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Tetrahedron Letters
Tetrahedron Letters 46 (2005) 7135–7138

Application of AM *SURE*™ resin to solid-phase peptide synthesis

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Received 18 July 2005; revised 11 August 2005; accepted 19 August 2005
Available online 6 September 2005

Abstract—A core-shell structure was found in AM *SURE*™ resin, in which most amino groups were located at the skin layer. The AM *SURE*™ resin revealed better synthetic efficiency, compared to the noncore-shell type resin in the synthesis of the fragment 65–74 of acyl carrier protein, the fragment 27–35 of HIV (human immunodeficiency virus) fusion inhibitor (T-20) and the Jung–Redemann 10-*mer*.

Since the advent of solid-phase synthesis,[1] various polymer supports have been developed to improve synthetic efficiency of peptides, nucleotides or small organic compound libraries. Among the various supports, 1% divinylbenzene (DVB) cross-linked polystyrene (PS) resin beads have proven to be the most suitable and so far have been widely used, due to their mechanical/chemical stability and facility of functionalization. However, they have been modified further in several ways to overcome some limitations in solid-phase peptide synthesis (SPPS).

For example, solvent compatibility or good swelling is needed for free diffusion of reagents into the polymer matrix. Sheppard and co-workers prepared a polyamide resin which was more compatible with the reaction solvent and proved that the polyamide resin gave better results compared to those obtained from the conventional PS resin in the synthesis of acyl carrier protein fragment 65–74 (ACP 65–74).[2] Rapp introduced polyethylene glycol (PEG)-grafted PS resin, TentaGel (PS-g-PEG), which is more suitable than PS resin as an amphiphilic support for a rapid peptide synthesis.[3] Janda and co-workers introduced another type of polystyrene resins, *J*anda*J*el™ resin, which contained flexible cross-linkers that rendered the resin with more 'solvent-like' properties. The increased swelling of *J*anda*J*el™ resin, compared to the conventional PS based resin, gave a higher yield in the synthesis of ACP 65–74.[4]

The density of the functional group in the resin is also important. To demonstrate the occurrence of the interchain interactions during the peptide synthesis, Tam et al. prepared the PS resins with clustered and branched functional groups at a similar level of peptide loading. The peptide-couplings in the clustered functional group resin were more difficult than those in the dispersed functional group resin because of the steric hindrance and the poor accessibility near the reactive sites, resulting from the interchain hydrogen bonding in the clustered peptidyl resins.[5]

In addition to the above mentioned factors, the spatial distribution of the functional groups within the resin affects the property of the resin. For easy access of the reagents from the bulk phase and good reaction kinetics, we have designed several core-shell type resins. A copolymer of PEG macromer and styrene, CutiCore (polyethylene glycol-surface grafted PS (PS-sg-PEG)) resin, possessed a core-shell structure. The resin was more efficient than PS or TentaGel (PS-g-PEG) resin in the coupling of amino acids during the early stages of SPPS and in the photo-cleavage of peptides from the resin.[6] Another core-shell type resin was also prepared from aminomethyl polystyrene (AM PS) resin by cross-linking it with 2,4,6-trichloro-1,3,5-triazine (cyanuric chloride) and grafting further with diamino PEG.[7] It also gave superior properties in SPPS. Recently, AM

Keywords: Core-shell type; Aminomethyl polystyrene resin; Solid-phase peptide synthesis.
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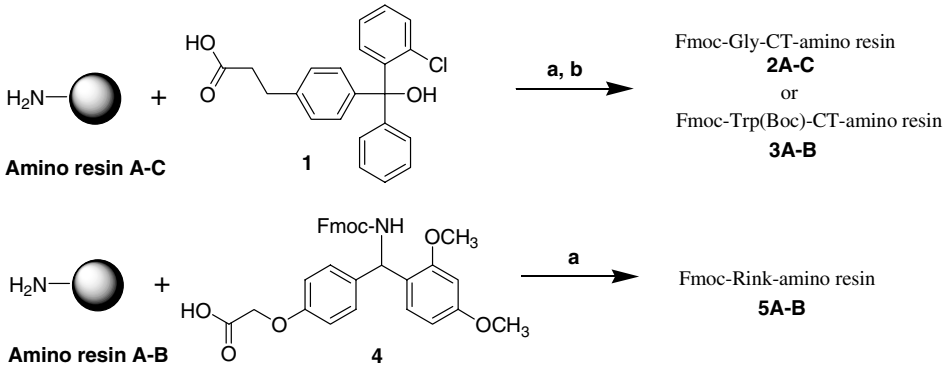
doi:10.1016/j.tetlet.2005.08.093



