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Core–Shell-Type Resins for Solid-Phase Peptide Synthesis: Comparison with Gel-Type Resins in Solid-Phase Photolytic Cleavage Reaction

Hanyoung Kim, Jin Ku Cho, Woo-Jae Chung, and Yoon-Sik Lee*

School of Chemical Engineering, Seoul National University, Seoul 151-744, Korea

yslee@snu.ac.kr

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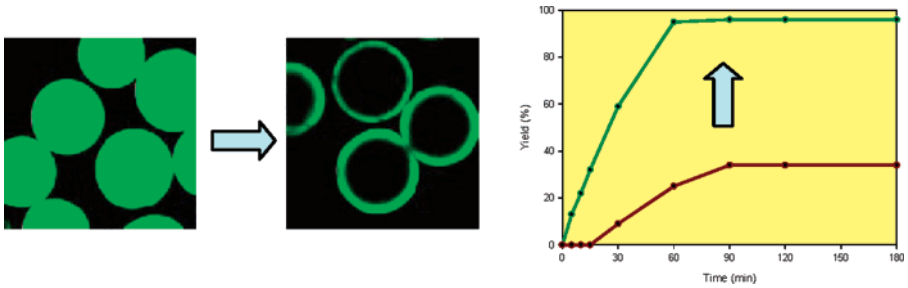
ABSTRACT



Novel core–shell-type resins with a rigid core and amino-functionalized flexible shell were prepared with 2,4,6-trichloro-1,3,5-triazine (CNC) and Jeffamine ED-600 starting from 1% cross-linked aminomethyl (AM) polystyrene resins. All of the amino groups were located outside the resin beads, and the loading capacity was 0.2–0.4 mmol/g. The amount of CNC treated was a determining factor in the properties of the final resins. The core–shell-type resins showed superior performances in terms of the initial loading of amino acid and the photocleavage reaction compared to the gel-type resins.

Since solid-phase synthesis was introduced by Merrifield, this protocol has been the standard for the preparation of peptides/oligonucleotides, and a huge number of solid-phase organic reactions have been developed for the purpose of combinatorial approach to find hits.[1] In general, lightly (1−2%) cross-linked gel-type resins are employed as the supports for solid-phase reactions. However, these gel-type resins require a suitable swelling ability for the diffusion of the reagents, which limits the choice of solvents and their usage for continuous flow systems.[2] Even if the resins are fullyswollen, they present an obstacle to the access of large molecules such as enzymes[3] or the penetration of light into the resin beads.[4]

In an effort to overcome these fundamental drawbacks, we previously proposed the concept of the core−shell type of resin, in which the rigid core can provide good mechanical properties and the functionalized flexible shell can allow facile contact of the reagents. These resins were prepared by suspension polymerization and exhibited successful behaviors in photolytic and enzymatic cleavage reactions.[5] The resins, however, suffered from relatively low loadings (0.05−0.2 mmol/g), and the preparation of the macromonomers was overly complicated. Here, we wish to report the

(1) (a) Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149. (b) Letsinger, R. L.; Mahadevan, V. *J. Am. Chem. Soc.* **1965**, *87*, 3526. (c) *Combinatorial Chemistry: Synthesis, Analysis, Screening*; Jung, G., Ed.; Wiley-VCH: Weinheim, 1999. (d) *The Combinatorial Index*; Bunin, B. A., Ed.; Academic Press: San Diego, 1998.

(2) (a) Kress, J.; Zanaletti, R.; Rose, A.; Frey, J. G.; Brocklesby, W. S.; Ladlow, M.; Bradley, M. *J. Comb. Chem.* **2003**, *5*, 28. (b) Yan, B.; Fell, J. B.; Kumaravel, G. *J. Org. Chem.* **1996**, *61*, 7467. (c) Hodge, P. *Synthesis and Separations using Functional Polymers*; Sherrington, D. C., Hodge, P., Eds.; John Wiley and Sons: New York, 1988. (d) Regen, S. L. *Macromolecules* **1975**, *8*, 689.

