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Controllable Core−Shell-Type Resin for Solid-Phase Peptide Synthesis

Hong-Jun Cho,[†] Tae-Kyung Lee,[‡] Jung Won Kim,[§] Sang-Myung Lee,*[,∥] and Yoon-Sik Lee*[,†]

[†]School of Chemical and Biological Engineering, Seoul National University, Seoul 151-744, Republic of Korea

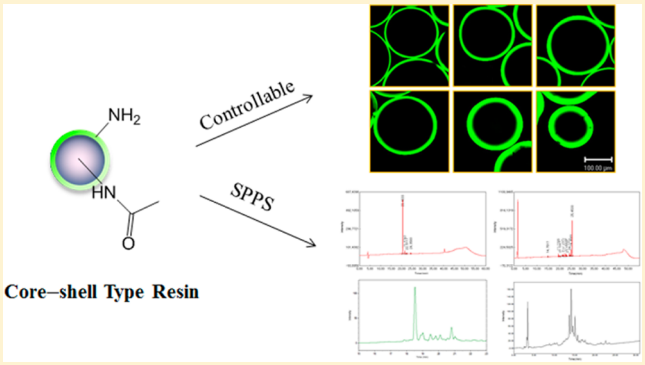
[‡]Department of Chemistry, University of Texas at Dallas, Richardson, Texas 75080, United States

[§]Department of Chemical Engineering, Kangwon National University, Kangwon-Do 245-711, Republic of Korea

[∥]Department of Chemical Engineering, Kangwon National University, Kangwon-Do 200-701, Republic of Korea

Supporting Information

ABSTRACT: A simple, mild, and inexpensive biphasic functionalization approach is attempted for preparing an ideal core−shell-type resin. The core−shell-type architecture was constructed by coupling Fmoc-OSu to the amino groups on the shell layer of an aminomethyl polystyrene (AM PS) resin. The shell layer thickness of the resin could be easily controlled under mild conditions, which was characterized by confocal laser scanning microscopy (CLSM). The efficiency of core−shell-type resin for solid-phase peptide synthesis (SPPS) was demonstrated by the synthesis of various peptides and compared with commercially available noncore−shell-type resins such as AM PS and poly(ethylene glycol)-based resins. The core−shell-type resin provided effective performance during the synthesis of hydrophobic peptide sequences, a disulfide-bridged cyclic peptide, and a difficult PNA sequence. Furthermore, a highly aggregative peptide fragment, MoPrP 105−125, was synthesized more efficiently on the core−shell-type resin under microwave conditions than AM PS and ChemMatrix resins.



INTRODUCTION

The synthesis of highly aggregative peptides, which are responsible for various diseases such as Alzheimer's, "Mad cow", Parkinson's, and Huntington's, is important to identify the causes of the diseases and to develop therapeutic agents.[1−3] Chemical and recombinant methods have been proposed to synthesize these difficult peptide sequences. Despite the merits of the recombinant method, it is still a complicated process and cannot produce unnatural and modified peptide sequences.[4] In contrast, the chemical method, particularly solid-phase synthesis, is a simple process and easy to scale up.[5] However, the chemical method has limitations in synthesizing long and aggregative peptide sequences. Since Merrifield introduced solid-phase peptide synthesis (SPPS) method,[6] various attempts have been made to synthesize difficult peptide sequences and complicated structural molecules, such as the development of a new synthetic strategy, new coupling reagents, new polymer supports, and the use of microwave reactors.[7]

One of the most pivotal factors for the success of solid-phase synthesis is choosing proper solid support. The performance of the solid support is closely related to its swelling property, accessibility, and compatibility with the solvent, reagents, and synthesized compound.[8,9] Polystyrene (PS) resin beads, which have been most commonly used as a solid support, are composed of a hydrophobic matrix and, therefore, are not compatible with peptides and biomolecules. Because of the many disadvantages of a hydrophobic polymer matrix, there has been a requirement for development of amphiphilic or hydrophilic polymer beads with both high stability and good swellability in various solvents.

As alternatives to hydrophobic PS resin, various gel-type poly(ethylene glycol) (PEG) grafted PS resins, such as TentaGel (PEG-PS), NovaGel (PEG-PS), and ArgoGel (PEG/PS), and polymer-grafted pins, such as SynPhase Lanterns, have been introduced.[10−16] To obtain a more hydrophilic PEG-based resin without PS, PEGA resin,[17,18] CLEAR resin,[19] SPOCC resin,[20,21] and ChemMatrix resin[22] have been developed for enzymatic reaction and for the synthesis of highly complex peptides. However, preparing a PEG-based solid support has several shortcomings, such as difficult synthetic procedures and high production costs. Therefore, the development of highly efficient and inexpensive solid supports still remains a challenge in peptide chemistry field.

