J|A|C|S
COMMUNICATIONS

Published on Web 04/17/2002

A Potent Polymer/Pyridoxamine Enzyme Mimic

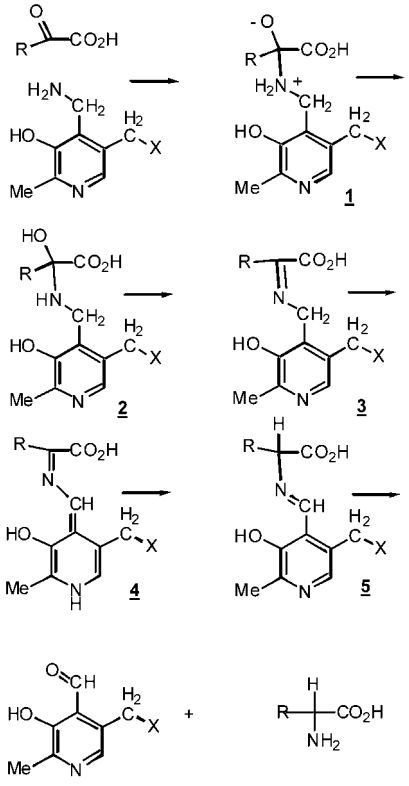
Lei Liu and Ronald Breslow*

Department of Chemistry, Columbia University, New York, New York 10027

Received February 11, 2002

Natural enzymes are macromolecules, while many enzyme models and mimics are small molecules. For example, we and others have done extensive studies on reactions related to those catalyzed by enzymes that use pyridoxal phosphate and pyridoxamine phosphate, the central coenzymes for amino acid metabolism.[1] As a typical example, they can catalyze the transamination between an amino acid and a keto acid, which is the most important form of nitrogen transfer in diverse biological systems. As part of this process, the pyridoxamine phosphate reacts with a ketoacid to form pyridoxal phosphate and the amino acid (Scheme 1).

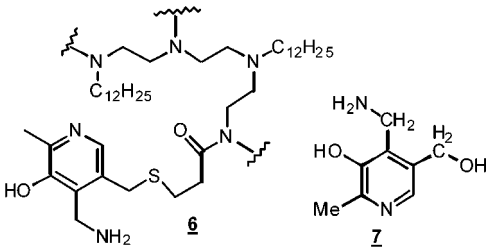
Scheme 1



By attaching appropriate basic side chains to pyridoxamine, we were able to enhance the rate of this transamination process by about 100 times.[2] When chiral side chains were rigidly mounted onto pyridoxamine, remarkable (ee % = 92) optical induction in transamination was also achieved.[3] Additionally, we attached pyridoxamine to cyclodextrins and observed good substrate selectivity in the transamination of a ketoacid, for example for the selective conversion of phenylpyruvic acid to form phenylalanine, competing with conversion of pyruvic acid to alanine.[4] Similar magnitudes of rate enhancement or optical induction were also seen in other artificial transamination systems based on catalytic antibodies,[5] peptides,[6] and proteins.[7]

The macromolecular character of enzymes may play a special role in catalysis. For example, reactions performed in the enzyme interior may be shielded from water, and the p*K*'s of acid and base groups can be significantly changed in the protein environment. Some synthetic polymers have shown interesting properties as enzyme mimics; in particular, polyethyleneimine and some of its modifications have proven to be effective hydrolysis catalysts.[8,9] Thus, we examined transamination by a reagent with pyridoxamine coupled to modified polyethyleneimine. The compound converted pyruvic acid to the corresponding amino acid alanine with remarkable acceleration.

