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Library Screening in Aqueous Media to Develop a Highly Active Peptide Catalyst for Enantioselective Michael Addition of a Malonate

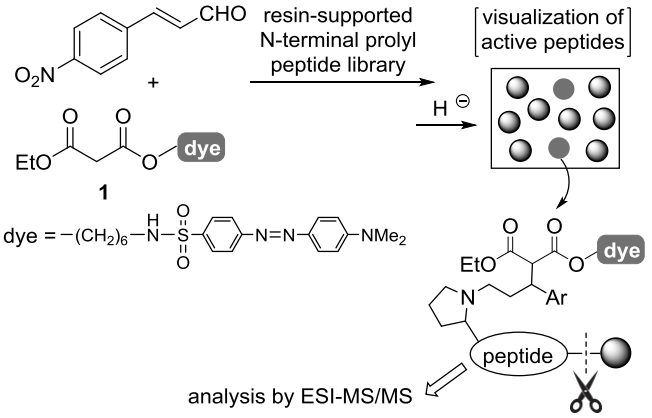
Kengo Akagawa,[a] Yumika Iwasaki,[a] and Kazuaki Kudo*[a]

Abstract: The screenings of peptide libraries with N-terminal L- or D-proline were performed under an aqueous condition to obtain capable catalysts for the asymmetric Michael addition of a malonate. From a D-prolyl peptide library, a consensus sequence, D-Pro-D-Pro-X-Trp-X_3, was found, and the peptide possessing lysine in this framework showed good reactivity and enantioselectivity. The presence of the tryptophan residue at the fourth position was essential, and replacing it to pyrenylalanine further improved the catalytic ability. With an optimum peptide, the reaction could be promoted efficiently in aqueous media at a low catalyst loading.

Introduction

Enzymes are the representative catalysts that promote selective reactions in water. The efficiency of enzymatic reactions is generally higher than that of conventional reactions with synthetic catalysts in organic solvents.[1] The key of the enzymatic catalysis is to construct a hydrophobic environment under aqueous conditions, where multiple functional groups of amino acid side chains collaboratively catalyze reactions. A highly active catalyst is expected to be developed by imitating such features of enzymes with an appropriately designed peptide.[2,3] While most of the reported peptide catalysts have been used in organic solvents, there are some successful examples of using peptides for stereoselective reactions in aqueous media. In those cases, relatively simple peptides, such as poly(amino acid)s for Juliá–Colonna epoxidation,[4] N-terminal prolyl origophenylalanine for an aldol reaction,[5] and a tripeptide, D-Pro-Pro-Glu for a Michael reaction,[6] have been employed, and there is no general principle to design a peptide with multiple functional groups for a stereoselective reaction in aqueous media.

Screening a peptide library constructed in a combinatorial manner is a powerful strategy to find a potent peptide catalyst from numerous combinations of amino acids.[7] We have recently established a facile library screening method to identify an efficient aminocatalyst[8] for Michael addition in organic solvent (Scheme 1).[9] In the screening, the catalytic activity is monitored by anchoring dye-labeled nucleophile **1** through in-situ reductive amination between the N-terminal amino group of peptides and the carbonyl group of a product. Because such a reaction is considered to be possible in the presence of water, it is expected that this method is applicable for exploring new peptide catalysts that function under aqueous conditions. Herein, we report the development of a highly active peptide for the enantioselective Michael addition of a malonate to an enal[10] through the library screenings in aqueous media.



Scheme 1. Procedure for the screening of a peptide library.

Results and Discussion

We initially tested the viability of the above-mentioned reductive amination chemistry in a water-containing solvent system by using ^{1}H NMR (Scheme 2). In the presence of a stoichiometric amount of an aminocatalyst, the Michael product predominantly existed as enamine **2**, and its hydrolyzed form was not detected. This indicates that the enamine state of the Michael adduct is more favorable in equilibrium than the hydrolyzed aldehyde even under the aqueous condition. Therefore, it is supposed that the Michael product generated by a resin-supported peptide can be retained within the resin bead during the library screening.[11] Upon the treatment of enamine **2** with a reductant, the Michael product was successfully anchored to the catalyst to give amine **3**. Thus, by using a dye-labeled malonate, the bead possessing an active peptide for the Michael addition is expected to be colored in aqueous media.

