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Model Systems for Flavoenzyme Activity. Molecular Recognition of Flavin at the Polymer−Liquid Interface

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Introduction

In biological processes molecular recognition and function are directly correlated. To gain a better understanding of these binding events and their concomitant activities, solution-phase model systems have been prepared.[1] These models allow the direct and detailed examination of the recognition event. An inherent problem with these constructs is their simpler architecture and lower complexity relative to the native biological systems.

To extend the viability of model studies, polymers have been used to probe recognition and catalytic events. The two most commonly used techniques for the preparation of these polymers are the processes of imprinting[2] and self-assembly.[3] These processes are limited by the fact that control of the structure of the recognition site is often difficult, making interpretation of results difficult.

In our continued efforts to probe the activity of flavoenzymes, we have functionalized a cross-linked polymeric bead to provide a preformed recognition site. This has the advantage that control of the active site geometry is complete. Also, the synthetic scheme is flexible enough that it may be modified easily to incorporate additional required functionality. We report here the formation of a triazine-functionalized rigid polymer as a steppingstone between solution-phase and solid-phase macromolecular recognition.[4]

Results and Discussion

The triazine-functionalized polymer **1** was prepared using a cross-linked, chloromethylated styrene−divinylbenzene copolymer (Scheme 1). Reaction of Merrifield's peptide resin **2** in *N,N*-dimethylformamide with an excess of sodium cyanide afforded the cyano-functionalized polymer **3** in ~90% conversion.[5] This was then reacted with dicyandiamide in a solution of potassium hydroxide dissolved in 1-pentanol to give the desired triazine-functionalized polymer **1** (~100% conversion, ~90% conversion overall).[6] To provide a control system for the flavin uptake studies, Merrifield's peptide resin **2** was reacted with potassium hydroxide dissolved in 1-pentanol under identical conditions to above, providing the alcohol-functionalized polymer **4**.

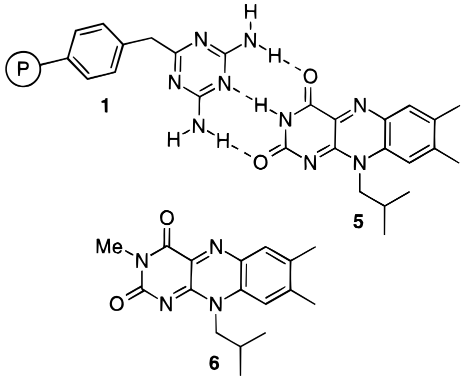
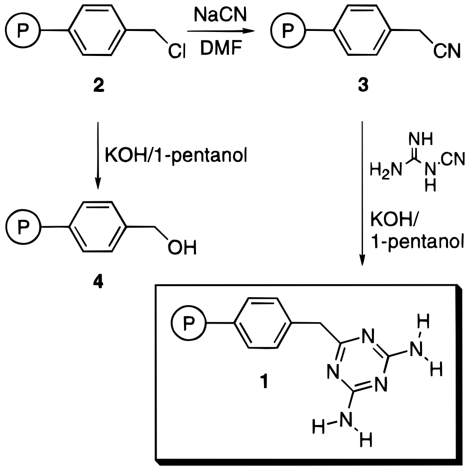


Figure 1. Hydrogen bonding between triazine-functionalized polymer **1** and flavin **5**; non hydrogen bonding control **6**.

Scheme 1



Flavin uptake experiments were then performed in which the polymers were shaken with a chloroform solution of either normal flavin **5** or N-methylated flavin **6** (Figure 1). The triazine-functionalized polymer **1** exhibited specific recognition for normal flavin **5**, removing it from solution, as shown by the drastic reduction in the observed absorption for the solution in the UV-vis spectrum (Figure 2).[7] In contrast, the triazine-functionalized polymer **1** showed no binding toward the sterically blocked N-methylated flavin **6**. As additional evidence for specific interaction, the alcohol-functionalized control polymer **4** exhibited no binding toward normal flavin **5**.

