Combinatorial Catalysis of an Elimination Reaction

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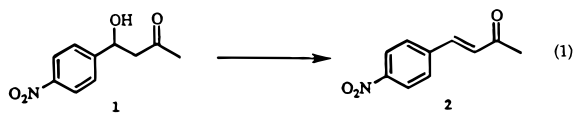
We have recently been engaged in the screening of randomly generated polymers for their catalytic or reactive potential.[1,2] Among thousands of such screenings (assisted by automation), a few polymers were found that are remarkably effective, and these were further "fine-tuned" to make them even better catalysts. The new approach was prompted, in part, by a feeling that the time has come to supplement, but by no means supplant, the traditional deterministic strategy in which complex enzyme models are synthesized often at great expense.

Previous work described a polymer that catalyzes a phosphate hydrolysis by a factor of 30 000 (exceeding that of a catalytic antibody for the same reaction).[1] In the present report, we develop polymers that catalyze a totally different reaction (an elimination), thereby demonstrating that the hydrolase activity was not simply a rare happenstance. As will be seen, the elimination catalysts display a slow induction period as if the polymers "learn" in situ how to promote the reaction.

An analogy between the combinatorial approach and that of Nature, although somewhat tongue-in-cheek, serves to illustrate our philosophical underpinnings: For billions of years Nature has also been evolving her catalysts, called enzymes, in a random manner. Thus, DNA molecules (and, hence, enzymes) were mutated, and once in great while, a mutation would create an improved enzyme that endowed the recipient with a survival advantage over those without it. By this means, new and improved enzymes were retained (just as we retain those randomly produced polymers that possess desirable properties). While Nature has utilized "survival of the fittest", our method invokes the "survival of the fastest" (with ourselves being the agent of selection). And while Nature amplifies successful enzymes via a process called life, we amplify our successful catalysts by producing them in large amounts once their formulas are discovered by the screening process. Among the more regretful failures of this analogy is the vast difference in time frame available to Nature and to the mortal chemist.

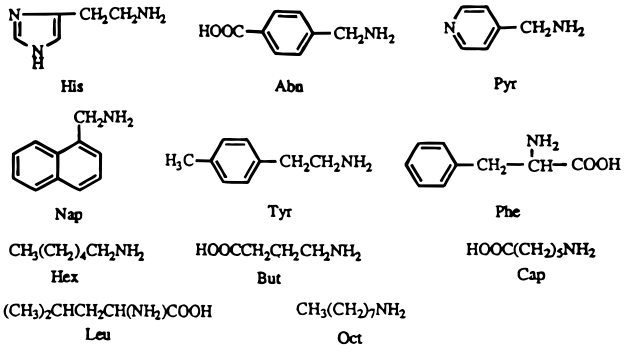
A recent objective was to develop a combinatorial polymer capable of catalyzing the dehydration of a β-hydroxy ketone (eq 1). This was an attractive reaction because it allowed



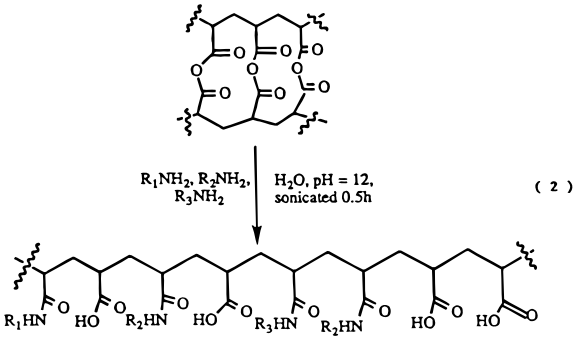
(1)

easy monitoring of multiple samples by an increase in UV absorbance. Moreover, related eliminations are found biologically (e.g., citrate to aconitate or malate to fumarate). As with the corresponding enzymes,[3] polymers that possess both acidic groups (to assist departure of the hydroxyl) and basic groups (to assist departure of the proton) would likely catalyze the dehydration—especially if the acid−base groups could adopt the proper spatial orientation. It was the purpose of this work described herein to develop such polymers.