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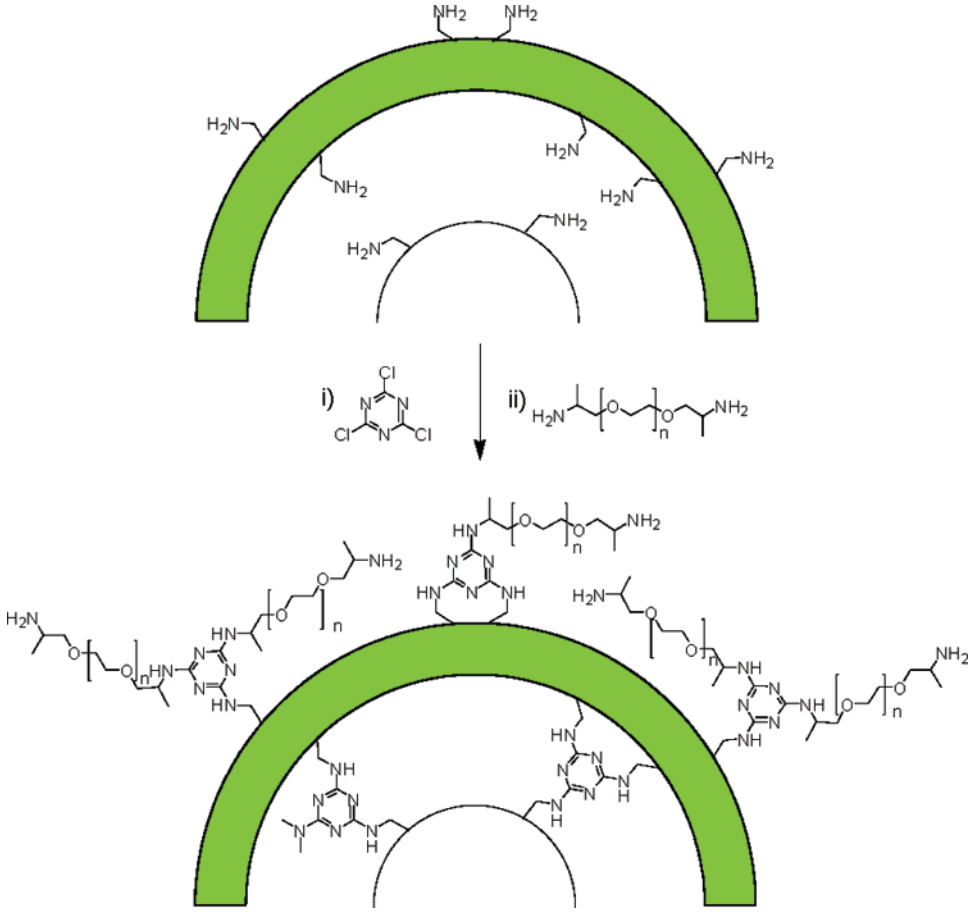
Scheme 1. Preparation of Core−Shell Resins



synthesis of novel core−shell (CS) resins readily prepared from commercially available aminomethyl polystyrene (AM-PS, BeadTech[6]) and their characteristics and performances in solid-phase reactions.

Initially, AM-PS resin (1% cross-linked) was further cross-linked with 2,4,6-trichloro-1,3,5-triazine (cyanuric chloride, CNC).[7] Then, the resultant resins were treated with an excess of *O*,*O*′-bis(2-aminopropyl) poly(ethylene glycol) 500 (Jeffamine ED-600, MW ∼600). At this stage, the long-chained Jeffamines were expected to selectively couple to the active chloride groups of the CNC outside the resin beads. Finally the remaining chloride on the resins was capped with dimethylamines (Scheme 1).

