Journal of Peptide Science
J. Pept. Sci. 2007; **13**: 655–661
Published online 22 August 2007 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/psc.897

JOURNAL OF PEPTIDE SCIENCE

Soft shell resins for solid-phase peptide synthesis

TAE-KYUNG LEE,[a] JEONG-HYUN CHOI,[a] SUN-JONG RYOO[b] and YOON-SIK LEE[a]*

[a] School of Chemical and Biological Engineering, Seoul National University, Seoul 151-744, Republic of Korea
[b] BeadTech Inc., Institute for Chemical Processes, Seoul National University, Seoul 151-744, Republic of Korea

Received 12 April 2007; Revised 2 June 2007; Accepted 4 June 2007

Abstract: Soft shell (SS) resins with a lightly or noncross-linked shell layer were prepared by reducing the amount of cross-linking agent, divinylbenzene (DVB), during seed suspension polymerization from polystyrene (PS) resin. These SS resins have a lower swelling volume than that produced by normal cross-linking. Despite its lower swelling, however, SS (10–00) resin, which consists of the 1% DVB-cross-linked core and the noncross-linked surface layer, showed higher efficiency in peptide synthesis compared with 1% DVB-PS resin and other SS resins. Copyright © 2007 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: soft shell resin; solid-phase peptide synthesis; polystyrene resin; seed suspension polymerization; core-shell type structure; 2-chlorotritylchloride resin

INTRODUCTION

Solid-phase synthesis has been widely employed as a tool for the preparation of polypeptides, oligonucleotides and small organic molecules owing to the merits of easy separation and recovery of the target compounds. For successful solid-phase peptide synthesis (SPPS), solid supports, such as polymer resin beads, are very important factors and significantly affect the synthetic efficiency [1]. Therefore, many attempts have been made to improve the synthetic performance by modifying various characteristics of the polymer supports, such as polarity [2–5], swelling property, cross-linking and flexibility [6–8] of the polymer chains and the linker systems [9].

The degree of cross-linking, in particular, is closely related to the swelling property of the resin bead and the flexibility of the polymer chains, and hence the synthetic performance. In polystyrene (PS) resins, the flexibility of the polymer chains and the strength of the resin are controlled by divinylbenzene (DVB), a cross-linker. Merrifield used 16 or 4% DVB, but this was decreased to 1–2% to increase the extent of the peptide coupling reactions [10]. Generally, when less cross-linking agent is employed, the polymer chains are more flexible and the resin beads are more swellable. Therefore, the functional groups become more accessible for the coupling reaction. However, the amount of cross-linking agent cannot be infinitely reduced because the resin becomes physically weak and causes difficulties during filtration.

To achieve both flexibility and robustness, resin morphology can be modified by grafting noncross-linked, linear polymer chains onto a rigid support. PS-grafted polyethylene (PE) film [11], Rasta resin [12] and DVB-PS resin with branched polymer chains [13] were prepared using this method. The synthetic performance with PS-grafted PE film was comparable to that obtained with the conventional DVB-PS resin bead. Similarly, poly(ethylene glycol) (PEG) chains have also been coupled or grafted onto PS resin in various ways. Thus, PS-PEG resin [14], TentaGel [15], ArgoGel [16], Champion resin [17] and CutiCore resin [18] were synthesized, in which the PEG chains were not cross-linked and remained flexible, ensuring high performance in SPPS.

On the other hand, the distribution of functional groups within the resin beads was also reported to affect the synthetic efficiency. Functional groups in the outer layer of resin beads are more accessible for the chemical reactions than those in the inner part. Thus, the so-called 'core–shell' type resins, in which most of the reactive sites are located in the shell layer, were prepared, which performed well in photolytic cleavage reactions and peptide synthesis [18–20].