Next, two types of peptide libraries were constructed (Figure 1). Library A consisted of N-terminal L-proline, variable AA^2 to AA^7 residues which were randomized with six kinds of amino acids, and a C-terminal octaethylene glycol linker and methionine.[7h] Library B had D-proline at the N-terminus, and the other constituents were same as library A. According to the procedure shown in Scheme 1, each library was screened in H_2O/THF (1:2). Detected sequences by the screenings are

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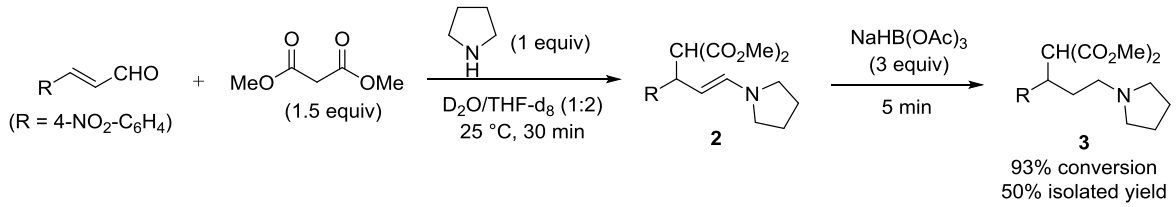


Scheme 2. Formation of the Michael adduct as an enamine state and reduction in aqueous media.

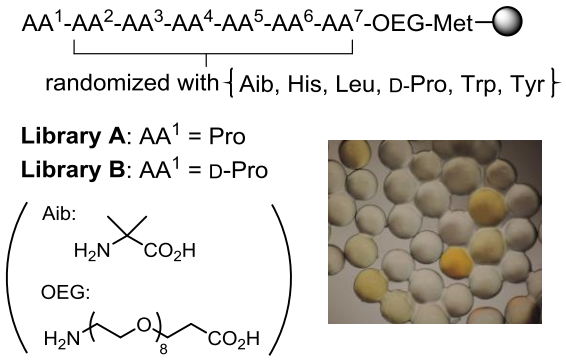


Figure 1. Design of libraries A and B. The inset picture shows the microscopic image of resin beads after coloring. AA = amino acid.

Library A (AA^1 = Pro)

Sample	AA^2	AA^3	AA^4	AA^5	AA^6	AA^7
1	Leu	Trp	Trp	Trp	D-Pro	Tyr
2	Leu	Trp	Trp	Trp	Tyr	D-Pro
3	Leu	Trp	Trp	Tyr	Trp	Trp
4	Leu	Trp	Trp	Tyr	Trp	Tyr
5	Leu	D-Pro	Trp	Leu	Tyr	Leu
6	Leu	Trp	D-Pro	Trp	Leu	Trp
7	Trp	Trp	Trp	Tyr	D-Pro	Tyr
8	Trp	Leu	Trp	Trp	D-Pro	Tyr
9	Trp	Trp	D-Pro	Leu	Aib	Trp
10	Trp	D-Pro	D-Pro	Trp	His	Leu

Library B (AA^1 = D-Pro)

Sample	AA^2	AA^3	AA^4	AA^5	AA^6	AA^7
11	D-Pro	Trp	Trp	Leu	Aib	Leu
12	D-Pro	Trp	Trp	Leu	Leu	Tyr
13	D-Pro	Trp	Trp	Aib	Leu	Tyr
14	D-Pro	Leu	Trp	Tyr	Trp	Trp
15	D-Pro	Leu	Trp	Leu	Trp	Trp
16	Aib	Tyr	Trp	D-Pro	Trp	Trp
17	Aib	Leu	Trp	D-Pro	Trp	Tyr
18	Leu	D-Pro	Trp	Trp	Leu	Leu
19	His	Aib	Tyr	D-Pro	Aib	Leu
20	Trp	D-Pro	Leu	Leu	Leu	Leu

