Polymer Complements to Nucleotide Bases. Selective Binding of Adenine Derivatives to Imprinted Polymers

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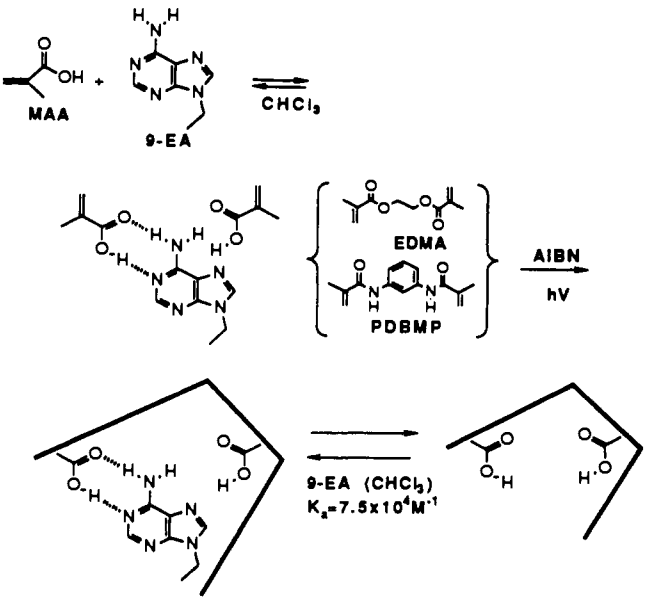
Recent developments in the design of small-molecule synthetic receptors for nucleotide bases has provided insight to DNA–DNA and protein–DNA recognition.[1] Binding sites for nucleotide bases have also been incoporated as part of macromolecular structures that include antibodies,[2] synthetic macromolecules,[3] and monolayers.[4]

We report that network polymers with strong binding sites to adenine can be prepared by copolymerization of cross-linking monomers such as ethylene glycol dimethacrylate (EDMA) and *N,N'*-1,3-phenylenebis(2-methyl-2-propenamide) (PDBMP) with methacrylic acid (MAA) in the presence of a templating molecule, 9-ethyladenine (EA).

The technique employed is template polymerization,[5-10] whereby functional monomers are preorganized about a template or imprinting monomer prior to their copolymerization with cross-linking monomers (Scheme I). Mixing of MAA and EA in $CHCl_3$ followed by photoinitiated copolymerization with EDMA and

Scheme I



PDBMP gives highly cross-linked network polymers.[11,12] EA is removed by Soxhlet extraction affording a polymer containing adenine recognition sites. Control polymers containing the same number of carboxylate groups but incorporating a "generic" template, benzylamine, P(BA), and materials prepared with EA template but no carboxylate groups, P(BL), were also prepared.

Batch rebinding studies in chloroform were performed to quantitatively evaluate the affinity of adenine and related derivatives to the polymers. The adsorption isotherm (C_b vs C_f) of EA to P(EA) and P(BA) reveals enhanced binding to polymers imprinted with EA compared with polymers imprinted with BA. Unfunctionalized polymer P(BL) exhibits no affinity for EA.

Assuming Langmuir type absorption, the binding was treated in a similar manner to data obtained from equilibrium dialysis experiments.[13] From plots of C_b/C_f versus C_b, the Eadie–Scatchard expression yields values for $\bar{K}_a$, an *average* association constant, from the slope and n, the number of binding sites, from the intercept.[14] The plots exhibit bimodal behavior with two distinct regions that can be fitted with straight lines.[16] The most selective sites exhibit an average $\bar{K}_a = 76\,000\ M^{-1}$. To compensate for the nonspecific weaker binding to carboxylate-functionalized polymers, differential binding data (ΔC_b), obtained by subtracting C_b(P(BA)) from C_b(P(EA)), is plotted to yield a linear plot. This treatment gives an average $\bar{K}_a$ of $79\,000\ M^{-1}$, which agrees well with the average value obtained for the most selective sites of the binding isotherm for P(EA). The average values for the association constant should be compared with the K_a of $160\ M^{-1}$ for the association in homogeneous solution between EA and butyric acid.[17]

