Peptide Catalysis

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Histidine-Containing Peptide Catalysts Developed by a Facile Library Screening Method**

*Kengo Akagawa, Nobutaka Sakai, and Kazuaki Kudo**

Abstract: *Although peptide catalysts have a high potential for the use as organocatalysts, the optimization of peptide sequences is laborious and time-consuming. To address this issue, a facile screening method for finding efficient amino-catalysts from a peptide library has been developed. In the screening for the Michael addition of a malonate to an enal, a dye-labeled product is immobilized on resin-bound peptides through reductive amination to visualize active catalysts. This procedure allows for the monitoring of the reactivity of entire peptides without modifying the resin beads beforehand. Peptides containing histidine at an appropriate position were identified by this method. A novel function of the histidyl residue, which enhances the binding of a substrate to the catalyst by capturing an iminium intermediate, was indicated.*

Peptides can be regarded as simplified forms of enzymes and possess high potential for organocatalysis.[1,2] Enzymes catalyze reactions efficiently and selectively by utilizing multiple functional groups of amino acids (AAs), which are spatially arranged in reaction pockets. In the development of peptide catalysts, it is important to choose appropriate AAs and allocate them at a suitable position. Extensive examination is necessary to identify such an ideal peptide sequence from the numerous possible combinations of AAs, and this is the most difficult task. Screening a peptide library constructed in a combinatorial manner is a powerful approach for solving this problem. The groups of Miller[3] and Wennemers[4] separately reported screening methods that could be used to find catalytically active peptides, in which either a fluorescent reporter molecule or a substrate is co-immobilized on resin beads along with the peptides. However, applicable reactions for such screenings are limited, because modifications on the resin are required to be compatible with solid-phase peptide synthesis. Another method is to evaluate individual peptides supported on beads by placing them in a well plate or tubes with reagents and then analyzing each sample in turn.[5,6] In this case, the evaluation step is time-consuming.

We have previously demonstrated that the resin-supported peptide **1** (Figure 1) having a prolyl residue at the N-terminus is an effective organocatalyst for Michael-type reactions of enals. The peptide consists of a β-turn motif, D-Pro–Aib (Aib: 2-aminoisobutyric acid),[7] and a helical section that stabilizes the entire peptide structure and accelerates the reactions.[8] Supporting this peptide on the resin allows a facile preparation of the catalyst and it can be used without considering the solubility of the hydrophobic peptide. By fine-tuning the Trp–Trp part, peptide **1** was successfully applied to stereoselective reactions that were not accessible using low-molecular-weight catalysts.[9] However, the optimization of peptide sequences required time and effort, because alterations of the AAs were conducted in a step-by-step manner. This situation led us to develop a new method for screening a peptide library.

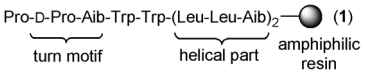

Figure 1. The resin-supported peptide catalyst.

Michael reactions catalyzed by cyclic secondary amines, such as proline, occur through the following steps:[10] 1) the formation of an iminium ion intermediate between the catalyst and the carbonyl group of a substrate, 2) the 1,4-addition of a nucleophile to the iminium intermediate, and 3) the hydrolysis of the resulting adduct to give the product. In most cases, the second step is rate-determining in the catalytic cycle. We chose the Michael addition of a malonate to an α,β-unsaturated aldehyde as a model reaction, as it typically proceeds through the above-mentioned reaction mechanism.[11]

First, we identified the rate-determining step for the reaction with a resin-supported prolyl catalyst. The reaction rate between 4-nitrocinnamaldehyde and dimethyl malonate was measured in the presence of a catalytic amount of resin-supported proline (Figure 2A). A first-order dependence on the concentration of the malonate was observed for the initial production rate of **2** (Figure 2B). This indicates that the step of the C–C bond formation is rate-determining in the catalytic cycle.[12,13]

Next, a strategy for screening the active peptides in a library was established. We envisaged that a catalytically active peptide could be visualized by anchoring the Michael product that was labeled with a dye chromophore. For this purpose, we attempted to covalently attach the product on the amine catalyst.[14] It was found that the reaction of a malonate and an enal with one equivalent of pyrrolidine afforded enamine **3** as the Michael adduct (Scheme 1). Although the hydrolysis of enamine **3** occurs much faster than the nucleophilic attack of the malonate,[15] the enamine formation between product **2** and the amine catalyst is thermodynami-