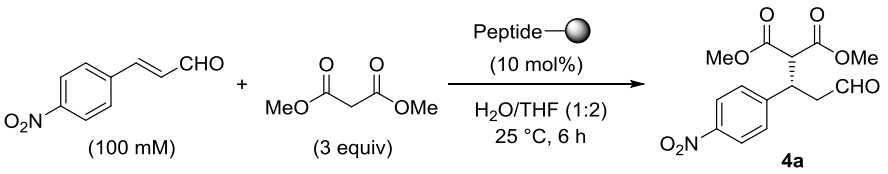
Figure 2. Detected sequences by the screenings of libraries A and B.

summarized in Figure 2. The noticeable features of the result are as follows: i) Trp was detected at a high ratio in the screening of both libraries, ii) the AA^2 position of the peptides from library A was either Leu or Trp, whereas the half of the peptides from library B had D-Pro at this position, and iii) most of the peptides detected from library B had Trp at the AA^4 position.

Some of these sequences were chosen, and the corresponding resin-supported peptides were synthesized and evaluated for the Michael addition of dimethyl malonate to an enal (Table 1). When this reaction was performed with the peptides obtained from negative hit beads in libraries A and B, the conversions were 11% and 13%, respectively (Table 1, Entries 1 and 2). The use of the peptides obtained from highly colored beads resulted in better conversions (Table 1, Entries 3 to 10). The enantiomeric preference of the reaction was not simply governed by the chirality of the N-terminal proline, and the ee of the product did not correlate the reaction rate. Among the examined sequences, the peptide with the consensus residues, D-Pro at AA^2 and Trp at AA^4, found in library B showed the highest reactivity and selectivity (Table 1, Entry 7, peptide **5**).[12] For this peptide catalyst, some control experiments were conducted. When tryptophan at the AA^3 position was replaced with alanine, a similar reactivity and slight increase in the enantioselectivity were observed (Table 1, Entry 11). In contrast, the change of tryptophan at the AA^4 position to alanine significantly decreased the reaction efficiency and selectivity (Table 1, Entry 12). This indicates that the side chain of the AA^4 tryptophan in D-Pro-D-Pro-X-Trp-X_3 sequence is close to the N-terminal prolyl group, and largely affects the reaction. The use of a truncated tetrapeptide, D-Pro-D-Pro-Trp-Trp, resulted in a low conversion (Table 1, Entry 13).[13] It is supposed that a certain length of a peptide chain is necessary for a high catalytic capability.

Because the peptides obtained from library B were more active than those from library A, an extended library with D-Pro at the N-terminus was constructed (Figure 3, library C). The variable part of the library was composed of ten kinds of amino acids including those with functional groups, such as Arg, Asp, Lys, and Thr.[14] The screening of this library was performed in the same aqueous solvent system, H_2O/THF (1:2). From the detected sequences shown in Figure 3, some interesting points were found: i) similar to the case of library B, many of the obtained peptides had D-Pro at the AA^2 position, ii) Trp residues were also observed more often than statistically expected, and iii) primary-amine-containing Lys was detected in four peptides, which is the most frequent among other amino acids with functional groups except for Tyr.

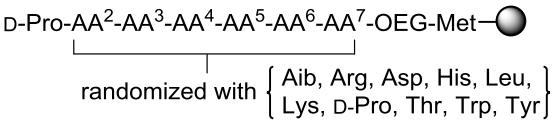
Table 1. Evaluation of peptide sequences from libraries A and B.



