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Preparation of a core–shell type MBHA resin and its application for solid-phase peptide synthesis

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ABSTRACT

MBHA (4-methylbenzhydrylamine) resin has been extensively used as a solid support for the synthesis of peptide amides. Herein, we prepared the core–shell-type MBHA resin by benzotriazole-catalyzed amidoalkylation and partial hydrolysis. The core–shell structure of the MBHA resin was confirmed by two-photon microscopy and its synthetic performance in solid-phase peptide synthesis (SPPS) was evaluated.

Recently various polymeric supports have been widely used in solid-phase peptide synthesis (SPPS) and their characteristics are important for improving synthetic efficiency. This is especially true for the distribution of the functional groups within the resin, since this characteristic greatly affects the diffusion of the reagents. Functional groups in the surface layer of the resin are more accessible to the reactants than those in the core layer. Therefore, so-called 'core–shell' type resins, in which most of the reactive sites are located in the surface layer, were developed for better solid-phase reactions. Several types of core–shell type resins have recently been developed and used in SPPS, such as *Cuti*Core resin,[1] *Hi*Core resin,[2] AM SURE™ resin,[3,4] and 2-chlorotrityl chloride (CTC) resin,[5] and these resins displayed better synthetic efficiency than conventional ones.

MBHA (4-methylbenzhydrylamine) resin is a well-known solid support for the synthesis of peptide amides and its industrial demand for peptide production is currently increasing. In order to prepare MBHA resin, many methods have been developed for introducing the benzhydrylamine (BHA) structure into the polystyrene resin, such as the oxime group reduction method,[6] Leuckart reduction method,[7] phthalimido linker method,[8] and imine group reduction method.[9,10] However, these methods have some drawbacks including low conversion and harsh reaction conditions.

In a previous study, we presented a simple method for the synthesis of the MBHA resin.[11] Compared to these other methods, MBHA resin was easily prepared by benzotriazole-mediated amidoalkylation. First, formamido PS resin, the precursor of MBHA resin, was synthesized from 1% DVB-PS resin using bis(formamide) and 1-benzotriazole. MBHA resin was efficiently obtained by removing the formamido groups and it displayed excellent properties as a solid support. In this Letter, we present the preparation of the core–shell-type MBHA resin and examine its novel characteristics, which result from the distribution of functional groups. Furthermore, we report the synthetic efficiency of the core–shell-type MBHA resin in SPPS compared to that of the non-core–shell type.

Formamido PS resin (**1**) was synthesized using our method for the preparation of MBHA resin, and the core–shell structure of the MBHA resin (**2**) was constructed by partial hydrolysis of the formamido groups in an aqueous solvent (Scheme 1). Since the core layer of the hydrophobic PS resin was not readily accessible to the hydrophilic reagents during hydrolysis, the formamido groups (**1**) were partially hydrolyzed in 6 N HCl/EtOH at 50 °C for 3 h, and converted into amino groups only on the surface layer of the resin. The loading capacity of the core–shell type MBHA resin (**2**) was determined to be 0.58 mmol/g by Fmoc-titration after loading Fmoc-Gly-OH onto the resin, and the existence of both amine and formamide groups was confirmed by FT-IR analysis.

To assess the distribution of the functional groups within the resin, we coupled a fluorescent dye (dansyl chloride) to the amino groups of the core–shell type MBHA resin, and imaged it by two-photon microscopy (TPM, emission 777 nm).[12–15] As shown in the TPM images of the core–shell type MBHA resin (Fig. 1), fluorescence was detected only at the shell layer and not in the core

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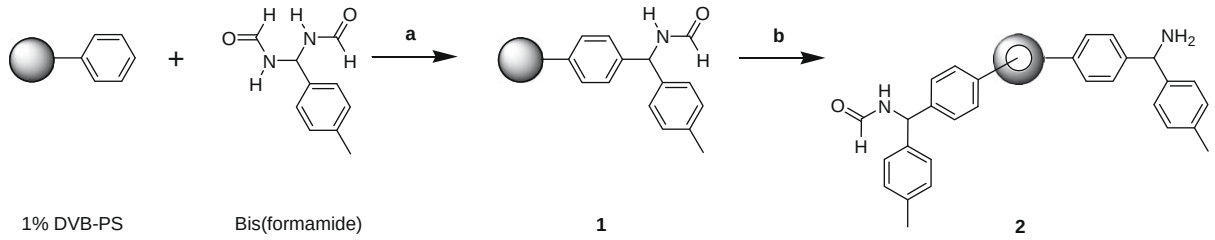