On the basis of these results, a flexible resin bead structure is preferred for solid-phase reactions, but some degree of rigidity in the resin is also mandatory for its physical strength. Therefore, we envisioned that PS resin could be modified to possess a flexible structure by reducing the amount of DVB in the shell region of the resin beads, while retaining the necessary rigidity by normal cross-linking in the core. Furthermore, such 'flexible–rigid' resin morphology is expected to perform better in peptide coupling reactions when combined with the core–shell type resin structure. For this, a core–shell type resin was designed with a flexible surface layer and a rigid core. The surface layer of the resin beads was less cross-linked or not cross-linked, and the core was cross-linked with DVB (1 or 2%) for rigidity of the resin beads. In relation to this concept, Merrifield reported PS-coated PS resin,

*Correspondence to: Yoon-Sik Lee, School of Chemical and Biological Engineering, Seoul National University, Seoul 151-744, Republic of Korea; e-mail: yslee@snu.ac.kr

wherein linear PS chains were coated onto a 2% DVB-PS resin. However the synthetic efficiency of the resin was not satisfactory [10,p339]. In this paper, we report the synthesis of soft shell (SS) resins, which consist of a lightly or noncross-linked shell layer and a cross-linked core, and their properties in SPPS.

MATERIALS AND METHODS

General

One percent DVB-PS resin (170–200 mesh), 2% DVB-PS resin (170–200 mesh), Fmoc amino acid derivatives, HOBt, HBTU and *p*-[(*R*, *S*)-α-[1-(9*H*-fluoren-9-yl)-methoxyform-amido]-2,4-dimethoxybenzyl]-phenoxyacetic acid (Fmoc-Rink linker) were obtained from BeadTech Inc. (Korea). Calcium phosphate, DVB (80%), styrene monomer (99%) and benzoyl peroxide (70%) were purchased from Sigma-Aldrich Co. (USA) and used without further purification. DCM was freshly distilled from CaH_2. Other solvents (NMP, THF and MeOH) were used as received. Hydroxyethyl cellulose was purchased from Dow Chemical Company (USA). The polymerization reactions were conducted by suspension polymerization using a mechanical stirrer (Panasonic M8GA3M, Japan), and the resin beads were separated using standard sieves (Cisa, Spain). Fluorescence images were obtained with an Olympus IX 70 microscope, and scanning electron microscope (SEM) pictures of the resin beads were taken using a JEOL JSM-T2000 instrument. The peptide fragments were analyzed on Younglin HPLC (Korea) equipped with an SP930D gradient solvent delivery pump and a UV 730D dual wavelength UV/vis absorbance detector, using a Waters Symmetry C_{18} column (5 μm, 3.9 × 150 mm) (USA). Mass spectra were acquired on a MALDI-TOF, Voyager-DE STR Biospectrometry Workstation (Applied Biosystems Inc., USA).

Soft Shell Resin

SS resins were prepared by seed suspension polymerization of 1% or 2% DVB-PS resins. The shell layer was or was not cross-linked with 0.5% DVB. Four types of SS resins were thus prepared and named as in Table 1. The procedure for the synthesis of the SS (10–00) resin is described as a representative example.

One percent DVB-PS (50 g, 170–200 mesh) was suspended in distilled water (600 ml). Calcium phosphate (6.0 g) and hydroxyethyl cellulose (2.0 g) were then added. Next, benzoyl peroxide (0.13 g) solution in styrene monomer (50 g) was added dropwise to the reaction mixture. The resulting suspension was stirred at 300 rpm at 90 °C under nitrogen atmosphere. After 3 h, the reaction mixture was cooled to room temperature, filtered, washed with distilled water (900 ml × 2), THF/3N HCl (3 : 1) (900 ml × 2), THF/water (3 : 1) (900 ml × 2), THF (900 ml) and MeOH (900 ml), and dried *in vacuo*. The resin was separated with standard sieves to yield 44 g of SS (10–00) resin (100–170 mesh).

