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Hydrophobic Effects on Rates and Substrate Selectivities in Polymeric Transaminase Mimics

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Recently we reported a great increase (ca. 2300-fold) of the transamination rate for the pyridoxamine−pyruvic acid system when we covalently attached pyridoxamine to polyethylenimine (PEI) carrying some attached lauryl groups.[1] We suggested that the excellent general acid−base catalysis exerted by the polymeric partially protonated amines is one of the reasons for the large rate enhancement.[1,2] We also found that the rate enhancement of the polymer over that of simple pyridoxamine **1** was a steep function of the length of the alkyl chains added in polymer with roughly the same percentage of alkylation and pyridoxamine attachment.[1] We suggested that the hydrophobic chains create regions in which the transamination can take place in a less than fully aqueous environment.

We have now explored this system further and discovered a number of important features. First of all, we find that the amination reaction of pyruvic acid with simple pyridoxamine is buffer-catalyzed but the reaction with our polymer-linked pyridoxamine shows no catalysis by external buffers. This supports the argument that the acid and base groups of the polymer are performing the catalytic proton transfers in the transamination process. Extrapolating [buffer] to zero, the PEI−pyridoxamine system is actually 10000-fold faster than is simple pyridoxamine with pyruvic acid.

Furthermore, we find that the polymer system shows saturation effects when titrated with substrates, Michaelis−Menten kinetics.[3] We report the k_2 and K_M values of different substrate−polymer reaction pairs in Table 1 (see footnote b). As the entries show, there are two striking effects of the added C-12 chains in the pyridoxamine−PEI hybrid. The k_2 constants are modified, and the substrate binding constants are modified as well. This latter effect shows up strongly with substrates that are themselves hydrophobic, such as the keto acids corresponding to phenylalanine and tryptophan.

We have also prepared a transaminase mimic **5** by linking pyridoxamine to polyallylamine (PAA) and leaving the nitrogens unmethylated.[4] As the data in Table 1 show, **5** shows a slightly smaller rate of transamination of pyruvic acid (k_2 and k_2/K_M) than is seen with the analogous **6**, derived from PEI. PAA is linear, while PEI is highly branched.[4] Complete methylation[5] decreased the k_2 of PEI-supported pyridoxamine with pyruvic acid and phenylpyruvic acid substrates (**6** vs **8**), while having only small effects on K_M. Bulky phenylpyruvic acid shows slightly lesser reactivity than pyruvic acid with **5**, **6**, and **8**.

Another mode of modifying PEI is alkylation with long hydrocarbon chains.[6] We have reported that partial (ca. 10%) laurylation of PEI-Methylated increases the transamination rate for pyruvic acid substrate by ca. 14-fold.[1] We now see that the effect of laurylation is due to a considerable increase in k_2 for pyruvic acid (**8** vs **9**), which overcomes the weaker binding of relatively non-hydrophobic pyruvic acid by **9**.

Apparently k_2 is increased by the lowered local dielectric constant which results from the presence of nonpolar lauryl groups.[7] In the more polar unmethylated PEI laurylation has a lesser effect on the rate for pyruvic acid transamination (**6** vs **7**).

Strikingly, for the phenylpyruvic acid case we see an impressive increase of rate, laurylation causing the k_2/K_M value to jump 190-fold for PEI-Methylated (**8** vs **9**), much more than the 12-fold increase seen for pyruvic acid. The 190-fold increase is due to both an increase of k_2 by 28 times, slightly smaller than the k_2 effect with pyruvic acid, and an increased binding strength reflected in a decrease of K_M by 7 times. We believe that the increased binding strength reflects hydrophobic interaction of the lauryl chains with the phenyl group of the substrate. For PEI-Unmethylated (**6** vs **7**) laurylation increases the transamination rate by 9 times for phenylpyruvic acid and 2 times for pyruvic acid, a less impressive demonstration of the hydrophobic effect. We then determined the effect of laurylation on the transamination rates for other hydrophobic substrates. (Table 2). The results show that the activity (k_2/K_m) of glyoxylic acid in transamination is similar to that of pyruvic acid. 4-Methyl-2-oxopentanoic acid, the precursor of leucine, shows only slightly higher reactivity as the lower K_M from some hydrophobic binding is compensated by a lower k_2. α-Ketoglutaric acid shows a 5-fold increase in k_2/K_m compared to glyoxylic acid and pyruvic acid, due primarily to stronger binding indicated in a smaller K_M. This likely reflects a carboxylate-ammonium salt bridge interaction. Haring and Distefano[9] have described a protein-based transaminase mimic with a k_2 of 4.8×10^{-3} min^{-1} for the α-ketoglutaric acid substrate and a K_M of 1.8 mM. The resulting k_2/K_M of 2.7 min^{-1} M^{-1} can be compared with the 16 min^{-1} M^{-1} k_2/K_M value for our polymer system.

