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Ionic liquid incorporated polystyrene resin for solid-phase peptide synthesis

Hong-Jun Cho [a], Sang-Myung Lee [a], Sungwon Jung [b], Tae-Kyung Lee [c], Hyo-Jin Yoon [a], Yoon-Sik Lee [a,b,*]

[a] School of Chemical and Biological Engineering, Seoul National University, Seoul 151-744, Republic of Korea
[b] Department of Nano Science and Technology, Graduate School of Convergence Science and Technology, Seoul National University, Suwon 443-270, Republic of Korea
[c] Department of Chemistry, University of Texas at Dallas, Richardson, TX 75080, USA

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ABSTRACT

Ionic liquid (IL) resins with an ionic liquid environment on solid support were prepared by immobilizing ionic liquid spacers on polystyrene (PS) resin. The properties of IL resins were dramatically changed as the anions of IL were exchanged. The performance of IL resins for solid-phase peptide synthesis (SPPS) was evaluated by measuring coupling kinetics of the first amino acid and synthesizing several peptides on IL resins.

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Polystyrene (PS) resin is one of the most commonly used polymer supports in solid-phase peptide synthesis (SPPS).[1] It has high mechanical and chemical stability, as well as good swelling properties in various solvents. However, PS resin presents some problems because of the hydrophobic matrix, which causes difficulties in the diffusion of reagents and aggregation of growing peptides suspended on resin.[2,3] For these reasons, polymer backbones composed of polyamide or polyethylene glycol (PEG) were introduced to provide a hydrophilic environment on polymer supports.[2,4] Furthermore, ethylene glycol units have been used as cross linkers to increase the flexibility of the polymer matrix.[3,5] Since hydrophilic polymer supports are fully solvated by polar solvents and compatible with peptides on resin, they can afford high yield and purity of peptide in SPPS. Previously, we developed core–shell type resins to circumvent the problems caused by the hydrophobic matrix.[6] Core–shell type resins can minimize the influence of hydrophobic environments on the resin due to their distinctive structures. Despite many efforts, the controversy over the interaction of polymer matrix, peptide chain and solvent is continuing with heated discussions.

Recently, as part of an effort to synthesize peptides more efficiently, IL was introduced as a solvent[7] and a soluble support.[8] In peptide synthesis, coupling reagents and amino acid derivatives can be effectively dissolved in IL and stabilized for selective reactions.[7] In addition, an attractive feature of ILs is that its polarity and solubility can be easily modulated by changing the anion species. This unique characteristic feature facilitates the generation of controllable environments when ILs are used as a soluble support or as reaction media. Therefore, introducing versatile IL groups to PS resin may address weaknesses in the PS resin and enhance the efficiency of in coupling reactions.

Immobilization of imidazole groups on a solid support is one way of introducing an IL environment on a PS resin. Previously, ILs have been immobilized on solid supports, such as silica particles[9] and PS resin,[10] to develop heterogeneous catalysts. However, to the best of our knowledge, there has been no report on the application of IL conjugation to SPPS. Herein, we report the preparation of imidazolium IL coupled resins, which are designed to allow variable IL environments with improved performance in SPPS.

The loading method of the imidazolium group on a polymer support is well known with respect to heterogeneous metals or Lewis acid catalysts.[9,10] However, imidazolium IL resin for solid-phase peptide synthesis requires an extra amine/hydroxy group, from which linkers or amino acids are elongated. Thus, 1-(3-aminopropyl)imidazole (API) was chosen as an imidazolium precursor and a linker for SPPS. Before introducing the imidazolium group on chloromethyl PS resin, the free amine group was protected by Boc group to obtain Boc-API (**1**) in 79% yield (Scheme 1). Then, CMPS resin was treated with an excess amount of compound **1**. The existence of imidazolium in the IL resin was verified by FT–IR (carbamate band: 1699 cm^{-1}, quaternary imidazolium band: 1162 cm^{-1}, Suppl. Fig. 1). The loading level of theimidazolium group on IL resin (**3A**) was determined to be 1.2 mmol/g by measuring nitrogen contents and Fmoc titration after coupling Fmoc-OSu. To obtain imidazolium IL resins which have different anions (BF_4^-, OTf^-, PF_6^- and $TFSI^-$), the IL resin (**3A**) was treated with an excess amount of $NaBF_4^-$, $NaOTf^-$, $NaPF_6^-$, or $LiTFSI^-$ in DMF/H_2O (1:1,