Although the resulting CS resins were less swellable than the gel-type resins as a result of their higher degree of cross-linking, their amphiphilic poly(ethylene glycol) shell permitted them to be compatible with a wide range of hydrophilic/hydrophobic solvents. In the procedure described above, we considered the amount of CNC added to be a determining factor in the final resin properties, because CNC plays the role of both cross-linkers and precursor of the amino-functional groups. Therefore, three types of resins were prepared from the AM-PS resin according to the amount of CNC (0.5−2, 3−5, 7−10 equiv of initial amino groups, respectively). After coupling with Jeffamine and coupling of the resulting amino groups of each CS resin with FTIC (5-fluorescein isothiocyanate), their cross-sectioned views were observed using a confocal fluorescent microscope.[8] TentaGel-NH_2 (0.3 mmol/g) and AM-PS resins (1.1 mmol/g) were both examined after FTIC coupling. From the confocal microscope images, we found that the TentaGel and AM-PS resin-bound FTIC was located across the whole region of the resin beads (Figure 1a and 1b, respectively). On the other hand, the CS resin-bound FTIC existed only on the surface area. In particular, the interior structures of the CS resins appeared to vary depending on the amount of CNC treated. When a small amount of CNC was used, the shape of the shell was irregular and some cracks were observed (CS resin I, Figure 1c). When higher amounts of CNC were used, thicker shell was formed (CS resin II and III, Figure 1d and 1e). The loading level of each CS resins was determined by means of the Fmoc photometric test after Fmoc-Leu coupling.[9] We found that the loadings of CS resin I were rather low, namely, 0.03−0.1 mmol/g. Such a low

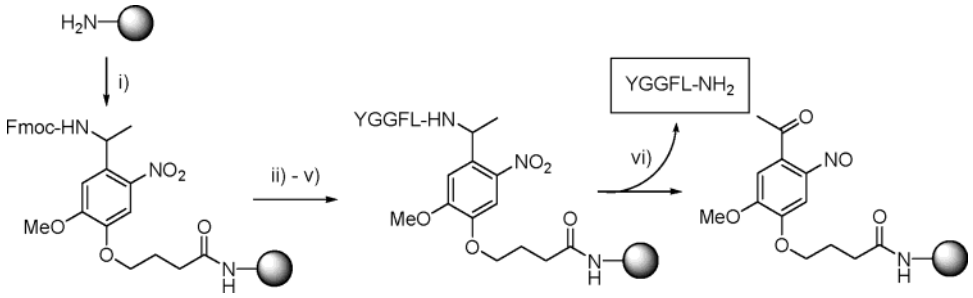
(6) http://www.beadtech.co.kr.

(7) No remaining amino group was left, which was confirmed using a qualitative ninhydrin test.

(8) (a) Kang, J.; Schwendeman, S. P. *Macromolecules* **2003**, *36*, 1324. (b) Heinemann, M.; Limper, U.; Buchs, J. *J. Chromatogr., A* **2004**, *1024*, 45.

(9) Average of three experiments (Fmoc-titration).

Scheme 2. SPPS and Photocleavage Reactions[a]



[a] Reagents and conditions: (i) Fmoc-protected 4-[4-(1-aminoethyl)-2-methoxy-5-nitrophenoxy]butyric acid, BOP, DIPEA, NMP; (ii) 25% piperidine in NMP; (iii) Fmoc-amino acid, BOP, HOBt, DIPEA, NMP; repeat (ii) and (iii); (iv) 25% piperidine in NMP; (v) 95% TFA, 2.5% triethylslane, 2.5% H_2O; (vi) *hν*, 360 nm, MeOH.

loading level proves that most of the CNC was used up in the cross-linking reactions and, consequently, there were very few active chlorides left to couple with the Jeffamine. The loadings of CS resin II and CS resin III were 0.2−0.4 and 0.5−0.7 mmol/g, respectively. However, when we tried to load Fmoc-Leu on the CS resin III, complete attachment was possible only after four repeated couplings, which indicates that the local density of amino groups on the shell was too high for the reagents to obtain free access. On the basis of the above results, we chose CS resin II as the optimal core−shell resin.