Phenylpyruvic acid shows a 10-fold increase in reactivity (k_2/K_M) over pyruvic acid, primarily due to a 5-fold decrease in K_M as a result of hydrophobic interactions between the lauryl groups on the PEI and the phenyl group on the ketoacid substrate. An even greater hydrophobic effect is seen in the case of indole-3-pyruvic acid, for which the hydrophobic interactions between the indole group and lauryl groups causes a 22-fold decrease in K_M and 24-fold increase in k_2/K_M when compared with the pyruvic acid case. Since amination of pyruvic acid by compound **9** showed a 10000-fold acceleration relative to pyridoxamine at [buffer] = 0 and since we had earlier found that the amination of pyruvic acid and of indolepyruvic acid by pyridoxamine had the same rate,[10] this means that our hybrid **9** accelerates the amination of indolepyruvic acid to tryptophan by ca. 240000-fold relative to pyridoxamine.

These substantial contributions of hydrophobic effect to the substrate selectivity of the polymeric transaminase mimic were confirmed by competition reactions with HPLC product analyses.

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Table 1: Michaelis Constants ($k_2 \times 1000$ (min^{-1}), K_M (mM)) of the Polymeric Pyridoxamine Reagents[a,b]

	pyruvic acid			phenylpyruvic acid		
reagent	k_2	K_M	k_2/K_M	k_2	K_M	k_2/K_M
PAA-Unmethylated, **5**	20 ± 1	15 ± 1	1.3	21 ± 4	43 ± 8	0.49
PEI-Unmethylated, **6**	55 ± 1	17 ± 1	3.2	49 ± 5	21 ± 5	2.3
PEI-lauryl-Unmethylated, **7**	70 ± 3	11 ± 0	6.4	50 ± 5	2.4 ± 0.6	21
PEI-Methylated, **8**	7.2 ± 0.7	12 ± 2	0.60	13 ± 1	27 ± 3	0.48
PEI-lauryl-Methylated, **9**	290 ± 60	42 ± 6	6.9	370 ± 30	4.0 ± 0.6	92

[a] All constants reported are averaged measurements from two trials and data analysis with Eadie−Hofstree and Hanes−Wolff[8] kinetic approximations. Five or six distinct substrate concentrations were used to define the kinetic curve for each polymer/substrate pair. Pyridoxamine concentrations in the polymer were determined by UV measurements (pyridoxamine, $\lambda = 324$ nm), ca. 1.0×10^{-4} M, T = 40 °C, pH = 7.5. Find details in Supporting Information. [b] k_2 is the rate constant for the reaction of an intermediate going to product. K_M has its usual meaning: $(k_2 + k_{-1})/k_1$, where k_1 and k_{-1} refer to the reversible formation of the reaction intermediate.

Scheme 1

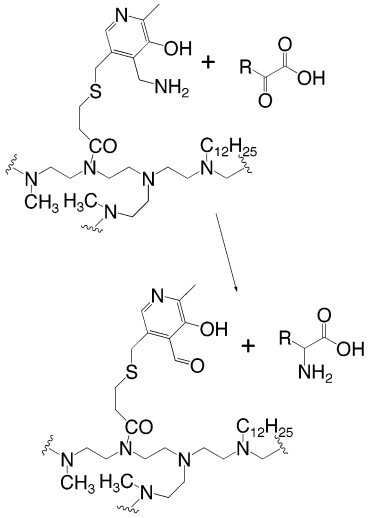

Table 2. Michaelis Constants ($k_2 \times 1000(min^{-1})$, K_M (mM)) of the PEI-lauryl-Methylated Transaminase Mimic, **9**[a]