* Corresponding author. Tel: +82 2 880 7073; fax: +82 2 876 9625.
E-mail addresses: yslee@snu.ac.kr, cho0122@snu.ac.kr (Y.-S. Lee).

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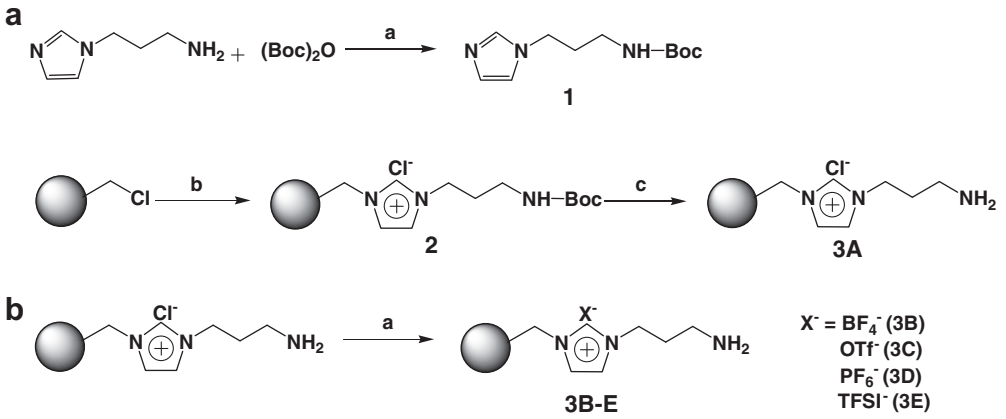

Scheme 1. Reagents and conditions: (a) synthesis of 1-[(*N*-*tert*-butoxycarbonyl)aminopropyl]-3-(3-triethoxysilylpropyl)imidazolium(**1**) and preparation of IL resin (**3A**): (a) $NaHCO_3$ in H_2O and THF, rt, 4 h; (b) compound **1**, NMP, 80 °C, 24 h; (c) TFA, DCM, rt, 2 h, and (b) process of anion exchange of IL resin: (a) $NaBF_4$, NaOTf, $NaPF_6$, LiTFSI in H_2O/DMF(1:1, v/v).

v/v) (Scheme 1b). The anions of imidazolium on the resin were easily identified by energy dispersive X-ray spectroscopy (EDS) (Suppl. Fig. 2). Traces of chloride ions in **3B–E** resins and fluoride ions in **3A** resin were observed, but a majority of original anions were mostly changed in each resin.

The swelling property of resins is the primary criterion for estimating solvent compatibility, which affects diffusion of the reagents into the resin and ultimately the synthetic efficiency in SPPS. When the swelling properties were compared in various solvents, there were considerable differences in each solvent depending on the anions (Suppl. Table 1). In general, IL resins with hydrophilic anions had a tendency to swell in polar solvents, while they were not completely compatible in less polar organic solvents such as DCM, THF, and hexane. It has been known that a polymer-supported ionic liquid system swells considerably in polar aprotic solvents such as DMF and DMSO and its swelling property can be controlled by anion.[11] In our cases, IL resins (**3B–E**) also swelled well in polar aprotic solvents (NMP and DMF), and especially **3E** resin displayed the highest swelling property in NMP. Because TFSI anion is the most hydrophobic among other anions (Cl^-, BF_4^-, OTf^- and PF_6^-), the ionic liquid with TFSI anion generates amphiphilic environment in polymer matrix which is presumably highly compatible with NMP or DMF.