[*] Dr. K. Akagawa, N. Sakai, Prof. Dr. K. Kudo
Institute of Industrial Science, University of Tokyo
4-6-1 Komaba, Meguro-ku, Tokyo 153-8505 (Japan)
E-mail: kkudo@iis.u-tokyo.ac.jp

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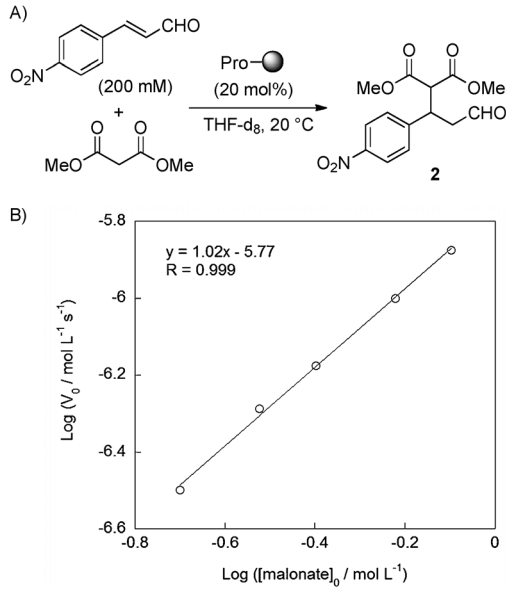
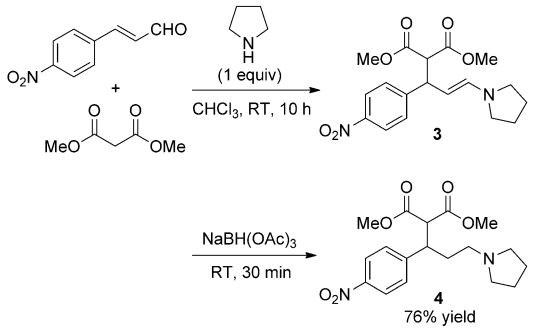
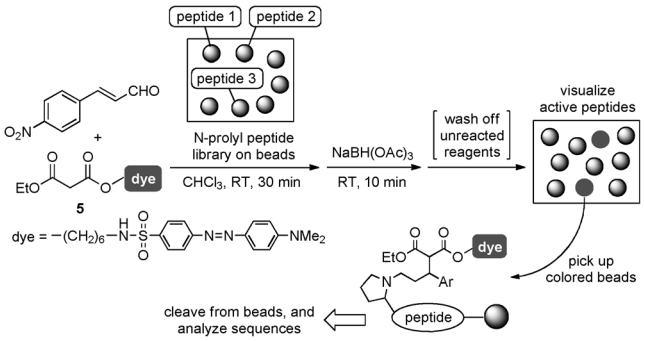


Figure 2. Michael addition of a malonate with a resin-supported prolyl catalyst: A) reaction conditions for measuring the conversion by ^{1}H NMR spectroscopy, and B) dependence of the malonate concentration on the initial reaction rate.



Scheme 1. Michael reaction with an equimolar amount of pyrrolidine followed by reduction.



Scheme 2. Procedure for screening a peptide library.

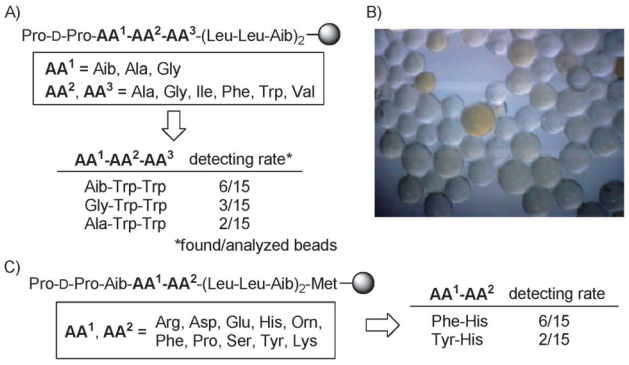


Figure 3. Results of the screening: A) initial peptide library and the multiply detected sequences, B) microscope image of the peptide library after the Michael reaction, reduction, and washing (average particle size = 90 μm), and C) second library including amino acids with functional groups and multiply detected sequences.

