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High Rates and Substrate Selectivities in Water by Polyvinylimidazoles as Transaminase Enzyme Mimics with Hydrophobically Bound Pyridoxamine Derivatives as Coenzyme Mimics

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We have studied enzyme mimics for many years,[1] including those that performed transamination of ketoacids to amino acids. Our systems normally focused on the first step, the reaction of a pyridoxamine with the ketoacid to form a pyridoxal and the amino acid, although we have also shown how this can be part of a complete catalytic cycle by using decarboxylative transamination of 2,2-disubstituted glycines to convert the pyridoxal back to the pyridoxamine form.[2] In our recent work, we used polyaziridines to mimic the transaminase enzyme itself.[3] Klotz,[4] Suh,[5] and Kirby[6] had used those polymers to mimic hydrolytic enzymes.

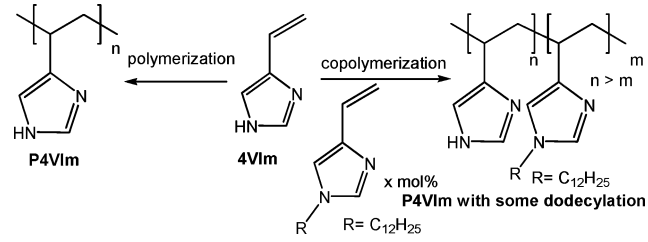
We used commercially available polyaziridines, as did Klotz, Suh, and Kirby, and compared the results for different sizes of the polymers, different N-alkylations of the nitrogens using alkyl groups from methyl to dodecyl, and different amounts of such alkylations. We used either covalently attached pyridoxamine derivatives or, even better, reversibly bound pyridoxamines with hydrophobic side chains as coenzyme mimics.[7] We saw that the most effective polymers had a limited amount of dodecylation; with enough we achieved a hydrophobic core in the polymer, while with too much the materials were insoluble in water.

We used water as the solvent to take advantage of hydrophobic binding of substrates and cofactors into the polymer as well as the higher rates achieved when the reaction is performed in an interior nonaqueous core of the polymer, as with natural enzymes. We achieved an increase by a factor of as much as 350 000 in the rate of transamination of hydrophobic phenylpyruvic acid to form phenylalanine relative to the rate of the same process and same pyridoxamine in water without the polymer. An even higher acceleration was observed with indolepyruvic acid to form tryptophan.

We also used polyaziridines with thiazolium and imidazolium coenzyme mimics carrying hydrophobic side chains to catalyze the benzoin condensation of two benzaldehyde molecules in water.[8] In the transaminase mimics, the polymer furnished general acid and general base catalysis of the reaction using the partially protonated main-chain amino groups. However, the benzoin condensation was not subject to general acid or base catalysis but simply to electrostatic catalysis by positive charges on the amino groups of the polyaziridines. In this report, we will describe the contrasting results and even better catalyses of the transamination reactions using a polymer series different from the polyaziridines.

Overberger[9] studied polyvinylimidazoles (PVIm's) formed by radical polymerization of either 4-vinylimidazole or 1-vinylimidazole. We were attracted to these compounds because they also incorporate base (imidazole) and acid (imidazolium) catalytic species and have side chains large enough to have some hydrophobic binding character of their own. We synthesized the polymer of 4-vinylimidazole (P4VIm, Scheme 1) according to the Overberger procedure (see the Supporting Information) and characterized the methanol soluble fraction by MALDI-TOF mass spectrometry. Using 1 mol % azobis(isobutyronitrile) (AIBN) for the polymers listed in Table 1 (except for 5 mol % AIBN for the polymers in entries 9 and 10 of the table, as noted), we obtained a mixture of 20- to 40-mer polymers along with some methanol-insoluble larger polymers (for the mass spectrum, see the Supporting Information) and used the entire polymer mixture to catalyze the reaction of pyridoxamine **A** (Scheme 2) with pyruvic acid to form alanine and pyridoxal in water. The rate of the reaction at pH 7.5 and 20 °C was followed by the appearance of the UV spectrum of the product pyridoxal (see Table 1 for the data and the reaction conditions), as we have described previously.[2] We saw that the reaction was 100-fold faster with a 37.5 mg/L P4VIm mixture (entry 2) than without the polymer (entry 1).

