	3.7	**8.4**	1.1
P(BzA)	3.4	1.0	0.0	0.2	0.3	1.4	0.3
P(9-EA)	3.5	3.7	4.9	2.7	9.4	4.9	*d*

[a] Mobile phase = 94/5/1 acetonitrile/water/acetic acid (apparent pH = 3.2). [b] Substrate abbreviations: an = aniline, py = pyridine, 2-apy = 2-aminopyridine, 2-(am)py = 2-(aminomethyl)pyridine, 4-apy = 4-aminopyridine, 2-(ma)py = 2-(methylamino)pyridine. [c] P(BzA) = polymer made with benzoic acid template. Other polymers (P) made using the substrate shown in parentheses as template. [d] Did not elute after 6 h.

Discussion

Factors that influence binding and specificity were explored using polymers imprinted with 9-EA. Photoinitiated polymerization at 5 °C afforded polymers with greater binding and specificity for 9-EA than polymers that were polymerized thermally at 60 °C. This appears to be general for imprinted polymers,[8,9] although a higher saturation capacity has been noted for thermal-initiated versus photochemically initiated polymers.[8] NMR experiments have shown that lower temperatures such as those found during photoinitiation result in an increase in the concentration of pre-polymerization complex.[9b] This is expected since the negative ΔS for formation of the pre-polymerization complex is favored at lower temperatures. The increase in the concentration of pre-polymerization complex, in turn, could be responsible for an increase in the numbers of specific binding sites created during polymerization. The end result would be an increase of specific binding sites that manifests in higher chromatographic retention. It is likely that even lower temperatures would result in better binding behavior. Perhaps as important is the association of 9-EA with MMA groups that have been incorporated into growing polymer chains. These interactions can result in induced polymer chain conformations that are subsequently "locked" in the network by further polymerization.

Table 5. Association Constants for 9EA with P(9EA) in Solvents of Different Polarity

polymer	solvent system	K_a (M^{-1})
P(9EA)	$CHCl_3$	7.6×10^4
P(9EA)	95:5, MeCN:H_2O	5.2×10^3
P(9EA)	92.5:5:2.5, ACN:AcOH/H_2O	7.0×10^2

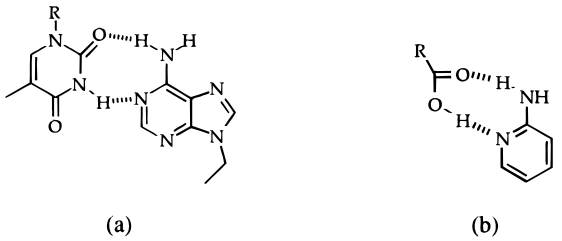
Investigation of solvent effects on binding site fidelity showed that the 9-EA imprinted polymer performs best in a mobile phase that is similar to the porogen utilized during polymerization. Kempe and Mosbach have suggested that using the same solvent in the mobile phase that was used as porogen would mimic, in the chromatographic mode, the interactions existing prior to and during the polymerization.[11] A possible explanation for this may lie in a link between conditions during polymerization and those during rebinding analysis on the product network polymer. The origins of specificity in the imprinted polymer are postulated to arise from the positioning of complementary functional groups which are then covalently locked into place during polymerization. Different swelling properties of different solvents, such as chloroform and acetonitrile, may play a role in determining shape and distance parameters that are locked into the forming polymer. In order to recreate and maintain these shape and distance parameters, it is possible that optimum rebinding conditions require the same, or very similar, swelling conditions used for polymerization. Swelling studies[8] on similar insoluble network polymers imprinted with L-phenylalanine anilide show swelling factors of 36% volume increase for materials made with acetonitrile as porogen, and 111% volume increase for materials made with chloroform as porogen. In support of swelling considerations for binding site fidelity, it should be noted that acetonitrile is more polar than chloroform, decreasing hydrogen bonding and electrostatic interactions both for the pre-polymerization complex and for rebinding studies. The effect of solvent polarity is demonstrated by batch rebinding experiments that show a decrease in binding affinity of substrate for the polymer as solvent polarity increases (Table 5).[12] Therefore, the polymer made with chloroform and analyzed using a chloroform mobile phase should show the highest binding affinity for substrate, which it does. By the same token, the polymer made with acetonitrile as porogen should also show higher binding affinity when chloroform is used instead of acetonitrile in the mobile phase. However, this is not the case; the acetonitrile-based mobile phase allows for higher chromatographic retention than the equivalent chloroform based mobile phase. Thus, re-creation of binding site fidelity must override polarity considerations.

