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Phosphatase Catalysis Developed *via* Combinatorial Organic Chemistry

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We have recently pointed out the possibility of developing new organic catalysts by searching through a multitude of randomly generated systems.[1] The method would, in principle, be similar to that of Nature, which has, over the course of 4.5 billion years, synthesized and examined innumerable compounds while progressively selecting only the most serviceable ones. This random search for improvement has led to amazingly effective catalytic proteins called enzymes. Chemists, in their attempt to emulate enzymes, have thus far preferred to do so *via* specific compounds that incorporate desired structural features. The problem with this "rational" strategy is that the multistep synthesis of complex molecules is a time-consuming business, and through no personal fault, chemists can survey only a limited number of educated guesses in the years allowed them. This paper departs from traditional bioorganic chemistry by utilizing a combinatorial approach to catalyst design.

The basic idea was to attach covalently onto polyallylamine, *via* a one-step amide bond formation illustrated in Figure 1, various combinations of eight functionalized carboxylic acids.[2] In addition, one of three metals (Mg^{2+}, Zn^{2+}, or Fe^{3+}) was complexed to the polymer. Thus, hundreds of potential polymeric catalysts were rapidly synthesized, each differing in the nature and number of its functional groups. One particular polymer, for example, possessed 15% Oct, 15% Imi, 15% Phe, and 5% Fe^{+3} where "%" refers to the percentage of amine groups on the polymer chain that had been derivatized. Each new polymer was then routinely screened for catalytic activity toward a phosphodiester. As will be shown, remarkable catalytic effects have thereby been uncovered. And the potential exists for even greater accelerations because only a small portion of the vast number of possible combinations has as yet been tested.

Specific directions for preparing the polymeric catalysts follow: Poly(allylamine hydrochloride),[3] with a molecular weight of 50 000−65 000, was converted into its free base by ion exchange over Amberlite IRA-400 resin (hydroxide form, 10-fold excess) followed by freeze-drying. A polyallylamine stock solution (100 μL, 0.5 M monomer equivalents in water) was added to 700 μL of dioxane−water (2:1 v/v) and mixed with 0−200 μL of stock solution of each compound in Figure 1 (0.05 M in dioxane or water) and 100 μL of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (0.5 M in water). The resulting solution was incubated at 50−60 °C for 20−40 h. (Preliminary kinetic data on polymer modification by Imi, obtained ^1H NMR, indicated that the amide coupling reaction was *ca.* 90% complete after 1.5 h. Analysis of the derivatization reactions by gel filtration (Sephadex G25) showed no unreacted carboxylic acid). The solvent was then removed under reduced pressure and the waxy residue redissolved in 10 mL of 0.05 M phosphate buffer (pH = 7.00) to give a solution containing 5 × 10^{-3} M monomeric equivalents or *ca.* 5 × 10^{-6} M polymer. Depending upon the proportions used, 5% to 45% of the polymer's amine groups had been derivatized. Since the arrangement of the functionalities was not controlled, a huge family of diverse polymers is formed. Sequences within the polymer chains reflect, in part, the localized nonbonded interactions among the functionalities and are "random" only in the sense that they are not predesigned.

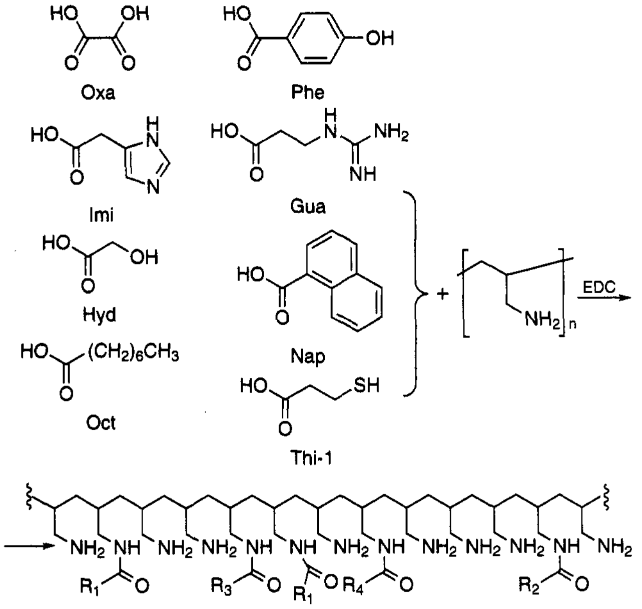

Figure 1. Eight functionalized carboxylic acids attached randomly to polyallylamine by means of amide bonds. The R groups on the polymer represent the various functionalities.

