공학석사학위논문

Preparation of PEGylated Core-Shell Type Polymer Supports for Peptide Synthesis and Immunoglobulin G Purification

펩타이드 합성과 면역글로불린 G의 정제를 위한 폴리에틸렌글리콜이 도입된 코어-쉘 형태의 고분자 지지체의 제조

2016년 2월

서울대학교 대학원

화학생물공학부

박 용 준

Preparation of PEGylated Core-Shell Type Polymer Supports for Peptide Synthesis and Immunoglobulin G Purification

펩타이드 합성과 면역글로불린 G의 정제를 위한
폴리에틸렌글리콜이 도입된 코어-쉘 형태의
고분자 지지체의 제조

지도 교수 이 윤 식

이 논문을 공학석사학위논문으로 제출함.

2016년 2월

서울대학교 대학원
화학생물공학부
박 용 준

박용준의 공학석사학위논문을 인준함.
2015년 12월

위 원 장 이 종 찬 (인)

부위원장 이 윤 식 (인)

위 원 한 지 숙 (인)

ABSTRACT

Preparation of PEGylated Core-Shell Type Polymer Supports for Peptide Synthesis and Immunoglobulin G Purification

Yong Jun Park

School of Chemical and Biological Engineering

The Graduate School

Seoul National University

Since Bruce Merrifield has introduced solid-phase peptide synthesis (SPPS) concept, various kinds of polymer supports have been developed for specific purpose. Polymer supports can be classified into gel type and core-shell type, according to the distribution of their functional groups. Reactions on the core-shell type resin are less influenced by diffusion of reagents because functional groups in the shell part are easily accessible. Therefore, core-shell type polymer support generally gives high purity of product. Meanwhile, hydrophilicity is also an important issue for bioassays, especially, on-bead ligand screening or protein immobilization.

In this thesis, water compatible core-shell type resins were prepared and their applications in SPPS and protein purification were described. Series of PEG grafted polystyrene resins were prepared from aminomethyl polystyrene (AM PS) resin. Swelling properties, physical shape of the core-shell type structure and the loading levels of PEG-PS resin were investigated. After characterization, the resins were tested for the purification of IgG, via immobilization of protein A onto the resins. The performance of IgG purification was also discussed. In order to assess the performance of the resin in SPPS, model peptide sequences were synthesized on the resin and their purity and the yields were compared to the ones on HiCore® resin.

***Keywords*:** core-shell type resin, water compatible resin, solid phase peptide synthesis, Protein A, IgG purification

Student number: 2014-20581

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SCHEMES

LIST OF ABBREVIATIONS

AcOH	Acetic acid
BOP	Benzotriazol-1-yloxy-tris(dimethylamino) Phosphonium hexafluorophsphate
BSA	Bovine serum albumin
DCM	Dichloromethane
DIC	Diisopropylcarbodiimide
DI water	Deionized water
DIPEA	*N*,*N*-Diisopropylethylamine
DMF	*N*,*N*-Dimethylformaide
EA	Ethyl acetate
ESI-MS	Electrospray ionization-mass spectrometry
EtOH	Ethanol
FITC	Fluorescein isothiocyanate
Fmoc	9-Fluorenylmethyloxycarbonyl
Fmoc-TEG-SA	*N*-1-(9-Fluorenylmethoxycarbonyl)-1,13-diamino-4,7,10-trioxatridecan-succinamic acid
FT	Fourier transform
HATU	1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate
HOBt	*N*-Hydroxybenzotriazole
HPLC	High-performance liquid chromatography
KCN	Potassium cyanide
MeCN	Methyl cyanide
MeOH	Methanol
MS	Mass spectrometry
NHS	*N*-hydroxysuccinimide
NMR	Nuclear magnetic resonance
Osu	*O*-Succinimide
PBS	Phosphate buffered saline

PBST	0.05 % TWEEN 20 in PBS buffer
Rbf	Round-bottom flask
SA	Succinic anhydride
SBP	Streptavidin binding peptide
SPPS	Solid-phase peptide synthesis
TFA	Trifluoroacetic acid
THF	Tetrahyrdofuran
TIPS	Triisopropylsilane
TWEEN 20	Polyoxyethylene-20-sorbitan monolaurate
UV	Ultraviolet
Vis	Visible

I. Introduction

I. 1 Polymer Supports for Solid-Phase Organic Synthesis

Since Bruce Merrifield has introduced solid-phase organic synthesis (SPOS) concept,[1] various reactions have been performed on polymer supports.[2-4] By using solid-phase synthesis strategy, products are synthesized and immobilized on the supports. This allows an easy removal of excess reagents and by-products by simple filtration. Therefore, excess amount of reagents can be used for high reaction yield, and high purity can be easily obtained without any complex separation process. Based on these merits, SPOS method has been chosen as a powerful tool in organic synthesis field.[5]

In order to increase the synthetic efficiency of SPOS, selection of good polymer supports is essential. A polymer supports must have proper chemical and physical stabilities. They must not be dissolved into solvents, and they must not be broken down during the reaction. Also, they must have good swelling properties to various solvents used during the reaction because the reaction occurs not only on the surface of the polymer supports, but also at the inner part of them. Among the

various polymer supports for SPOS, divinylbenzene-crosslinked polystyrene (1 % DVB-PS) based resin has been mainly used because it shows good swelling properties in organic solvents, stable in reaction conditions and easy to handle. Besides, various kinds of functional groups could be easily introduced to the PS resin.[6-8]

I. 2 Core-Shell Type Polymer Supports

Although various solid supports could be used for SPOS, polymer supports have been mainly used because of their good chemical stability and physical property. Polymer supports can be classified into swelling type (gel type) and non-swelling type. Swelling type can be classified into non-core-shell type and core-shell type, according to the distribution of their functional groups (**Figure 1**). Functional groups of gel type resin are located throughout the entire part of resin, whereas those of core-shell type resin were only at the outer layer. Because the reagent molecules hardly reach to the functional groups at the inner part of the resin, reactions on gel type resin are sometimes hard to be completed, which causes low purity of products. On the other hand, functional groups at the outer part are easily accessible, and the reactions which occur on the core-shell type resin are less influenced by diffusion. Thus, each reaction steps on the resin could be easily completed, which then leads to high purity of final.[9] Based on this advantage, various core-shell type resins, such as AM SURE™ resin and ReSure resin, have been developed by Lee *et. al.*[9-11] **(Figure 2**).

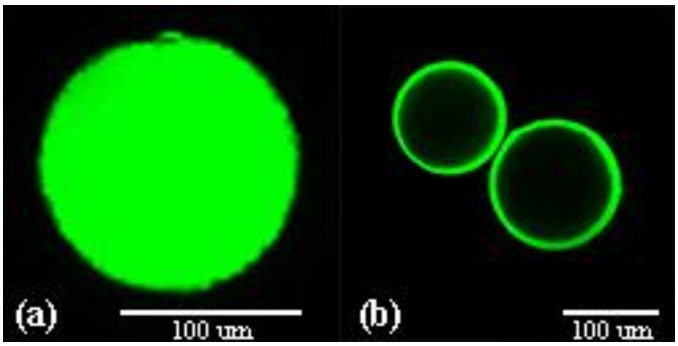


Figure 1. CLSM images of (a) gel type polymer resin; (b) core-shell type polymer resin.