cally favorable. Addition of $NaBH(OAc)_3$ to this mixture gave the three-component assembly **4**.[16] Based on this reaction scheme, it was considered that the resin beads were colored, depending on their catalytic activity, when a dye-labeled nucleophile was employed. The procedure for screening a peptide library is shown in Scheme 2: 1) stirring of a mixture of the α,β-unsaturated aldehyde, dye-labeled malonate **5**, and the peptide library in chloroform, 2) anchoring the product to the resin by addition of the reductant and washing-off any unreacted reagents, 3) collecting the colored beads, while observing them under the microscope, and 4) detaching peptides from the beads and analyzing their sequences using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and electrospray ionization (ESI) MS/MS. The initial peptide library was constructed by randomizing the third to fifth residues of peptide **1** (Figure 3A).[17] AAs suitable for the formation of a turn structure were introduced at the third position.[7b] AAs possessing side chains with different sizes and structures were chosen for the fourth and fifth positions. A standard split and mix method was employed to provide a one-bead-one-peptide library.[18] With this library containing 108 different peptides, the above screening was conducted. After the Michael addition, reduction, and washing[19] it was observed that some beads were colored (Figure 3B). Mass spectrometry analysis of peptides cleaved from the positive beads revealed that the Aib–Trp–Trp sequence, the same sequence as in peptide **1**, was the most frequent sequence (Figure 3A). Interestingly, other multiply detected peptides had the Trp–Trp sequence at the fourth and fifth residues. These results are consistent with our previous observation that the introduction of tryptophans to these positions was effective for promoting reactions. With the aim of achieving a marked enhancement of the catalytic activity by the participation of functional groups of the AAs in the reaction cycle, a second-generation library was constructed (Figure 3C).[20] After the screening with this library containing 100 different peptides, a Phe–His sequence was detected with a high frequency, and a similar sequence, Tyr–His, was also observed multiple times (Figure 3C).

The catalytic activity of each peptide identified in the screening was evaluated. Peptide **1** containing Trp–Trp at the fourth and fifth positions,[21] peptides **6** and **7** with Phe–His and Tyr–His sequences, respectively, and peptide **8** with

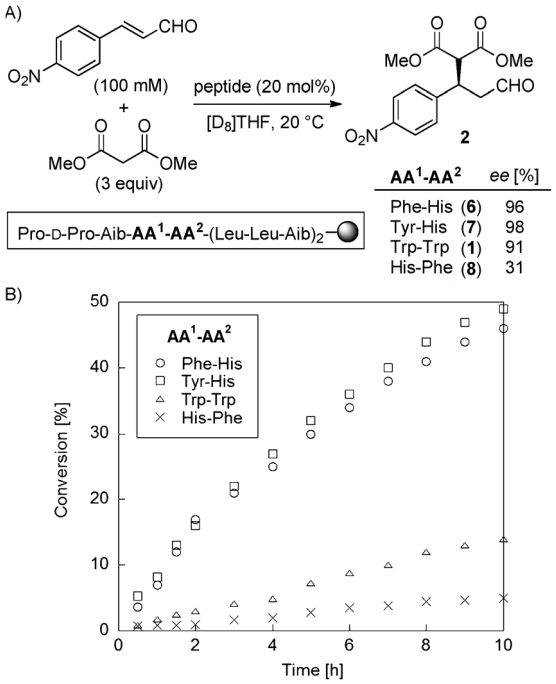


Figure 4. Evaluation of peptide catalysts: A) reaction conditions for measuring the conversion by ^{1}H NMR spectroscopy, and the enantioselectivity for each catalyst, and B) time course of the reactions.