The derived number of selective binding sites on P(EA) is 20

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(1) (a) Rebek, J., Jr.; Askew, B.; Ballester, P.; Buhr, C.; Costero, A.; Jones, S.; Williams, K. *J. Am. Chem. Soc.* **1987**, *109*, 6866–6867. (b) Rebek, J., Jr.; Askew, B.; Ballester, P.; Buhr, C.; Jones, S.; Nemeth, D.; Williams, K. *J. Am. Chem. Soc.* **1987**, *109*, 5033–5035. (c) Williams, K.; Askew, B.; Ballester, P.; Buhr, C.; Jeong, K. S.; Jones, S.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1989**, *111*, 1090. (d) Zimmerman, S. C.; Wu, W. *J. Am. Chem. Soc.* **1989**, *111*, 8054–8055. (e) Zimmerman, S. C.; Zeng, Z. *J. Org. Chem.* **1990**, *55*, 4789–479. (f) Goswami, S.; Hamilton, A. D.; Van Engen, D. *J. Am. Chem. Soc.* **1989**, *111*, 3425. (g) Adrian, J. C.; Wilcox, C. S. *J. Am. Chem. Soc.* **1989**, *111*, 8055. (h) Mack, D. P.; Iverson, B. L.; Dervan, P. B. *J. Am. Chem. Soc.* **1988**, *110*, 7572. (i) Protein–DNA Interactions; Sauer, R. T., Ed. In *Methods in Enzymology*; Academic Press, Inc.: San Diego, 1991; Vol. 208. (j) Barton, J. K. *Chem. Eng. News* **1988**, *66*, 30. (k) Pyle, A. M.; Rehmann, J. P.; Meshoyrer, R.; Kumar, C. V.; Turro, N. J.; Barton, J. K. *J. Am. Chem. Soc.* **1989**, *111*, 3051. (l) Strobel, S. A.; Dervan, P. B. *J. Am. Chem. Soc.* **1989**, *111*, 7286. (m) Dervan, P. B. *Science* **1986**, *232*, 464. (n) Prakash, G.; Kool, E. T. *J. Chem. Soc., Chem. Commun.* **1991**, 1161.

(2) (a) Lavayre; Jacques; Leng; Mark. *Biochemie* **1977**, *59*, 33–42. (b) Stollar, B. D. *Nucleic Acid Antigens*; Sela, M., Ed.; Academic Press: New York, 1973; Vol. 1.

(3) (a) Schott, H.; Greber, G.; Bucsis, L. *Makromol. Chem.* **1970**, *136*, 303. (b) Schott, H.; Greber, G. *Makromol. Chem.* **1970**, *136*, 307. (c) Schott, H.; Greber, G. *Makromol. Chem.* **1971**, *144*, 333. (d) Akashi, M.; Tokiyoshi, T.; Miyauchi, N.; Mosbach, K. *Nucleic Acids Res. (Symp. Ser.)* **1985**, *16*, 41. (e) Nielsen, R. E.; Egholm, M.; Berg, R. H.; Buchart, O. *Science* **1991**, *254*, 1497.

(4) Sasaki, D. Y.; Kurihara, K.; Kunitake, T. *J. Am. Chem. Soc.* **1991**, *113*, 9685–9686.

(5) For a review, see: Wulff, G. In *Polymeric Reagents and Catalysts*; Ford, W. T., Ed.; ACS Symposium Series 308; American Chemical Society: Washington, DC, 1986.

(6) (a) Wulff, G.; Minarik, M. *J. Liq. Chromatog.* **1990**, *13*, 2987–3000. (b) Wulff, G.; Vesper, W.; Grobe-Einsler, R.; Sarhan, A. *Makromol. Chem.* **1977**, *178*, 2799.