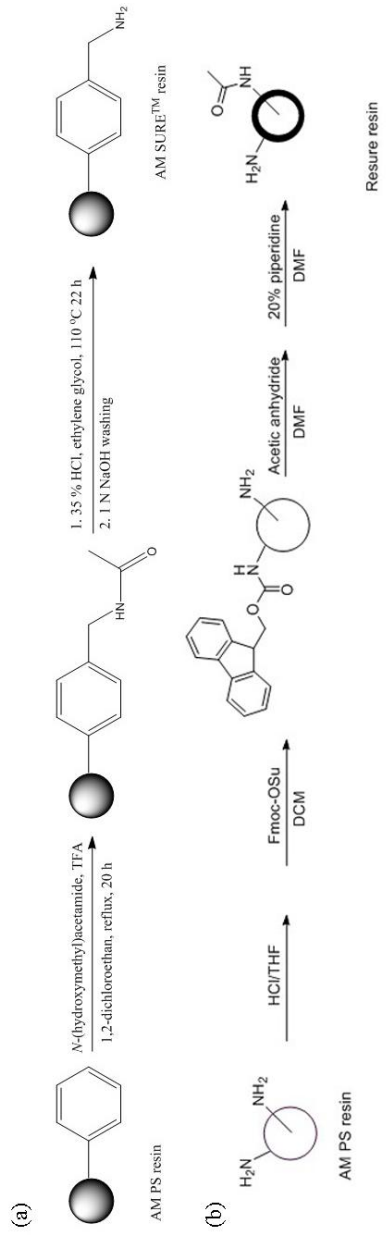


Figure 2. Procedures for preparation of various core-shell type resins: (a) AM SURE™ resin; (b) ReSure resin.

AM SURE™ resin was prepared by partial hydrolysis of acetamidomethyl PS resin. First, polystyrene (crosslinked with 1% DVB) resin was acetamidomethylated with *N*-(hydroxymethyl) acetamide. After that, acetyl groups on surface layer were hydrolyzed by treating with concentrated hydrochloric acid. As a result, AM SURE™ resin has free amino groups on the surface while protected amino groups exist in the core part.[12]

On the other hand, ReSure resin was prepared by partial protection and deprotection of aminomethyl (AM) PS resin. Amino groups of AM PS resin were converted into hydrophilic ammonium salt form by treating with diluted hydrochloric acid. Less than stoichiometric amount of Fmoc-OSu in DCM was introduced to induce partial protection of amino groups from outer part of the resin. Core-shell ratio and loading level of ReSure resin could be controlled at this step by changing the amount of Fmoc-OSu. Remaining free amino groups of inner part of the resin were acetylated by treating with acetic anhydride. Finally, Fmoc groups were deprotected for ready to use.[11] These core-shell type resins showed good performance in peptide synthesis compared to the conventional gel type resins.[11,13]

I. 3 Water Compatibility of Polymer Supports for On-bead Bioassays

Since the compounds which were bound to the polymer supports can be easily separated and collected via simple filtration and elution, SPOS can be applied to bioassay fields without isolating them from the polymer supports. For example, one-bead one-compound (OBOC) peptide library was prepared for screening substrates or inhibitors of enzymes.[14] In this method, various kind of peptide sequences were synthesized on resin beads by split and mix method (**Figure 3**). Then, target protein was treated with OBOC peptide libraries to screen the peptide ligands which are strongly interacted with the target protein. When peptide ligands which have high affinity to a target protein are synthesized on resins, the target protein could be easily separated and purified from protein mixture.

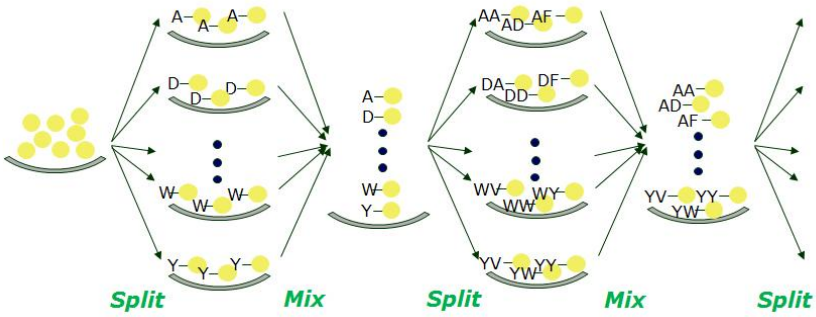


Figure 3. Split and mix method for synthesis of OBOC peptide library.

Choice of appropriate polymer support is a very important factor for on-bead assays. PS based resin, for example, has been commonly used for peptide synthesis because of its good swelling property to organic solvents used in peptide synthesis. Although PS based resin showed good performance in peptide synthesis, it is not appropriate for on-bead bioassays because of its hydrophobic character. To overcome this drawback, hydrophilic parts such as poly(ethylene glycol) (PEG) derivatives have been introduced to hydrophobic polymer supports to give water compatibility.[15] Various water compatible solid supports such as Tentagel resin and HiCore® resin have been developed for use in water condition.[10,16,17]

Tentagel resin was prepared by PEG grafting to PS resin (**Figure 4**). Because it is made up of about 70 % of PEG by weight, it shows good swelling properties to polar protic solvents such as water and methanol. Due to this reason, it gives better accessibility of hydrophilic reagent than PS resin[19].

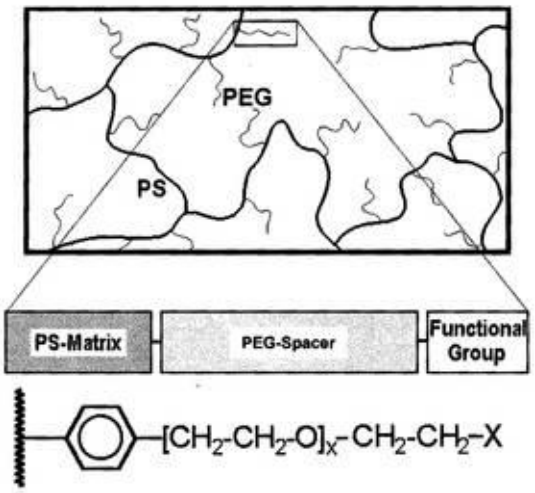


Figure 4. Structure of Tentagel resin.[18]

HiCore® resin was prepared by crosslinking of AM PS resin with cyanuric chloride (CNC), followed by introduction of Jeffamine ED-600 (O,O'-bis(2-aminopropyl) polypropylene glycol-block-polyethylene glycol-block-polypropylene glycol 500). By using CNC, rigid core is formed by crosslinking the internal amino groups of AM PS resin, and at the same time, provides reaction sites for introducing PEG layer which contains amino groups (**Figure 5**)[10]. HiCore® resin showed good swelling property in various solvents including water and showed good performance in on-bead substrate screenings.[20,21]

But these water compatible polymer supports have their own drawbacks. Tentagel resin is not core-shell type resin and HiCore® resin has rather low swelling property and auto-fluorescence which are originated from CNC groups. To overcome these drawbacks, PEGylation of AM SURE™ resin has been tried.[22] The succinylated AM SURE™ resin was activated with HBTU or DIC and HOBt, and then treated with Jeffamine ED-600. But this PEGylated AM SURE™ resin gave poor reproducibility in preparation.