From the above experiment, the apparent association constant (K_a) was calculated to be 25 M^{-1}.[8] To study the transition between solution-phase and polymer−liquid interface binding, the binding of a soluble monomeric equivalent **7** was obtained via NMR titration. This was

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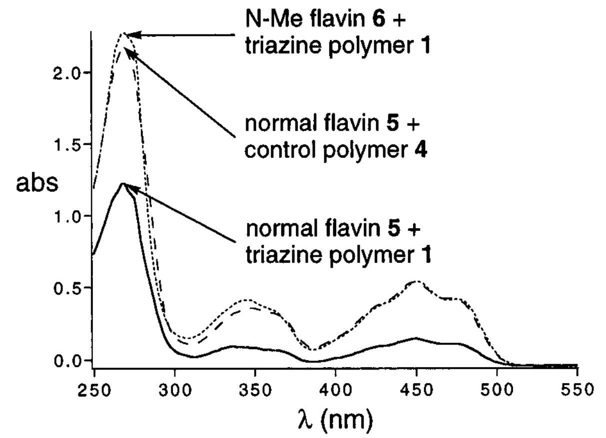
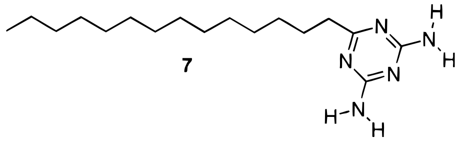


Figure 2. UV-vis spectra showing removal of flavin **5** from a $CHCl_3$ solution, and control studies. Initial concentration of flavin **5**/**6** 0.011 M in 0.5 mL of $CHCl_3$, 25 mg of resin used. After shaking 24 h at rt, 30 μL aliquots were removed and then diluted with 8.5 mL of $CHCl_3$.

found to have an association constant (K_a) = 555 ± 8 M^{-1}, higher than that obtained for **1** consistent with previous studies.[6]



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In conclusion, we have developed a triazine-functionalized polymer **1** which has shown specific recognition for flavin **5**. Binding study calculations have shown the triazine-functionalized polymer **1** to be an efficient and specific binder for solution-phase normal flavin **5**. Applications of this synthetic scheme for the general preparation of a variety of functionalized polymers utilizing specifically attached redox-active and synthetically useful groups are underway and will be reported in due course.[9]

Experimental Section

General Methods. Chemicals were purchased from Aldrich, Fisher, and Janssen Chimica, and used as received. Thin layer chromatography was carried out on glass precoated plates with silica gel 60. All reactions were performed under an argon atmosphere. Microanalyses were performed by the University of Massachusetts (Amherst) Microanalysis Service. Infrared spectra were measured on a Perkin Elmer Model 1600 FT-IR Spectrophotometer. UV-vis spectra were measured on a Hewlett Packard Model 8452A diode array spectrophotometer. ^{1}H NMR spectra were recorded on a Bruker/IBM AC200 (200 MHz) spectrometer using $CDCl_3$ as solvent.

Cyano-Functionalized Polymer 3. Sodium cyanide (490 mg, 10 mmol) was added to a suspension of Merrifield's resin (1 g, chloromethylated styrene−divinylbenzene copolymer, 2% cross-linked, 2.78 mequiv of Cl/g) in *N*,*N*-dimethylformamide (10 mL). After 10 days, the suspension was filtered and washed with *N*,*N*-dimethylformamide (10 mL), water (10 mL), methanol (10 mL), and finally dichloromethane (10 mL) to give a pale yellow solid (911 mg). Elemental analysis for Merrifield's resin **2**: C, 83.06; H, 7.09; Cl, 9.32. Elemental analysis for the cyano-functionalized polymer **3**: C, 87.58; H, 7.25; N, 3.49; Cl, 0.13; FT-IR (KBr) 3013, 2919, 2249, 1602, 756, 691. This corresponds to approximately 90% conversion of available sites.

Triazine-Functionalized Polymer 1. Dicyandiamide (127 mg, 1.5 mmol) was added to a suspension of **3** (0.5 g, ~2.50 mequiv of CN/g) in a solution of potassium hydroxide (14 mg, 0.25 mmol) dissolved in 1-pentanol (5 mL). The suspension was heated under reflux for 24 h, filtered, and washed with water (10 mL) and then dichloromethane (10 mL) to give a tan solid (649 mg). Elemental analysis for the triazine-functionalized polymer **1**: C, 76.99; H, 6.89; N, 11.65; FT-IR (KBr) 3131, 2919, 2155, 1649, 1549, 1431, 691. This corresponds to approximately 100% substitution of available sites.