For efficient SPPS, we have proposed several core−shell-type polymer beads in which functional groups are mainly

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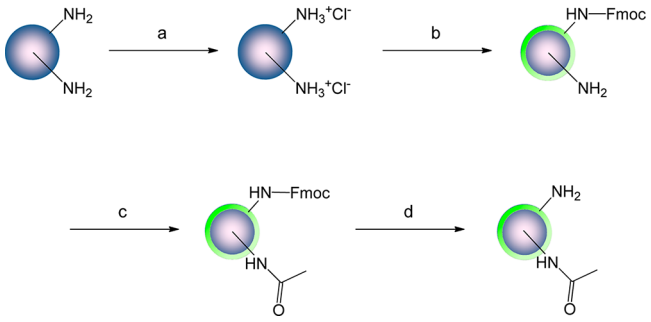
distributed on the outer layer of the resin.[23−29] The core−shell structure was designed to overcome the diffusion problem of reagents and for increased accessibility. Because of this distinctive structural feature, the core−shell-type resins afford high performance in peptide synthesis and photolytic cleavage reactions. But the use of two-step polymerization,[23,24] cross-linking of core domain,[25,29] and partial hydrolysis[26−28] to prepare the core−shell structure on polymer beads requires complicated procedures and harsh conditions, such as a higher reaction temperature than the glass transition temperature of PS. As a result, core−shell polymer beads are not prepared reliably and consistently and they do not always show good performance in SPPS.

Here, we describe a robust method for preparing a core−shell-type resin, which can be facilely and inexpensively prepared from aminomethyl polystyrene (AM PS) resin using a biphasic functionalization method which was developed by Lam.[30−34] The resulting resin possesses good swelling properties and a clear core−shell-type structure which can be easily controlled and reproduced. To evaluate the performance of the core−shell-type resin, difficult peptide sequences, a disulfide-bridged cyclic peptide, and a peptide nucleic acid (PNA) were synthesized, and the results were compared with those of noncore−shell-type AM PS resin, TentaGel resin, and CLEAR resin, which are widely used for SPPS as controls. Furthermore, a long peptide sequence which contains many hydrophobic sequences was synthesized by a microwave-assisted method, and the results were compared with those of AM PS resin and ChemMatrix resin.

RESULTS AND DISCUSSION

Preparation of Core−Shell Type Resin. As shown in Scheme 1, AM PS resin was treated with 1 N HCl/THF to prepare a hydrophilic HCl salt form. After washing with water, Fmoc-OSu in CH_2Cl_2 was added to the resin and was coupled to the amino groups on the shell layer. During this step, Fmoc groups start to couple with the amino groups on the outermost interface between the aqueous and organic phases and gradually react with the amino groups at the inner part of the resin due to the movement of the interface to the inside of the resin during Fmoc coupling. The remnant amino groups in the core domain of the resin were capped by acetyl groups. After deprotection of the Fmoc groups, we finally obtained the core−shell-type resin, which provided clear surface morphology without any physical changes (see Figure S1a in the Supporting Information). Chemical changes from amine to amide were confirmed by FT-IR (amide band, 1652 cm^{-1}; see Figure S1b in the Supporting Information).

Scheme 1. Preparation of Core−Shell-Type Resin[a]



[a]Reagents and conditions: (a) 1 N HCl/THF, 2 h; (b) Fmoc-OSu, DIPEA, CH_2Cl_2, rt, 12 h; (c) acetic anhydride, DIPEA, DMF, rt, 2 h; (d) piperidine/DMF (1:4), rt, 1 h.

Swelling Properties. The swelling properties of the core−shell-type and AM PS resins were measured in various solvents (Table 1). The core−shell-type resin exhibited good swelling properties in most aprotic polar solvents. In particular, the core−shell-type resin swelled much better than AM PS resin in NMP, DMF, and CH_2Cl_2. Thus, this resin was expected to afford good coupling efficiency during the early stage of peptide synthesis.[35]

Table 1. Swelling Volume of Core−Shell-Type Resin and AM PS Resin in Various Solvents

	swelling volume (mL/g)							
resin	dry	H_2O	NMP	DMF	MeOH	THF	CH_2Cl_2	Hex
core−shell-type resin	1.7	1.5	7.5	6.6	2.3	5.5	6.2	1.9
AM PS resin	1.6	1.4	6.0	5.5	2.6	3.9	4.5	2.1

Core−Shell Structure. The distribution of reactive amino groups on the resin was clearly visualized after fluorescent dye coupling.[36] After FITC (fluorescein 5(6)-isothiocyanate) was covalently attached to the resin, the FITC-labeled resin was analyzed by confocal laser scanning microscopy (CLSM) to confirm the core−shell structure. As shown in the CLSM images (Figure 1), the core−shell (a) (b) (c) type resin revealed reactive amino groups on the shell layer, whereas the AM PS resin had reactive amino groups in the entire region of the resin. In addition, the fluorescence intensity profile of the core−shell-type resin in Figure 1c demonstrates quite clearly that the functional groups were concentrated on the shell layer.