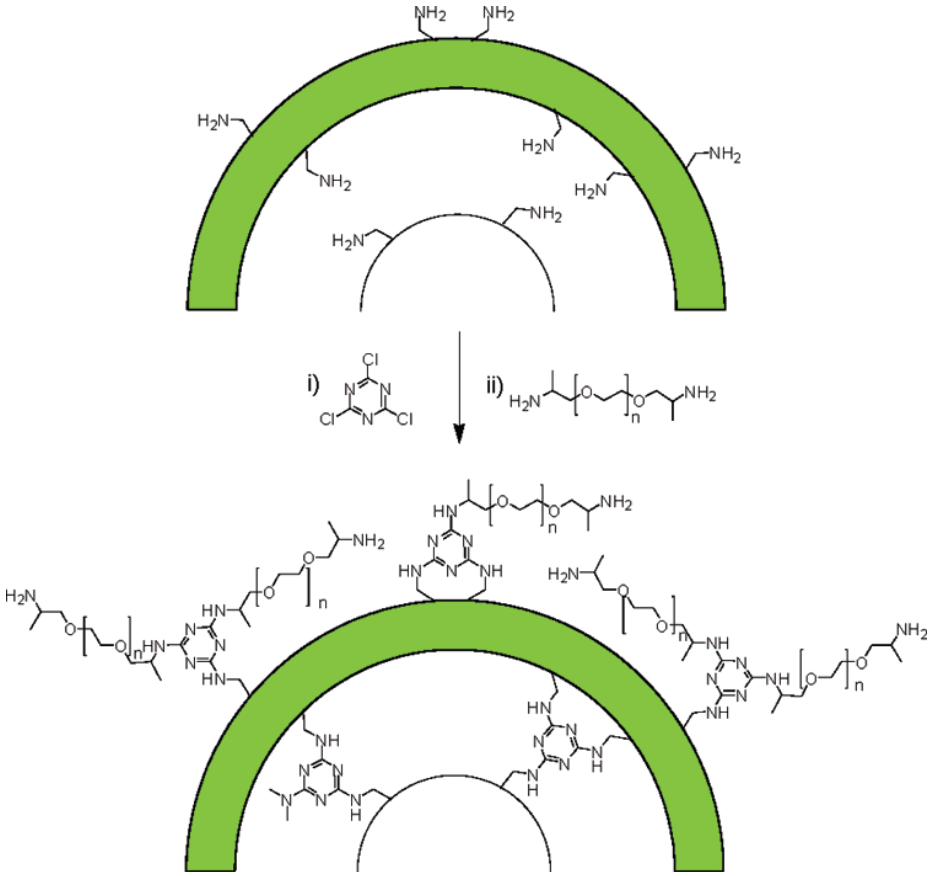


Figure 5. Procedures for preparation of HiCore® resin.

I. 4 Research Objectives

In this thesis, preparation, characterization, and application of water compatible core-shell type resin are described. Poly(ethylene glycol) derivatives were grafted on core-shell type AM PS (ReSure) resin under various conditions. In order to verify that the prepared resin is applicable to on-bead bioassay, IgG purification is performed by using protein A immobilized resin. Also, several model peptide sequences were synthesized on the prepared resin and performance data such as yield and purity will be discussed.

II. Experimental Section

II. 1 General

Materials

Aminomethyl polystyrene resin (100-200 mesh, 1.78 mmol/g), 9-fluorenylmethyloxycarbonyl succinimide (Fmoc-OSu), (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP), 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU), 1-hydroxybenzotriazole (HOBt) anhydrous and filtered reactors (Libra tube) were purchased from BeadTech Inc. (Korea). Diethylene glycol bis(3-aminopropyl)ether was purchased from Fluka (USA). Ninhydrin, *O,O'*-bis(2-aminopropyl)polypropylene glycol-block-polyethylene glycol-block-polypropylene glycol 500 (Jeffamine® ED-600), succinic anhydride, *N*,*N*-diisopropylcarbodiimide (DIC), *N*-hydroxysuccimide (NHS), molecular sieves (4 Å, 1.6 mm diameter pellet), human immunoglobulin G (IgG), glutaraldehyde solution (grade II, 25 % in H_2O), triisopropylsilane (TIPS), bovine serum albumin (BSA) and sodium cyanoborohydride were purchased from Sigma-Aldrich Co.

(USA). Phenol was purchased from Junsei Chemical Co. Ltd. (Japan). Fmoc-Lys(Fmoc)-OH was purchased from Bachem (Switzerland). Benzene, magnesium sulfate ($MgSO_4$) anhydrous, hydrochloric acid (HCl), trifluoroacetic acid (TFA), tetrahydrofuran (THF), ethyl acetate (EA), methyl cyanide (MeCN), dichloromethane (DCM), *N*,*N*-dimethylformamide (DMF), methanol (MeOH), ethanol (EtOH), acetic anhydride, *N*,*N*-diisopropylethylamine (DIPEA), piperidine, pyridine and thionyl chloride were purchased from Daejung Chemicals & Metals Co. (Korea). Potassium cyanide (KCN) was purchased from DC Chemical Co. Ltd. (China). SPHERO^{TM} Streptavidin coated fluorescent yellow particles (0.44 um, 0.1 % w/v) was purchased from Spherotech Inc. (USA).

Instruments

UV absorbance was measured by using Optizen 2120UV (Mechasys, Korea). Fluorescent images were taken by using LSM 780 confocal laser scanning microscope (Carl zeiss, Germany). Libra tubes were shaken by using SI-600R shaker (Jeio tech., Korea). Purity of synthesized peptide was analyzed by using LCQ LC/MS system (Thermo Finnigan, USA).

Ninhydrin Color Test (Kaiser Test) [23]

Three kinds of stock solutions were made by (1) adding 2 mL of 1 mM KCN solution to 98 mL of pyridine, (2) dissolving 5 g of ninhydrine to 100 mL of EtOH, and (3) mixing 80 g of phenol with 20 mL of EtOH. A small amount of resin was placed in a small glass test tube (~1 mL) and 2-3 drops of each stock solution were added sequentially. The mixture was placed in a heat block and heated at 100 ℃ for 5 min. The color of amine containing resin was turned to dark blue, while that of amine-free resin remained yellow.