Binding in 9-EA imprinted polymers is strongly influenced by electrostatic interactions. This is seen in the pH profile for the retention of 9-EA on its own imprinted polymer versus a control polymer (Figure 1). Enhanced binding at pH = 4.3 may be accounted for by protonation of the N-2 nitrogen (Scheme 3; the pK_a was found to be 3.6 in 95/5 ACN/H_2O, and 4.15 in water), forming a positively charged 9-EA molecule that binds well to the carboxylate groups on the polymer.

Maximum retention occurs at pH = 4.3, a value close to the pK_a of N-2 on 9-EA, which suggests that the retention is controlled by an ion-exchange process. This is supported by a

(11) Kempe, M.; Mosbach, K. *Anal. Lett.* **1991**, *24*, 1137.

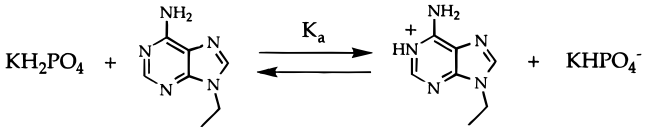
(12) Association constants were obtained as described in ref 5. Templated polymers are not homogeneous materials, and it necessarily follows that neither are binding sites. The K_a's obtained from this treatment follow a mono-site Langmuir adsorption model. However, there are clear strong and weak (nonspecific) binding domains present, suggesting that there is not a continuum of binding sites. Mathematical evaluation of the distribution of binding sites in imprinted polymers will be treated elsewhere.



(a) (b)

Figure 4. Watson−Crick binding mode between (a) thymine and adenine and (b) Watson−Crick-type binding between a carboxylic acid and 2-aminopyridine.

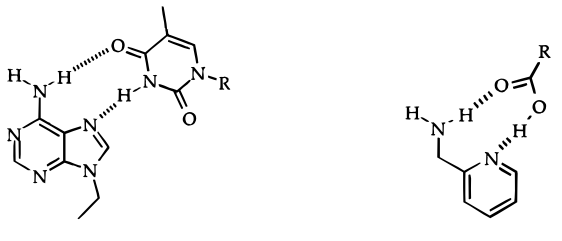
Scheme 3. First Protonation of 9-EA



theoretical ion-exchange model, described in the literature[13] for imprinted polymers, that showed a correlation between the pK_a of solute and maximum chromatographic retention. In looking at the control polymer imprinted with benzoic acid, there is little or no dependence of retention on pH (Figure 1). The reason for this may be that, although the optimum binding conditions of the polymer can be controlled by the pH of the external solvent system, the selectivity is controlled by the *imprinting process*. The selectivity of the imprinted polymers is due to the shape-selective cavity built into the polymer matrix and the preorganization of functional groups complementary to the template molecule. This is further substantiated by Figure 2, which shows pH-dependent selectivity by the 9-EA imprinted polymer for 9-EA only and not for other DNA base derivatives. This particular study also demonstrates a possible link between binding and specificity in imprinted polymers. The specificity of the imprinted polymer appears to be enhanced as binding affinity increases. The specificity is determined by the complementarity of functional groups as well as the shape of the binding cavity. These factors are not affected equally by changing conditions at the rebinding stage. In other words, the binding cavity is only available for 9-EA and not the other DNA bases under these (and possibly any) conditions. This is supported by Figure 2 which shows the pH dependence of 9-EA on the 9-EA imprinted polymer but little or no dependence for the other DNA base substrates. However, even though there seems to be a link between binding affinity and optimum specificity in the imprinted polymers, binding affinity is *not the origin of specificity*.

Finally, quantitative structure−binding relationship studies were employed to determine the major binding interactions of the imprinted polymer with 9-EA. Although there are many trends that can be gleaned from the data in Table 4, the numbers in bold highlight two important observations. First, the polymer imprinted with 2-aminopyridine shows the highest binding for the 9-EA substrate, followed closely by the polymer imprinted with 2-(aminomethyl)pyridine (see bold type in 9-EA column). The polymer imprinted with 2-aminopyridine mimics the Watson−Crick binding mode of adenine with thymine found in the double helix of DNA (see Figure 4); thus the imprinting method can actually elicit major binding motifs, *without prior knowledge of actual receptor design.* In the same fashion, the polymer imprinted with 2-(aminomethyl)pyridine mimics the Hoogsteen binding mode found in crystals grown with adenine and thymine (see Figure 5).[14]

(13) Sellergren, B.; Shea, K. J. *J. Chromatogr.* **1993**, *654*, 17.