Control of Shell Layer Thickness. Previous approaches for the preparation of core−shell structure on PS resins led to the realization that consistent production is difficult because it requires elaborate controls of the reaction under harsh conditions. However, in our new method, we can precisely control the number of amino groups and the thickness of the shell layer by regulating the amount of Fmoc-OSu and DIPEA under mild conditions. Thus, various classes of core−shell-type resins could be obtained by varying the amount of reagents (Fmoc-OSu and DIPEA), and the loading levels of each resin were then determined by Fmoc titration. For example, when 0.51 mmol of reagents were treated with 1 g of resin, we finally obtained 0.43 mmol/g of core−shell-type resin analyzed by Fmoc titration. From this result, we found that the loading level of the reactive amino group on the resin was linearly correlated to the amount of added reagent and gave ca. 82% yield (Figure 2a). The changes in the thickness of the shell layer and the distribution of amino groups on the resins were visualized by FITC. As shown in the CLSM images (Figure 2b), shell layer thickness was increased as the amine loading levels increased. These results indicate that the core−shell structure can be prepared precisely and consistently using this method, with the whole range of amine loading levels appropriate for target specific SPPS.

Peptide Synthesis. *Synthesis of Acyl Carrier Protein (ACP)(65−74) and JR 10-mer.* ACP(65−74) and JR 10-mer were chosen as model peptides and were synthesized using Fmoc/tBu strategy on Rink amide linker coupled core−shell-type and AM PS resins.[37,38] The peptides are noted as difficult

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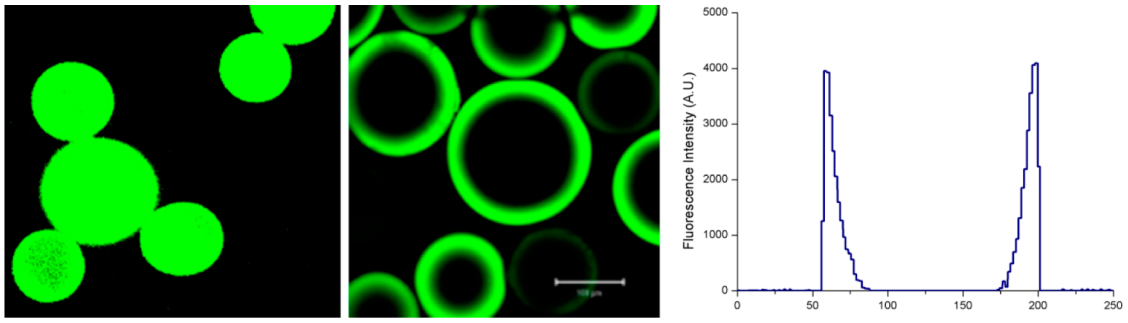

Figure 1. Visualizing the distribution of amino group on resins: CLSM images of (a) AM PS resin and (b) core−shell-type resin coupled with FITC. (c) Fluorescence profile graph of a line passing through the center of core−shell-type resin.

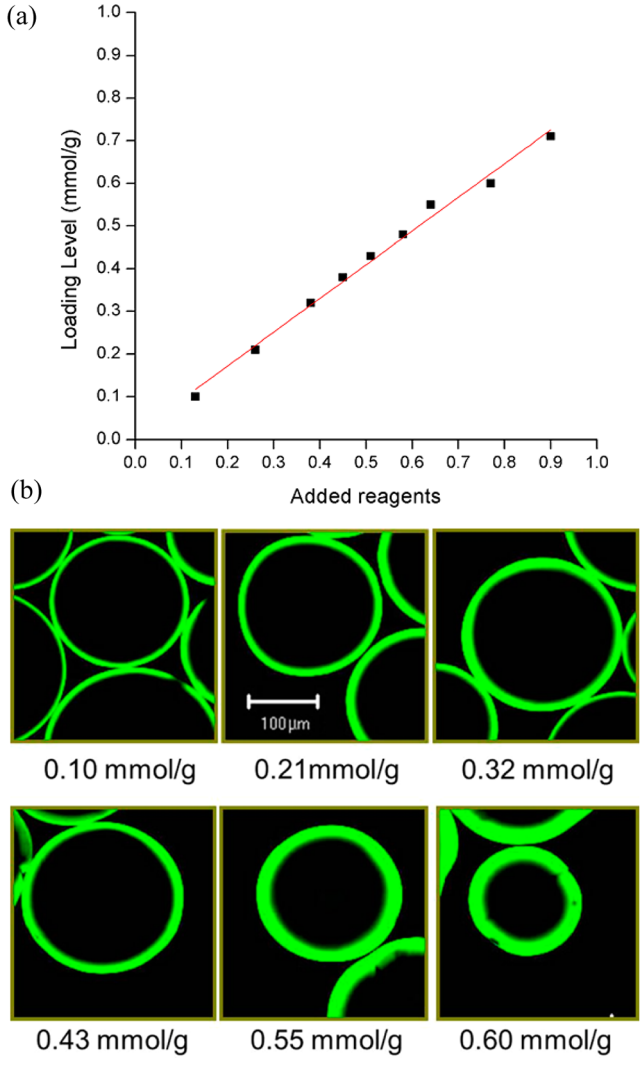

Figure 2. Controlling the thickness of the shell layer of the core−shell-type resin: (a) Correlation between the amount of added reagents (Fmoc-OSu and DIPEA: 0.13, 0.26, 0.38, 0.45, 0.51, 0.58, 0.64, 0.77, and 0.90 mmol) and the loading level of reactive amino groups on the resin. (b) CLSM images of the core−shell-type resins with 0.10, 0.21, 0.32, 0.43, 0.55, 0.60 mmol/g.