Entry	Peptide	Conversion [%]	*ee* [%]
1	Pro-D-Pro-D-Pro-Leu-His-Aib-Tyr[a]	11	–26[b]
2	D-Pro-Aib-His-Aib-D-Pro-D-Pro-D-Pro[a]	13	29
3	Pro-Leu-Trp-Trp-Trp-D-Pro-Tyr (Sample 1)	22	30
4	Pro-Leu-D-Pro-Trp-Leu-Tyr-Leu (Sample 5)	24	27
5	Pro-Leu-Trp-D-Pro-Trp-Leu-Trp (Sample 6)	24	32
6	Pro-Trp-D-Pro-D-Pro-Trp-His-Leu (Sample 10)	24	–3[b]
7	D-Pro-D-Pro-Trp-Trp-Leu-Aib-Leu (Sample 11) (**5**)	61	68
8	D-Pro-Aib-Tyr-Trp-D-Pro-Trp-Trp (Sample 16)	25	43
9	D-Pro-Aib-Leu-Trp-D-Pro-Trp-Tyr (Sample 17)	27	40
10	D-Pro-His-Aib-Tyr-D-Pro-Aib-Leu (Sample 19)	41	12
11	D-Pro-D-Pro-**Ala**-Trp-Leu-Aib-Leu	60	75
12	D-Pro-D-Pro-Trp-**Ala**-Leu-Aib-Leu	20	31
13	D-Pro-D-Pro-Trp-Trp	21	52

[a] The sequence was obtained from a faintly colored bead during the screening. [b] The negative value indicates that the major enantiomer was in (*R*)-configuration.

Library C

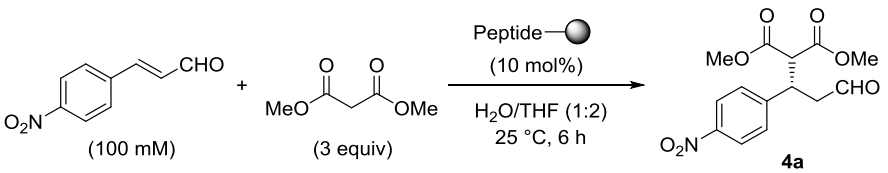


Sample	AA^2	AA^3	AA^4	AA^5	AA^6	AA^7
21	D-Pro	Trp	Tyr	Tyr	Leu	Lys
22	D-Pro	Trp	Thr	D-Pro	Leu	Leu
23	D-Pro	Aib	Trp	Aib	Tyr	Lys
24	D-Pro	Aib	Trp	Lys	Leu	Tyr
25	D-Pro	Aib	Leu	Trp	Tyr	Leu
26	D-Pro	Leu	Trp	Leu	Tyr	Trp
27	D-Pro	Leu	Trp	His	Trp	Thr
28	Leu	Aib	Trp	D-Pro	His	Leu
29	Leu	Trp	Lys	Arg	D-Pro	Trp
30	Aib	Trp	Leu	Trp	Trp	Leu

Figure 3. Design of library C and detected sequences by the screening.

Some peptide sequences detected from library C were picked up and evaluated in the asymmetric Michael reaction (Table 2). Compared to catalyst **5**, the peptides possessing a lysine residue within the D-Pro-D-Pro-X-Trp-X_3 structure showed a higher reactivity (Table 2, Entries 1 and 2). The use of other peptides that lacked Trp at the AA^4 position (Table 2, Entry 3) or D-Pro at the AA^2 position (Entry 4) resulted in lower conversions than the case with peptide **5**. To check the importance of each amino acid in the most active peptide **6**, alanine scanning was conducted. When the lysine residue at the AA^7 position was changed to alanine, the reaction efficiency markedly decreased (Table 2, Entry 5). This is of interest, because the C-terminal amino acid is seemingly far from the reaction center at the N-terminus; however, the presence of the residue with an amino group accelerates the reaction. The introduction of 2,3-diaminopropionic acid (Dap) having a primary amino group in the side chain somewhat improved the reaction rate (Table 2, Entry 6) compared to the case of alanine, but Dap was not so effective as lysine. Similar to peptide **5**, changing tryptophan at the AA^4 position of peptide **6** to alanine dramatically lowered the reactivity and enantioselectivity (Table 2, Entry 7), while the replacement of the AA^6 residue did not affect the reaction so significantly (Table 2, Entry 8). The hydrophobic and bulky nature of the AA^4 tryptophan was assumed to be important; thus, this amino acid was replaced to 3-(1-pyrenyl)alanine (Pya).[15] Along with enhanced enantioselectivity, the highest catalytic activity was attained (Table 2, Entry 9, peptide **7**).[16] Compared to the result obtained with previously developed peptide catalyst **8**[9] under the same conditions (Table 2, Entry 10), the catalytic activity of peptide **7** was remarkably enhanced. It should be noted that the use of the aqueous solvent was essential for the high catalytic efficiency of peptide **7**; the reaction hardly proceeded in THF (Table 2, Entry 11).