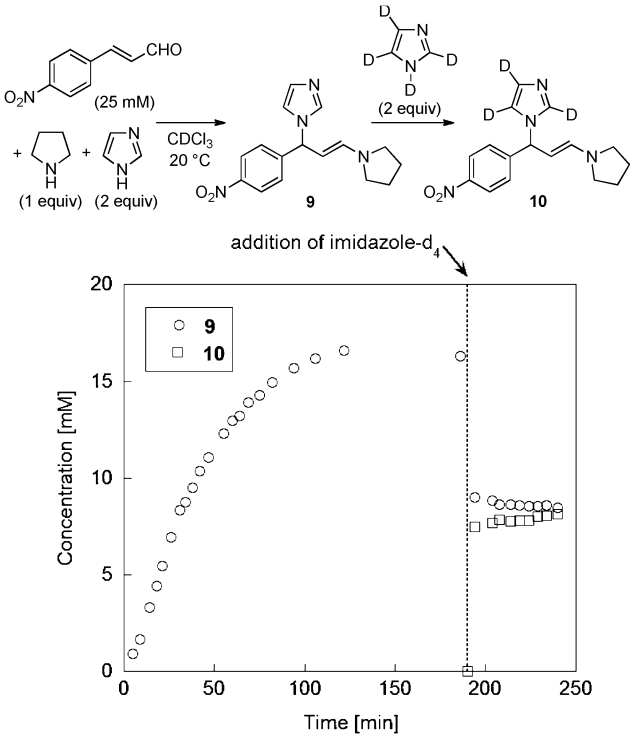


Figure 5. Reaction profile for the generation of imidazole adduct **9** and the exchange with deuterated imidazole.

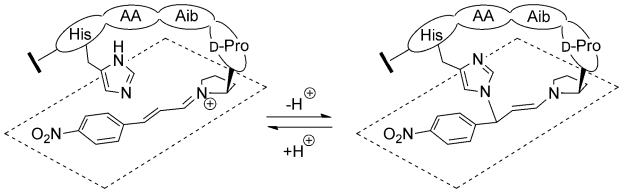


Figure 6. Proposed model for the reversible addition of the histidyl side chain.

a His–Phe sequence as a negative control for peptide **6** were tested in the Michael reaction of dimethyl malonate (Figure 4A). Compared to peptide **8**, a slightly higher reactivity was observed for peptide **1**, and the reaction was markedly enhanced by peptides **6** and **7** possessing a histidyl residue at the fifth position (Figure 4B).[22] A high enantiomeric excess was obtained using the peptide catalysts detected in the screening, whereas control peptide **8** only achieved a poor enantioselectivity. Although the goal of the present screening was not to identify stereoselective catalysts, this method provided peptides generating a high enantioselectivity in the investigated reaction. It should be noted that the groups of Miller and Wennemers also reported the common finding that reactivity correlates with stereoselectivity in the screening of peptide libraries.[3,4]

To clarify the reason for the high reactivity of the peptides with histidine at the fifth position, the following experiments were conducted.[23] When the enal, pyrrolidine, and imidazole were mixed, the Michael addition of imidazole took place to give enamine **9** (Figure 5).[24] The rapid formation of enamine **10** was observed upon addition of deuterated imidazole to this mixture, and the reaction reached equilibrium at which the concentrations of enamines **9** and **10** were identical. The facile exchange of the imidazole group indicates that the addition reaction of imidazole is reversible, and that the adduct quickly interconverts with the iminium ion intermediate. It is assumed that a peptide with a histidyl residue at an appropriate position captures an enal by the Michael addition of the imidazole group (Figure 6). Because this state can provide the reactive iminium intermediate through retro Michael addition, an acceleration of the reaction is expected due to the increased amount of the substrate in the resin. To obtain supporting evidence for this hypothesis, the enal and peptide catalysts were mixed, and the ratio of the enal taken up by the resin was estimated by ^{1}H NMR spectroscopy (Figure 7). Whereas peptide **1** with the Trp–Trp sequence and the control peptide **8** did not show a clear interaction with the enal, its uptake into the resin was observed with peptides **6** and **7**, which both had a histidine residue at the fifth position. The entrapment of the substrate into the resin supposedly leads to the increase of the amount of the iminium ion intermediate, which results in the enhancement of the Michael reaction.