peptide sequences due to the β-sheet structure and, hence, are suitable targets for evaluating the synthetic performance of the resins in SPPS. High-loaded and low-loaded resins (ca. 0.50, 0.25 mmol/g) were prepared to determine the differences in synthetic performance between the core−shell- and noncore−shell-type resins. The results of the peptide synthesis revealed that the core−shell-type resin afforded ACP(65−74) and JR 10-mer in high crude yield and purity, as compared to those obtained from the AM PS resin (Table 2). Noticeable differences in synthetic performance were exhibited on the high-loaded resin. In particular, from the results of ACP(65−74), we found that the desired product was obtained without significant deletion sequences from the core−shell-type resin, whereas many deletion sequences were detected from the AM PS resin, as shown in the high-performance liquid chromatography (HPLC) chromatogram. Although no critical differences in the purity and HPLC patterns were found from the low-loaded resins during JR 10-mer synthesis, we noticed that the core−shell-type resin provided better synthetic efficiency than the AM PS resin at a high loading level (Table 2). Based on these results, we can say that many deletion sequence peptides and side products during SPPS might come from the core domain of the resins. Therefore, it is noteworthy that the core−shell structure offered a significant advantage to overcome the diffusion problem because the accessibility of the functional groups located on the shell layer is better than that in the core domain for the incoming reagents.

Table 2. Crude Yield and Purity of Peptides (ACP 65−74 and JR 10-mer) Obtained Using the Core−Shell-Type Resin and AM PS Resin

		ACP(65−74)		JR 10-mer	
loading level	resin	crude yield (%)	purity (%)	crude yield (%)	purity (%)
high loading	core−shell -type resin	99	88	90	45
	AM PS resin	60	28	83	35
low loading	core−shell -type resin	99	88	99	54
	AM PS resin	85	37	89	51

Synthesis of Cyclic Peptide (iRGD). A disulfide-bridged cyclic peptide, triethylene glycol (TEG) grafted iRGD (c-(CRGDRGPDC)-TEG-K),[39,40] was synthesized to show that the peptide could be well-prepared on the core−shell-type resin (0.33 mmol/g) as on TentaGel resin (0.30 mmol/g). Peptide synthesis was followed by the same procedure as above, and on-resin cyclization was performed using thallium(III) trifluoroacetate ($Tl(tfa)_3$).[41] After cleavage, the peptides were recovered in similar crude yields (40−42%) and purities (44%) from both of the resins. As shown in Figure 3, the HPLC pattern of the peptide from the core−shell-type resin looked quite similar to that from TentaGel. Linear peptide

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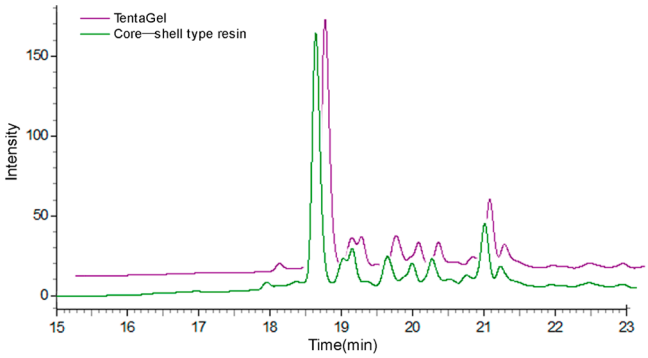

Figure 3. HPLC analysis of the crude iRGD peptide prepared on Tentagel and the core−shell-type resin.

sequences containing free cysteine residues were not detected in the HPLC peaks assigned by the matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) mass spectrometry. The cyclization reaction was carried out effectively on the core−shell-type resin. These results indicated that the core−shell-type resin showed effective synthetic performance as good as TentaGel, even for the synthesis of a disulfide-bridged cyclic peptide.

Synthesis of PNA. PNA oligomer was synthesized on the core−shell-type resin, and the result was compared with that of CLEAR resin, which is generally used as a solid support in the field of PNA oligomer synthesis. The PNA probe for HPV 31 genotyping (Bts-ctgcaattgcaaacagtg), which is known as one of the difficult sequences of the HPV types, was prepared by benzothiazole-2-sulfonyl (Bts) strategy using self-activated Bts protected PNA monomer.[42] The core−shell-type resin gave similar results to the CLEAR resin (see Figure S6 in the Supporting Information). During synthesis, trans-acylation and other side reactions were not observed on the core−shell-type resin. PNA synthesis on the core−shell-type resin could be further optimized, and is expected to replace the CLEAR resin for the synthesis of PNA oligomers.