(a) (b)

Figure 5. Hoogsteen binding mode between (a) thymine and adenine and (b) Hoogsteen-type binding between a carboxylic acid and 2-(aminomethyl)pyridine.

Second, the polymer imprinted with 2-aminopyridine binds its own template best (bold type in 2ampyr column), while the polymer imprinted with 2-(aminomethyl)pyridine binds its template best (bold type in 2(am)pyr column). This indicates that high specificity is obtained with polymers imprinted with molecular structures that have two nitrogens positioned α or β to each other, one being part of an aromatic ring. The importance of the exocyclic amine on binding was verified by chromatographic studies on *N*-acetyl-9-propyladenine. Both 9-EA and *N*-acetyl-9-propyladenine were run on HPLC using the column packed with 9-EA imprinted polymer. The mobile phase used was 92.5/5/2.5 ACN/AcOH/H_2O, and injections of 10 μL of 1.0 mM of each compound gave k' values of 39.7 versus 1.6 for 9-EA and *N*-acetyl-9-propyladenine, respectively. Essentially all recognition is lost upon acylation of the 2-amino nitrogen.

Summary and Conclusions

Several experiments performed on polymers imprinted with 9-EA have provided insight on the nature of binding and specificity of imprinted polymers. A comparison of lower temperature photoinitiated versus higher temperature thermally initiated polymers showed best results were obtained by the lower temperature photoinitiation method. This agrees with earlier reports that polymerization under conditions of low temperature give optimal rebinding results.[8,9] It is reasonable to suggest that better chromatographic retention may be linked to an increase in high-fidelity binding sites arising from an increase in template−carboxylate complexes both prior to and during polymerization. Furthermore, it was shown that rebinding to the 9-EA imprinted polymer is optimized when solvated in a mobile phase that is most similar to the porogen used for polymerization. The porogen influences the timing of polymer phase separation and the degree of polymer chain solvation in the phase-separated solid. Thus, during polymerization, the microenvironment of the binding sites in the developing polymer adjusts to solvation by the porogen. Maintaining the site fidelity is optimal when the mobile phase contains the same solvent as that used for porogen. Finally, the retention of 9-EA on its imprinted polymer was found to be pH dependent. From these results, it can be concluded that electrostatic forces play an important role in rebinding interactions of 9-EA in imprinted polymers. The retention of the other DNA and RNA base derivatives showed little or no dependence on pH. Thus it was concluded that, although specificity for 9-EA was enhanced by an increase in binding affinity, the origins of specificity must lie in the complementarity built into the polymer, not just a differential binding affinity among the different bases.

(14) (a) Watson, J. D.; Crick, F. H. C. *Nature* (*London)* **1953**, *171*, 737. (b) Hoogsteen, K. *Acta Crystallogr.* **1963**, *16*, 907.

(15) *Synthetic Procedures in Nucleic Acid Chemistry*; Zorbach, W. W.; Tipson, R. S.; Eds.; Interscience: New York, 1968; Vol. 1 and Vol 2.

The binding affinity and specificity of the polymers is dependent not only on polymerization parameters but also on characteristics of the substrate as well. A quantitative structure−binding relationship study was designed to determine which substructural elements of 9-EA contribute to the binding and specificity of the 9-EA imprinted polymer. This was accomplished by imprinting a number of molecules that mimic substructures of 9-EA. It was found that structures that have two nitrogens positioned α or β to each other, one being part of an aromatic ring, appear to be mainly responsible for the binding and specificity of 9-EA to its imprinted polymer. Interestingly, these substructures also provide the major binding interactions found for adenine in nature, as well as for small molecule receptors designed to bind other target molecules. Future work will extend the method of molecular imprinting to the other DNA and RNA bases and assess the binding properties of the polymers obtained.

Experimental Section

General Procedure. Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded on a Bruker WM-250 (250 MHz), General Electric QE-300 (300 MHz), or General Electric GN-500 (500 MHz) spectrometers. The chemical shifts are reported on the δ scale in parts per million downfield from TMS (0.00 ppm) or with deuterated solvent as internal references ($CDCl_3$, 7.26 ppm; CD_3OD, 4.78 ppm; D_2O, 4.80 ppm; DMSO-d_6, 2.49 ppm). Coupling constants (J) are reported in hertz; abbreviations used are as follows: s, singlet; t, triplet; q, quartet; quin, quintet; m, multiplet; br, broad; dd, doublet of doublets. Carbon nuclear magnetic resonance spectra (^{13}C NMR) were obtained using the General Electric QE-300 at 75.4 MHz or the General Electric GN-500 at 125.8 MHz with deuterated solvent as reference ($CDCl_3$, 77.7 ppm; CD_3OD, 49.3 ppm; DMSO-d_6, 43.5 ppm). UV spectra were recorder on a Perkin-Elmer Lambda 4A UV/vis spectrophotometer or a Hewlett Packard 8452A diode array spectrophotometer. HPLC analyses were performed with a Millipore Waters 501 or 6000A solvent delivery system equipped with a Waters 484 tunable absorbance detector and a Hewlett-Packard 3396A integrator; or a Shimadzu LC-10AS dual pump gradient solvent delivery system equipped with an SPD-10AV UV/vis detector and a Hewlett-Packard 3396A integrator.

