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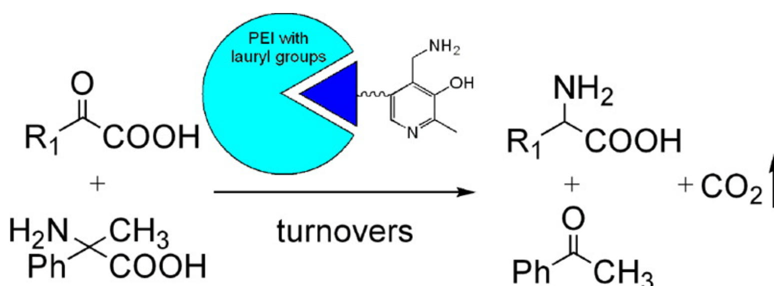

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Transamination Reactions with Multiple Turnovers Catalyzed by Hydrophobic Pyridoxamine Cofactors in the Presence of Polyethylenimine Polymers

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Natural enzymes are macromolecules, but most enzyme models are small molecules.[1] To mimic the role of the macromolecular character of enzymes in catalysis, we have recently studied some polymeric and dendrimeric enzyme models.[2−4] We found a great increase of transamination rate for the pyridoxamine/ketoacid system when we covalently linked pyridoxamine to polyethylenimine (PEI) carrying some attached lauryl groups, or covalently located one pyridoxamine unit at the core of poly(amidoamine) (PAMAM) dendrimers.

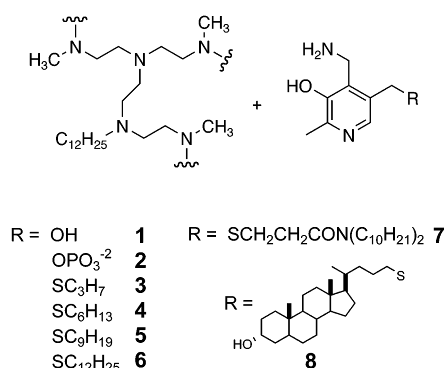
In our polymeric and dendrimeric mimics the pyridoxamine cofactor was *covalently* attached to PEI or PAMAM. However, in the real transaminases the pyridoxamine cofactor forms a noncovalent complex with the enzyme protein matrix.[5] Thus we have now developed some noncovalent polymer−pyridoxamine systems as better transaminase mimics, in which the coenzyme reversibly binds into the polymer. We find that they are even more potent than the covalently linked analogues, since they bind into the hydrophobic region of the polymer. Furthermore, we have now developed a novel catalytic cycle that recycles the pyridoxal cofactor to the pyridoxamine, and for the first time achieves high turnovers in transamination in such enzyme mimics.

Our noncovalent polymer−pyridoxamine systems consist of two components. The first is a fully methylated PEI polymer[6] carrying some attached lauryl groups (Scheme 1). This is used to mimic the holoenzyme environment. The second part is a pyridoxamine cofactor carrying a hydrophobic side chain as the binding group. Details of the synthesis can be found in the Supporting Information. The transamination reactions producing racemic amino acids from the ketoacids were monitored using the HPLC method (see Supporting Information). We found that all these reactions follow good pseudo-first-order kinetics (Table 1).

The background transamination rate between **1** (1.5×10^{-4} M) and pyruvic acid (5.0×10^{-3} M) was measured to be 1.4×10^{-6} min^{-1} at 20 °C, pH 7.5. Addition of 2.5×10^{-5} M of PEI polymer with 8.7% laurylation increased this rate by ca. 9-fold, which we attributed to the intermolecular general acid/base catalysis effects of the polymer. We expected that larger enhancement would be achieved if pyridoxamine could form a stable complex with the polymer. Thus, we examined pyridoxamine 5-phosphate (**2**), which indeed exhibited a 300-fold rate enhancement in the presence of the PEI that had 0% laurylation. The PEI with 8.7% laurylation was also effective for **2**, but the transamination rate under this condition was only 140 times higher than the background. This suggested that the major attraction between **2** and PEI polymer was the ammonium-phosphate salt-bridge interaction, which did not require any lauryl groups.