Scheme 1. Preparation of the Fmoc-Gly-OH loaded amino resins (**2A**–**C**), Fmoc-Trp(Boc)-OH loaded amino resins (**3A**–**B**) and Fmoc-Rink-amino resins (**5A**–**B**): (a) HBTU, HOBt, DIEA, NMP, 30 °C, 2 h; (b) (1) $SOCl_2$, MC; (2) Fmoc-Gly-OH or Fmoc-Trp(Boc)-OH, DIEA, MC; DIEA/MeOH (1:9).

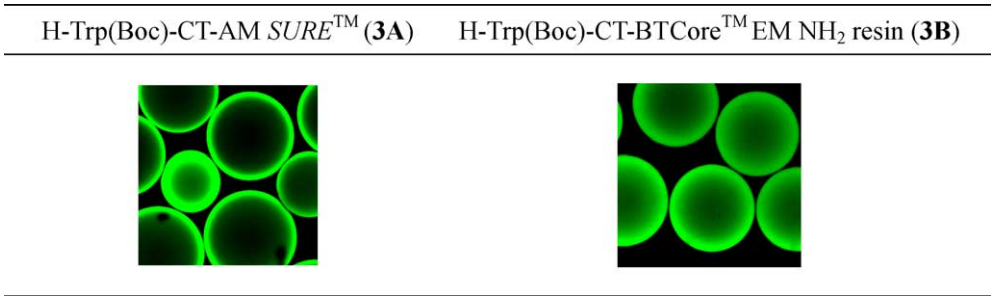


Figure 1. Confocal fluorescence images of FITC-coupled H-Trp(Boc)-CT-amino resins.

SURE™ (AminoMethyl SUrface-layered polystyrene REsin)[8] was prepared from PS (1% DVB cross-linked) resin by introduction and partial hydrolysis of the acetamidomethyl groups. All of the amino groups in the AM *SURE*™ were located at the skin layer, which was confirmed by a confocal microscopy (optical-slicing) and a physical slicing after fluorescence staining.[8] Until now, however, the correlation of a spatial distribution of the functional group to the synthetic efficiency in SPPS has not been studied thoroughly. In this letter, the advantages of the AM *SURE*™ resin over the noncore-shell type PS resins in SPPS will be described.

As a core-shell type resin, AM *SURE*™ (**A**), was prepared from PS (1% DVB cross-linked) resin.[8] BTCore™ EM NH_2 resin (2-aminoisopropyl PS resin) (**B**)[9] and aminomethyl polystyrene (AM PS) resin (**C**)[10] were used as the reference resins. To test the performance of the resins in SPPS, a 2-chlorotrityl type linker (**1**)[11] was coupled to the amino groups to give 2-chlorotrityl linker-coupled amino resins (CT-amino resins). After chlorination with thionyl chloride, Fmoc-Gly-OH and Fmoc-Trp(Boc)-OH were loaded to give Fmoc-Gly loaded amino resins (**2A**–**C**) and Fmoc-Trp(Boc) loaded amino resins (**3A**–**B**), respectively (Scheme 1). The distributions of the functional groups in the Fmoc-Trp(Boc)-CT-amino resins (**3A**–**B**) were investigated by the confocal microscopy after removing the Fmoc moiety and coupling with fluorescein isothiocyanate (FITC). As shown in Figure 1, the amino groups of H-Trp(Boc)-CT-AM *SURE*™ resin (**3A**), which originated from the core-shell type amino resin (**A**), were located at the shell layer. In the H-Trp(Boc)-CT-BTCore™ EM NH_2 (**3B**), which were derived from the noncore-shell type resin (**B**), however, the amino groups were located both in the core and the shell of the resins even though the amino groups seemed more concentrated at the shell layer.

