A combinatorially developed reducing agent

Fredric M. Menger,* Craig A. West and Julia Ding

Department of Chemistry, Emory University, Atlanta, Georgia 30322, USA

An automated search of thousands of combinatorially-functionalized polymers bearing dihydropyridine groups defines a few active examples that can reduce a ketone in water.

Recently we introduced a new approach to reaction dynamics in which a huge library of randomly-functionalized polymers was screened for catalytic activity toward phosphate ester hydrolyses.[1] Rate enhancements as large as 3×10^4 were thereby uncovered (exceeding those reported for a corresponding catalytic antibody). It was argued that the approach provides an alternative to the classical method of synthesizing enzyme models in which multiple functional groups are in the proper juxtaposition to effect catalysis. Such synthetic work can be extremely time consuming, and there is no guarantee, even when the desired compound is in hand, that it will perform as hoped.

The details are simple. Two commercially available polymers, poly(allylamine) (PAA, MW = 50–60 kD) and poly(ethylenimine) (PEI, MW = 25 kD), were randomly derivatized, *via* amide bonds, by a mixture of three or four carboxylic acids selected from Fig. 1. EDCI, a water-soluble carbodiimide, was used as the coupling agent. From 20–60% of the polymer amino groups were functionalized, the remainder being left untouched. For example, a polymer might be constructed with 10% naphthyl groups, 15% imidazole groups, 5% octyl groups and 5% butanol groups (using Nap, Imi, Oct and BuOH in Fig. 1, respectively). All kinetic runs were carried out at pH 7.2 where the free amino groups, being largely protonated, solubilize the polymer in water. In addition, any unprotonated amino groups could act as ligands for metals (*e.g.* Cu^{+2}) that were added to the system.

Aqueous solutions of polymer, EDCI, and the various carboxylic acids were made available to an automatic pipeting device. A computer program directed the device to dispense the reactants (including 96 different concentration ratios of carboxylic acids) into a set of test tubes for a 24 h incubation at 55–60 °C. No unreacted carboxylic acid was detected by TLC after this time. The resulting polymers were 'randomly' substituted in the sense that no control was exercised over the functionalizations. In actual fact, however, the positioning of the four substituents, and the subsequently added metal ion, may not be 'random' in the strict meaning of the word. For example, when one amino group is derivatized with an octanoyl group, the adjacent amino groups may become more prone to receiving other octanoyl groups owing to hydrophobic attraction. To preclude confusion about the meaning of 'random', compounds will henceforth be called 'combinatorial polymers'. Each combinatorial polymer is, of course, a complex system[2] composed of a mixture of polymeric variations that is impossible to separate. Even if we could isolate a pure component, it would be extremely difficult to sequence it because (unlike proteins) the polymer chain cannot be subdivided. Fortunately, our goals are directed primarily toward function. A polymer system that serves a useful function is of great interest even in the absence of detailed structural information.

It was clear that, in order for combinatorial polymers to become something other than a curiosity, they must be able to do more than catalyse the ever-popular ester hydrolysis. For this reason we have been examining eliminations, condensations and redox systems of synthetic utility. This article focuses on the conversion of ketones to alcohols *via* combinatorial polymers having a reductive capacity. Reducing capabilities were imparted to the polymers by incorporating (simultaneously with the other diverse functionalities) a 5 or 10% content of the dihydropyridine DHP. Dihydropyridines are well known from NADH models[3–8] to convert activated ketones into alcohols. The hope, therefore, was to construct active sites that promote enzyme-like reductions within their confines.

Combinatorial polymers were screened for their ability to homogeneously reduce benzoylformic acid ($PhCOCO_2^-$) to