9-Ethyladenine, 1-propylcytosine, and 1-propylthymine were all synthesized according to procedures found in ref 15. 1-Cyclohexyluracil was obtained from Aldrich Chemicals and used without further purification. Ethylene glycol dimethacrylate (EGDMA, Polysciences) was first washed twice with aqueous 1 M NaOH and once with aqueous saturated NaCl solution to remove inhibitor and further dried with anhydrous $MgSO_4$. The monomer was then filtered away from the solids and distilled under reduced pressure (10 mmHg, 60 °C). Methacrylic acid (MAA, Aldrich) was distilled over $CaCl_2$ (10 mmHg, 80 °C). AIBN (Fluka) was recrystallized from methanol. All solvents were obtained from commercial suppliers and purified prior to use. THF and diethyl ether were dried over sodium−benzophenone ketyl and distilled. Benzene and acetonitrile were dried by refluxing over CaH_2 and then distilled. DMF was dried over 4 Å molecular sieves and distilled under reduced pressure.

Polymer Preparation. A typical example is given here, all polymers were prepared in identical fashion. To a solution 0.4 mmol of 9-EA (65.2 mg, 0.4 mmol) in acetonitrile (9.3 mL) was added methacrylic acid (0.4 mL, 4.8 mmol), ethylene glycol dimethacrylate (6.6 mL, 34.8 mmol), and AIBN (66 mg, 0.4 mmol). The reaction mixture was transferred to a 50 mL thick-walled tube. The tube was degassed by freeze−thaw three times under vacuum, then flame sealed. The polymerization was initiated photochemically by a standard laboratory UV light source (a Hanovia medium-pressure 500 W mercury arc lamp) at 5 °C and allowed to proceed for 24 h. The polymerization tubes were turned 180° after the first 10 min, 20 min, and 10 h of polymerization. After crushing, the polymers were Soxhlet extracted in methanol overnight, then dried under vacuum at 50 °C. The extract was evaporated and redissolved in MeOH (10 mL) and a portion diluted 1000× in $CHCl_3$. The splitting yield (amount of extracted template) was determined spectrophotometrically at 255 nm using a calibration curve for quantification. The substrates were recovered in greater than 95% yield. The particles were ground by mortar and pestle and sized; particles sized 25−38 μm were used to slurry pack stainless steel columns (length, 10.0 cm; i.d., 4.6 mm) to full volume (approximately 0.6 g of polymer) for chromatographic experiments, particles in the 38−150 μm size range were used for batch rebinding studies.

Chromatographic Experiments. The polymers were ground by mortar and pestle or by mechanical mill (Janke & Kunkel IKA WERK grinding mill type A 10 S, 20 000 rpm with water circulating temperature control) and sized using U.S.A. Standard Testing Sieves (A.S.T.M. E.-11 specification). The particles (25−38 μm size range, unless otherwise indicated) were slurry packed in stainless steel chromatographic columns (length, 100 mm; i.d., 4.6 mm) using a Waters 501 or 6000A solvent delivery system. The rest of the polymer (<25 and >38 μm) was saved in 20 mL scintillation vials. Once slurry packed, the columns were then washed on line (in addition to previous Soxhlet extraction) with acetonitrile or 7/3 acetonitrile/water until a stable baseline was obtained. HPLC analyses were performed isocratically at room temperature using a Waters 501 or 6000A solvent delivery system and a Waters 484 UV/vis tunable absorbance detector; flow rate, mobile phase, and substrate conditions are indicated in the text for each experiment. The void volume was determined by injecting a small amount of an inert substance, namely acetone, acetonitrile, or sodium nitrate; of the three, the void volume marker utilized was that with the smallest retention volume. The capacity factor (k') was determined by the relation $k' = (R_v - D_v)/D_v$, where R_v is the retention volume of the substrate and D_v is the void volume.

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