Catalytic activity toward bis(*p*-nitrophenyl) phosphate was determined by mixing the following items: (a) 1.0 mL of a buffered functionalized polymer; (b) an aliquot of metal chloride solution; (c) 20 μL of 0.05 M stock solution of phosphodiester dissolved in water. Initial hydrolysis rates were then obtained by periodic monitoring of the *p*-nitrophenolate absorption at 400 nm using a Varian 200 spectrophotometer. Most absorption *vs.* time plots showed an induction period of unknown origin;[4] rates were calculated from the linear slopes subsequent to this induction period. Repeat runs, carried out by two different investigators starting from the very beginning of the procedure, were generally reproducible within a factor of two.

It was immediately clear that certain combinations, among the hundreds investigated, were far more effective catalysts than others.[5] For example, Figure 2 shows kinetic runs with two different polymer catalysts. The fast run contained a polymer with 7.5% Oct, 15% Imi, 10% Phe, and 10% Fe^{3+}; the slow run contained a polymer with 8% Oct, 2.5% Imi, 15% Phe, and 10% Fe^{3+}.

Several mixtures composed of unmodified polyallylamine, compounds in Figure 1, and metals[6] failed to

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[1] Menger, F. M.; Fei, Z. X. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 346.

[2] For other approaches to multifunctional polymers see: Chan, W.; Chen, Y.; Peng, Z.; Yu, L. *J. Am. Chem. Soc.* **1993**, *115*, 11735. Kiefer, H. C.; Congdon, W. I.; Scarpa, I. S.; Klotz, I. M. *Proc. Natl. Acad. Sci., U.S.A.* **1972**, *69*, 2155. Sundell, M. J.; Näsman, J. H. *Chemtech* **1993**, 16. Suh, J.; Cho, Y.; Lee, K. J. *J. Am. Chem. Soc.* **1991**, *113*, 4198.

[3] Polymer and other compounds were purchased from Aldrich.

[4] Slow, substrate-induced conformational changes in the polymer may be occurring. Note that each metal atom and thiol group is a potential site for cross-linking that would help rigidify the polymer.

[5] The search resembles the selection of catalytic antibodies from a combinatorial library. See: Janda, K. D.; Lo, C. L.; Li, T.; Barbas, C. F., III; Wirsching, P.; Lerner, R. A. *Proc. Natl. Acad. Sci, U.S.A.* **1994**, *91*, 2532.

[6] Typical inactive controls consisted of polyallylamine plus (a) 7.5% Oct, 15% Imi, 10% Phe, and 5% Fe^{3+} or (b) 8% Imi, 32% Gua, and 10% Zn^{2+}. The presence of carbodiimide does not affect the reaction rate.

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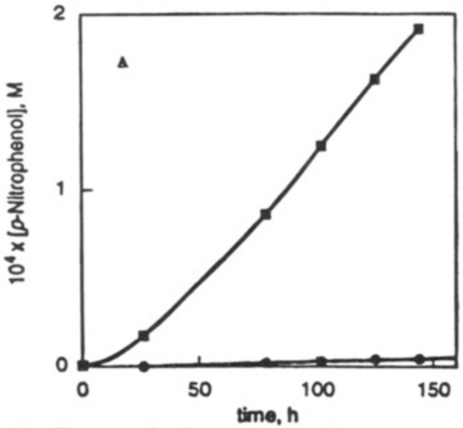

Figure 2. Fast and slow runs with two combinatorial catalysts. Fast: 7.5% Oct, 15% Imi, 10% Phe, and 10% Fe^{3+}. Slow: 8% Oct, 2.5% Imi, 15% Phe, and 10% Fe^{3+}.

Table 1. Rate Enhancements in the Hydrolysis of Bis(*p*-nitrophenyl) Phosphate at pH = 7.00 and Ambient Temperature Induced by Combinatorial Catalysts

% Oct	% Imi	% Phe	% Thi	% Fe^{3+}	k_{cat}/k_{uncat}
10	0	0	0	10	<500
0	10	0	0	10	<500
0	0	10	0	10	<500
5	5	0	5	10	<500
0	5	5	5	10	<500
5	0	5	5	10	<500
5	5	5	5	10	<500
7.5	15	10	0	5	31 400
2.5	15	10	0	5	25 100
2.5	15	10	0	7.5	20 100
2.5	15	10	0	15	22 700
15	15	15	0	5	19 300

[a] A value less than 500 indicates that there was no catalysis within experimental error.

promote the hydrolysis. Attachment of the functionalities to the polymer seems, therefore, necessary for catalysis.