Table 1 Soft shell (SS) resins with differing degrees of cross-linking in the core and shell layer

SS resin	Degree of cross-linking (DVB wt%)	
	Core part	Shell layer
SS (10–00)	1.0	0.0
SS (10–05)	1.0	0.5
SS (20–00)	2.0	0.0
SS (20–05)	2.0	0.5

Aminomethyl Soft Shell (AM-SS) Resin

N-(Chloromethyl)phthalimide (1.13 g) and aluminum chloride (0.77 g) in nitrobenzene (6.0 ml) were added to a suspension of SS (10–00) resin (3.0 g) in DCM (37.5 ml) at room temperature. The resulting mixture was stirred for 4 h at 25 °C. After quenching with water and 3N HCl, the resin was filtered, washed with THF/3N HCl (3 : 1) (40 ml × 2), THF/water (3 : 1) (40 ml × 2), THF (40 ml) and MeOH (40 ml × 2), and dried *in vacuo* to yield 3.4 g of phthalimidomethyl SS (10–00) resin. The resin was suspended in THF (20 ml) and 40% methylamine (10 ml) was added [17]. The resulting mixture was shaken for 72 h at room temperature. The resin was filtered, washed with THF/water (3 : 1) (40 ml × 2), THF (40 ml × 2) and MeOH (40 ml × 2), and dried *in vacuo*. The loading level was 0.90 mmol/g resin, as calculated from Fmoc-titration after coupling with Fmoc-Gly-OH.

Fmoc-Rink Linker-loaded Aminomethyl Soft Shell (Fmoc-Rink-AM-SS) Resin

AM-SS (10–00) resin was suspended in NMP, and a solution of Fmoc-Rink linker (2 equiv. based on the loading level of resin), HBTU (2 equiv.), HOBt (2 equiv.) and DIEA (4 equiv.) in NMP was added. The resulting mixture was shaken in a shaking incubator for 2 h at 25 °C. After the reaction was complete (negative Kaiser ninhydrin test), the resin was filtered, washed with NMP (×2), DCM (×2) and MeOH (×3), and dried *in vacuo* to yield the Fmoc-Rink-AM-SS (10–00) resin. The loading level was 0.60 mmol/g resin by Fmoc-titration.

2-Chlorotrityl Soft Shell Resin

To a suspension of SS (10–00) resin (20 g) in DCM (200 ml) was added a solution of aluminum chloride (23.1 g) in nitrobenzene (60 ml). A solution of 1-chloro-2-[dichloro(phenyl)methyl]benzene (95%, 52 g) in dichloroethane (52 g) was slowly added, and the resulting mixture was stirred for 4 h at 25 °C. After cooling to 0 °C, the reaction was quenched by adding water, THF and 3N HCl. The resin was collected by filtration, washed with THF/3N HCl (3 : 1) (400 ml × 2), THF/water (3 : 1) (400 ml × 2), THF (400 ml × 2) and MeOH (400 ml × 2), and dried *in vacuo* to yield 36.6 g of 2-chlorotrityl soft shell (CT-SS) (10–00) resin [21]. To determine the loading level, the resin was loaded with excess Fmoc-Gly-OH (see below for the procedure). The loading level was 1.1 mmol/g resin by Fmoc-titration after Fmoc-Gly-OH was loaded onto the CT-SS (10–00) resin.

DOI: 10.1002/psc

Fmoc-Gly-OH and Fmoc-Trp(Boc)-OH-loaded 2-Chlorotrityl Soft Shell (Fmoc-Gly-O-CT-SS and Fmoc-Trp(Boc)-O-CT-SS) Resin

To a mixture of CT-SS (10–00) resin (2.0 g) and DCM (20 ml) was added slowly thionyl chloride (99%, 0.88 ml), and the resulting mixture was stirred for 2 h at 25 °C. The resin was filtered, washed with DCM (30 ml × 3) and dried *in vacuo* to yield 2-chlorotrityl chloride (CTC)-SS resin. Fmoc-Gly-OH (0.39 g) and DIEA (1.8 ml) were then added to the resin suspension in DCM (20 ml). The resulting mixture was shaken in a shaking incubator for 2 h at 25 °C. DIEA/MeOH (1 : 9) solution (2 ml) was added, and the resulting mixture was shaken for 30 min. The resin was filtered, washed with DCM (30 ml × 3) and MeOH (30 ml × 3), and dried *in vacuo* to yield Fmoc-Gly-O-CT-SS (10–00) resin (loading level: 0.42 mmol/g resin). The same method was used to obtain Fmoc-Trp(Boc)-O-CT-SS (10–00) resin (loading level: 0.58 mmol/g resin).