Scheme 1. Preparation of the formamido PS resin (**1**) and core–shell type MBHA resin (**2**). Reagents and conditions: (a) 1-benzotriazole (0.2 equiv based on the bis(formamide) linker), $AlCl_3$, DCE, reflux, 72 h; (b) 6 N HCl, EtOH, 50 °C, 3 h.

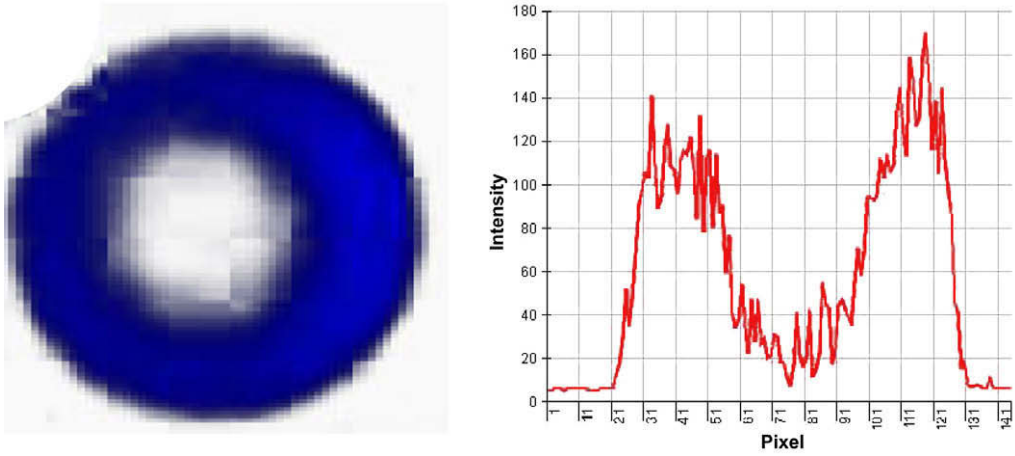

Figure 1. Distribution of functional groups in core–shell type MBHA resin: Two-photon microscopic fluorescence images of dansyl chloride-coupled resin; Images on the lefthand side are taken from a single bead. The graph on right-hand side is the corresponding intensity values for a horizontal line passing through the center of the resin.

region, indicating that the resin (**2**) contained the core–shell structure. In contrast, fluorescence was observed in all regions of the non-core–shell type MBHA resin. This indicates that functional groups were distributed within the core layer of the non-core–shell type resin, which may cause kinetic problems when bulky reagents are added during the reaction (see Supplementary data).

We investigated the chemical stability of the formamido groups that remained in the core layer of the core–shell type MBHA resin. The formamido PS resin (**1**) was subjected to several strong acidic and basic conditions including a typical peptide synthesis condition, and any changes in the functional groups were determined by FT-IR analysis. The intensity of the carbonyl band (1684 cm^{-1}) was maintained after all treatment conditions, and there were no significant loss or change in the formamido groups. Therefore, we concluded that the formamido groups do not cause any side reactions during peptide synthesis.

To determine the loading efficiency of Fmoc-amino acids (Fmoc-AAs) onto the core–shell type MBHA resin (**2**), 20 different Fmoc-AAs were introduced onto resin (**2**) using HBTU and HOBt for 2 h, and the resulting resins were checked by Fmoc-titration. Their loading levels were 0.45–0.58 mmol/g and the loading yields were 78% to quantitative (Table 1). These results indicate that resin (**2**) had a high loading capacity and excellent performance in the first loading step.