To evaluate IL environment effects on peptide synthesis, the coupling reaction kinetics of the first amino acid most affected by polymer matrix on the resins was measured in NMP. Fmoc-His(Trt)-OH and Fmoc-Phe-OH, which were known to have poor and good loading yield, respectively,[12] were selected as model coupling amino acids. During the time course of the reaction, small portions of the resin were withdrawn from the reaction mixture and the coupling kinetics was examined by Fmoc titration. As shown in the line graph (Fig. 1a), coupling kinetics of Fmoc-His(Trt)-OH exhibited similar trends on IL resins and on AMPS resin except for**3A** resin. Because of difficulty in coupling Fmoc-His(Trt)-OH on most of the polymer supports, the environmental change of IL resins gave little influence on coupling kinetics. In the case of **3A** resin, the low swelling property might contribute to low coupling kinetics. However, in the Fmoc-Phe-OH coupling (Fig. 1b), the yield and coupling kinetics on IL resins were proportional to swelling property. In particular, **3D** and **3E** resins had much better coupling efficiency than the AMPS resin. Therefore, IL resins with TFSI or PF_6 anion and NMP as a solvent give the optimal condition for coupling of the first amino acid in SPPS. Based on these results, we confirmed that the initial coupling kinetics was mostly affected by swelling properties originated from the IL environments.

Finally, we applied imidazolium IL resins to SPPS to evaluate their synthetic performance. As a model pentapeptide, Leu-enkephalin (H-YGGFL-NH_2) was synthesized using Fmoc/*t*-Bu chemistry after anchoring Fmoc Rink amide linker on the IL resins and AMPS resin, respectively. Then, the peptide was cleaved and analyzed by HPLC and MALDI-TOF mass spectroscopy (Suppl. Figs. 3 and 4). Leu-enkephalin was recovered quantitatively in high purity (93–95% purity, Table 1) on IL resins (**3B–3E**). We found that **3A** resin showed relatively low yield (82%) and low purity (87%) due to the intrinsic property of IL[Cl^-]. However, IL resins generally exhibited better synthetic performance than AMPS resin (Table 1) and solution-phase synthesis[13] in which ionic liquid was used as a soluble support (overall yield: 50%, purity: 90%). As a result of synthesis of Leu-enkephalin, we can conclude that the synthesis of peptide on IL resins is more efficient than on AMPS resin or in ionic liquid, although the synthetic performance of IL resins can not be precisely compared with an easy sequence to be synthesized.

To further investigate the clear differences on the effect of anions and the IL environment in SPPS, Jung-Redemann 10-mer[14] (JR 10-mer, H-WFTTLISTIM-NH_2), which was known as one of the most difficult sequence to be synthesized, was synthesized on IL resins using Fmoc/*t*-Bu chemistry. JR 10-mer is a suitable peptide model for examining the efficiency of solid support in SPPS.[15] Peptide synthesis showed that JR-10mer was obtained in higher purities from IL resins (**3A–E**) than from AMPS resin (Table 1). In particular, the best resin for SPPS was IL[$TFSI^-$] resin (**3E**, 57% purity). The performance of IL resins appeared to depend on the swelling properties, especially loading of the first amino acid on the resin. However, despite having a similar swelling property to **3D** resin, AMPS resin exhibited the lowest performance in SPPS. These results are attributed to not only the swelling property of resins but also to the IL spacer group which can furnish the growing peptide with an adequate ionic environment within the polymer matrix and may inhibit the peptide on the resin from self-aggregation. Based on this, we demonstrated that the IL spacer could create an ideal environment for efficient SPPS.