Fmoc Quantitation [24,25]

About 30 mg of well-dried resin with Fmoc group was weighed in a Libra tube. Three mL of 20 % (v/v) piperidine in DMF was applied to the resin, and the mixture was shaken at room temperature for 1 hour. After that, 10 μL of the reaction solution was withdrawn and diluted 100 times by adding DMF. With this solution, UV absorbance at 290 nm was measured using quartz cuvette and DMF was used as a blank solution. Loading level was calculated by using following formula.

$$\textit{Loading level (mmol/g)} = (55.206 \times Abs_{290} - 1.0223) / \textit{wt of resin (mg)}$$

Generally, Fmoc quantitation test was done 3 times for its accuracy.

Protein Concentration Measurement [26]

Concentrations of protein in solutions were measured by using spectrophotometer. The absorbances of protein solutions with known concentrations (0.125, 0.250, 0.500, and 1.00 mg/mL) at 280 nm were measured for set a standard curve. The absorbances of protein solution with unknown concentrations at 280 nm were measured and the concentration was calculated by the standard curve.

Preparation of Core-shell Type AM PS (ReSure) Resin

ReSure resin was prepared by the reported method.[11] AM PS resin (1 g, 1.78 mmol/g) was treated with 1 *N* HCl in THF to form a hydrophilic ammonium salt for 3 hours, and washed with water for 3 times. Then, Fmoc-Osu (0.8 equiv., 480 mg) and DIPEA (1.6 equiv, 0.47 ml) in DCM was introduced to the resin. The reaction was proceeded for overnight. After reaction, the remaining amino groups of inner part were acetylated by treating with acetic anhydride and DIPEA (1 ml each) in DMF (10 ml) for 3 hours. Loading level was calculated after

deprotecting Fmoc groups of ReSure resin. The prepared resin was stored under *vacuo.*

II. 2 Preparation of PEG-ReSure Resin

PEGylation on ReSure resin was performed in the following 2 ways. First, Jeffamine ED-600 was grafted onto the resin, and TEG grafted ReSure resin. In this case, we expected that short PEG molecules might act as a mediator for hydrophilic PEG chain and hydrophobic PS backbone. Second, short PEG molecules were grafted several times onto ReSure resin.

II. 2. 1 Preparation of TEG Grafted ReSure Resin

II. 2. 1. 1 Synthesis of N-1-(9-Fluorenylmethoxycarbonyl)-1,13-diamino-4,7,10-trioxatridecan-succinamic acid (Fmoc-TEG-SA)

Fmoc-TEG-SA was prepared by using a modified reported method.[27] Diethyleneglycol bis(3-aminopropyl)ether (2.18 mL, 10 mmol) was poured into 250 mL Rbf and dissolved in MeCN (25 mL). The Rbf was placed in an ice bath and stirred. Succinic anhydride (1.0 g, 10 mmol) was dissolved in 50 mL of MeCN and dropped into the Rbf

for 30 minutes. After an hour, ice bath was removed and reaction was proceeded for additional 2 hours at room temperature.

After the reaction, solution was discarded and remaining residue was gently washed with MeCN for 3 times. After washing, mixture of MeCN and deionized water (1:2 v/v, 75 mL) and DIPEA (2.46 mL, 13 mmol) were introduced to the Rbf. The Rbf was shaken gently until all the residue was dissolved. After that, the Rbf was placed in an ice bath and stirred. Fmoc-Osu (4.4 g, 13 mmol) was dissolved in 50 mL of MeCN and was dropped into the Rbf for 30 minutes. After an hour, ice bath was removed and reaction was proceeded overnight at room temperature.

After the reaction, MeCN was removed by using rotary evaporator and 50 mL of deionized water was added. The pH of solution was adjusted at 8-9 by adding 5 g of $NaHCO_3$ to the aqueous solution. The aqueous phase was washed with EA (50 mL) by using separatory funnel. Upper layer (EA) was discarded and same amount of fresh EA was poured and washed 2 more times. After the final washing, EA was discarded, and then aqueous phase was acidified to pH 2 with 1 N HCl. The acidified aqueous phase was extracted with EA (50 mL) for 3 times and the organic layer was gathered and dried with anhydrous $MgSO_4$.

Dried organic layer was filtered, and evaporated by using rotary evaporator. The remaining residue was dried *in vacuo.* Synthesized Fmoc-TEG-SA was analyzed with NMR and MS assignment.

^{1}H NMR (400 MHz, DMSO): δ 7.91-7.24 (m, 8H, Ar-H), 7.80 (t, 1H, N-H), 7.25 (t, 1H, N-H), 4.30 (d, 2H, OCH_2-CAr), 4.21 (t, 1H, Ar-CH), 3.50 (m, 8H, OCH_2-CH_2-O), 3.37 (m, 4H, OCH_2), 3.06 (m, 4H, N-CH_3), 2.43-2.27 (m, 4H, CO-CH_2-CH_2-CO), 1.62 (m, 4H, CH_2)

MS (ESI): calculated for $C_{29}H_{39}N_2O_8$ $[M+H]^+$ 542.3, found 542.1.

II. 2. 1. 2 Introduction of Fmoc-TEG-SA to ReSure Resin

Fmoc-TEG-SA (3 equiv., 239 mg), HOBt (3 equiv., 60 mg), BOP (3 equiv., 195 mg) and DIPEA (6 equiv., 0.17 mL) in DMF were added to ReSure resin (1.47 mmol/g, 100 mg) in a Libra tube. The tube was shaken for 3 hours at room temperature by using a shaking incubator. After the reaction, solution was filtered out from the tube by using aspirator. The resins were washed with DMF, DCM and MeOH for 3 times each. Disappearance of all amino groups of the resins was confirmed by ninhydrin color test. Additional Fmoc-TEG-SA was also coupled to the Fmoc-TEG-SA ReSure resin by repeating Fmoc

deprotection and the same coupling step. The resins were named as TEG_1-ReSure resin and TEG_2-ReSure resin after the number of coupling times.

II. 2. 2 PEGylation of ReSure Resin

II. 2. 2. 1 PEGylation with Jeffamine ED-600

ReSure resin (0.9 mmol/g, 200 mg), TEG_1-ReSure resin (0.45 mmol/g, 200 mg), and TEG_2-ReSure resin (0.19 mmol/g, 200 mg) were added to Libra tube separately. After that, succinic anhydride (3 equiv.) and DIPEA (6 equiv.) in DMF were added to each Libra tubes. The tubes were shaken for 3 hours at room temperature by using a shaking incubator. After the reaction, the resins were filtered and washed with DMF, DCM and MeOH for 3 times each. Disappearance of free amino groups on the resins was confirmed by ninhydrin color test.