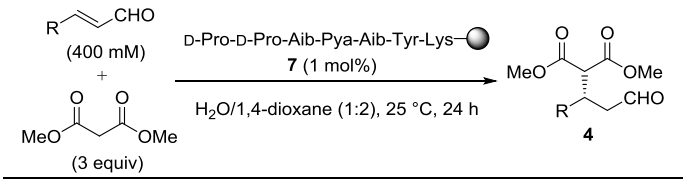
Table 2. Evaluation of peptide sequences from library C.



Entry	Peptide	Conversion [%]	*ee* [%]
1	D-Pro-D-Pro-Aib-Trp-Aib-Tyr-Lys (Sample 23) (**6**)	78	75
2	D-Pro-D-Pro-Aib-Trp-Lys-Leu-Tyr (Sample 24)	69	73
3	D-Pro-D-Pro-Aib-Leu-Trp-Tyr-Leu (Sample 25)	29	71
4	D-Pro-Leu-Aib-Trp-D-Pro-His-Leu (Sample 28)	39	27
5	D-Pro-D-Pro-Aib-Trp-Aib-Tyr-**Ala**	48	77
6	D-Pro-D-Pro-Aib-Trp-Aib-Tyr-**Dap**[a]	67	76
7	D-Pro-D-Pro-Aib-**Ala**-Aib-Tyr-Lys	18	39
8	D-Pro-D-Pro-Aib-Trp-Aib-**Ala**-Lys	62	73
9	D-Pro-D-Pro-Aib-**Pya**-Aib-Tyr-Lys[b] (**7**)	95	87
10	Pro-D-Pro-Aib-Tyr-His-(Leu-Leu-Aib)$_2$ (**8**)	30	–91[c]
11[d]	**7**	3	n.d.[e]

[a] Dap indicates L-2,3-diaminopropionic acid. [b] Pya indicates L-3-(1-pyrenyl)alanine. [c] The negative value indicates that the major enantiomer was in (*R*)-configuration. [d] The reaction was performed in THF. [e] Not determined.

Table 3 Peptide-catalyzed Michael addition of a malonate



Entry	R	**4**	Yield [%]	*ee* [%]
1	4-NO_2-C_6H_4	**a**	77	87
2[a]	4-NO_2-C_6H_4	**a**	72	87
3	4-CN-C_6H_4	**b**	72	81
4	4-CF_3-C_6H_4	**c**	71	80
5	3-NO_2-C_6H_4	**d**	72	84
6	3,5-Cl_2-C_6H_3	**e**	75	82
7	3,5-Br_2-C_6H_3	**f**	82	84
8	3,5-$(CF_3)_2$-C_6H_3	**g**	73	85

[a] The reaction was performed with 0.3 mol% of peptide catalyst **7** for 48 h.