Synthesis of MoPrP(105−125) Using Microwave-Assisted SPPS. Because microwave irradiation enhances coupling efficiency during SPPS,[43] several PEG-based resins, such as Tentagel, ChemMatrix and other PS-PEG resins, have provided satisfactory outcomes following microwave-assisted SPPS, due to the versatile properties of PEG. The synthesis of MoPrP(105−125) was carried out on the core−shell-type resin to ascertain whether the core−shell structure could afford better results during microwave-assisted SPPS, as compared to those of AM PS and ChemMatrix resins which have similar loading levels (0.40−0.45 mmol/g). MoPrP(105−125) (^{105}KTNLKH^{111}VAGAAAAGAVVGG LG^{125}), a peptide fragment of a mouse prion protein, has a high aggregation tendency which is caused by the presence of long hydrophobic sequences at its C-terminus (111−125).[44,45] Mild protocols (70 °C for 300 s, maximum power 20 W) were followed during microwave irradiation in every coupling and deprotection step, so as to determine the differences in performance among resins. Although the crude yields are similar among the resins (core−shell-type resin: 59%, AM PS resin: 52% and ChemMatrix resin: 61%), the HPLC pattern showed dramatic differences between the core−shell-type and noncore−shell-type resins (Figure 4). The HPLC data (Figure 4a) showed that the peptide from the AM PS resin was accompanied by many deletion sequences and that the main peak was an

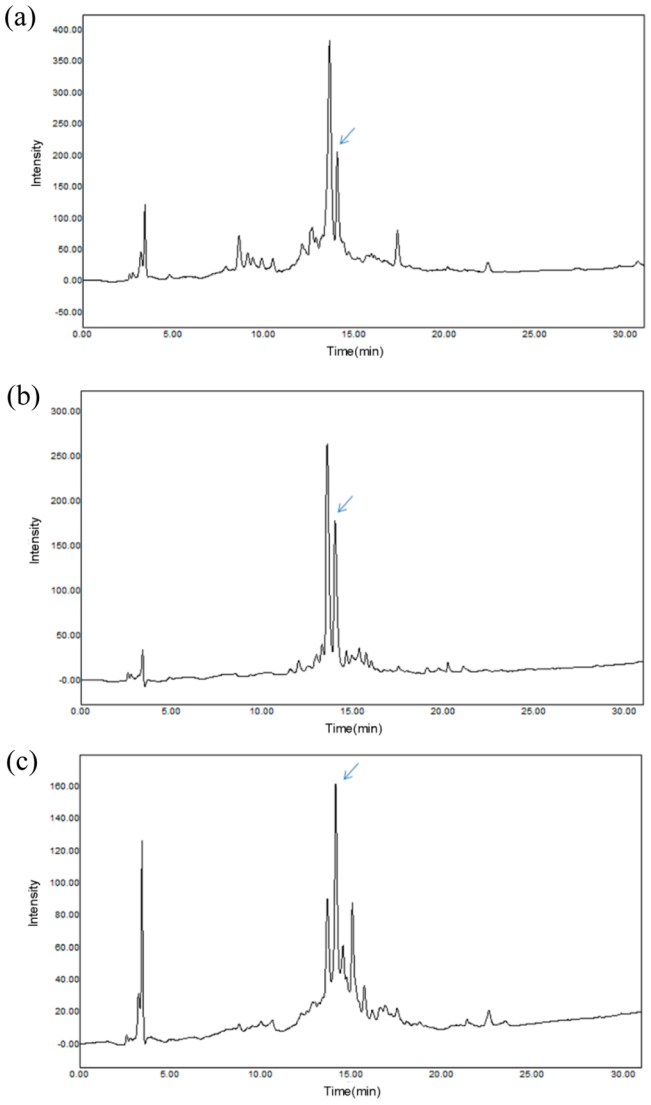

Figure 4. HPLC analysis of crude MoPrP 105−125 prepared by automatic microwave-assisted peptide synthesizer on (a) AM PS, (b) ChemMatrix resin, and (c) core−shell-type resin. Desired product is marked with an arrow.

undesired product with a low molecular weight ([M + H] = 939.5, [M − Ala + H] = 868.5, t_R = 13.6 min; see Figure S11 in the Supporting Information). The desired product appeared in low purity (13%, t_R = 14.1 min) next to the main peak. The HPLC data from ChemMatrix, which is similar to that from the AM PS resin, revealed a few side products due to the PEG chain, which can minimize self-aggregation of the growing peptide chains on the resin. However, it still yielded the same undesired product as the major one of the AM PS resin (Figure 4b, purity = 26%). By Edman degradation and MALDI-TOF mass analysis, we found that this undesired product was KTNKKHAGG or KTNKHAGAGG ([M + H] = 939.5, [M − Ala + H] = 868.5), which did not possess most of the hydrophobic residues in the MoPrP(105−125) sequences. Thus, it appears that the reagents could not diffuse freely into the core domain of the resins due to the aggregated fragments formed during the initial stages of SPPS with hydrophobic sequences. Because of this, the noncore−shell-type resins led to serious deletion during MoPrP(105−125) synthesis, even with the PEG-based resin. However, the peptide synthesized on the

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core−shell-type resin gave higher purity than other resins and afforded the desired product as a major one (Figure 4c, purity = 31%). Because of the excellent reagent accessibility of the core−shell structure, the synthesis of hydrophobic sequences was well carried out, which led to an increased yield and higher purity. Although the unexpected side product with a deletion sequence was detected in the HPLC ([M + H] = 1781.07, t_R = 15.0 min, see Figure S11 in the Supporting Information), we believe that this could be easily overcome by optimizing the reaction conditions, as it involved only one or two sequences. Based on these results, we can conclude that the core−shell-type resin can offset the hydrophobic environment which causes severe deletion sequences during peptide synthesis by enhancing reagent accessibility and can therefore enable more efficient peptide synthesis than other noncore−shell-type resins.