To compare the synthetic efficiency of the core-shell type resin with noncore-shell resins, ACP 65–74 sequence was selected. ACP 65–74 is a well-known difficult sequence and its main by-product is a *des*-Val[65] sequence. The ACP 65–74 sequence was synthesized on the Fmoc-Gly-CT-amino resins (**2A**–**C**) using Fmoc/*t*-Bu chemistry, and then the peptides were cleaved with 95% TFA from the resins and analyzed with HPLC (Fig. 2).[12] The desired ACP 65–74 was obtained in 99% purity from the core-shell type resin (**2A**), while in 79%, and 77% purity from **2B** and **2C**, respectively. Furthermore, the common by-products, *des*-Val and *des*-Ala sequences, were detected in front of the ACP 65–74 peak in the noncore-shell type resin (**2B**) (Fig. 2b), but the core-shell type resin (**2A**) showed only the desired sequence without any truncated peptides (Fig. 2a).[13]

In another case, the synthetic efficiency of the fragment 27–35[†] of T-20 (T-20 27–35)[14] was investigated with the

[†]The structure of protected fragment 27–35 is Fmoc-Asp(*t*-Bu)-Lys(Boc)-Trp(Boc)-Ala-Ser(*t*-Bu)-Leu-Trp(Boc)-Ans(Trt)-Trp(Boc)-OH.

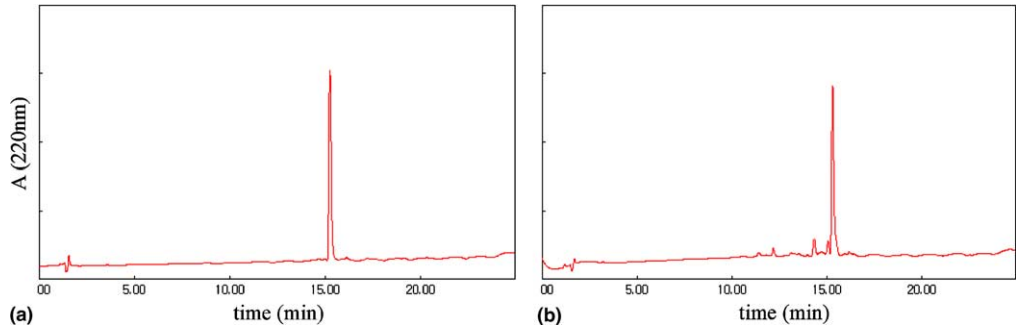


Figure 2. HPLC analyses of the crude product (ACP 65–74) cleaved from (a) AM *SURE*™ and (b) BTCore™ EM NH_2 resin.

Fmoc-Trp(Boc)-CT-amino resins (**3A**–**B**). The desired nonapeptide was synthesized using the Fmoc/*t*-Bu chemistry and cleaved with 1% TFA from the resin. The results of HPLC analyses of the product showed that the nonapeptide was obtained in 83% purity from the core-shell type resin (**3A**), but with 60% purity from the noncore-shell type resins (**3B**).[15]

We also applied AM *SURE*™ to the synthesis of the Jung–Redemann 10-*mer* (J-R 10-*mer*, H-WFTTLI-STIM–NH_2), which has been reported to be another difficult sequence.[16] Fmoc-Rink linker (**4**) was anchored onto the amino resins to give Fmoc-Rink-AM *SURE*™ (**5A**) and Fmoc-Rink-BTCore™EM NH_2 (**5B**), respectively (Scheme 1). After peptide elongations, the Jung–Redemann 10-*mer* was prepared in higher yield with **5A**.[17] From these results, we have confirmed that the AM *SURE*™ resin gave more satisfactory results in the synthesis of three peptides. This could be attributed to the core-shell structure of the resin since the functional groups at the surface are more accessible than those in the interior to the incoming amino acid derivatives. These results suggest that the spatial distributions of the functional groups within the resin are important to give better performance of the resin in SPPS.

