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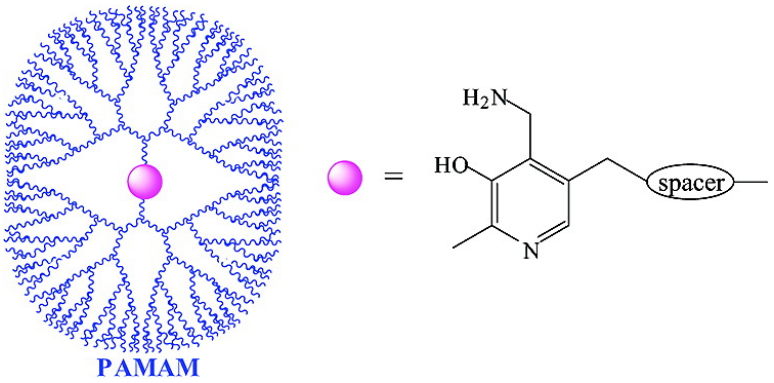

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Dendrimeric Pyridoxamine Enzyme Mimics

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Natural enzymes are macromolecules. Their macromolecular structures offer ideal frames for construction of versatile and robust catalytic sites. Strong and selective binding of the substrate is attained through a combination of the hydrophobic effect and specific substrate−enzyme interactions such as hydrogen bonding. The macromolecular structure can also create regions in which the catalyzed reactions occur in a less than fully aqueous medium.

In comparison with natural enzymes, most enzyme models are small molecules. Although many features of the real enzymes have been well mimicked by these models, it remains to mimic the role of the macromolecular character of enzymes in catalysis.[1] Thus, we recently studied some polymeric enzyme models. We reported a great increase of transamination rate for the pyridoxamine−keto acid system when we attached pyridoxamine to polyethylenimine (PEI) carrying some attached lauryl groups.[2] We also reported that hydrophobic effects exert profound effects on rates and substrate selectivities in the PEI−pyridoxamine transaminase mimics.[3]

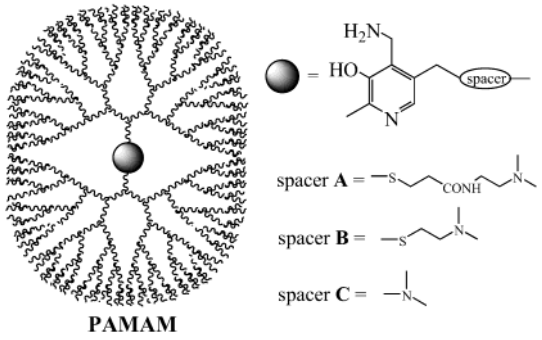
We saw that the excellent general acid−base catalysis exerted by the polyamines is one of the reasons for the large rate enhancement. We also saw that the lauryl chains attached to the polymers create regions in which the rate constants are substantially increased and into which hydrophobic keto acid substrates selectively bind. Nevertheless, those polymeric enzyme models do not have a well-defined structure, and each polymer molecule contains more than one coenzyme unit.

To make more defined macromolecular transaminase models we have now synthesized a number of water-soluble poly(amidoamine) (PAMAM) dendrimers[4] in which we located one pyridoxamine unit at the core. Unlike PEIs whose structures are poorly defined, PAMAM dendrimers are known to be good mimics of globular proteins.[5] We have tuned the structure and size of the dendrimeric enzyme models, which has greatly affected the transamination rates.

The synthesis of the dendrimers began with the *N*-Boc and phenolic-*O*-MOM protected pyridoxamine, to which a spacer was covalently attached. The free NH_2 group of the spacer was then used as the starting point for the PAMAM dendrimer synthesis, which basically involves a two-step iterative sequence: (a) branching double alkylation of the terminal NH_2 groups with methyl acrylate followed by (b) amidation of the terminal esters with excess ethylenediamine.[4] The ester-terminated, which are referred to as "half-generations", were converted to the NMe_2-terminated full generations using excess *N*,*N*-dimethylethylenediamine. Alternatively, the NMe_2-terminated full generations can be made from the NH_2-terminated full generations through complete methylation using $HCHO/NaBH(OAc)_3$. Furthermore, we can also cap the NH_2-terminated full generations with acetyl groups using Ac_2O. The last step in the synthesis is the deprotection of both Boc and MOM groups using HCl (1 N). All the final pyridoxamine dendrimers were completely water soluble.

The synthesized dendrimers were characterized with ^1H NMR and FAB or MALDI MS. The NMe_2 or NHAc-terminated full generations are referred to as **Gn-Me** or **Gn-Ac**. Three spacers were used in the synthesis, and the corresponding dendrimers are referred to as **A**, **B**, and **C** (see Scheme 1). Gn possesses 2^n terminal NMe_2 or NHAc groups. According to Tomalia's studies,[5] the diameters of the PAMAM dendrimers without the pyridoxamine core are about 1.5, 2.2, 2.9, 3.6, and 4.5 nm for G2, G3, G4, G5, and G6, respectively. In comparison with these values, the respective diameters of insulin, cytochrome *c*, and hemoglobin are about 3.0, 4.0, and 5.5 nm. The sizes of the G4−6 pyridoxamine dendrimers are close to those of natural globular enzymes.