CONCLUSION

A core−shell-type resin was prepared by a biphasic functionalization method under relatively mild conditions. The procedure was beneficial for controlling both the thickness of the shell layer and loading level of the resin. The core−shell-type resin exhibited good swelling properties in various solvents and afforded a uniform core−shell structure. In SPPS, the core−shell-type resin provided effective synthetic performance during the synthesis of hydrophobic peptide sequences, a disulfide-bridged cyclic peptide, and a difficult PNA sequence. Furthermore, a highly aggregative peptide fragment was synthesized effectively on the core−shell-type resin under microwave-assisted synthesis. These results demonstrate that the core−shell-type resin can compensate for the synthetic loss caused by the hydrophobic environment by abandoning the core part of the resin, which is responsible for the deletion sequences. Therefore, the core−shell-type resin has comparative advantages over other PEG-based resins in terms of synthetic performance. Furthermore, we expect that the core−shell-type resin lend itself to other applications in organic synthesis as a polymer support, polymer-supported reagent, or as a polymer-supported catalyst.

EXPERIMENTAL SECTION

General Information. Unless otherwise noted, all solvents and reagents were obtained from commercial suppliers and used without further purification. Aminomethyl polystyrene (AM PS) resin (1% DVB-PS, 100-200 mesh) was obtained from BeadTech, Inc. (Korea). CLEAR Resin was purchased from Peptide International, Inc. (Louisville, KY). TentaGel HL NH_2 was from Rapp Polymere (Germany). H-Rink Amide-ChemMatrix was from PCAS BioMatrix, Inc. (Canada). Bts (Benzothiazole-2-sulfonyl)-PNA monomer reagents were supported by Panagene, Inc. (Korea). The resins were characterized by FT-IR (Bomem, FTLA2000) and elemental analysis (Leco, CHNS-932). Confocal laser scanning microscope (CLSM, Carl Zeiss-LSM510) was used to verify coreshell-type structure. The morphologies of the resin were investigated by field emission scanning electron microscopy (FE-SEM, Jeol Inc. JSM-6700F). The peptide fragments were analyzed on Younglin HPLC (Korea) using a SPIRIT PEPTIDE 120 C18 column (5 μm, 250 mm × 4.6 mm) (Louisville, KY) and Waters symmetry C18 (5 μm, 150 mm × 3.9 mm) (Milford, MA). Mass spectra were acquired on MALDI-TOF, Voyager-DETM STR Biospectrometry Workstation (Applied Biosystems, Inc., USA). Prion peptide was synthesized on a microwave-assisted peptide synthesizer (CEM).

Preparation of Core−Shell-Type Resin. Aminomethyl polystyrene resin (10 g, 2.3 mmol/g) was treated with 1 N HCl/THF (80 mL, 1/1, v/v) for 2 h, and the resin was washed with water (8 × 100 mL). After the water was removed by filtration, Fmoc-OSu (2.87 g, 8.5 mmol) and DIPEA (1.48 mL, 8.5 mmol) were dissolved in CH_2Cl_2 (80 mL) and were added to the resin with vigorous shaking overnight at room temperature. The resin was washed with DMF, CH_2Cl_2, and MeOH (3 × 100 mL, each). To cap the amino group in the core part of the resin, the resin was treated with acetic anhydride (4.25 mL, 45 mmol) and DIPEA (7.84 mL, 45 mmol) in DMF for 2 h at room temperature. The Fmoc group was removed with 20% piperidine/DMF for 1 h. The resin was washed with DMF (3×), CH_2Cl_2 (3×), and MeOH (3×) and were dried in vacuo. The loading level of the resin was determined to be 0.67 mmol/g by Fmoc titration (the Fmoc group was removed by 20% piperidine/DMF for 1 h. The resulting fulvene−piperidine adduct was quantified by UV absorbance at 290 nm).[46] To identify the surface uniformity and morphology of the resin, core−shell-type resin was examined by FE-SEM (JSM-6700F, JEOL) operated at an accelerating voltage of 5 kV. For high-resolution images, the resin was placed on carbon tape and was coated with Pt via sputtering.

Coupling FITC on Core−Shell-Type Resin for CLSM Analysis. FITC (2 equiv) and DIPEA (4 equiv) were dissolved in DMF (2 mL) and added to the core−shell-type resin (50 mg) which was swollen in DMF. After being shaken for 2 h at room temperature, the resin was washed with DMF (3×), CH_2Cl_2 (3×), and MeOH (3×) to remove residual FITC and then dried in vacuo. As a control group, AM PS resin was coupled with FITC using the same procedure as the core−shell-type resin. To investigate the distribution of amino groups in the resins by CLSM, the fluorescent-labeled resins were placed on a standard microscope glass slide. These resins were examined on CLSM (Carl Zeiss LSM-510) with a 20× power lens using an argon ion (λ = 488 nm) for excitation.