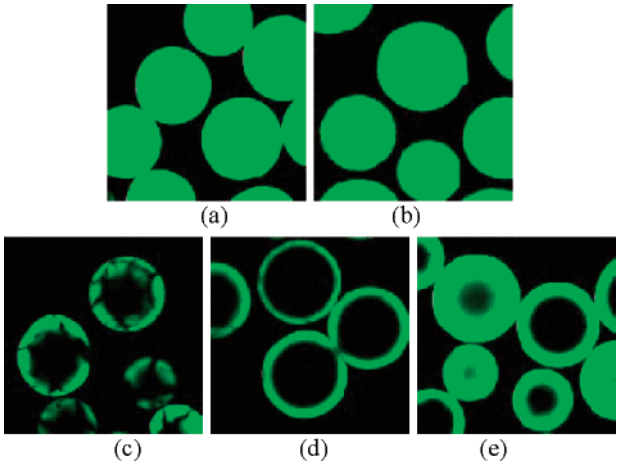


Figure 1. Cross-sectioned confocal microscope images of FITC-coupled resins: (a) TentaGel resin (0.3 mmol NH_2/g); (b) AM-PS resin (0.3 mmol NH_2/g); (c) CS resin I (0.5−2 equiv of CNC used, 0.03−0.1 mmol NH_2/g); (d) CS resin II (3−5 equiv of CNC used, 0.2−0.4 mmol NH_2/g); (e) CS resin III (7−10 equiv of CNC used, 0.5−0.7 mmol NH_2/g).

To confirm the performance in solid-phase synthesis, CS resins were applied to the photocleavage reaction, which is often preferable to using acidic or basic conditions owing to its orthogonality. The *o*-nitrobenzyl photocleavable linker, Fmoc-protected 4-[4-(1-aminoethyl)-2-methoxy-5-nitrophenoxy]butyric acid was attached to the resin and Leu-enkepalinamide was synthesized by Fmoc-chemistry.[10] The final peptide was released by UV light at 360 nm (Scheme 2).[11] The results with TentaGel-NH_2 resin (0.30 mmol/g) and AM-PS (1% cross-linked) resin were compared with those of CS resin (0.37 mmol/g). For the sake of obtaining a fair comparison, the original loadings of PS resin (1.1 mmol/g) were reduced by controlling the equivalence of the photocleavable linker during the coupling. Finally, 0.27 and 0.58 mmol/g of the PS resin-bound photolinker were obtained, and the remaining free amino groups were capped by acetylation.

Interestingly, in the course of assembling the peptide on the resins, we found that the loading of the first amino acid on the CS resin proceeded much more efficiently than that on the other resins. In the solid-phase organic synthesis, the initial loading is known to be troublesome step because of the matrix effect of the polymer backbone.[12] However, this problem could be minimized on the CS resins.

To examine the detailed kinetics, the coupling yields of Fmoc-Leu were investigated according to the coupling time.

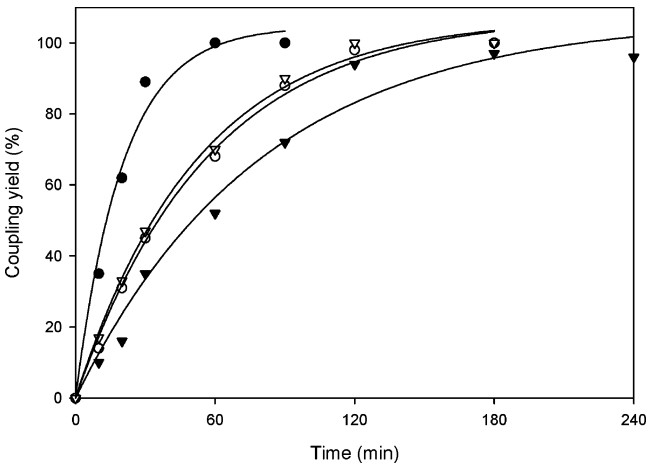


Figure 2. Initial loading time of Fmoc-Leu on the resins: (●) CS resin, (▽) TentaGel resin, (○) LL PS resin, (▼) HL PS resin.