Scheme 1



The transamination reactions forming racemic alanine and phenylalanine from the ketoacids were monitored using the UV and HPLC methods.[2,3] We found that all these reactions follow good pseudo-first-order kinetics (see Supporting Information). Since we also found that most of the dendrimer systems show saturation effects when titrated with substrates (Michaelis−Menten kinetics), we report the k_2 and K_M values of different substrate−dendrimer reaction pairs in Table 1.

The k_2/K_M value for simple pyridoxamine was measured to be 0.004 min^{-1} M^{-1} at 60 °C with both pyruvic and phenylpyruvic acid substrates.[6] Compared to this value, the NMe_2-terminated dendrimers show significant rate enhancements (see Figure 1). At the first, third, and sixth generations, the rate enhancements are about 100-, 300-, and 1000-fold, respectively, for pyruvic acid. Simple PEI−pyridoxamine also shows a rate enhancement of about 1000-fold.[3] However, the rate enhancement seen for the laurylated-PEI-pyridoxamine was about 10000-fold, reflecting an increase in k_2.[3]

We studied the buffer effects of HEPES on the transamination reactions (for details see the Supporting Information). The amination reaction of pyruvic acid with simple pyridoxamine is strongly buffer catalyzed,[3] but all the dendrimers show much smaller buffer effects on the transamination. In particular, G5 and G6 dendrimers show nearly no catalysis by external buffers. These observations suggest that that the amino groups of the PAMAM moiety are performing the catalytic proton transfers in the transamination process.

However, not all the catalytic effects come from the general acid−base mechanism. For the NMe_2-terminated dendrimers, building from G1 to G6, the k_2 values increase by about 2−3 times, whereas the K_M values decrease by 2−3 times with pyruvic acid and are even smaller with phenylpyruvic acid. Therefore, the substrate binding effect also plays a role in the dendrimer catalysis.

Compared with the NMe_2-terminated dendrimers, NHAc-

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Table 1. Michaelis Constants [$k_2 \times 1000$ (min^{-1}), K_M (mM), and k_2/K_M (min^{-1} M^{-1})] of the Dendrimer Pyridoxamine Reagents[a,b]

		pyruvic acid			phenylpyruvic acid		
	dendrimer	k_2	K_M	k_2/K_M	k_2	K_M	k_2/K_M
	pyridoxamine	–	–	0.004 ± 0.001	–	–	0.004 ± 0.001
G1	A-Me	–	–	0.52	16 ± 2	17 ± 1	0.94
	B-Me	58 ± 4	85 ± 5	0.68	20 ± 2	21 ± 2	0.95
	C-Me	–	–	0.48	36 ± 1	47 ± 0	0.77
	C-Ac	–	–	0.038	1.5 ± 0.1	35 ± 2	0.042
G2	A-Me	–	–	0.61	18 ± 1	7.8 ± 0.7	2.3
	B-Me	64 ± 4	58 ± 3	1.1	32 ± 4	16 ± 3	2.0
	C-Me	110 ± 10	110 ± 10	1.0	51 ± 2	30 ± 1	1.7
	C-Ac	–	–	0.087	2.1 ± 0.1	21 ± 0.1	0.10
G3	A-Me	46 ± 2	56 ± 2	0.82	20 ± 0	5.8 ± 0.2	3.4
	B-Me	89 ± 5	62 ± 4	1.4	42 ± 1	15 ± 0	2.8
	C-Me	66 ± 1	56 ± 1	1.2	66 ± 3	28 ± 1	2.4
	C−Ac	–	–	0.13	3.4 ± 0.1	18 ± 0.2	0.19
G4	A-Me	62 ± 10	60 ± 6	1.0	38 ± 1	9.4 ± 0.2	4.0
	B-Me	93 ± 1	43 ± 1	2.2	55 ± 0	11 ± 1	5.0
	C-Me	74 ± 1	48 ± 1	1.5	73 ± 1	19 ± 1	3.8
	C-Ac	14 ± 1	53 ± 4	0.26	7.6 ± 0.3	15 ± 1	0.51
G5	A-Me	76 ± 12	30 ± 5	2.5	53 ± 1	4.2 ± 0.1	12.6
	B-Me	99 ± 2	28 ± 1	3.5	58 ± 1	4.3 ± 0.3	13.5
	C-Me	130 ± 10	41 ± 1	3.2	70 ± 1	6.9 ± 0.3	10.1
	C−Ac	5.3 ± 0.6	23 ± 3	0.23	5.5 ± 0.3	11 ± 1	0.23
G6	A-Me	130 ± 10	30 ± 1	4.3	81 ± 1	3.3 ± 0.2	24.5
	B-Me	110 ± 10	18 ± 2	6.1	53 ± 1	1.8 ± 0.1	29.4
	C-Me	180 ± 10	39 ± 1	4.6	58 ± 1	2.7 ± 0.1	21.5
	C-Ac	5.1 ± 0.1	17 ± 0	0.30	5.2 ± 0.1	6.9 ± 0.2	0.75

[a] All constants reported are averaged measurements from two trials and data analysis with Eadie−Hofstee and Hanes−Wolff kinetic approximations. $T = 60$ °C, pH = 7.5. [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.