Activation of succinylated resin was performed in 3 ways. The succinylated resin in Libra tube was treated with i) DIC (5 equiv.) and *N*-hydroxysuccimide (5 equiv.) in DMF for 1 hour, or ii) thionyl chloride solution (33 % in DMF, v/v) for 30 minutes or iii) HATU (5

equiv.) and DIPEA (10 equiv.) in DMF for 3 hours at room temperature. The tubes were shaken by using a shaking incubator. After the reaction, the resins were filtered and washed with dried DMF, DCM and THF for 3 times each.

Jeffamine ED-600 (10 equiv.) and DIPEA (20 equiv.) in various solvent were introduced to the activated resin in Libra tube. The tubes were shaken for overnight at room temperature. After that, the resins were filtered and washed with DMF, DCM and MeOH for 3 times each. Appearance of amino groups was confirmed by ninhydrin color test.

II. 2. 2. 2 PEGylation with Fmoc-TEG-SA

ReSure resin (1.01 mmol/g, 200 mg) was swallen in DMF for 10 minutes in a Libra tube. Fmoc-TEG-SA (3 equiv., 329 mg), HOBt (3 equiv., 82 mg), BOP (3 equiv., 268 mg), DIPEA (6 equiv., 0.21 mL) and 3 mL of DMF were added to the resin in the tube. The tube was shaken for 3 hours at room temperature by using shaking incubator. After the reaction, the resin was filtered and washed with DMF, DCM and MeOH for 3 times each. Disappearance of free amino groups was confirmed by ninhydrin color test. Fmoc group was removed by 20 %

(v/v) piperidine in DMF (3 ml) for 30 minutes and appearance of amino groups was confirmed by ninhydrin color test. After that, Fmoc-TEG-SA was introduced again via the same procedure. After that Fmoc-Lys(Fmoc)-OH (3 equiv., 358 mg) was introduced to increase the loading level of the resin. After removing Fmoc groups, Fmoc-TEG-SA was introduced to the resulting resin again to give an equal number of ethylene glycol residues with Jeffamine ED-600.

II. 2. 3 Characterization of PEG-ReSure Resin

II. 2. 3. 1 Swelling Property Test

Small portions of dried AM PS resin, ReSure resin, HiCore® resin and PEG-ReSure resin (1.0 g each) were weighed and added into a graduated cylinder (ID, 0.8 cm; length, 20 cm) respectively. The resin was submerged in chosen solvents and the cylinder was sealed and stood for overnight at room temperature. The swelling volume was measured and recorded as mL/g resin.

II. 2. 3. 2 Confocal Laser Scanning Microscopy (CLSM) Analysis

In order to observe an auto-fluorescence signal, PEG-ReSure resin was exposed to the light at a wavelength of 488 nm. In order to confirm the core-shell structure of PEG-ReSure resin, FITC was conjugated to amino groups of the resin and analyzed by CLSM.

II. 3 Application of PEG-ReSure Resin to Solid-Phase Peptide Synthesis

Leu-enkephalin amide (YGGFL-NH_2) and streptavidin binding peptide amide (IQHPQ-NH_2) were synthesized on PEG-ReSure resin as a performance test for the resin in peptide synthesis. The loading level of each resin (100 mg, PEG- ReSrue, and HiCore®) was adjusted to about 0.2 mmol/g after Rink amide linker was coupled.

II. 3. 1 Synthesis of Leu-enkephalin (YGGFL-NH_2)

Fmoc amino acid (5 equiv.), BOP (5 equiv.), HOBt (5 equiv.), and DIPEA (10 equiv.) were dissolved into 3 ml of DMF, and added to the Rink amide PEG-ReSure resins. The mixture was shaken for 1.5 hours at room temperature. After the reaction, the resin was filtered and washed with DMF, DCM, and MeOH for three times each. Fmoc groups of the resin were removed by using 3 mL of 20 % (v/v) piperidine/DMF for 30 minutes, and the resin was washed by the same method above. After coupling of the first amino acid residue, coupling and deprotection steps were repeated with Fmoc-amino acids

(sequentially, Leu, Phe, Gly, Gly, and Tyr). After all the amino acid residues were coupled on the resin, cleavage and global deprotection were performed by using the modified cleavage cocktail (TFA : H_2O : TIPS = 95 : 2.5 : 2.5, volume ratio) for 2 hours. Cleavage cocktail solution was collected by filtration. The resin was washed with MeCN for three times, and the filtrates were combined to the cleavage cocktail solution. The collected solution was evaporated with reduced pressure, and 20 mL of cold diethyl ether was added to precipitate the peptide product. After precipitation, diethyl ether was decanted after centrifugation (5000 rpm, 5 min), and the peptide was washed with cold diethyl ether for three times, and dried with N_2 blowing. The peptide was analyzed with LC-MS. Calculated for $C_{28}H_{39}N_6O_6$ $[M+H]^+$ 555.3, found 555.3

II. 3. 2 Synthesis of Streptavidin Binding Peptide (SBP, IQHPQ-NH_2)

Fmoc-amino acids (sequentially, Gln, Pro, His, Gln, and Ile) were coupled to the resins by the same methods above. After all the amino acid residues were coupled on the resin, cleavage and global

deprotection were performed by the same method as above. The crude peptide was washed with cold diethyl ether, dried with N_2 blowing, and analyzed with LC-MS. Calculated for $C_{27}H_{45}N_{10}O_7$ $[M+H]^+$ 621.3, found 621.3

II. 3. 2. 1 Application of PEG-ReSure Resin to On-bead Bioassay

Streptavidin binding peptide (SBP, IQHPQ-NH_2) was synthesized on PEG-ReSure resin (0.47 mmol/g, 100 mg) via the same Fmoc chemistry described above. Amino acid coupling was confirmed by ninhydrine color test after each coupling and deprotection step. N terminal of the final amico acid was acetylated. After the last coupling reaction, global deprotection was performed with cleavage cocktail (TFA : H_2O : TIPS = 95 : 2.5 : 2.5, volume ratio) for 2 hours.

Streptavidin coated fluorescent yellow particles (SPHERO^{TM}, 0.44 um, 0.1 % w/v) in PBS buffer were introduced to Ac-IQHPQ-PEG-ReSure and Ac-PEG-ReSure, respectively. The mixture was shaken for 3 hours at room temperature by using a shaking incubator. The resin was filtered out and washed with PBST (0.05 % TWEEN 20 in PBS buffer) and PBS buffer (25 mM, pH 7.4) for 3 times each. The resin

was dried under *vacuo*. Binding of streptavidine to SBP coupled resin was confirmed by taking CLSM images of each dried resins.