Peptide Synthesis

The fragment 65–74 of the acyl carrier protein [22] (H-Val-Gln-Ala-Ala-Ile-Asp-Tyr-Ile-Asn-Gly-R, R=NH_2 or OH) was synthesized using Fmoc-Rink-AM resins (Table 4) and Fmoc-Gly-O-CT resins (Table 6). Gln and Asn side-chains were protected with Trt; Bu^t was used for the protection of Asp and Tyr side-chains. The Fmoc moiety was removed using 20% piperidine/NMP (1 × 5 min, 1 × 10 min). The peptide was elongated with the preformed activated ester, which was prepared using Fmoc amino acid (3 equiv.), HBTU (3 equiv.), HOBt (3 equiv.) and DIEA (6 equiv.) in NMP. The resin and the Fmoc amino acid mixture were shaken for 1 h at 30 °C. The peptide was cleaved by shaking the peptidyl resin with TFA/triisopropylsilane/H_2O (95 : 2.5 : 2.5) solution for 1 h. For HPLC analyses, a flow rate of 1.0 ml/min and a 20-min gradient from 10 to 40% solvent B (solvent A, 0.1% TFA in water; solvent B, 0.1% TFA/acetonitrile) was used with a Waters Symmetry C_{18} column (5 μm, 3.9 × 150 mm). Absorbance was measured at 220 nm.

JR 10-mer [23] (H-Trp-Phe-Thr-Thr-Leu-Ile-Ser-Thr-Ile-Met-NH_2) was prepared using Fmoc-Rink-AM resins (Table 4) in the same way as ACP (65–74). Trp side-chain was protected with Boc; Bu^t was used for the protection of Thr and Ser side-chains. The peptide was cleaved with TFA/thioanisole/1,2-ethanedithiol/anisole (90 : 5 : 3 : 2) solution for 1 h. For HPLC analyses, a flow rate of 1.0 ml/min and a 30-min gradient from 10 to 50% solvent B (solvent A, 0.1% TFA in water; solvent B, 0.1% TFA/acetonitrile) was used with a Waters Symmetry C_{18} column (5 μm, 3.9 × 150 mm). Absorbance was measured at 220 nm.

The fragment 27–38 of T1249 [24] (H-Gln-Lys-Leu-Asp-Lys-Trp-Ala-Ser-Leu-Trp-Glu-Trp-OH) was synthesized using Fmoc-Trp(Boc)-O-CT resins (Table 6) as in the synthesis of ACP (65–74), but using 2 equiv. of Fmoc amino acid. Trp and Lys side-chains were protected with Boc; Bu^t was used for the protection of Glu and Asp side-chains. The peptide was recovered by treating the peptidyl resin with TFA/triisopropylsilane/H_2O (95 : 2.5 : 2.5) solution for 1 h. For HPLC analyses, a flow rate of 1.0 ml/min and a 30-min gradient from 10 to 50% solvent B (solvent A, 0.1% TFA in water; solvent B, 0.1% TFA/acetonitrile) was used with a Waters Symmetry C_{18} column (5 μm, 3.9 × 150 mm). Absorbance was measured at 220 nm.

Swelling Measurement

Dry resin samples (0.50 g) were weighed and placed in a 10-ml graduated cylinder (i.d., 0.8 cm; length, 20 cm). Excess solvent was then added. The samples were allowed to swell for 1 h at 25 °C. After removing air bubbles with a small spatula and sealing the cylinder, the samples were allowed to stand for 24 h at 25 °C. The swelling volume was recorded as milliliter per gram of the resin.