Scheme 1. Synthesis of P4VIm and Partially Dodecylated P4VIm



We then turned to the use of hydrophobic derivatives of pyridoxamine and copolymers of 4-vinylimidazole with 1-dodecylated-4-vinylimidazole (obtained from 4-vinylimidazole and dodecyl iodide; the NMR spectrum indicated that it was 83% 4-vinyl-1-dodecylimidazole and 17% 5-vinylimidazole). We prepared the pyridoxamine derivatives **B**−**E** (Scheme 2) as described previously[2] and examined them with simple P4VIm (entries 3−6 in Table 1); we also used cofactor **E** with copolymers of 4-vinylimidazole and its dodecylated monomer (entries 11−15). We also examined the use of the reversible addition−fragmentation chain transfer (RAFT)-modified radical polymerization of 4-vinylimidazole, which normally affords a more monodisperse polymer, and saw by MALDI-TOF that this changed somewhat the distribution of polymer but did not lead to significantly less polydispersity. The modified (entry 10) and unmodified polymers

Scheme 2. Structures of P1VIm (Table 1, Entry 7) and Pyridoxamine Derivatives **A**−**E**

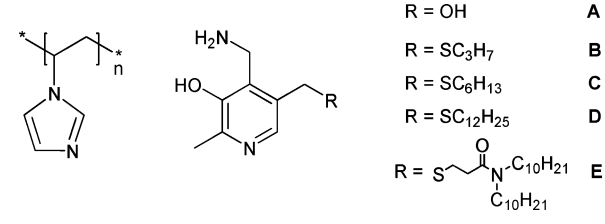


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Table 1. Kinetic Study of the Transamination Reaction under Various Conditions[a]

entry	pyridoxamine	polymer	$k_{transamination}$ (min^{-1})	$k_{relative}$
1	**A**	no polymer	$(1.4 \pm 0.3) \times 10^{-6}$	1.00[b]
2	**A**	P4VIm	$(1.4 \pm 0.2) \times 10^{-4}$	100
3	**B**	P4VIm	$(7.0 \pm 0.2) \times 10^{-4}$	500
4	**C**	P4VIm	$(1.3 \pm 0.1) \times 10^{-2}$	9300
5	**D**	P4VIm	$(3.3 \pm 0.2) \times 10^{-2}$	23600
6	**E**	P4VIm 0% dodecylated	$(1.7 \pm 0.1) \times 10^{-1}$	121400
7	**E**	P1VIm 0% dodecylated	$(31.2 \pm 0.1) \times 10^{-3}$	22300
8	**E**	PEI 0% dodecylated	$(1.2 \pm 0.1) \times 10^{-2}$	8600[b]
9	**E**	P4 VIm 0% dodecylated[c]	$(1.4 \pm 0.1) \times 10^{-1}$	100000
10	**E**	P4 VIm 0% dodecylated[d]	$(1.5 \pm 0.1) \times 10^{-1}$	107200
11	**E**	P4VIm 2.3% dodecylated	$(1.2 \pm 0.1) \times 10^{-1}$	85700
12	**E**	P4VIm 4.5% dodecylated	$(1.3 \pm 0.1) \times 10^{-1}$	92900
13	**E**	P4VIm 10.1% dodecylated	$(1.1 \pm 0.1) \times 10^{-1}$	78600
14	**E**	P4VIm 14.8% dodecylated	$(6.9 \pm 0.1) \times 10^{-2}$	49300
15	**E**	P4VIm 30% dodecylated	–	–

[a] Reaction conditions: 1.5×10^{-4} mol/L pyridoxamine, 37.5 mg/L polymer, 5.0×10^{-3} mol/L pyruvic acid, 2.0×10^{-3} mol/L EDTA, 20 °C, pH 7.5. [b] Data from ref 2. [c] Polymerization was done with 5 mol % AIBN instead of the normal 1 mol %. [d] Polymerization was done with 5 mol % AIBN instead of the normal 1 mol % and 8 mol % chain-transfer agent (CTA) reagent.

(entry 9) had similar rates with cofactor **E**. We found that simple P4VIm with cofactor **E** (entry 6) was 14-fold more effective as a catalyst than our polyaziridine without dodecyl groups under the same conditions (entry 8). Poly(1-vinylimidazole) (P1VIm) was also prepared (see the Supporting Information), and the transamination rate was determined to be enhanced 22 300-fold (entry 7), meaning that P1VIm is only 2.6-fold more effective than the polyaziridine (entry 8).