Scheme 1. Library of Amines Used to Form Combinatorial Polymers



Polymers were formed by reacting poly(acrylic anhydride) simultaneously with three or four amines,[4] taken from a library of the 11 amines in Scheme 1, according to eq 2. The



poly(acrylic anhydride), prepared from acrylic anhydride and AIBN,[4] had an average molecular weight of about 4500 (MALDI/TOF mass spectrometry). Fourteen combinations of amines (e.g., His/Abn/Hex; His/Pyr/Phe/Cap) were examined in 96 different ratios each for a total of 1344 polymer variations. Twenty percent or less of the carboxyl groups in any given poly(acrylic acid) sample were derivatized (e.g., 5% His/5% But/5% Nap). Those polymers that were water-insoluble in pH = 7.0 buffer were discarded. A few experimental details below will clarify the procedure.

Liquid deliveries were assisted by an ICN QuadFlex computer-controlled automatic pipetting system. Thus, poly(acrylic anhydride) (100 μL, 0.05 M monomer units in DMSO) was pipetted into 96 test tubes (12 × 75 mm), whereupon the DMSO was removed at 40 °C by speed evaporation (Jouan RC 10-10). The following was then added to each test tube: (a) 500 μL of pH = 12 buffer and (b) three or four amine solutions (25−150 μL of 5 mM each) as dictated by 96 computerized combinations. After bath sonication for 30 min (sufficient, according to NMR, to complete the aminolysis), the water was removed by speed evaporation, leaving a waxy residue that was dissolved in 1 mL of pH = 7.0 phosphate buffer (0.05 M) to make a solution 5 mM in monomer units. The following were then pipetted into the 96 400 μL wells of a microtiter plate: (a) 50 μL of the derivatized polymer solution; (b) 125 μL of 2×10^{-4} M

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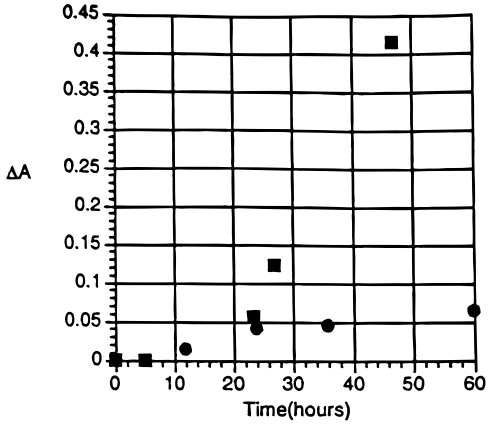

Figure 1. Change in absorbance at 340 nm for eq 1 (pH = 7.0, 23 °C) catalyzed by polymer with 5% His/5% Oct/5% Phe/5% Cap (squares) as compared to a polymer with 5% His/7.5% But/2.5% Nap (circles). These are examples of "fast" and "slow" polymers, respectively. Less than 1% of the screened polymers are "fast".

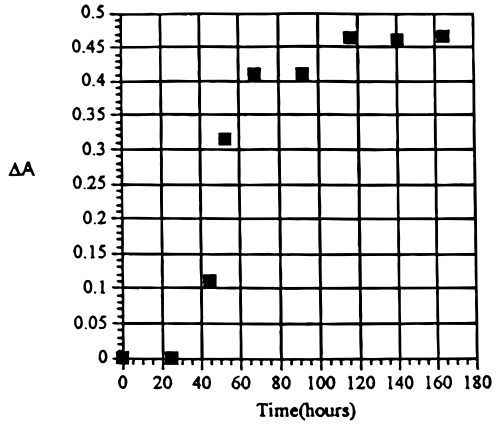

Figure 2. Change in absorbance at 340 nm for eq 1 (pH = 7.0, 23 °C) catalyzed by combinatorial polymer with 5% His/5% But/5% Nap. Note the striking induction period in which there is no detectable reaction for the first 24 h.

substrate **1** in water−acetonitrile (9.5/0.5 v/v); (c) 75 μL of a pH = 7.0 phosphate buffer. Thus, the catalysis was screened with solutions whose final concentrations were 1.2×10^{-5} M polymer (assuming a MW = 6100 for the derivatized material) and 1.0×10^{-4} M substrate. Monitoring of the 96 wells was carried out periodically at 340 nm for the appearance of the β-aryl-α,β-unsaturated ketone using an ICN MCC/340 scanner. Reactions run to completion gave infinity absorbances consistent with a calculated infinity and with an absorbance measured on a plate with purified product **2**. The presence of **2** in an actual run was also verified by GC−MS.