In SPPS, this first amino acid loading step is rather slow due to the matrix effect of the polymer backbone. However, if the core–shell type resin can minimize this problem, the coupling reaction could be performed much more efficiently. Thus, we examined whether the coupling rate of the first amino acid to the core–shell type resin was faster than that of other resins. In order to determine this, Fmoc-His(Trt)-OH and Fmoc-Phe-OH were selected because Fmoc-His(Trt)-OH had the lowest loading yield (78%) and

Table 1
Loading levels and yields of Fmoc-amino acids onto the core–shell type MBHA resin (**2**): Fmoc-amino acids were loaded onto the resin using HBTU, HOBt, and DIPEA in NMP for 2 h at 25 °C

Fmoc-AA-OH	Loading level (mmol/g)	Loading yield (%)	Fmoc-AA-OH	Loading level (mmol/g)	Loading yield (%)
Ala	0.53	91	Leu	0.57	98
Arg(Pbf)	0.45	78	Lys(Boc)	0.55	94
Asn(Trt)	0.46	79	Met	0.57	98
Asp(*t*Bu)	0.49	84	Phe	0.58	Quantitative
Cys(Trt)	0.48	82	Pro	0.55	95
Gln(Trt)	0.47	81	Ser(*t*Bu)	0.57	99
Glu(*t*Bu)	0.51	88	Thr(*t*Bu)	0.55	94
Gly	0.53	91	Trp(Boc)	0.56	96
His(Trt)	0.45	78	Tyr(*t*Bu)	0.54	93
Ile	0.51	88	Val	0.56	96

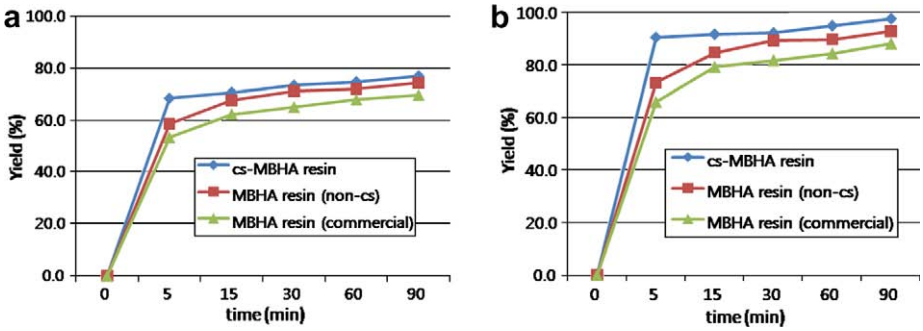

Figure 2. Coupling kinetics of Fmoc-amino acid loading onto the resins: The coupling was proceeded with HBTU, HOBt, and DIPEA in NMP during 5 min, 15 min, 30 min, 60 min, and 90 min. (a) Fmoc-His(Trt)-OH loading; (b) Fmoc-Phe-OH loading.

Fmoc-Phe-OH showed the highest loading yield (quantitative). Three types of MBHA resins, core–shell type (0.58 mmol/g), non-core–shell type (0.74 mmol/g), and a commercially available one (1.20 mmol/g), were used in the coupling kinetics study. In order to set similar capacities of resins, Fmoc-Rink amide linker was anchored onto each of the MBHA resins on different conditions, resulting in Fmoc-Rink-amide-MBHA-cs-resin (0.40 mmol/g) and Fmoc-Rink-amide MBHA resin (0.43 and 0.50 mmol/g). After adjusting almost similar capacities, Fmoc-amino acids were loaded onto the resins to check the first amino acid coupling rate. The coupling yields were examined by Fmoc-titration. As expected, the coupling rate of the core–shell type resin was faster than that on the non-core–shell resin (Fig. 2). These results clearly demonstrate that the first amino acid coupling reaction was faster and more efficient in the core–shell type MBHA resin (**2**) than the non-core–shell type resin (see Supplementary data).