In conclusion, we have prepared AM *SURE*™ resin in which the aminomethyl groups are located mostly at the surface layer. The core-shell type structure of AM *SURE*™ resin gave higher yields in the synthesis of three types of peptides, ACP 65–74, T-20 27–35 and J-R 10-*mer*, which proves that the distribution of functional groups within the resin beads is another important factor in determining the synthetic efficiency of peptides.

Acknowledgement

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

Supplementary data

Supplementary data associated with this article can be found, in the online version at doi:10.1016/j.tetlet.2005.08.093.

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8. Lee, T. K.; Ryoo, S. J.; Byun, J. W.; Lee, S. M.; Lee, Y. S. *J. Comb. Chem.* **2005**, *7*, 170–173, AM *SURE*™ is commercially available from BeadTech. Inc. (Seoul, Korea; http://beadtech.com).
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10. Aminomethyl resin (Advanced ChemTech, 1.1 mmol/g resin, 100–200 mesh, 1% DVB).
11. 2-Chlorotrityl type linker (**1**) is also available from BeadTech Inc.
12. ACP 65–74 was synthesized from Fmoc-Gly-CT amino resins (**2A**: 0.36, **2B**: 0.47, **2C**: 0.30 mmol/g resin). To the peptidyl resin was added the pre-formed activated ester, which was prepared using Fmoc-protected amino acid (2 equiv), HBTU (2 equiv), HOBt (2 equiv) and DIEA (4 equiv) in NMP. The resulting mixture was shaken for 1 h at 30 °C. Fmoc moiety was removed using 20% piperidine in NMP (3 × 7 min). The peptide was cleaved by shaking the resin with TFA/triisopropysilane/H_2O (95:2.5:2.5) solution for 1hr. For HPLC analyses, a flow rate of 1.0 mL/min and a 20 min gradient of 10–40% of solvent B (solvent A, 0.1% TFA in water; solvent B, 0.1% TFA/acetonitrile) were used with a Waters Symmetry® C_{18} column (5 μm, 3.9 × 150 mm). Absorbance was measured at 220 nm.
13. Three sequences were analyzed with ESI/MS along retention time of HPLC chromatogram (Fig. 2b): *des*-Val ($[M+Na]^+$ 986.4, calcd 986.5, 14.37 min), *des*-Ala ($[M+Na]^+$ 1014.5, calcd 1014.5, 15.10 min), ACP 65–74 ($[M+Na]^+$ 1085.4, calcd 1085.5, 15.33 min).
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15. The fragment 27–35 of T-20 was synthesized from Fmoc-Trp(Boc)-CT-amino resins (**3A**: 0.34, **3B**: 0.35 mmol/g resin) as in Ref. 12. The peptide was cleaved by washing

the resin with 1% TFA/MC solution and the cleaved mixture was collected in pyridine. For HPLC analyses, a flow rate of 1.0 mL/min and a 15 min constant flow of 99% solvent B followed by a 20 min gradient of 70–99% of solvent B (solvent A, 0.1% TFA in water; solvent B, 0.1% TFA/acetonitrile) were used with a Waters Symmetry® C_{18} column (5 μm, 3.9 × 150 mm). Absorbance was measured at 260 nm. ESI/MS of the protected fragment 27–35: $[M+H]^+$ 2184.5, calcd 2182.1.

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17. The J-R 10-*mer* was prepared with Fmoc-Rink-amino resins (**5A**: 0.46, **5B**: 0.48 mmol/g resin) as in Ref. 12, but using 3 equiv of Fmoc-protected amino acid. 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 of 10–50% of 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 J-R 10-*mer* was obtained in 40% yield with **5A** and 35% yield with **5B**. MALDI-TOF of J-R 10-*mer*: $[M+Na]^+$ 1233.7, calcd 1233.6.