In conclusion, we have prepared IL resins by introducing amine functionalized an imidazolium group to a PS resin. By changing the anions (Cl^-, BF_4^-, OTf^-, PF_6^- and $TFSI^-$) of the IL, IL resins revealed different properties based on the characteristics of each IL spacer group. For example, the swelling properties of IL resins were dramatically changed in different solvents. In particular, IL[PF_6^-] and IL[$TFSI^-$] resins swelled the most in NMP and performed well in initial loading of amino acids. They also achieved higher purity in solid-phase peptide synthesis than AMPS resin.

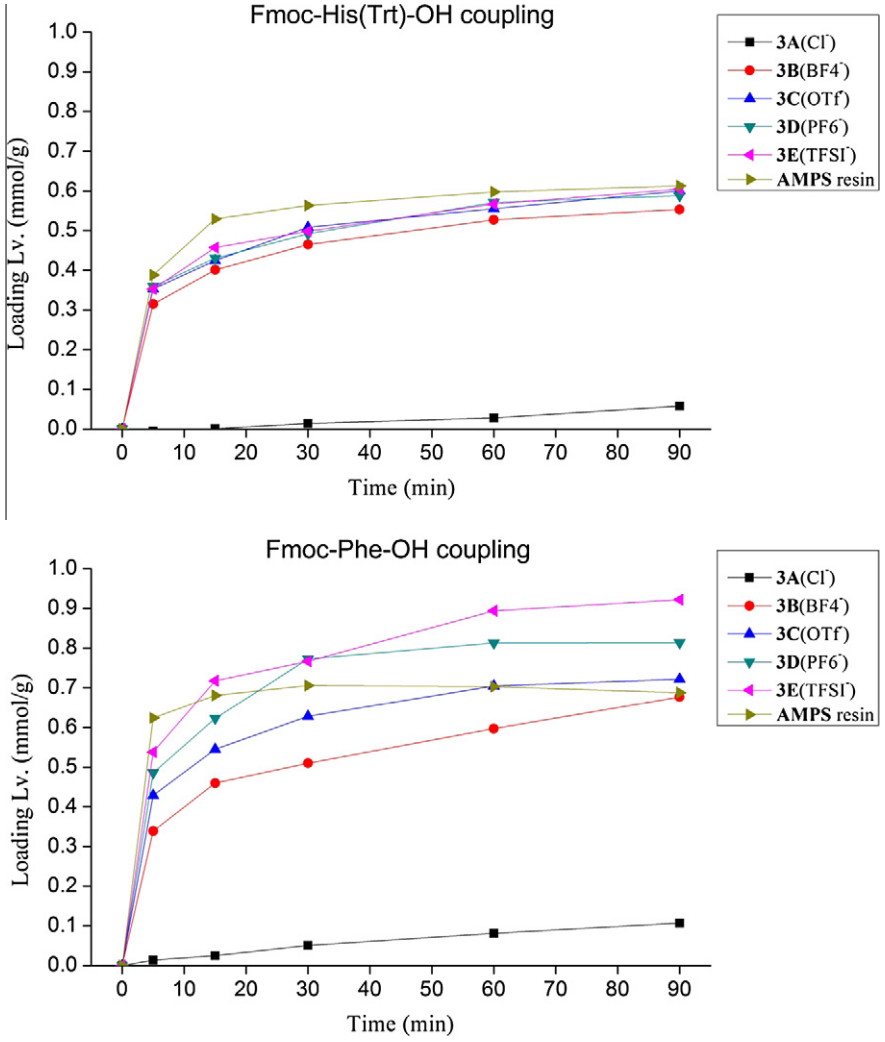

Figure 1. Coupling kinetics of Fmoc-His(Trt)-OH (a) and Fmoc-Phe-OH (b) on IL resins.

Table 1
Purity of peptides (Leu-enkephalin and JR 10-mer) using IL resins and AMPS resin

Resin	Leu-enkephalin (%)	JR 10-mer (%)
3A (Cl^-)	87	42
3B (BF_4^-)	95	48
3C (OTf^-)	93	43
3D (PF_6^-)	94	51
3E ($TFSI^-$)	94	57
AMPS resin	86	33

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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.2011.01.067.

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