We used commercial polyethylenimine (PEI).[8,10] It is highly branched, has a molecular weight of about 60 000, and contains about 1400 monomer residues. About 25% of the amino groups of PEI are primary, about 50% secondary, and the remaining tertiary. Chemical modification of PEI is possible.[9] We alkylated the polymer with lauryl iodide, acylated the product with the *N*-hydroxysuccinimide ester of 3,3′-dithio-dipropionic acid, and methylated the remaining primary and secondary amino groups using formaldehyde and sodium cyanoborohydride. The disulfide bonds of the polymer were reduced by sodium borohydride to liberate thiol groups, which were then S-alkylated with 5-(bromomethyl)-pyridoxamine to yield the final water-soluble polymeric pyridoxamine reagent **6**.



Proton NMR indicated that 12% of the nitrogen atoms of the polymer carried lauryl groups, and 29% carried propionylpyridoxamine groups. The elemental analysis (C/N ratios) indicated 11 and 22%, respectively, in reasonable agreement with the NMR results.

The polymer was treated with an excess of pyruvic acid in various buffer solutions, without added metal ions and with an excess of added EDTA,[11] and the UV spectrum for pyridoxamine (λ_{max} 324 nm) rapidly decreased and was replaced by the characteristic spectrum of pyridoxal (λ_{max} 385 nm). The pseudo-first-order rate constant with the polymer was $(1.0 \pm 0.1) \times 10^{-2}$ min^{-1} at 30 °C and pH 5.0, while with simple free pyridoxamine **7** under the same conditions it was $(1.5 \pm 0.4) \times 10^{-6}$ min^{-1}. Thus, the attached polymer increases the rate of pyridoxamine transamination with pyruvic acid by a factor of 6700.

* To whom correspondence should be addressed. E-mail: rb33@columbia.edu.

10.1021/ja025895p CCC: $22.00

An HPLC analysis of the reaction products under the same conditions (as a function of time) detected alanine whose rate of formation had a rate constant of $(1.5 \pm 0.3) \times 10^{-2}$ min^{-1} with the polymer, and $(1.8 \pm 0.6) \times 10^{-6}$ min^{-1} with pyridoxamine, a rate constant ratio of 8300. Furthermore, the final yield of alanine corresponded to 80% of the original pyridoxamine in the polymer, indicating that essentially all the pyridoxamine units in the polymer are of comparable reactivity, with no special "hot spots." We believe that the unreactive 20% of the pyridoxamine units in the polymer are trapped by the pyridoxals that are formed. At pH 7.0 the rate enhancement by the polymer is still 2300 times, while at pH 8.0, the optimum for pyridoxamine itself, it is 1900 times (full data in Supporting Information).

Transamination by simple pyridoxamine shows strong metal ion catalysis—adding 1 equiv of $CuCl_2$ per pyridoxamine unit to the pH 5.0 solution (without added EDTA) increases the free pyridoxamine rate constant to 1.1×10^{-2} min^{-1}, a 6000-fold rate increase; however, the rate for the polymer increases by only 30-fold. The transaminase enzymes do not use metal ions.

The rate enhancement of the polymer over that of simple pyridoxamine was a steep function of the length of the alkyl chains added, in polymers with roughly the same percentage of alkylation and of pyridoxamine attachment. At pH 7.0 and 30 °C, the acceleration over the rate with pyridoxamine, from kinetics by UV, was 160 for C-1 chains, 180 for C-3, 500 for C-6, 1000 for C-9, 2300 for C-12, and 2500 for the C-15 and C-18 normal alkyl chains.

This chain effect seems unlikely to involve hydrophobic binding of a substrate as hydrophilic as pyruvic acid. Instead we believe that the hydrophobic chains modify the p*K*'s of the amino groups in the polymer, as seen previously,[12] and also create a cavity in which the transamination can take place in a less than fully aqueous environment.[13]

The transamination sequence involves several steps in which general acid and general base catalyses are involved. This includes addition of the amino group of a pyridoxamine unit to the carbonyl group of the ketoacid, in which a proton must be removed from zwitterionic intermediate **1** and a proton must be added to the oxyanion of that intermediate to form the neutral carbinolamine **2**. Then a general acid must protonate the hydroxyl group of **2** and later a proton must be removed from the nitrogen to form the imine **3**. At some point the pyridine nitrogen is protonated or at least hydrogen-bonded by a general acid, and then the proton of the methylene group must be removed by a general base to produce intermediate **4**. A general acid must protonate the α carbon of the ketoacid unit, and the resulting Schiff base **5** must then be hydrolyzed with the assistance of general acids and bases, reversing the sequence by which imine **3** was originally formed.