II. 4 Application of PEG-ReSure Resin to IgG Purification

II. 4. 1 Immobilization of Protein A

II. 4. 1. 1 Introduction of Aldehyde Group on PEG-ReSure Resin

Glutaraldehyde (10 equiv., 0.59 mL) and sodium cyanoborohydride (3 equiv., 47 mg) in a mixture of DMF and AcOH (5:2, v/v) was introduced to PEG-ReSure resin (0.47 mmol/g, 330 mg) in a Libra tube. The tube was shaken for 3 hours at room temperature by using a shaking incubator. After the reaction, the resin was filtered and washed with DMF, DCM, MeOH and PBS buffer (25 mM, pH 7.4) for 3 times each. Disappearance of amino groups was confirmed by ninhydrin color test.

II. 4. 1. 2 Immobilization of Protein A

Protein A in PBS buffer (1 mg/mL, 3 mL) was prepared by diluting protein A stock solution (50 mg/mL) with PBS buffer. The protein A buffer solution was introduced to the resin (1 mL) in Libra tube. The

tube was shaken for 4 hours at room temperature by using a shaking incubator. The resin was filtered and washed with PBST and PBS buffer for 3 times each. Filtrate and washing solutions were gathered and the concentration of protein A was calculated by measuring Abs_{280}.

Sodium cyanoborohydride (3 equiv., 47 mg) in PBS buffer was introduced to the protein A immobilized PEG-ReSure resin in Libra tube. The tube was shaken for 6 hours at room temperature by using a shaking incubator. After the reaction, the resin was filtered and washed with PBS buffer for 3 times. The resin were soaked in PBS buffer containing 0.02 % of sodium azide and stored at 4 ℃ before use.

II. 4. 2 Stability Test Under Elution Condition

Acid stability during elution condition was tested by the following method. Gly-HCl buffer (pH 2.7, mM, 3 mL) which is used for IgG elution was introduced to the protein A immobilized resin (200 mg) in Libra tube. The tube was shaken for 30 minutes at room temperature by using a shaking incubator. After the incubation, the resin was filtered and washed with PBST and PBS buffer for 3 times each. Filtrate and

washing solutions were gathered and the concentrations of protein A in each solution were measured.

II. 4. 3 Purification of IgG

II. 4. 3. 1 Protein Binding

BSA or IgG in PBS buffer (1 mg/mL, 3 mL) was prepared by diluting stock solution (10 mg/mL) with PBS buffer. The protein solution was introduced to the resin in Libra tube. The tube was shaken for 30 minutes at room temperature by using a shaking incubator. After the introduction, the resin was filtered and washed with PBST and PBS buffer for 3 times each. Filtrate and washing solutions were gathered and the concentration of protein in each solution was calculated by measuring Abs_{280}.

II. 4. 3. 2 Elution and Regeneration

Gly-HCl buffer (100 mM, pH 2.7, 3 mL) was introduced to the protein bound resin in a Libra tube. The tube was shaken for 30 minutes at room temperature by using a shaking incubator. The resin was

filtered and treated with 3 mL of Gly-HCl buffer once again for 30 minutes at room temperature. The resin was filtered and washed with PBST and PBS buffer for 3 times each. The filtrate and washing solution was gathered after the elution for protein concentration measurement, and the resin was soaked in PBS buffer containing 0.02 % of sodium azide and stored at 4 ℃ before reuse.

II. 4. 3. 3 Protein Selectivity Test

In order to confirm that protein A immobilized resin had specific affinity to IgG, the following experiments were performed. At first, IgG solution in PBS buffer (1 mg/mL, 3 mL) was introduced to the acetylated PEG-ReSure resin to confirm that IgG had no affinity to bare PEG-ReSure resin. BSA in PBS buffer (1 mg/mL, 3 mL) was also introduced to the protein A immobilized PEG-ReSure resin in order to confirm that protein A immobilized PEG-ReSure resin had no binding affinity to BSA.

III. Results and Discussion

III. 1 Preparation of PEG-ReSure Resin

PEGylation of ReSure resin was tried with various reaction conditions; method A) direct grafting of Jeffamine ED-600 onto succinylated ReSure resin, method B) grafting of Jeffamine ED-600 onto TEG-SA grafted ReSure resin, method C) grafting of TEG-SA with Fmoc chemistry. (**Scheme 1**) The results of loading level were summarized in (**Table 1**). In entry 1-9, Jeffamine ED-600 was directly conjugated onto succinylated ReSure resin with various coupling reagents. In entry 10-11, Jeffamine ED-600 was conjugated onto the succinylated TEG-SA_n-ReSure resin (n = 1, 2) which was expected to have more compatibility to Jeffamine molecules than bare ReSure resin. To mimic the structure of Jeffamine ED-600, Fmoc-TEG-SA was introduced several times. In entry 12, Fmoc-TEG-SA was introduced sequentially via conventional Fmoc chemistry. Because the length of ethylene glycol (EG) unit of Jeffamine ED-600 is almost four times that of TEG, Fmoc-TEG-SA was coupled four times to adjust the length of EG unit. After introduction of Fmoc-TEG-SA twice, Fmoc-Lys(Fmoc)-

OH was introduced to increase the final loading level value of the resin, and then Fmoc-TEG-SA was introduced once again.

Scheme 1. Various schemes for preparation of PEG-ReSure.

Table 1. Final Loading Levels of Amino Groups on the Resins According to Various Coupling Conditions

Entry	Resin	Initial loading level (mmol/g)	Activation Reagent/Activation time (h)	PEGylation solvent	Final loading level (mmol/g)	Yield[a] (%)
1	ReSure	1.47	DIC, NHS / 1	DMF	0.10	14
2	ReSure	1.47	DIC, NHS / 1	DCM	0.05	7
3	ReSure	1.47	DIC, NHS / 1	DMF/DCM (50:50, v/v)	0.11	15
4	ReSure	1.47	$SOCl_2$ / 0.5	DMF	0.06-0.19[b]	8-26
5	ReSure	1.47	HATU / 3	DMF	0.09	13
6	ReSure	1.47	HATU / 3	DCM	0.11	15
7	ReSure	1.47	HATU / 3	DMF/DCM (50:50, v/v)	0.12	17
8	ReSure	1.47	HATU / 3	MeCN	0.11	15
9	ReSure	1.47	HATU / 3	THF	0.14	19
10	TEG_1–ReSure	0.45	HATU / 3	THF	0.05	14
11	TEG_2-ReSure	0.19	HATU / 3	THF	0.03	18
12	ReSure	1.01	HOBt, BOP / 3	DMF	0.47	59

[a] Yield was calculated based on theoretically calculated loading level.

[b] Results were fluctuated

When Jeffamine ED-600 (entry 1-9) was directly introduced to the ReSure resin, almost all trials showed low yield. Among them, use of HATU as a coupling reagent and THF as a solvent (entry 9) showed the best loading level (0.14 mmol/g). It was supposed that THF provided good compatibility to both resin and Jeffamine ED-600. On the other hand, use of thionyl chloride showed fluctuating results with poor reproducibility, probably because of its low stability. Introduction of Jeffamine ED-600 to TEG-SA grafted ReSure resin showed almost the same result.