Fmoc-Titration

Resin sample (30.0 mg) was suspended in 3.0 ml of 20% piperidine/DMF at 25 °C for 50 min. Aliquots (0.10 ml) of the mixture were withdrawn and diluted to 10.0 ml with DMF. The absorbance of the diluted solution was measured at 290 nm using DMF as blank solution [25].

RESULTS AND DISCUSSION

The SS resin, which consists of a flexible shell layer and a rigid core, was obtained by lightly cross-linking the shell layer onto 1 or 2% DVB-cross-linked PS resin. To construct this resin structure, styrene or styrene–DVB (0.5%) mixture was polymerized onto the 1 or 2% DVB-PS resin of 170–200 mesh size. Thus, four SS resins were prepared with varying degrees of cross-linking in the core and in the shell layer (Table 1). Although the resin beads partially aggregated during the suspension polymerization, the final resin was mostly obtained in fine bead form with a 40–50% yield after 100–170 mesh sieving. The resin beads increased in size and retained their original shape after polymerization and sieving (Figure 1). Furthermore, SEM images revealed a clean surface morphology of the SS resins (Figure 2).

This architecture has previously been reported as 'interpenetrating polymer networks' and the swelling volume of such resins was reported to be lower than that of primary polymer networks. The reduced swelling

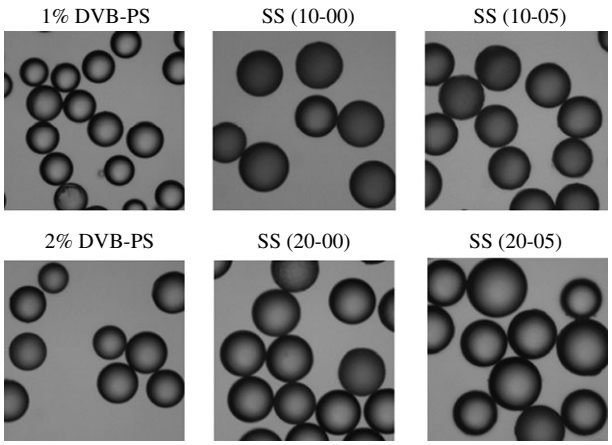

Figure 1 Microscopic images of DVB-PS resins (170–200 mesh) and soft shell (SS) resins (100–170 mesh) (equal magnification).

DOI: 10.1002/psc

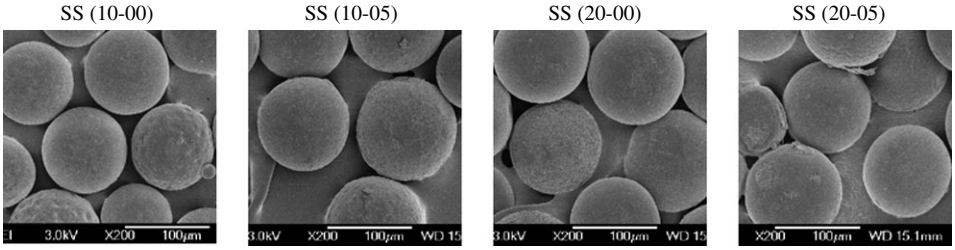

Figure 2 SEM images of soft shell resins (100–170 mesh).

property is attributed to the entanglement of the growing polymer chains within the seed resin beads during polymerization, thus increasing 'physical cross-linking' in the resin beads, without affecting covalent cross-linking [26]. Likewise, SS resins exhibited a similar swelling behavior; SS (10–00) resin and SS (10–05) resin had a lower swelling volume than 1% DVB-PS in DCM. SS (20–00) resin and SS (20–05) resin also had lower swelling volumes than 2% DVB-PS (Table 2). In addition, their functionalized resins (aminomethyl resins) showed similar swelling tendencies (Table 2).