Table 1 further illustrates the distinction between good and poor "combinatorial" catalysts. Thus, rate enhancements (k_{cat}/k_{uncat}) are listed as a function of polymer composition. The uncatalyzed rate constant, k_{uncat}, was assumed to be 4.7×10^{-8} h^{-1}, a number taken from the literature.[7] Values of k_{cat}/k_{uncat} in Table 1 less than 500 indicate that no reaction was observed in 2 weeks (the limit of 500 reflecting the uncertainty in the spectrophotometer readings over that time period). Two features of Table 1 are particularly noteworthy: (a) large rate accelerations of up to 3×10^4 can be achieved with the best of our "combinatorial" catalysts. This was elevated to 1.5×10^5 when the polymer concentration was increased to 5×10^{-5} M. Other combinations, not in Table 1, can also be highly effective catalysts (e.g., 8% Imi, 32% Gua, and 10% Zn^{2+} for which $k_{cat}/k_{uncat} = 6 \times 10^4$). (b) Derivatization of polyallylamine with only a single functional group never produced a catalytic polymer. As with enzymes, catalysis is predicated upon *multiple* interactions.

Figure 3 shows "reactivity histograms" in which rates (varying from (1 to 120) $\times 10^{-4}$ absorbance units/hour) have been plotted as a function of the percentages of Imi, Phe, Oct, and Fe^{3+}. The rates were selected after a preliminary screening had eliminated inactive combinations. Such histograms lead to the following conclusions: (a) The higher the Imi, the faster the rates within the range examined. (b) No benefit accrues be elevating Fe^{3+} above 5%. (c) Optimum activity occurs with a polymer having 15% Imi, 10% Phe, 7.5% Phe, and 5% Fe^{3+}. Shifting to lower or higher Phe and Oct percentages serves only to diminish the rates. As additional data accumulate in the future, it may be necessary to adopt a simplex optimization or similar computer routine to locate efficiently the very best catalysts.

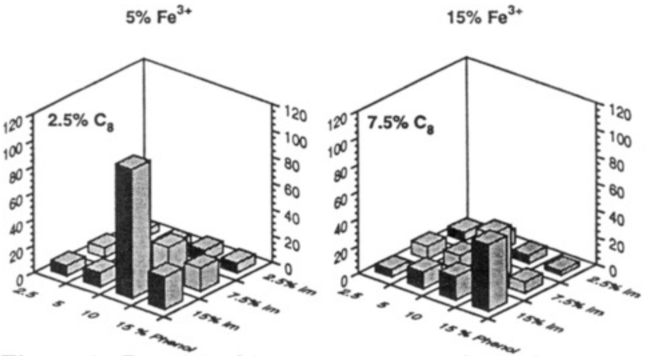

Figure 3. Reactivity histograms; see text for explanation.

While screening various combinations of four functionalities (10% each) from the list in Figure 1, we found polymers that hydrolyze a phosphomonoester, *p*-nitrophenyl phosphate. This substrate was of interest because it had been used previously in a study of antibody-catalyzed hydrolyses,[8] and thus, it presented an opportunity to compare biologically and combinatorially-based catalysts. We found that our best polymer (10% Oxa, 10% Imi, 10% Sal, 10% Thi-1, and 10% Zn^{2+}) catalyzes the phosphomonoester under the standard conditions with an observed rate constant of 2.1×10^{-3} h^{-1}. This is about five times larger than the k_{obs} reported for the antibody system.[8,9]

The structure of most catalytic antibodies is unknown, and the same is true for our "combinatorial" polymers. It seems unlikely that we will ever be able to separate and characterize the active components among the ensemble of countless polymeric variations. Fortunately, our goals focus upon function rather than structure. Developing catalysts for organic reactions in aqueous systems is a profitable enterprise apart from the availability of structural information. In the future, we anticipate the use of combinatorial chemistry to prepare catalysts from polymers that possess a removable transition state analog. The hope, of course, is that the functional groups subsequently bonded to such a polymer will properly interact with a substrate once the corresponding transition-state analog is detached. The present work also opens up the intriguing possibility of chiral syntheses *via* chiral polymers (*e.g.*, catalysts based upon poly-L-lysine or upon polyallylamine bearing chiral functionalities).

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(7) Vance, D. H.; Czarnik, A. W. *J. Am. Chem. Soc.* **1993**, *115*, 12165. Note that the substrate, although activated by nitro groups, is nonetheless an extremely unreactive compound at pH = 7.0, 25 °C ($t_{1/2}$ = 2 millenia).

(8) Scanlan, T. S.; Prudent, J. R.; Schultz, P. G. *J. Am. Chem. Soc.* **1991**, *113*, 9397.

(9) See the following (and references therein) for combinatorial chemistry as applied to equilibrium processes: Borchardt, A.; Still, W. C. *J. Am. Chem. Soc.* **1994**, *116*, 373. Jung, G.; Beck-Sickinger, A. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 367. Zuckerman, R. N.; Martin, E. J.; Spellmeyer, D. C.; Stauber, G. B.; Shoemaker, K. R.; Kerr, J. M.; Figliozzi, M.; Goff, D. A.; Siani, M. A.; Simon, R. J.; Banville, S. C.; Brown, E. G.; Wang, L.; Richter, L. S.; Moos, W. H. *J. Med. Chem.* **1994**, *37*, 2678.