(7) (a) Sellergren, B.; Ekberg, B.; Mosbach, K. *J. Chromatog.* **1985**, *347*, 1. (b) O'Shannessy, D. J.; Ekberg, B.; Andersson, L. I.; Mosbach, K. *J. Chromatogr.* **1989**, *399*, 391. (c) Sellergren, B.; Ekberg, B.; Mosbach, K. *J. Chromatog.* **1990**, *516*, 313. (d) Sellergren, B.; Lepistö, M.; Mosbach, K. *J. Am. Chem. Soc.* **1988**, *110*, 5853. (e) Anderson, L. I.; Mosbach, K. *J. Chromatog.* **1990**, *516*, 313.

(8) Fischer, L.; Müller, R.; Ekberg, B.; Mosbach, K. *J. Am. Chem. Soc.* **1991**, *113*, 9358.

(9) (a) Wulff, G.; Heide, B.; Helfmeier, G. *J. Am. Chem. Soc.* **1986**, *108*, 1089. (b) Shea, K. J.; Dougherty, T. K. *J. Am. Chem. Soc.* **1986**, *108*, 1091.

(10) (a) Shea, K. J.; Sasaki, D. Y. *J. Am. Chem. Soc.* **1989**, *111*, 3442. (b) Wulff, G.; Kirstein, G. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 684–686.

(11) Shea, K. J.; Stoddard, G. J.; Shavelle, D. M.; Wakui, F.; Choate, R. M. *Macromolecules* **1990**, *23*, 4497.

(12) (a) Guyot, A. *Synthesis and Separations Using Functional Polymers*; Sherrington, D. C., Hodge, P., Eds.; John Wiley & Sons: New York, 1989; p 1. (b) Guyot, A.; Bartholin, M. *Prog. Polym. Sci.* **1982**, *8*, 277. (c) Heitz, W. *Adv. Polym. Sci.* **1977**, *23*, 1.

(13) (a) Segel, I. H. *Enzyme Kinetics*; Wiley: New York, 1975. (b) Sellergren, B. *Makromol. Chem.* **1989**, *190*, 2703.

(14) Rebinding selectivities of templated polymers vary as a function of the fraction of sites bound.[15] This finding is interpreted in terms of the formation of a distribution of binding sites. The experimentally observed binding constant (K_{app}) is therefore an average value, $\bar{K}_a$. We do not at present have information regarding the breadth of this distribution.

(15) (a) Shea, K. J.; Sasaki, D. Y. *J. Am. Chem. Soc.* **1991**, *113*, 4109. (b) Wulff, G.; Grobe-Einsler, R.; Sarhan, A. *Makromol. Chem.* **1977**, *178*, 2817.

(16) Tsapis, A.; Rogard, M.; Alfsen, A.; Mihaesco, C. *Eur. J. Biochem.* **1976**, *64*, 369.

(17) Lancelot, G. *J. Am. Chem. Soc.* **1977**, *99*, 7037.

0002-7863/93/1515-3368$04.00/0

μmol/g, or 35% of the theoretical number of sites (57 μmol/g).[18] From studies of the time dependence of binding we conclude that equilibrium is reached for both specific and nonspecific binding within ca. 2 min with a $t_{1/2}$ of 20–30 s.

The binding and selectivity of the polymers was also evaluated from their performance as chromatographic supports. The retention volumes of various substrates using 25–38-μm polymer particles packed in 10-cm HPLC columns are summarized in Table I. In all cases, polymers imprinted with 9-ethyladenine, P(EA), retain adenine bases over other purine or pyrimidine bases. The phenomena is quite general and is observed in mobile phases that range from chloroform to aqueous KH_2PO_4/CH_3CN (3:7, pH = 4.5). That the specificity is due to the EA imprinting molecule is clear from a comparison with polymers that contain the same number of carboxylic acid groups but were imprinted with benzylamine, P(BA) (Table I). These supports show no preference for the adenine derivatives over other purine or pyrimidine bases. The adenine specificity is dramatically revealed by a chromatographic trace of a mixture of adenine, guanine, cytosine, uracil, and thymine which are injected on a P(EA) column (mobile phase $CH_3CN/AcOH/H_2O$ (92.5:5:2.5)). All bases with the exception of EA elute close to the void volume (2.0 min); despite a relatively broad peak, the retention time for EA is 26.8 min!