From these results, it was concluded that Jeffamine ED-600 was hardly coupled to ReSure resin directly. It seemed that amino groups of Jeffamine ED-600 had low accessibility to the functional groups of the resin, because Jeffamine has long ethylene/propylene glycol chain. Comparing these results, hydrophilicity of the resin's surface may not play a key factor (entry 9-11).

From the results of consecutive introduction of Fmoc-TEG-SA (entry 12), we can conclud that multiple coupling of small size spacer molecules was more efficient than single grafting of large size spacer molecule.

III. 1. 2 Characterization of PEG-ReSure Resin

III. 1. 2. 1 Swelling Property

Generally, swelling property of resins is an important factor for successful solid-phase peptide synthesis, because the reaction occurs in both outer part and inner part of the resin. Swelling property of AM PS resin, ReSure resin and PEG-ReSure resin were measured under various solvent conditions and the results were summarized in **Table 2**. AM PS resin and ReSure resin were swollen well in non-polar solvents, but less or not swollen in ploar solvents such as MeOH and H_2O. PEG-ReSure resin showed even swelling properties to all the solvents which are commonly used in solid-phase peptide synthesis. It is probably due to the fact that inner part of the resin provided high swelling property to non-polar solvents and layer part proveded high swelling property to hydrophilic solvents. Compare to HiCore® resin, PEG-ReSure resin showed better swelling properties in hydrophobic solvents, because core structure of the resin was not crosslinked.

Table 2. Swelling Property of AM PS, ReSure and PEG-ReSure Resin in Various Solvents

Resins	Swelling property (mL / g resin)						
	Dry	**H_2O**	**MeOH**	**DCM**	**ACN**	**THF**	**DMF**
AM PS	2.0	-	2.6	7.8	3.1	5.5	5.9
ReSure	2.3	-	2.7	6.1	2.9	5.2	5.7
HiCore®	2.2	2.3	3.0	4.8	3.2	4.2	4.2
PEG-ReSure	2.4	3.0	3.6	5.9	3.8	4.0	5.2

III. 1. 2. 2 CLSM Analysis of PEG-ReSure Resin

To find out the distribution of amino groups and the existance of autofluorescence of PEG-ReSure resin, CLSM images were taken (**Figure 6.**). In order to confirm the distribution of functional groups on the AM PS resin, ReSure resin, and PEG-ReSure resin, FITC was coupled to the resin. After FITC coupling, AM PS showed fluorescent signal from entire part of the resin (**Figure 6. (a)**). ReSure resin and PEG-ReSure resin showed strong fluorescent signal from the surface layer while the inner part did not (**Figure 6. (b)** and **(c)**).

From this CLSM analysis, core-shell structure of PEG-ReSure resin as well as ReSure resin was confirmed. The size of the resins were not changed while the thickness of the shell layer was slightly increased after PEGylation. Inner part of PEG-ReSure resin showed no fluorescent sinal at 488 nm, which means that the prepared resin gave no auto-fluorescence signal (**Figure 7.**)

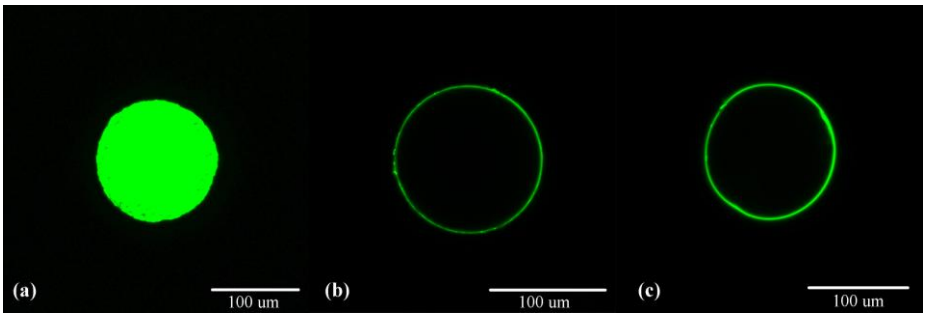


Figure 6. CLSM images of (a) FITC conjugated AM PS resin, (b) FITC conjugated ReSure resin, and (c) FITC conjugated PEG-ReSure resin.

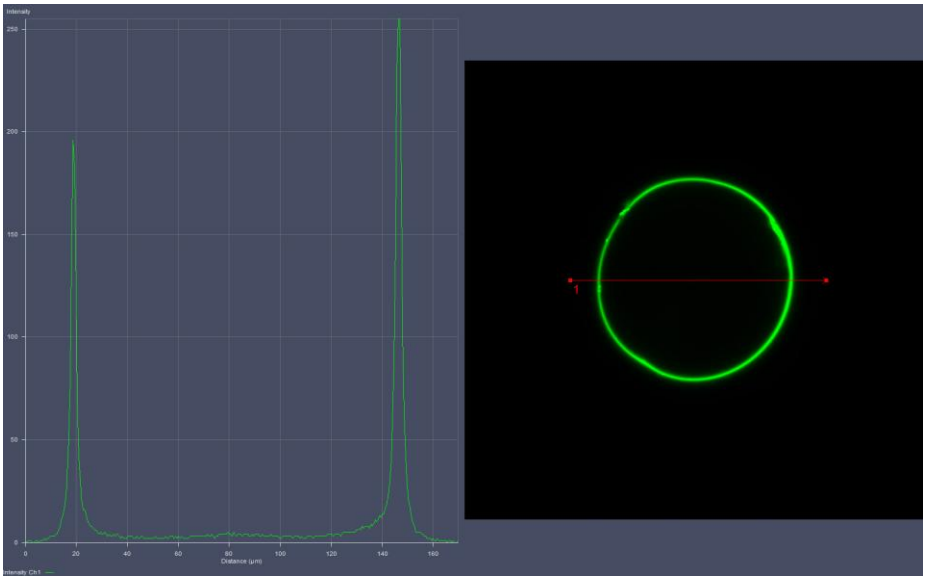
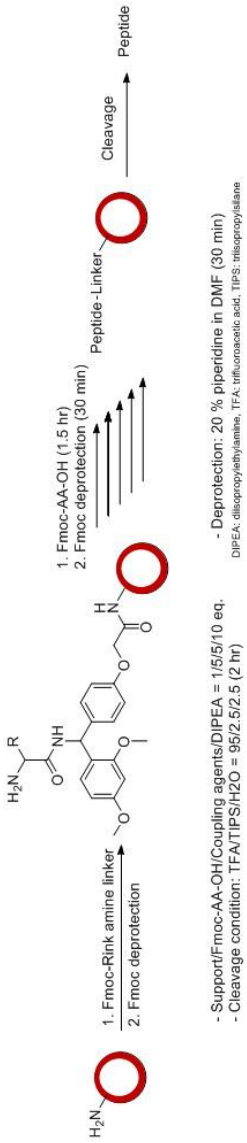


Figure 7. Fluorescence profile of FITC conjugated PEG-ReSure resin.