substrate	k_2	K_M	k_2/K_M
glyoxylic acid	92 ± 6	30 ± 5	3.1
pyruvic acid	100 ± 0	28 ± 1	3.6
4-methyl-2-oxo-pentanoic acid	55 ± 4	13 ± 3	4.2
α-ketoglutaric acid	120 ± 0	7.4 ± 0.9	16
phenylpyruvic acid	200 ± 1	5.5 ± 0.6	36
indole-3-pyruvic acid	110 ± 2	1.3 ± 0.2	85

[a] Reported constants have been determined from UV measurement, $T = 30$ °C, pH = 7.5. All constants reported are averaged measurements from two trials and data analysis with Eadie−Hofstree and Hanes−Wolff[8] kinetic approximations. Five or six distinct substrate concentrations were used to define the kinetic curve for each reagent/substrate pair. See footnote b of Table 1.

Competition reactions were run with 20:20:1 (pyruvic acid: phenylpyruvic acid:pyridoxamine unit, or pyruvic acid:indole-3-pyruvic acid:pyridoxamine unit) ratios at 28 °C, pH 7.5, for 1 h. Amino acid product ratios determined from HPLC analysis were 1:8.5, for the alanine−phenylalanine case and 1:20 for the alanine−tryptophan case, consistent with the results from the kinetic studies in Table 2 under slightly different conditions.

Thus, we have shown that these polyamine-supported pyridoxamine transaminase mimics demonstrate Michaelis−Menten kinetics and substrate selectivity. Laurylation of the polymers both increases k_2 by producing a less aqueous reaction environment and selectively binds the more hydrophobic substrates, decreasing K_M. The result is high rate acceleration for all substrates, and particularly high acceleration for hydrophobic substrates.

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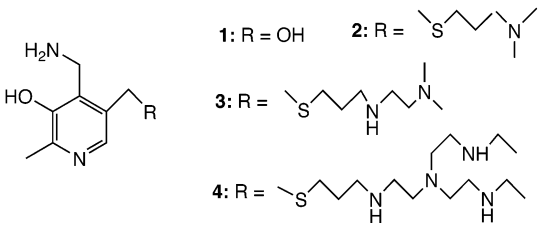
Supporting Information Available: Procedures for the synthesis of the polymeric transaminase mimics and model compounds **2**−**4**; detailed kinetic data for the buffer effect and acetonitrile effect. (PDF) This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Liu, L.; Breslow, R. *J. Am. Chem. Soc.* **2002**, *124*, 4978.
(2) We have made pyridoxamine model compounds **2**−**4** containing one, two, and four amino groups in the side chain. The transamination rates of these compounds were found to be 12, 36, and 68 times higher than the free simple pyridoxamine **1** at pH 7.0 in aqueous HEPES buffer. (See the Supporting Information.) These are much smaller accelerations than we have shown with the polymer-based reagents.



(3) Experiments were carried out under conditions of excess substrate, that is $S_0 \gg C_0$. C_0 represents one reaction site (i.e., pyridoxamine unit) on the polymer and S_0 the ketoacid substrate. Under these circumstances initial observed rate constants k_{obs} are measured which can fit into equation: $k_{obs} = k_2 S_0/(K_M + S_0)$. For detailed kinetics see Supporting Information. For another example of Michaelis−Menten kinetics in a simple polymeric system see: Suh, J.; Scarpa, I. S.; Klotz, I. N. *J. Am. Chem. Soc.* **1976**, *98*, 7060.
(4) Poly(allylamine) $[-CH_2CH(CH_2NH_2)-]_n$ is a linear polymer, average M_w ca. 17000; polyethylenimine is a branched polymer which has a molecular weight of ca. 60000, containing ca. 1400 monomeric ethylamine residues. About 25% of the amino groups are primary, about 50% are secondary, and about 25% are tertiary. For more details see: Suh, J. *Synlett* **2001**, 1343.
(5) Polymers were completely methylated using reductive amination with HCHO and $NaBH_3CN$.
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