We believe that this sequence of general acid and general base catalyses can be particularly well accomplished by the polyethyleneimine species. The proximity of the many nitrogens means that even at low pH there will be some unprotonated nitrogens able to act as bases, and indeed bases strong enough that they are just too weak to be protonated. These are the strongest bases that can exist in the system at a given pH. Similarly, there are protonated amine cations almost strong enough as acids to lose their protons, the strongest general acids that can exist at equilibrium. Combined with the somewhat nonpolar environment that the long alkyl chains provide, the general acids and bases with p*K*'s close to the operating pH are features that are common to many enzymes.

With phenylglycine as the sacrificial reagent, we see catalytic formation of alanine with our polymeric catalyst, but with only 2.5 turnovers. It remains to be seen whether such polymers carrying coenzyme groups can perform other enzymelike processes, including stereoselective reactions. However, our early results reported here do indicate that such artificial polymer-based enzyme/coenzyme systems have interesting potential.

Acknowledgment. We thank Mary Rozenman and Wenjun Zhou for some experimental studies, and the NIH and NSF for financial support of this work

Supporting Information Available: Details of the polymer catalyst syntheses and characterization, and the kinetic studies and data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Martell, A. E. *Acc. Chem. Res.* **1989**, *22*, 115. (b) Breslow, R. *Acc. Chem. Res.* **1995**, *28*, 146.
(2) Zimmerman, S. C.; Czarnik, A. W.; Breslow, R. *J. Am. Chem. Soc.* **1983**, *105*, 1694.
(3) Zimmerman, S. C.; Breslow, R. *J. Am. Chem. Soc.* **1984**, *106*, 1490.
(4) (a) Breslow, R.; Canary, J. W.; Varney, M.; Waddell, S. T.; Yang, D. *J. Am. Chem. Soc.* **1990**, *112*, 5212. (b) Fasella, E.; Dong, S. D.; Breslow, R. *Bioorg. Med. Chem.* **1999**, *7*, 709.
(5) Cochran, A. G.; Pham, T.; Sugasawara, R.; Schultz, P. G. *J. Am. Chem. Soc.* **1991**, *113*, 6670.
(6) (a) Imperiali, B.; Roy, R. S. *J. Am. Chem. Soc.* **1994**, *116*, 12083. (b) Roy, R. S.; Imperiali, B. *Tetrahedron Lett.* **1996**, *37*, 2129.
(7) (a) Huang, H.; Brown, M. L.; Davies, R. R.; Young, E. C.; Distefano, M. D. *J. Am. Chem. Soc.* **1996**, *118*, 10702. (b) Kuang, H.; Distefano, M. D. *J. Am. Chem. Soc.* **1998**, *120*, 1072.
(8) Suh, J. *Synlett* **2001**, 1343 and references therein.
(9) Johnson, T. W.; Klotz, I. M. *Macromolecules* **1974**, *7*, 149.
(10) From Sigma Chemical Co., product P-3143. Experimental details are in Supporting Information.
(11) 01 or 0.001 M EDTA was added to the reaction solution to block the effect of any possible metal ion contaminants. The EDTA, at 10 and 100 times the concentration of the pyridoxamine units, completely suppressed the accelerations otherwise seen when copper chloride was added.
(12) Suh, J.; Park, H. J.; Hwang, B. K. *Bioorg. Chem.* **1994**, *22*, 318.
(13) Hollfelder, F.; Kirby, A. J.; Tawfik, D. S. *J. Org. Chem.* **2001**, *66*, 5866.

JA025895P