Generally, higher resin swelling results in a more facile solid-phase reaction. However, the SS resin, except for SS the (20–05) resin, exhibited comparable or higher performance in the aminomethylation reaction compared to PS resins, despite their low swelling properties (Tables 2 and 3), indicating that the SS resins possess more flexible polymer chains at the shell layer and therefore easily accessible to the aminomethylation.

To evaluate SS resins in SPPS, JR 10-mer [23] and ACP (65–74) [22] were synthesized on Fmoc-Rink-AM resins using Fmoc/Bu^t chemistry. The synthetic yields from SS (10–00) and SS (10–05) resins were comparable to that from 1% DVB-PS resin in JR 10-mer synthesis, while SS (20–00) resin produced lower yields (Table 4). The highest yield was obtained using the SS (20–05) resin. However, this could be attributed to its lower loading level (i.e. 0.16 mmol/g resin). In the synthesis of ACP (65–74), all resins exhibited comparable efficiencies (Table 4). Among the SS resins, the synthetic performance of SS (10–00) resin was almost equal to 1% DVB-PS despite its lower swelling. SS resin has more cross-linking than 1% DVB-PS mainly because of 'physical cross-linking', which results in decreased resin swelling. Nevertheless, the polymer chains are more freely accessible for chemical reactions because the physically cross-linked polymer chains can move freely within the local region.

In addition to the aminomethyl group, the CT group was also introduced into the SS resins using the 'pseudo-activated linker' method [21]. This method was reported to mainly functionalize the surface layer of resin beads, owing to the sterically hindered character of the pseudo-activated linker. The CT-SS resins were

Table 2 Swelling properties of PS resins and aminomethyl (AM) resins

Resin	Swelling volume (ml/g resin)					
	PS resin		Soft shell resin			
	1% DVB	2% DVB	10–00	10–05	20–00	20–05
PS (in DCM)	7.8	5.6	7.3	6.4	4.9	4.8
AM resin (in DMF)	7.6	5.2	7.1	6.2	4.8	4.4

Table 3 Loading levels of aminomethyl (AM) resins (mmol/g resin)[a]

	PS resin		Soft shell resin			
	1% DVB	2% DVB	10–00	10–05	20–00	20–05
Loading level of AM resin	0.88	0.47	0.90	0.91	0.62	0.17

[a] Calculated from Fmoc-titration of Fmoc-Gly-OH coupled AM resin.

Table 4 Yields of peptide fragments synthesized using Fmoc-Rink-AM resins[a]

Peptide fragment	Yield (%)[b]				
	PS resin	Soft shell resin			
	1% DVB	10–00	10–05	20–00	20–05
JR 10-mer	34	31	30	18	40
ACP(65–74)-NH_2	79	80	77	81	76

[a] Loading levels of Fmoc-Rink-AM resins were 0.53 (1% DVB-PS), 0.54 (SS 10–00), 0.62 (SS 10–05), 0.47 (SS 20–00) and 0.16 (SS 20–05) mmol/g resin.
[b] Yields are calculated from crude yield by multiplying HPLC purity.

DOI: 10.1002/psc

prepared by 2-chlorotritylation, and Fmoc-Trp(Boc)-O-CT-SS and Fmoc-Gly-O-CT-SS resins were obtained after coupling Fmoc-Trp(Boc)-OH and Fmoc-Gly-OH to the activated CT-SS resins, respectively. Only SS (10–00) and SS (10–05) were used to generate CT resins, because SS (20–00) and SS (20–05) had relatively low swelling volumes and loading capacities (Tables 2 and 3).

CT-SS (10–00) resin had the same swelling volume as CT 1% DVB-PS resin, while CT-SS (10–05) resin had lower swelling. However, tritylation of SS (10–00) resin was slightly more efficient than that of the other resins (Table 5). Notably, despite the similar swelling, SS (10–00) resin had higher loading in tritylation than 1% DVB-PS resin, as was also observed in aminomethylation.