In conclusion, the data show that molecular imprinting may be used for the preparation of polymers with high affinity for adenine derivatives. The strength of binding ($\Delta G^* \approx 6.5$ kcal/mol)[19] and the selectivity are comparable to those of the best "small-molecule" *designed* receptors.[20] Moreover, the simplicity involved in the preparation of the polymers should make them attractive for use as nucleoside selective separation matrices. Presently we are investigating the extension of this technique for the recognition of both natural and unnatural nucleotide bases and their oligomers.

Table I. Retention Volumes for Purine and Pyrimidine Bases on P(EA) and P(BA) Templated Polymers[a]

substrate[b]	P(EA)[c]	P(BA)[d]	P(EA)[e]	P(BA)[f]
9-ethyladenine (EA)	**48.65**	2.50	**5.30**	1.59
benzylamine	1.72	**3.05**	1.42	**2.45**
1-cyclohexyluracil	2.11	1.84	1.88	1.75
1-propylcytosine	1.75	1.86	1.47	1.50
1-propylthymine	1.88	1.68	1.60	1.56
adenine	**23.94**		**2.64**	1.41
guanine	1.76	1.62	1.18	1.23
uracil	1.99	1.72	1.25	1.30
cytosine	2.82	2.00	1.30	1.30
thymine	2.06	1.76	1.31	1.35
adenosine	**14.73**	2.96	**1.34**	1.25
guanosine	4.89	3.47	1.08	1.17
uridine	1.98	1.73	1.13	1.22
cytidine	2.16	2.57	1.09	1.19
thymidine	1.92	1.70	1.20	1.27
2′-deoxyadenine	**16.79**	2.80	**1.51**	1.28
2′-deoxyguanine	3.93	2.66	1.10	1.18
2′-deoxyuridine	1.95	1.71	1.16]	1.22
2′-deoxycytidine	2.11	2.02	1.12	1.19
2′,5′-dideoxycytidine	1.88	1.85	1.23	1.28

[a] The polymers (25–38-μm particle size) were slurry packed in stainless steel chromatographic columns (length: 100 mm, i.d. 4.6 mm). The chromatographic investigations were performed using two mobile phases: (1) $CH_3CN/AcOH/H_2O$, 92.5:5:2.5; (2) 0.1 M KH_2PO_4 + K_2HPO_4 (aqueous, pH 4.5)/CH_3CN, 3:7, 1 mL/min. [b] Ten microliters of a 1.0 mM solution of each substrate was used. [c] Polymer was prepared using 9-ethyladenine (0.4 mM) as template. Mobile phase 1. [d] Polymer was prepared using benzylamine (0.4 mM) as template. Mobile phase 1. [e] Same polymer as in *c*. Mobile phase 2. [f] Same polymer as in *e*. Mobile phase 2.

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Supplementary Material Available: Background information and experimental details (11 pages). Ordering information is given on any current masthead page.

(18) The theoretical number of binding sites is determined from the number of millimoles of template (EA) per gram of polymer. The recovery of template following polymerization is typically ~90%. The high yield of recovered template implies that most of the binding sites are accessible to bulk solvent. This result does not distinguish between surface and interior sites, but fluorescence labeling studies suggest that the sites are distributed throughout the material. (Shea, K. J.; Sasaki, D. Y.; Stoddard, G. J. *Macromolecules* **1989**, *22*, 1722.)

(19) The ΔG value is from $\Delta G = -RT \ln K_a$, where K_a is the association constant derived from the batch rebinding experiments, in chloroform at 25 °C.

(20) K_a's in chloroform for designed adenine receptors: 11 000 M^{-1} (Rebek);[1c] 25 000 M^{-1} (Zimmerman);[1d] 45 000 M^{-1} (Wilcox);[1g] and 120 000 M^{-1} (Zimmerman).[1c]