The coupling rate on the CS resin was approximately 2−4 times faster than that on TentaGel or the low-loading (LL) PS resin and high-loading (HL) PS resin (Figure 2).[13]

After the solid-phase peptide synthesis, photocleavage reactions were carried out on each resin. As expected, compared to the other resins, the CS resin exhibited an excellent performance in the photocleavage reaction. The CS resin released the product rapidly and gave more than 95% yield within 1 h. In contrast, TentaGel resin released just 72% of the product, and LL PS resin and HL PS resin released only 41% and 34% of the product, respectively, even after 3 h (Figure 3). The difference in yield between TentaGel and PS resins may be partly due to the swelling difference in methanol.[14] Unlike the gel resins, the performance of the CS resin was not related to the swelling ability.

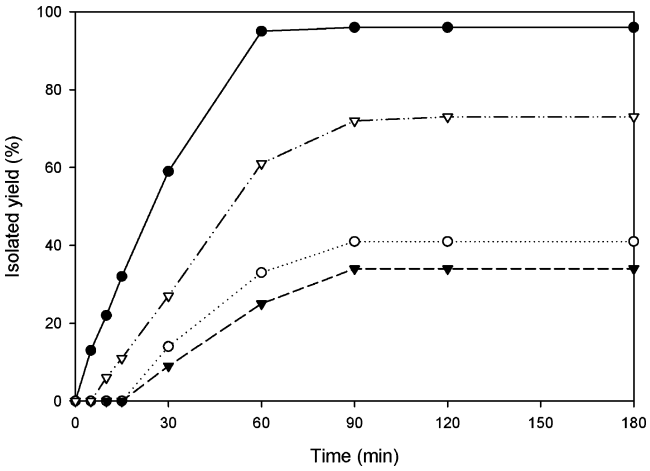


Figure 3. Kinetic profiles of photolytic cleavage on each resin: (●) CS resin, (▽) TentaGel resinm (○) LL PS resinm (▼) HL PS resin.

In conclusion, we developed novel core−shell-type resins, in which the functional groups were located on the shell layer. They were readily prepared from 1% cross-linked PS resins via two reaction steps. Regardless of the swelling ability, the core−shell resin showed excellent results in the photocleavage reaction. In addition, the initial loading onto the CS resins progressed more efficiently than that on the other gel-type resins. Further studies on the enzyme compatibility of the CS resin and the optimization of the length of the PEG chains are underway.

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Supporting Information Available: Experimental procedures for the preparation of CS resins, solid-phase peptide synthesis, swelling properties of resins, HPLC chromatogram, and MS spectra of peptides. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) The product was isolated with cold ether and analyzed with HPLC and MALDI-TOF MS.
(12) (a) Li, W.; Yan, B. *J. Org. Chem.* **1998**, *63*, 4092. (b) Basso, A.; Evans, B.; Pegg, N.; Bradley, M. *Tetrahedron Lett.* **2000**, *41*, 3763.
(13) We used rather stringent condition (equimolar addition of Fmoc-Leu, HOBt, BOP, and DIEA, 2 equiv each) on purpose for comparison of the CS resin and TentaGel. When we used an excess amount of DIEA (1.5 equiv of other reagents), the CS resin and TentaGel showed similar properties in the first amino acid coupling.
(14) Usually, methanol or PBS buffer has been used as a solvent in this type of reaction, because the cleaved product is water- or methanol-soluble free peptide.[10]