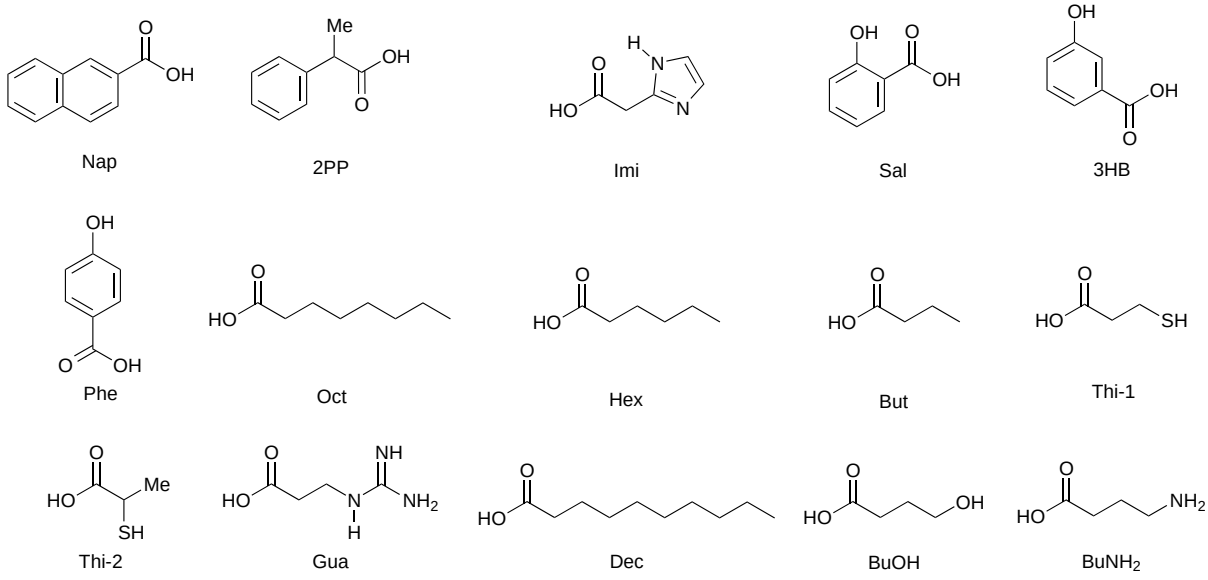

Fig 1 Carboxylic acids used to derivatize the polyamines

Table 1 Five active polymers taken from a library of over 8000, most of which were inactive[a]

Polymer	Side-chains[b]	Activity (%)
PEI	2.5% Imi, 5% Hex, 7.5% Nap, 2.5% Thi-1, 5% Zn^{+2}	30
PEI	15% Hex, 2.5% Gua, 2.5% BuOH, 5% Mg^{+2}	33
PEI	2.5% Imi, 15% Nap, 2.5% Hex, 5% Mg^{+2}	34
PAA	10% Imi, 2.5% Hex, 10% Nap, 10% Thi-2, 10% Zn^{+2}	42
PAA	2.5% Gua, 7.5% Hex, 7.5% Nap, 2.5% Thi-2, 10% Cu^{+2}	28

[a] Each activity is an average from three polymers created *de nova*. The uncertainty in the values is 6 in absolute %. [b] Symbols are defined in Fig. 1. Percentages represent the composition of solutions from which the polymers were made.

mandelic acid [$PhCH(OH)CO_2^-$]. A total of 96 runs at a time were carried out side-by-side within the 400 μl wells of a microtiter plate. Each well contained the following components dissolved in a pH 7.2 TRIS buffer at 24 °C: 50 μl of a functionalized polymer (corresponding to 2.4 mm of the dihydropyridine); 100 μl of 1.25 mm Cu^{+2}, Mg^{+2} or Zn^{+2}; and 150 μl of 0.021 m benzoylformic acid. Reactions were monitored periodically, with an ICN MCC/340 scanner, at 340 nm for the disappearance of the dihydropyridine chromophore. Data, recorded as percentage yield, refer to the percent of oxidized dihydropyridine after 5 days when all the reactions had ceased. The percentage yield varied from 0–50% depending upon the polymer functionalization. A yield of 23%, for example, indicates that 23% of the 25–100 dihydropyridine sites per polymer chain were active. A larger scale reaction, worked up by column chromatography on silica (MeOH–$CHCl_3$–H_2O, 30 : 60 : 4) gave a product identified as mandelic acid by ^1H NMR spectroscopy. GLC analysis of mandelic acid (as the methyl ester) gave a percentage yield that corresponded to that calculated from the dihydropyridine chromophore.