Reactions range from "slow" (ΔA = 96 h absorbance change = 0.07−0.15) to "fast" ($\Delta A \geq 0.4$ corresponding to $>2t_{1/2}$'s). Figure 1 gives an example of each in which 5% His/7.5% But/2.5% Nap is slow, whereas 5% His/5% Oct/5% Phe/5% Cap is fast. Less than 1% of the 1344 runs can be categorized as "fast". The catalytic ability of a polymer depends not only upon the types of substituents but upon the ratio of substituents within one particular substituent set. For example, $\Delta A = 0.35$ for 5% His/2.5% Cap/7.5% Pyr/5% Leu, while $\Delta A = 0.14$ for 5% His/2.5% Cap/2.5% Pyr/10% Leu. Deletion of one substituent, but with no other change, can have a substantial effect upon the rate. For example, removing Pyr from 5% His/10% Pyr/5% Phe/5% Hex diminishes ΔA from 0.36 to 0.18. Fast rates were duplicated independently by two people who prepared the polymers identically. Preliminary attempts to purify fast polymers (Sephadex G-75 and Superdex 30 Prep) failed to give fractions with additional activity. Since control experiments with suitable mixtures of free amines were found to give no rate enhancement, catalysis is predicated upon attachment of the functionalities onto the polymer framework (a conclusion that also mitigates any concern that catalysis arises from traces of unreacted amine).

Antibody-catalyzed dehydration of our substrate at pH = 7.0 (37 °C) affords a catalysis of 1200 above the background reaction.[5] Our best dehydrations are comparable, i.e., a catalysis of 920 at pH = 7.0 (23 °C) on the basis of the nearly linear rate ensuing after 24 h. Thus, combinatorial polymers (or antibodies for that matter) do not measure up to an enzyme. Yet it should be borne in mind that we have hardly begun to sample the available polymer combinations including those containing phenols, thiols, metals, etc. And combinatorial polymers have an advantage over antibodies in that they are readily prepared with no input of biotechnology.

One of the most interesting aspects of our investigations relates to the presence of an induction period evident in Figures 1 and 2. It is as if the polymers "learn" to catalyze after, roughly, a 24 h time period of inactivity. To better define the system, a variety of experiments were carried out with 5% His/5% Oct/5% Phe/5% Cap, and the results are now briefly summarized: (a) Rates are twice as fast at pH = 6.0 as at pH = 7.0, while rates at pH = 5.0 are very slow. (b) A remarkable temperature sensitivity was observed: The fast rates at 23 °C are killed at both 0 and 40 °C. Normal kinetic curves are restored when a substrate/polymer solution at 40 °C is cooled back to 23 °C. (c) Sonication of substrate/polymer solutions also destroys activity. (d) Cross-linking the polymer via addition of 1% 1,7-diaminoheptane (along with the four substituent amines) greatly retards the rate. (e) Induction periods persisted even when the polymers were allowed to remain in solution for 4 days prior to addition of the substrate.

The above observations are most easily explained in terms of a substrate-induced transformation into a catalytically active conformation. At 0 °C, the polymer transformation is slow, while heating to 40 °C or sonication disrupts the active conformation. Cross-linking rigidifies the polymer and impedes its conformational rearrangement necessary to achieve catalysis. It appears, therefore, that we are observing a type of nonbiological "induced fit".[6]

Combinatorial polymeric catalysis is a new field, and naturally there remain a host of unanswered questions (e.g., catalyst specificity, substrate binding, laboratory-to-laboratory reproducibility, scale-up behavior, etc.). Yet our preliminary results affirm our conviction that, at a time when water is becoming more and more desirable as an industrial solvent, "fortuitous" catalysis with water-soluble polymers systems is an attractive area for further investigation.[7]

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(6) Koshland, D. E., Jr., In *The Enzymes*; Academic Press: New York, 1959, Vol. 1, Chapter 7.

(7) For an entirely different approach to the subject, see: Brocchini, S.; James, K.; Tangpasuthadol, V.; Kohn, J. *J. Am. Chem. Soc.* **1997**, *119*, 4553−4554.