To test the synthetic efficiency of CT resins, T1249 (27–38) [24] and ACP (65–74) were synthesized using the Fmoc/Bu^t strategy (Table 6). In the synthesis of T1249 (27–38), CT-SS (10–00) resin was more efficient than CT-1% DVB-PS and CT-SS (10–05) resins. Moreover, CT-SS (10–00) resin produced ACP (65–74) in much higher purity than CT-1% DVB-PS (90% *vs* 75%), while CT-SS (10–05) resin was not satisfactory. The higher performance of CT-SS (10–00) resin is due to the flexibility of the linear PS chains in the surface layer. As the polymer chains in SS (10–00) resin are physically cross-linked, and not chemically cross-linked, they are more flexible and accessible than those of 1% DVB-PS and other SS resins. On the other hand, the chains of SS (10–05) resin are restricted in motion because of more physical cross-linking, even though the polymer chains in the surface layer were less cross-linked than in 1% DVB-PS. These results indicate that chain entanglement (physical cross-linking) was severe during seed suspension polymerization.

Table 5 Swelling volumes and loading levels of 2-chlorotrityl (CT) 1% DVB-PS and soft shell (SS) resins

	PS resin	SS resin	
	1% DVB	10–00	10–05
Swelling volume of CT resin in DCM (ml/g resin)	5.6	5.6	5.2
Loading level[a] of CT resin (mmol/g resin)	1.0	1.1	0.9

[a] Calculated from Fmoc-titration of Fmoc-Gly-O-CT resin.

Table 6 Yields of the peptide fragment synthesized using Fmoc-Trp(Boc)-O-CT resins and Fmoc-Gly-O-CT resins

Peptide fragment		PS resin	SS resin	
		1% DVB	10–00	10–05
T1249(27–38)	Yield (%)[a]	80	87	78
	Loading level[b]	0.47	0.58	0.54
ACP(65–74)	Yield (%)[a]	75	90	60
	Loading level[c]	0.60	0.42	0.41

[a] Yields are calculated from crude yield by multiplying HPLC purity.
[b] Loading level of Fmoc-Trp(Boc)-O-CT resins (mmol/g resin).
[c] Loading level of Fmoc-Gly-O-CT resins (mmol/g resin).

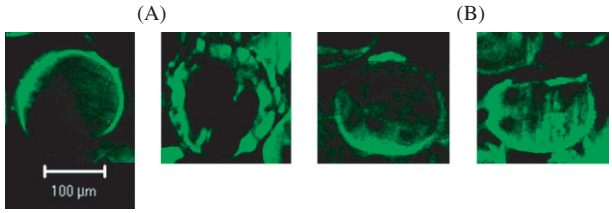

Figure 3 Confocal microscopic images of cross-sectioned resin beads: (A) FITC-Trp(Boc)-O-CT-1% DVB-PS resin and (B) FITC-Gly-O-CT-SS (10–00) resin.

Besides the above-mentioned factors related to resin performance, the distribution of reactive sites in the resin beads is also important in SPPS [18–20]. Thus, fluorescence-labeled CT resins were analyzed by confocal microscopy after physically slicing the resin beads [27,28]. As shown in Figure 3, the functional groups of CT-SS (10–00) resin and CT-1% DVB-PS resin are more densely populated in the surface layer. Although both are core-shell type resins, CT-SS (10–00) resin was better than CT-1% DVB-PS resin in peptide synthesis. The flexibility of the polymer chains in the SS resin is thought to contribute to the higher performance (Table 6).

Comparing the results from aminomethyl resins, we found that CT resins exhibited greater differences in synthetic performance. It is believed that the synthetic efficiency could be improved by the flexibility of the polymer chains and the accessibility originating from the core–shell structure. Among the resins studied, SS (10–00) resin showed the highest efficiency in peptide synthesis. HPLC data and mass analyses of the peptides fragments obtained from SS (10–00) resin are shown in Figure 4.