Among a total of 8198 polymers tested, 92% were considered inactive (< 10% yield). Activities of 10–20, 20–40 and > 40%, were found in 7, 1 and 0.3% of the polymer population, respectively. Two of the most active polymers (> 25%) were: (*a*) PEI; 5% DHP; 2.5% Imi; 15% Nap; 2.5% Thi-1; 5% Zn^{+2}. (*b*) PAA; 10% DHP; 10% Imi; 2.5% Hex; 10% Nap; 10% Thi-2; 10% Zn^{+2}. By contrast, the following two were inactive: (*a*) PEI; 5% DHP; 5% Imi; 5% BuOH; 5% Nap; 5% Sal; 5% Zn^{+2} or 5% Mg^{+2}; (*b*) PAA; 10% DHP; 5% Dec; 5% Gua; 7.5% 2PP; 5% Sal; 10% Zn^{+2}. Table 1 lists five additional reactive polymers; note that recipes for PAA and PEI are, in general, not the same.

The multitude of inactive systems can be regarded as 'controls' in that, apart from polymer composition, they duplicate the reaction conditions. Moreover, mixtures of individual components that simulated the high-activity polymers were all found to be inert. Thus, a polymer was functionalized only with the DHP. No reducing capabilities were observed with this polymer when (after purification from EDCI by GPC) it was mixed with an appropriate combination of free acids in Fig. 1 plus a metal ion. Clearly, a polymeric ensemble of functionalities is necessary for activity. Lack of disappearance of the DHP chromophore in the control also demonstrated the stability of DHP to hydrolysis. A single run with poly(ethylenimine) of low molecular weight (7 kD) was also carried out. This polymer, endowed with a 'favourable' substituent ratio with respect to the 25 kD polymer, gave no reaction. Although a useful combinatorial polymer of low molecular weight might well exist, one would have to discover its recipe in a separate and distinct set of screenings.

To assess the reproducibility of the combinatorial polymers, two people each prepared the ten best polymers discovered by the other. Yields were reproduced within a factor of two and usually much better. The same was true when duplicating a full set of 96. Although not all the variables affecting the polymer functionalizations have been systematically investigated, it is known that the reaction conditions are critical. For example, the presence of a dioxane cosolvent in the functionalization mixtures has a profound effect upon the activity of the resulting polymer. This is hardly surprising; polymer sequences would be expected to have a high solvent sensitivity. Sequences could also be sensitive to the order in which functionalities are placed onto the polymer, but this point has not yet been tested.

Thousands of activity data are best summarized by the following generalizatons. (*a*) A metal ion is necessary for activity. (*b*) All high-activity polymers possess at least one hydrophobic side-chain (Nap Oct, *etc.*). (*c*) Imi or Gua is found in most but not all reactive polymers. (*d*) Percentages within a single set of functionalities can be important. For example, PEI with 2.5% Imi, 15% Nap and 2.5% Thi-1 (plus 10% Mg^{2+}) had an activity of 40%. The same system but with 10% Imi, 2.5% Nap, and 7.5% Thi-1 had an activity of only 1.3%. (*e*) No active poly(allylamines) were observed when they contained oxalic or malonic acid even in concert with other seemingly 'beneficial' combinations. (*f*) A thiol or hydroxy group often assists the reduction. Coincidentally, no doubt, alcohol dehydrogenase also requires a dihydropyridine (NADH), a metal ion (Zn^{2+} coordinated to two thiolates), a histidine and a serine.[9]

The half-life of our better reductions is about 2 h. This is fast relative to many other 'NADH models'[3] which, in order to obtain any reaction at all, require aprotic solvents rather than water, as used in our experiments.

The potential for combinatorial reactivity seems unlimited. In the specific case at hand, one can contemplate: (*a*) achieving true catalysis *via* an external reducing agent that regenerates the dihydropyridine units of high-activity polymers; (*b*) discriminating between two ketones (*e.g.* reducing $PhCOCO_2^-$ but not $PhCOCO_2Me$) or between two similar ketones in the same molecule [*e.g.* reducing $MeCO(CH_2)_2CO(CH_2)_3Me$ to $MeCO(CH_2)_2CHOH(CH_2)_3Me$]; (*c*) stereoselectively reducing ketones with chiral polymers (*e.g.* polymers based on poly-l-lysine or on PAA with chiral substituents).

Low cost is an important virtue of combinatorial polymers. Once an active polymer is discovered, it can be prepared in large amounts and in a single step without specialized biochemical methods.

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