Much more significant rate enhancement was observed when pyridoxamines with hydrophobic side chains were used. With C_3, C_6, C_9, and C_{12} side chains, the transamination rates for **3**, **4**, **5**, and **6** were respectively measured to be 33, 2800, 13000, and 16000 times faster than the background. When two C_{10} side chains were

Scheme 1



R = OH **1**
OPO_3^{-2} **2**
SC_3H_7 **3**
SC_6H_{13} **4**
SC_9H_{19} **5**
$SC_{12}H_{25}$ **6**

R = $SCH_2CH_2CON(C_{10}H_{21})_2$ **7**

R = [steroid] **8**

Table 1. Transamination Rates under Different Conditions[a]

pyridoxamine	PEI laurylation (%)	$k_{transamination}$ (min^{-1})	$k_{relative}$
1	*b*	$(1.4 \pm 0.3) \times 10^{-6}$	1.00
1	8.7	$(1.3 \pm 0.3) \times 10^{-5}$	9.3
2	0	$(4.2 \pm 0.2) \times 10^{-4}$	300
2	8.7	$(2.0 \pm 0.3) \times 10^{-4}$	140
3	8.7	$(4.6 \pm 0.9) \times 10^{-5}$	33
4	8.7	$(4.0 \pm 0.1) \times 10^{-3}$	2800
5	8.7	$(1.8 \pm 0.1) \times 10^{-2}$	13000
6	8.7	$(2.2 \pm 0.2) \times 10^{-2}$	16000
7	0[c]	$(1.2 \pm 0.1) \times 10^{-2}$	8600
7	5.0	$(8.7 \pm 0.2) \times 10^{-3}$	6200
7	8.7	$(3.5 \pm 0.2) \times 10^{-2}$	25000
7	18.0	$(2.2 \pm 0.1) \times 10^{-2}$	16000
7	27.0	$(9.0 \pm 0.6) \times 10^{-3}$	6400
8	8.7	$(3.9 \pm 0.2) \times 10^{-2}$	28000

[a] Reaction conditions: 1.5×10^{-4} mol/L pyridoxamine, 2.5×10^{-5} mol/L polymer, 5.0×10^{-3} mol/L pyruvic acid, 2.0×10^{-3} mol/L EDTA, $T = 20$ °C, pH = 7.5. [b] No polymer was used for this entry. [c] The reaction mixture was not homogeneous for this entry.

attached to pyridoxamine (**7**), the transamination rate was increased to 25000-fold. The highest transamination rate (28000-fold) was observed for compound **8** that possessed a steroid side chain. Our previous PEI−pyridoxamine reagent with ca. 10% laurylation, in which pyridoxamine was covalently attached to PEI, exhibited only a 10000-fold rate enhancement over the background.[2,3] Thus, the present noncovalent system is more powerful than the previous covalent one, possibly because the noncovalent, dynamic system can be self-organized more suitably for the reaction.

Part of the rate enhancement must stem from the noncovalent attraction between the pyridoxamines and the PEI polymers, resulting in more efficient general acid/base catalysis by the polymer. When PEI with 0% laurylation was utilized, compound **7** could not dissolve into the reaction medium. However, **7** could form clear, homogeneous solutions with PEIs that were 5.0, 8.7, 18, and 27% laurylated. Some rate enhancement must also arise as the transamination reaction occurrs in a less than fully aqueous medium. This must explain why **7** was more reactive in the presence

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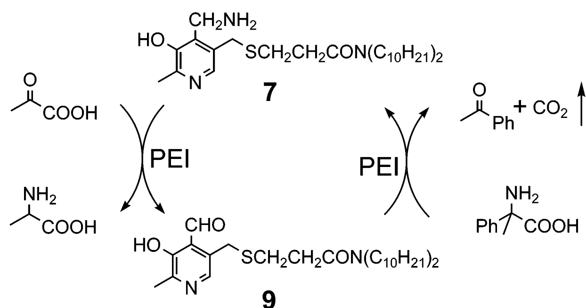
Scheme 2



of the PEI with 8.7% laurylation than with 5.0% laurylation, even though under both conditions the reaction solution was completely homogeneous. Nevertheless, too much laurylation decreased the reactivity possibly because of some loss of general acid/base catalysis.

Competition reactions were run with 30:30:1 (pyruvic acid−phenylpyruvic acid−**7**, or pyruvic acid−indole-3-pyruvic acid−**7**) ratios at 20 °C, pH 7.5 in the presence of the PEI with 8.7% laurylation. Amino acid product ratios determined from HPLC analysis were 1:(14±2) for the alanine−phenylalanine case, and 1:29(±1) for the alanine−tryptophan case. These ratios were slightly higher than those (1:8.5 for pyruvic acid−phenylpyruvic acid, and 1: 15 for pyruvic acid−indole-3-pyruvic acid) obtained previously with the covalent PEI−pyridoxamine reagent,[3] but both the covalent and noncovalent PEI−pyridoxamine systems show substrate selectivities favoring the hydrophobic ketoacid substrates. Since amination of pyruvic acid by compound **7** showed a 25000-fold acceleration relative to the background reaction and since we had earlier found that the amination of pyruvic acid and of indolepyruvic acid by simple pyridoxamine had the same rate,[7] this shows that **7** accelerates the amination of indolepyruvic acid to tryptophan by ca. 725000-fold relative to the background.