CONCLUSIONS

SS resins with a lightly or noncross-linked surface layer were prepared by decreasing the amount of DVB in seed suspension polymerization using 1or 2% DVB-PS resin as the seed particle. The swelling volume of the resulting resins was less than that produced by normal cross-linking (1 or 2%), owing to the entanglement of the polymer chains. In peptide synthesis, however, the SS (10–00) resin that has linear chains in the surface layer exhibited higher efficiency than 1% DVB-PS and other

DOI: 10.1002/psc

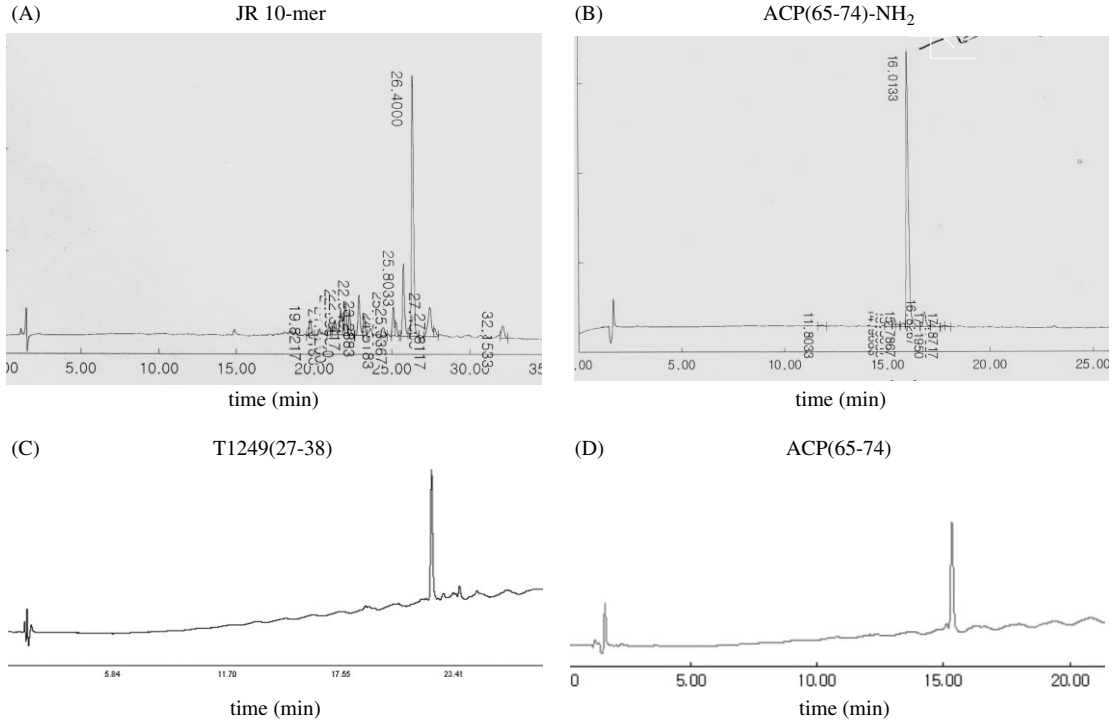

Figure 4 HPLC chromatograms of peptide fragments synthesized using Fmoc-Rink-AM-SS (10–00) resin (A and B) or CT-SS (10–00) resin (C and D): (A) JR 10-mer ($[M + Na]^+$ 1233.7, calcd 1233.6), (B) ACP(65–74)-NH_2 ($[M + Na]^+$ 1083.5, calcd 1084.5), (C) T1249(27–38) ($[M + H]^+$ 1588.4, calcd 1589.8) and (D) ACP(65–74) ($[M + Na]^+$ 1085.4, calcd 1085.5).

SS resins. This can be attributed to the flexibility of the polymer chains in the surface layer. The combination of the flexible, linear chains at the surface layer and the core–shell structure of the resin improved the synthetic efficiency in SPPS.

Acknowledgements

This study was supported by a grant from the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (A050432).

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