Pentadecyldiaminotriazine 7. To a solution of potassium hydroxide (41 mg, 0.74 mmol) in 1-pentanol (5 mL) were added dicyandiamide (373 mg, 4.43 mmol) and the starting nitrile (1 mL, 3.69 mmol). The reaction mixture was then stirred for 24 h at 140 °C. After cooling, the resulting solid was suspended in boiling water, filtered, dried, and recrystallized using boiling methanol/hexanes. **7** (34%): mp 120−121 °C; FT-IR (KBr) 3323, 2913, 2851, 1841, 1544, 1446 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.05 (4H, br s), 2.48 (2H, t, $J = 7.8$ Hz), 1.70 (2H, m), 1.25 (22H, m), 0.88 (3H, t, $J = 6.5$ Hz). Anal. Calcd for $C_{17}H_{33}N_5$: C, 66.39; H, 10.82; N, 22.79. Found: C, 65.96; H, 11.04; N, 21.94.[10]

UV-vis Binding Studies. Five samples were prepared: (i) 0.5 mL of normal flavin **5** solution (0.011 M, $CHCl_3$); (ii) 0.5 mL of *N*-Me flavin **6** solution (0.011 M, $CHCl_3$) (iii) 0.5 mL of normal flavin **5** solution (0.011 M, $CHCl_3$) + 24.95 mg of the triazine-functionalized polymer **1**; (iv) 0.5 mL of normal flavin **5** solution (0.011 M, $CHCl_3$) + 25.03 mg of the alcohol-functionalized control polymer **4**; (v) 0.5 mL of *N*-Me flavin **6** solution (0.011 M, $CHCl_3$) + 25.01 mg of the triazine-functionalized polymer **1**. These were sealed and shaken for 24 h at room temperature after which a 30 μL aliquot was removed, diluted by the addition of 8.5 mL of $CHCl_3$, and a UV-vis spectrum obtained.

^{1}H NMR Titration. NMR complexation studies were performed in $CDCl_3$, a noncompetitive solvent, to allow the observation of specific hydrogen bonds. Association constants were determined through nonlinear least-squares curve fitting with the resulting curve providing a good fit to the 1:1 binding isotherm.[11]

Triazine 7-Flavin 5 Binding. To a host solution of normal flavin **5** (5×10^{-3} M), a guest solution of triazine **7** (5×10^{-2} M) was added. This resulted in a smooth downfield shift in the resonance of H(3) of flavin **5**. Final concentrations: $[\text{guest}]_{\text{total}} = 0.01875$ M; $[\text{host}]_{\text{total}} = 0.003125$ M. This provided an association constant (K_a) = 555 ± 8 M^{-1}.

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Supporting Information Available: IR spectra for polymer resins **1** and **3**, UV-vis spectra for the binding studies, and NMR, IR, and titration graph (association) for the diaminotriazine **7** (9 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(8) The uptake of normal flavin **5** was based on the relative absorbencies (at 450 nm) of the runs involving the triazine-functionalized polymer **1** and the alcohol-functionalized control polymer **4**. This value was then used to calculate the association constant (K_a), assuming all triazine binding sites to be homogeneous and equally available to the solution.

(9) For examples of synthetically useful functionalized polymers see: Inoue, K.; Nishikawa, Y.; Tanigaki, T. *J. Am. Chem. Soc.* **1991**, *113*, 7609. Inoue, K.; Nakahara, H.; Kawata, K.; Tanigaki, T. *Makromol. Chem., Rapid Commun.* **1992**, *13*, 485.

(10) The elemental analysis for the receptor **7** always showed low nitrogen content, a common feature in nitrogen-containing heterocycles.

(11) To provide K_a, the data were fitted to the equation

$$\delta_{\text{obs}} = \delta_{\text{H}} + \frac{(\delta_{\text{HG}} - \delta_{\text{H}})\left(\left([H_t] + [G_t] + \frac{1}{K_a}\right) - \left(\left([H_t] + [G_t] + \frac{1}{K_a}\right)^2 - 4[H_t][G_t]\right)^{1/2}\right)}{2[H_t]}$$

where the experimentally determined parameters are as follows: $[G_t]$ and $[H_t]$, the total guest and host concentrations, respectively, δ_{obs} the observed shift, and δ_{H}, the shift of the host in the absence of guest. Parameters determined through fitting are K_a, the host−guest association constant, and δ_{HG}, the chemical shift of the host−guest complex.