Despite the remarkable rate enhancement in the half-transamination reaction achieved in the above noncovalent PEI−pyridoxamine systems, very few turnovers of the catalyst were observed when we attempted to use various amino acids to convert the pyridoxal produced in situ back to pyridoxamine. Thus, we synthesized compound **9** (Scheme 2), which was the proposed product from the half-transamination between pyruvic acid and **7**. We found that **9** showed very low reactivity toward a number of amino acids including phenylalanine and phenylglycine at neutral pH, room temperature, in the presence of PEI but without any metal assistance.[8]

We observed that **9** could react with amino acids at elevated temperatures, but the reaction pathway was not the conventional transamination to provide ketoacid as the product. What actually occurred at high temperatures was an oxidative decarboxylation that yielded pyridoxamine, aldehyde (for amino acids with α-H) or ketone (for amino acids without α-H), and CO_2 (See Scheme 2).[9] Using 2-amino-2-phenylpropionic acid as the substrate, we measured the decarboxylation rates by monitoring the formation of acetophenone with HPLC (Table 2). We found that the decarboxylation rate increased by about 2-fold from 0% to 27% laurylation.[10]

Table 2. Decarboxylation Rates and Turnovers[a]

PEI (%)	$k_{decarboxylation}$ (min^{-1})[a]	TOF (h^{-1})[b]	TON[b]
0[c]	0.031 ± 0.3	0.054 ± 0.010	5 ± 2
5.0	0.042 ± 0.2	0.16 ± 0.03	12 ± 2
8.7	0.055 ± 0.4	0.42 ± 0.06	81 ± 19
18.0	0.080 ± 0.1	0.29 ± 0.04	14 ± 3
27.0	0.082 ± 0.3	0.051 ± 0.014	8 ± 3

[a] Reaction conditions: 1.0×10^{-4} mol/L **9**, 2.5×10^{-5} mol/L polymer, 1.0×10^{-2} mol/L 2-amino-2-phenyl-propionic acid, $T = 60$ °C, pH 7.5. [b] Reaction conditions: 2.5×10^{-5} mol/L **7**, 2.5×10^{-5} mol/L polymer, 5.0×10^{-3} mol/L pyruvic acid, 1.0×10^{-2} mol/L 2-amino-2-phenylpropionic acid, $T = 60$ °C, pH 7.5.

Taking advantage of the above findings, we designed a novel, full transamination pathway as shown in Scheme 2. 2-Amino-2-phenylpropionic acid was sacrificed in the reaction,[11] but new amino acids could be produced in a multiple-turnover fashion from ketoacids as monitored by HPLC analyses. Both the TOF (turnover frequency) and TON (turnover number) measurements indicated that PEI with 8.7% laurylation provided the optimum reaction condition. The highest TON (81) is still modest, but it is the highest turnover number reported thus far for a nonenzymatic pyridoxamine-catalyzed transamination. The next challenge is to evolve systems that can transaminate with higher TON and with enantioselectivity. Some progress in this area will be reported elsewhere.

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

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(6) Polyethylenimine used in this study 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.
(7) Breslow, R.; Hammond, M.; Lauer, M. *J. Am. Chem. Soc.* **1980**, *102*, 421
(8) The transmination rate between alanine (1.0 M, a very high value!) and a pyridoxal attached to peptide and was measured to be $1-2 \times 10^{-3}$ min^{-1} at 25 °C, pH 4.0, in the presence of $CuCl_2$ (see: Imperiali, B.; Roy, R. S. *J. Org. Chem.* **1995**, *60*, 1891).
(9) Oxidative decarboxylation has also been studied very recently. See: Zabinski, R. F.; Toney, M. D. *J. Am. Chem. Soc.* **2001**, *123*, 193.
(10) It was previously proposed that an apolar environment could greatly enhance the decarboxylation reactivity. (Askley, J. A.; Lo, C.-H. L.; McElhaney, G. P.; Wirsching, P.; Janda, K. D. *J. Am. Chem. Soc.* **1993**, *115*, 2515.)
(11) We also tried dimethylglycine and diphenylglycine as sacrificial reagent. The former one showed too low reactivity. The latter one reacted with ketoacid, directly yielding diphenyl ketone in the absence of pyridoxal. This caused a significant background reaction. Additionally, the resulting diphenylmethyleneimine of **7** did not hydrolyze readily under the reaction conditions.

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