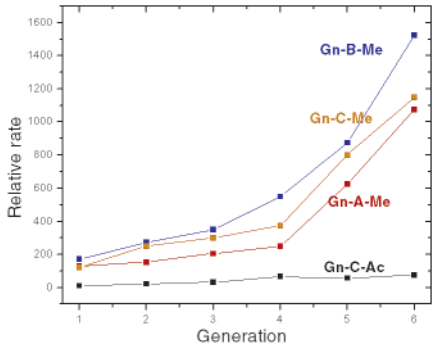

Figure 1. Relative rates (k_2/K_M) of the amination reaction of pyruvic acid with dendrimer pyridoxamines compared with that of simple pyridoxamine.

terminated dendrimers show much smaller reactivities (k_2/K_M). Since K_M values for the NHAc-terminated dendrimers are close to those for the NMe_2-terminated dendrimers, this suggests that the terminal NMe_2 groups provide better general acid−base catalysis than the internal tertiary amines.

In the previous studies we found that addition of acetonitrile to an aqueous pyridoxamine/pyruvic acid system increases the transamination rate significantly.[3] We suggested that a medium effect exerted by the lauryl groups may cause the rate enhancement from PEI−pyridoxamine to laurylated PEI−pyridoxamine. Thus, we laurylated 10% of the terminal NH_2 groups of the G6 dendrimer and then completely methylated the remaining primary and secondary amines. We found that this laurylated G6 dendrimer shows nearly the same transamination rate as the unlaurylated case. The local environment at the core of the G6 dendrimer is already very different from a fully aqueous medium due to the relatively hydrophobic amidoamine groups.

Previous studies have demonstrated that PAMAM dendrimers can selectively bind hydrophobic guest molecules.[7] Similar hydrophobic binding effects are fully indicated by our results concerning the transamination reactivities (k_2/K_M) between the dendrimeric pyridoxamines and phenylpyruvic acid substrate. At the first, third, and sixth generation (NMe_2-terminated), k_2/K_M values for phenylpyruvic acid are about 0.5-, 1-, and 5-fold larger than those for pyruvic acid. These rate enhancements come mainly from the much smaller K_M values with phenylpyruvic acid than those with pyruvic acid substrate, indicating the hydrophobic binding effects of the PAMAM dendrimers.

In summary, we find that a single pyridoxamine in the core of a dendrimer is comparable to a pyridoxamine unit linked to M_n 60 000 PEI in transaminating pyruvic and phenylpyruvic acids to alanine and phenylalanine, but it is less effective than the laurylated PEI−pyridoxamine. Also, our second-order rate constants k_2/K_M are comparable to the value of 2.7 min^{-1} M^{-1} at 37 °C reported for α-ketoglutarate and pyridoxamine linked to a protein.[8] However, in the dendrimer, as with the previous PEI pyridoxamines, we see less than five turnovers using sacrificial amino acids such as phenylglycine. Thus, there are still problems to be solved in this new class of enzyme mimics.[9]

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Supporting Information Available: Synthesis procedures; detailed kinetic data; pertinent spectra (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Hollfelder, F.; Kirby, A. J.; Tawfik, D. S. *J. Org. Chem.* **2001**, *66*, 5866. (b) Suh, J. *Synlett* **2001**, 1343.
(2) Liu, L.; Breslow, R. *J. Am. Chem. Soc.* **2002**, *124*, 4978.
(3) Liu, L.; Rozenman, M.; Breslow, R. *J. Am. Chem. Soc.* **2002**, *124*, 12660.
(4) Tomalia, D. A.; Baker, H.; Hall, M.; Kallos, G.; Rock, J.; Smith, P. *Polym. J.* **1985**, *17*, 117.
(5) Tomalia, D. A.; Huang, B.; Swanson, D. R.; Brothers, H. M., II; Klimash, J. W. *Tetrahedron* **2003**, *59*, 3799.
(6) Compare: Distefano's k_2/K_M value for pyridoxamine-α-keto glutarate pair is 9.6×10^{-4} min^{-1} M^{-1} at 37 °C. See Huang, K.; Distefano, M. D. *J. Am. Chem. Soc.* **1998**, *120*, 1072.
(7) Beezer, A. E.; King, A. S. H.; Martin, I. K.; Mitchel, J. C.; Twyman, L. J.; Wain, C. F. *Tetrahedron* **2003**, *59*, 3873.
(8) Häring, D.; Distefano, M. D. *Bioorg. Med. Chem.* **2001**, *9*, 2461.
(9) For a review and prospectus of other dendrimer enzyme mimics, cf. Kim, Y.; Zimmerman, S. *Curr. Opin. Chem. Biol.* **1998**, *2*, 733−742.

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