Synthesis of Peptides. ACP fragment 65−74 (VQAAIDYING) was synthesized manually on Fmoc-Rink resins (core−shell-type resin: 0.25, 0.51 mmol/g, AM PS resin: 0.28, 0.48 mmol/g) using an Fmoc/tBu solid-phase procedure. For this, Fmoc-Rink amide linker (3 equiv) was coupled on the core−shell-type resins (50 mg) with HBTU (3 equiv), HOBt (3 equiv), and DIPEA (3 equiv). In the case of AM PS resin, Fmoc-Rink amide linker (0.8 mmol) was coupled on AM PS (1 g, 2.3 mmol/g) with HBTU (0.8 mmol), HOBt (0.8 mmol), and DIPEA (0.8 mmol) in DMF (15 mL) for 2 h. The remnant of the amino group was capped with excess acetic anhydride and DIPEA for 2 h. The loading level of Fmoc-Rink-AM PS resin was determined to be 0.48 mmol/g (when 0.5 mmol of reagents was used, 0.28 mmol/g of Fmoc-Rink-AM PS resin was obtained in the same procedure). After Fmoc deprotection with 20% piperidine/NMP for 1 h, the resins (core−shell-type resin: 0.25, 0.51 mmol/g, AM PS resin: 0.28, 0.48 mmol/g, 50 mg each) were treated with preactivated amino acid solution, which was prepared with Fmoc-amino acid (3 equiv), HBTU (3 equiv), HOBt (3 equiv), and DIPEA (6 equiv) in NMP (2 mL). All amino acid couplings were performed for 1 h at 25 °C. After the coupling reaction, the resin was washed with NMP (5×). The Fmoc group was removed using 20% piperidine/NMP (2 mL, 5 min + 10 min). Completion of each coupling step was monitored by the Kaiser test. The final peptide was cleaved with TFA/TIS/H_2O (95:2.5:2.5) solution for 1 h and was recovered by ether precipitation. White solids were obtained, and the crude yield was calculated based on the initial loading level of resins (crude yields: 99% (13.15 mg, low-loaded core−shell-type resin), 99% (27.00 mg, high-loaded core−shell-type resin), 85% (12.57 mg, low-loaded AM PS resin), 60% (15.41 mg, high-loaded AM PS resin)). For the HPLC analysis, a flow rate of 1.0 mL/min and a 30 min gradient of 10−50% of solvent B followed by a 10 min constant flow of 100% solvent B (solvent A, 0.1% TFA in water; solvent B, 0.1% TFA in acetonitrile) was used with spirit peptide C18 column (5 μm, 250 mm × 4.6 mm). Absorbance was measured at 220 nm (purities: 88% (low-loaded core−shell-type resin), 88% (high-loaded core−shell-type resin), 37% (low-loaded AM PS resin), 28% (high-loaded AM PS resin)). ACP 65−74 was analyzed by MALDI-TOF (calculated exact mass = 1061.6 for $C_{47}H_{75}N_{13}O_{15}$ (ACP 65−74), $[M + Na]^+$, found 1084.6).

JR 10-mer (WFTTLISTIM) was synthesized by the same procedure as used for ACP 65−74. The peptide was cleaved from the resin by

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TFA/thioanisole/1,2-ethanedithiol/anisole (90:5:3:2) solution for 1 h and was recovered by ether precipitation. White solids were obtained (crude yields: 99% (15.01 mg, low-loaded core−shell-type resin), 90% (27.76 mg, high-loaded core−shell-type resin), 85% (12.64 mg, low-loaded AM PS resin), 60% (15.30 mg, high-loaded AM PS resin)). For HPLC analysis, a flow rate of 1.0 mL/min and a 30 min gradient of 10−60% solvent B followed by a 10 min constant flow of 100% solvent B (solvent A, 0.1% TFA in water; solvent B, 0.1% TFA in acetonitrile) was used with a Waters symmetry C18 column (5 μm, 150 mm × 3.9 mm). Absorbance was measured at 220 nm (purities: 54% (low-loaded core−shell-type resin), 45% (high-loaded core−shell-type resin), 51% (low-loaded AM PS resin), 35% (high-loaded AM PS resin)). The JR 10-mer peptide was identified by MALDI-TOF (calculated exact mass = 1210.6 for $C_{58}H_{90}N_{12}O_{14}S$ (JR 10-mer), $[M + Na]^+$, found 1233.8).