Our copolymers with dodecyl groups are more effective with phenylpyruvic acid than with simple pyruvic acid. This reflects the better binding of a hydrophobic substrate into the more hydrophobic polymer. The reaction of phenylpyruvic acid was too rapid to follow by the UV method under our conditions, so we determined the selectivity of a competition reaction using 30:30:1 phenylpyruvic acid/pyruvic acid/pyridoxamine derivative mixture to form a phenylalanine/alanine ratio that was determined by HPLC, as previously.[2] We observed a ratio of 3:1 for the reaction using simple P4VIm and (19 ± 1):1 for the reaction with the copolymer having 4.5% dodecylated monomer. The latter is slightly higher than the 14:1 ratio we had seen previously with the dodecylated polyaziridine.[2] Since amination by this imidazole copolymer (entry 12) was 92 900-fold more rapid than the reaction without polymer, and since we had shown previously that simple pyridoxamines aminate pyruvic acid and phenylpyruvic acid at the same rate,[10] this means that the acceleration of the amination of phenylpyruvic acid by the polymer with cofactor **E** is $(1.77 \pm 0.1) \times 10^6$ (i.e., almost 2-million-fold). The amination of indolepyruvic acid, forming tryptophan, was accelerated $(3.9 \pm 0.1) \times 10^6$-fold (i.e., 4-million-fold) under the same conditions.

The use of these polyvinylimidazoles in the benzoin condensation with thiazolium and imidazolium coenzyme mimics is under investigation. We are also pressing forward with an attempt to produce polymers with smaller polydispersity, including the use of oxazoline ionic polymerization, as we employed previously with phenylalanine derivatives.[11] Since those ionic polymers are not only almost monodisperse but also isotactic, retaining the chirality of the parent amino acids, analogues derived from histidine could well be polyimidazoles with useful chiral selectivities for products and starting materials.

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Supporting Information Available: Synthesis procedures and pertinent spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) *Artificial Enzymes*; Breslow, R., Ed.; Wiley-VCH: Weinheim, Germany, 2005.
(2) Liu, L.; Zhou, W. J.; Chruma, J.; Breslow, R. *J. Am. Chem. Soc.* **2004**, *126*, 8136.
(3) (a) Zhou, W. J.; Liu, L.; Breslow, R. *Helv. Chim. Acta* **2003**, *86*, 3560. (b) Liu, L.; Breslow, R. *Bioorg. Med. Chem.* **2004**, *12*, 3277.
(4) Delaney, E. J.; Wood, L. E.; Klotz, I. M. *J. Am. Chem. Soc.* **1982**, *104*, 799.
(5) Suh, J. *Synlett* **2001**, 1343, and references therein.
(6) Hollfelder, F.; Kirby, A. J.; Tawfik, D. S. *J. Org. Chem.* **2001**, *66*, 5866.
(7) (a) Liu, L.; Breslow, R. *J. Am. Chem. Soc.* **2002**, *124*, 4978. (b) Liu, L.; Rozenman, M.; Breslow, R. *J. Am. Chem. Soc.* **2002**, *124*, 12660.
(8) Zhao, H.; Foss, F. W.; Breslow, R. *J. Am. Chem. Soc.* **2008**, *130*, 12590.
(9) (a) Overberger, C. G.; Vorcheheimer, N. *J. Am. Chem. Soc.* **1963**, *85*, 951. (b) Overberger, C. G.; Corett, R.; Salamone, J. C.; Yaroslavsky, S. *Macromolecules* **1968**, *1*, 331. (c) Overberger, C. G.; Shen, C.-M. *Bioorg. Chem.* **1971**, *1*, 1. (d) Overberger, C. G.; Glowaky, R. C.; Vandewyer, P. H. *J. Am. Chem. Soc.* **1973**, *95*, 6008.
(10) Breslow, R.; Hammond, M.; Lauer, M. *J. Am. Chem. Soc.* **1980**, *102*, 421.
(11) Bandyopadhyay, S.; Zhou, W. J.; Breslow, R. *Org. Lett.* **2007**, *9*, 1009.

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