Finally, the substrate scope was examined with peptide catalyst **7** after optimizing the reaction conditions. Regardless of the nature of the substituents on the phenyl ring, the use of aromatic enals afforded the products in good yield and enantioselectivity (Table 1, entries 1–5). Substrates with naphthyl and thienyl groups gave similar results (entries 6 and 7). As a nucleophile, also dibenzyl malonate could be employed (Table 1, entries 8 and 9). In the case of aliphatic aldehydes, the use of *S*,*S*′-dibenzyl dithiomalonate was effective for obtaining products in a good yield, although

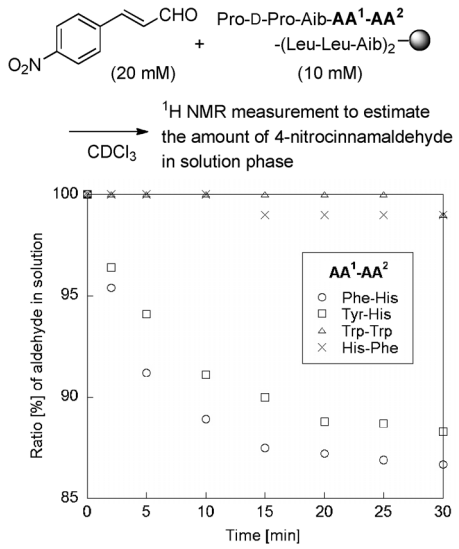
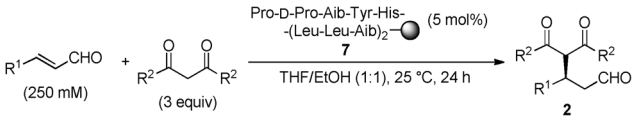


Figure 7. Evaluation of the substrate-binding capability of a peptide.

Table 1: Peptide-catalyzed Michael addition of malonates to α,β-unsaturated aldehydes.



Entry	R^1	R^2	**2**	Yield [%][a]	*ee* [%]
1	4-$NO_2C_6H_4$	OMe	**a**	89	98
2	4-ClC_6H_4	OMe	**b**	84	94
3	4-BrC_6H_4	OMe	**c**	88	96
4	4-$MeOC_6H_4$	OMe	**d**	82	92
5	C_6H_5	OMe	**e**	84	94
6	2-naphthyl	OMe	**f**	80	94
7	2-thienyl	OMe	**g**	83	94
8[b,c]	4-$NO_2C_6H_4$	OBn	**h**	70	98
9[b,c]	4-$MeOC_6H_4$	OBn	**i**	76	96
10[b,d]	Et	SBn	**j**	80[e]	78
11[b,d,f]	c-Hex	SBn	**k**	77[e]	78

[a] Yields of isolated products. [b] The reaction was performed with 400 mM of an enal at 0 °C. [c] The ratio of THF/EtOH used was 1:2. [d] The amount of a malonate was 1.1 equiv. [e] The product was isolated as the corresponding carboxylic acid after oxidizing the formyl group. [f] The amount of catalyst **7** was 10 mol%.

the enantioselectivity was somewhat lower (Table 1, entries 10 and 11). However, because only a poor selectivity was attained with a representative low-molecular-weight catalyst for these substrates,[25] the superior applicability of the peptide catalyst was demonstrated.

In conclusion, a new peptide catalyst was developed by a library screening method. It was shown that histidine at the fifth position plays a critical role in the enhancement of the reaction by trapping a substrate through the reversible Michael addition of an imidazole group. In the screening, a dye-labeled product was immobilized by reducing the enamine form of the Michael adduct, which allowed us to monitor the catalytic activity of an entire peptide mixture in a single event. Thus, the screening could be performed easily, and is potentially applicable to other amine-catalyzed reactions. The development of highly active peptide catalysts is expected to be accelerated by this screening method.

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[19] Because the Michael product was not detected in the filtrate solution, it was confirmed that the hydrolysis of the Michael adduct from the peptide did not occur.

[20] In this library, methionine was inserted between the helical part and the resin to detach peptides through treatment with cyanogen bromide. For the resin beads, TentaGel S–NH_2 (AnaSpec, Inc., product number: 22798, 0.24 mmol g^{-1} amine loading) was used. In the following experiments for the evaluation of catalysts and the reaction scope, this resin was used.

[21] The peptides with Gly–Trp–Trp and Ala–Trp-Trp for the third to fifth residues showed a reactivity comparable with that of peptide **1**.

[22] To check that the coloring of beads in the screening conditions corresponds to the catalytic activity, a coloring test was conducted with catalysts **7** and **8**. See Figure S5 in the Supporting Information.

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[25] For the results with a low-molecular-weight catalyst, see page S7 in the Supporting Information.