We also found that the core–shell type MBHA resin (**2**) was less swollen in typical solvents such as DMF, NMP, DCM, THF, and MeOH than in the non-core–shell type MBHA resin (Table 2). In general, the high swelling properties of the resin in organic solvents are important factors because it is closely related to the diffusion of reagents within the resin and hence affects the reaction efficiency in SPPS. However, during the manufacturing processes for peptide synthesis, this causes an excessive consumption of solvents and problems associated with waste disposal. Therefore, the lower swelling properties of the core–shell type MBHA resin are advantageous in that they will consume less solvent during peptide production. In addition, the core–shell type MBHA resin displayed better synthetic performance due to the fast kinetics of reagent diffusion.[3,4]

Table 2
Swelling volume of the MBHA resins in typical solvents

	Swelling volume (mL/g resin)				
	DMF	NMP	CH_2Cl_2	THF	CH_3OH
Core–shell type MBHA resin	5.6	6.4	4.4	5.2	2.7
Non-core–shell type MBHA resin	6.0	7.2	6.4	6.8	2.8

To test the synthetic performance of the core–shell type MBHA resin (**2**), the human myelin basic protein fragment (MBP (87–99)), which has been industrially synthesized from a MBHA resin, was selected as a model protein fragment. A Fmoc-Rink amide linker was anchored onto the MBHA resins such that the Fmoc/*t*Bu strategy could be used for peptide synthesis. After MBP (87–99) was synthesized on the Fmoc-Pro-NH-Rink amide-MBHA-cs-resin (0.40 mmol/g resin), the peptide fragments were cleaved with TFA/TIS/H_2O (95:2.5:2.5), and the purity was determined by HPLC (Fig. 3). The product showed one major peak, which was confirmed to be MBP (87–99) by MALDI-TOF Mass. The desired peptide was obtained in 83% purity from the core–shell type MBHA resin (**2**), which was slightly higher than that obtained from the non-core–shell type resin (79%).

In summary, the core–shell type MBHA resin (**2**) was efficiently prepared by partial hydrolysis of the formamido groups on the surface layer, and resin (**2**) displayed good physical and chemical properties as a solid support in SPPS. It also had a better synthetic

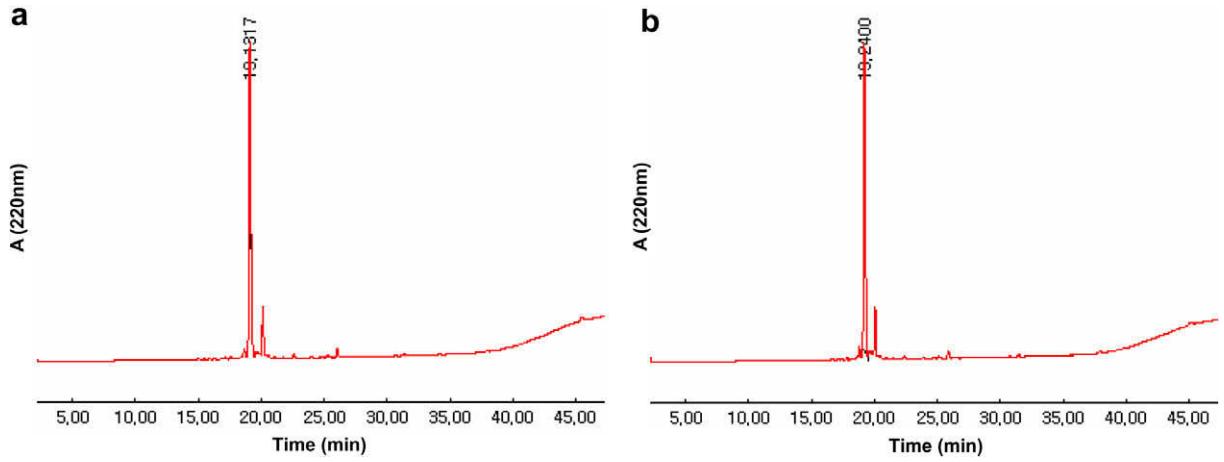

Figure 3. HPLC analyses of the crude products MBP (87–99) synthesized from (a) core–shell type MBHA resin; (b) non-core–shell type MBHA resin (MALDI-TOF: calcd 1554.9 for VHFFKNITPRTP-NH_2 $[M+H]^+$, found 1555.0).

performance than the non-core–shell type resin in the synthesis of the model peptide, MBP (87–99).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.05.009.

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