Finally, with the optimum peptide, the asymmetric Michael addition of dimethyl malonate to enals was examined at a low catalyst loading (Table 3). Here, the organic co-solvent was changed to 1,4-dioxane because of a slightly better result compared to THF. In the presence of 1 mol% of peptide **7**, the reaction proceeded smoothly in the aqueous solvent system, and the product was obtained in good yield and enantioselectivity (Table 3, Entry 1). Although the yield was somewhat decreased, the catalyst loading could be reduced to as low as 0.3 mol% by elongating the reaction time (Table 3, Entry 2). As for the enal, the substrate with an electron withdrawing group was necessary for good enantioselectivity.[17] The use of such enals afforded the Michael products in good yield and selectivity (Table 3, Entries 3 to 8).

Conclusions

By the library screening under an aqueous condition, a highly active peptide catalyst for the asymmetric Michael addition of a malonate was developed. Compared to the prior work where the screening had been performed in organic solvent, the following points are noteworthy: i) the D-form of proline at the N-terminus was favorable for the catalytic ability, in contrast to the previous L-prolyl catalyst, ii) hydrophobic and bulky pyrenylalanine in an appropriate peptide framework played a critical role for the aqueous reaction, whereas nucleophilic histidine was effective in organic solvent, and iii) the presence of primary-amine-containing lysine at the C-terminus accelerated the reaction. By using such a multifunctional peptide catalyst, the Michael reaction at a low catalyst loading was achieved. A further application of the screening method to other reactions in aqueous media is expected.

Experimental Section

To a mixture of resin-supported peptide 7 (3.9 mg, 0.8 μmol of the terminal prolyl group), an α,β-unsaturated aldehyde (80 μmol), 1,4-dioxane (132 μL), and water (66 μL), dimethyl malonate (240 μmol) was added. After stirring the mixture at 25 °C for 24 h, the peptide catalyst was filtered off and washed with chloroform. The solvent was removed under reduced pressure, and the residue was purified by flash column chromatography on silica gel (ether/hexanes 3:7 to 9:1) to afford the Michael product. To determine the enantioselectivity, the Michael products were converted to the corresponding methyl esters according to previous reports.[9,10b]

Acknowledgements

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Keywords: Asymmetric catalysis • Combinatorial chemistry • Library screening • Michael addition • Peptides

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[11] In our previous study (ref. 9), it was demonstrated that the condensation and dissociation between the product aldehyde and amine catalyst rapidly take place within a resin bead, but such a reaction beyond resin beads is sluggish.

[12] Such a phenomenon that an efficient catalyst also possesses high enantioselectivity has been observed in the screenings of peptide libraries (ref. 7a, 7d, and 9).

[13] When the Leu-Aib-Leu part of peptide **5** was replaced with a known helical sequence, $(Leu\text{-}Leu\text{-}Aib)_2$, comparable reactivity and selectivity to those of peptide **5** were observed (55% conversion, 71% ee).

[14] The possible combinations of amino acids in library C are 10^6. A part of these sequences (approximately 10^4 beads) were subjected to the screening.

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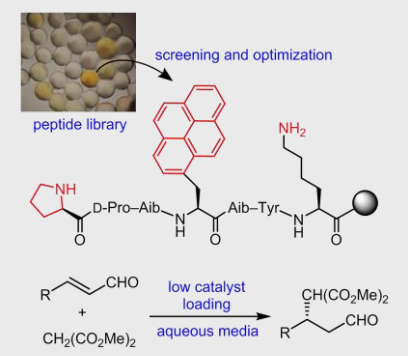
[16] The calculated structure of peptide **7** is shown in Supporting Information.

[17] When 4-methoxycinnamaldehyde was used, the reaction speed was comparable to the cases of the substrates with electron withdrawing groups; however, the selectivity was 54% ee.

COMMUNICATION

The screening of peptide libraries under an aqueous condition afforded capable catalysts for the asymmetric Michael addition of a malonate. An optimized D-prolyl catalyst having pyrenylalanine and lysine residues within an appropriate peptide framework efficiently promoted the reaction in aqueous media at a low catalyst loading.



Library Screening*

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Library Screening in Aqueous Media to Develop a Highly Active Peptide Catalyst for Enantioselective Michael Addition of a Malonate