iRGD (c(CRGDRGPDC)-TEG-K) was synthesized on an Fmoc-Rink amide core−shell-type resin and Fmoc-Rink amide TentaGel resin (0.33 mmol/g, respectively) by the same method. After the last amino acid coupling, the linear peptide containing the S-Acm protecting groups was treated with 2 equivalents of $Tl(tfa)_3$ in NMP (2 mL) for 2 h. The final cyclic peptide was cleaved from the resin with TFA/thioanisole/TIS/H_2O (85:5:5:5) solution for 1 h and was recovered by ether precipitation. White solids were obtained (crude yields: 40% (10.79 mg, core−shell-type resin) and 42% (11.19 mg, Tentagel)). For the HPLC analysis, a flow rate of 1.0 mL/min and a 30 min gradient of 10−60% of solvent B followed by a 10 min constant flow of 100% solvent B (solvent A, 0.1% TFA in water; solvent B, 0.1% TFA in acetonitrile) was used with spirit peptide C18 (5 μm, 250 mm × 4.6 mm). Absorbance was measured at 260 nm (purities: 47% (core−shell-type resin) and 47% (Tentagel)). The peptide was identified by MALDI-TOF (calculated exact mass = 1626.7 for $C_{70}H_{106}N_{20}O_{21}S_2$ (Fmoc-iRGD-TEG-K) $[M + H]^+$, found 1627.5).

PNA Oligomer Synthesis. Synthesis of the PNA oligomer (PNA probe for HPV 31, Bts-ctgcaattgcaaacagtg) was kindly tested by Panagene, Inc., using a Bts strategy. Briefly, Fmoc-PAL-resins were prepared by coupling the Fmoc-Gly-OH and Fmoc-PAL linker sequentially on core−shell-type and CLEAR resins. To adjust for the same loading level (0.15 mmol/g) of resins (core−shell-type resin and CLEAR resin), 0.15 mmol (per 1 g of each resin) of Fmoc-PAL linker was coupled on the resins, and the remnant of amino groups were then capped with excess acetic anhydride and DIPEA. After deprotection of the Fmoc group, the resins were treated with a 0.3 M solution of appropriate cyclic Bts-PNA monomer in DMF for 1.5 h at 40 °C. The remnant amino group was capped by treating 5% acetic anhydride and 6% lutidine in DMF for 3 min. To detach overreacted acetyl group on the Bts-protected amine, 1.0 M piperidine in DMF was used for 3 min. Then, the Bts protecting group was deprotected by treatment with 1 M 4-methoxybenzenethiol and 1 M DIPEA in DMF for 10 min at 40 °C. This cycle was repeated for every Bts-PNA monomer coupling step. After the last monomer was coupled, the final PNA oligomer was cleaved from the resins with 25% *m*-cresol in TFA for 1.5 h and was characterized by HPLC (purities: 37% (CLEAR resin) and 36% (core−shell-type resin)).

Microwave-Assisted Synthesis. MoPrP 105−125 (KTNLKHVAGAAAAGAVVGGLG) was synthesized using Fmoc/tBu-based SPPS strategy on a Liberty microwave-assisted peptide synthesizer (CEM). Three kinds of Rink amide linker loaded resins were prepared (core−shell-type resin: 0.40 mmol/g, AM PS: 0.45 mmol/g, ChemMatrix: 0.41 mmol/g). These resins (100 mg each) were transferred to a Teflon vessel, and peptide synthesis was carried out under nitrogen bubbling. Each coupling reaction was performed with Fmoc-amino acid (5 equiv), HBTU (5 equiv) and DIPEA (10 equiv) in DMF by irradiating at 70 °C for 300 s (maximum power 20 W). Double Fmoc deprotection with 20% piperidine/DMF was performed at 75 °C for 30 s (maximum power 40 W) for the first deprotection and 70 °C for 180 s (maximum power 35 W) for the second deprotection. After the last amino acid coupling and deprotection, the final peptide was cleaved from the resins with TFA/TIS/H_2O (95:2.5:2.5) solution for 1 h, and was recovered by ether precipitation. White solids were obtained (crude yields: 52% (43.44 mg, AM PS), 61% (46.45 mg, ChemMatrix) and 59% (43.98 mg, core−shell-type resin)). For the HPLC analysis, a flow rate of 1.0 mL/min and a 30 min gradient of 10−60% of solvent B followed by a 10 min constant flow of 100% solvent B (solvent A, 0.1% TFA in water; solvent B, 0.1% TFA in acetonitrile) was used with spirit peptide C18 (5 μm, 250 mm × 4.6 mm). Absorbance was measured at 230 nm (purities: 13% (AM PS), 26% (ChemMatrix) and 31% (core−shell-type resin)). The peptide was analyzed by MALDI-TOF (calculated exact mass = 1860.1 for $C_{81}H_{141}N_{27}O_{23}$ (MoPrP(105−125)) $[M + H]^+$, found 1861.1).

■ ASSOCIATED CONTENT

Supporting Information

HPLC chromatogram, MALDI-TOF mass, Edman degradation data. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*(S.-M.L.) Tel: +82-33-250-6335. Fax: +82-33-251-3658. E-mail: sangmyung@kangwon.ac.kr. (Y.-S.L.) Tel: +82-2-880-7073. Fax: +82-2-880-1604. E-mail: yslee@snu.ac.kr.

Notes

The authors declare no competing financial interest.

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