III. 2 Application of PEG-ReSure Resin to Solid-Phase Peptide Synthesis

In order to examine the synthetic performance of PEG-ReSure resin, peptides were synthesized on PEG-ReSure resin via Fmoc chemistry and the results were compared with that of HiCore® resin, which has been previously reported to give good performance in solid-phase peptide synthesis. Model peptides were Leu-enkephalin (H-Tyr-Gly-Gly-Phe-Leu-NH_2) and SBP (H-Ile-Gln-His-Pro-Gln-NH_2). (**Scheme 2**)

Synthesized peptide fragments were cleaved and deprotected with modified cleavage cocktail (TFA : H_2O : TIPS = 95 : 2.5 : 2.5, v/v). The yield of synthesized peptide was determined by comparing calculated and measured weight of peptide, and the purity was determined by HPLC and ESI-MS. Overall, better yield was obtained from PEG-ReSure resin, and purity of synthesized peptides was varied according to the sequence of peptides. (**Table 3**)



Scheme 2. Scheme for solid-phase peptide synthesis.

Table 3. The Results of Solid-phase Peptide Synthesis

Resin	Sequence	Yield (%)	Purity (%)
PEG-ReSure	YGGFL-NH_2	96	98
	IQHPQ-NH_2	95	79
HiCore®	YGGFL-NH_2	84	87
	IQHPQ-NH_2	94	88

III. 2. 1 Application of PEG-ReSure Resin to On-bead Bioassay

Streptavidin coated fluorescent yellow particles were treated to Ac-IQHPQ-PEG-ReSure and Ac-PEG-ReSure resins to examine the possibility of on-bead bioassay with PEG-ReSure resin in water conditions. CLSM images of the resins were taken. As a result, streptavidin coated fluorescent yellow particles were only detected from Ac-IQHPQ-PEG-ReSure resin (**Figure 8. (a)**), while Ac-PEG-ReSure showed no fluorescence (**Figure 8. (b)**).

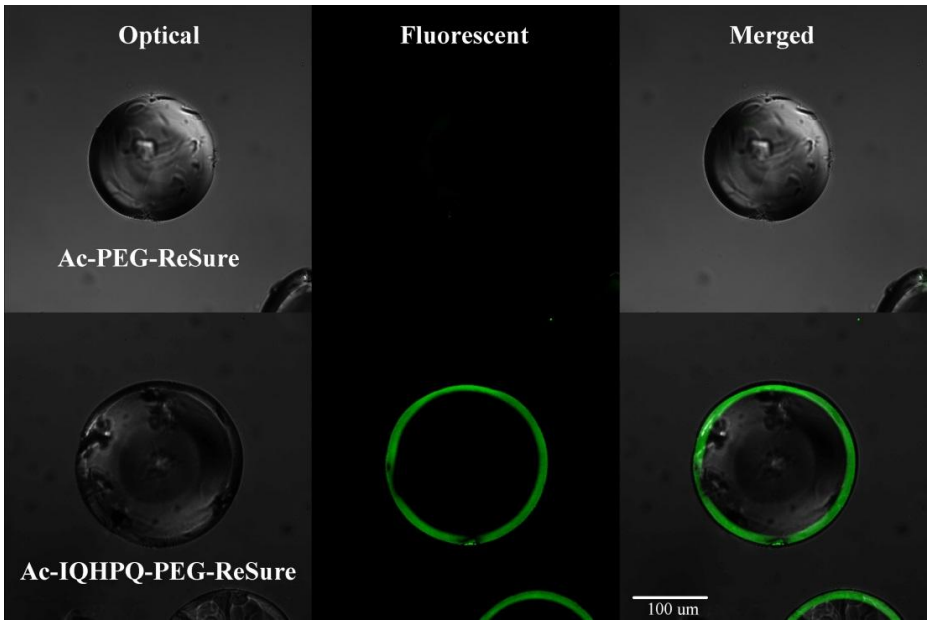


Figure 8. CLSM images of Streptavidin coated fluorescent yellow particle treated Ac-IQHPQ-PEG-ReSure resin, and Ac-PEG-ReSure resin.

III. 3 Application to IgG Purification

III. 3. 1 Protein A Conjugation

Conventionally, IgG was purified from serum or cell lysate by affinity column chromatography with protein A immobilized agarose beads. Agarose is a polysaccharide polymer, which has been widely used in bio-field because of its water compatibility. However, agarose beads utilized for affinity column chromatography for IgG purification have some drawbacks such as low flow rates, poor mechanical properties and low stability in acidic elution condition.

Protein A has been immobilized onto solid supports via Schiff base formation and reduction.[21] Therefore, functional groups of PEG-ReSure were converted to aldehyde groups by treating with glutaraldehyde and sodium cyanoborohydride. After that, protein A solution (1 mg/mL, 3 ml) was introduced to the aldehyde group ended PEG-ReSure resin (1 mL). The amount of conjugated protein A was calculated by measuring Abs_{280} of protein A solutions. In order to confirm that the immobilized protein A is stable in elution condition, protein A immobilized resin was treated with elution buffer without IgG complexation. After the

treatment, no leaching of immobilized protein A was detected. The results of the analysis were summarized in **Table 4**. The amount of immobilized protein A was 0.6 mg from 1 mL of resin and almost no protein A was leached out in elution condition.

Table 4. The Result of Protein A Loading

Entry	Amount of Protein A (mg)		
	Initial	Immobilized	Leached
PEG-ReSure resin (1 mL)	3	0.6	0

III. 3. 2 IgG Purification

IgG was purified by using protein A immobilized PEG-ReSure resin. At first, protein A immobilized resin was treated with IgG to find out whether it can be applicable in purification of IgG. Next, BSA was introduced to the resin to confirm that protein A immobilized resin had selectivity toward IgG. At last, IgG was introduced to acetylated PEG-ReSure resin to confirm that bare PEG-ReSure resin had no affinity toward IgG. The results aare summarized in **Table 5.**

As expected, protein A immobilized PEG-ReSure resin showed specificity to IgG (**Table 5. Entry 1** and **2**), and PEG-ReSure resin showed no non-specific binding property (**Table 5. Entry 3**).

Table 5. Results of Affinity Profiles Between Resins and Proteins

Entry	Resin (1 mL)	Introduced Protein	Amount of Protein (mg)	
			Bound	**Eluted**
1	Protein A-PEG-ReSure	IgG	0.5	0.5
2	Protein A-PEG-ReSure	BSA	0.0	0.0
3	Ac-PEG-ReSure	IgG	0.0	0.0

IV. Conclusion

PEG-ReSure resin was prepared from ReSure resin by several methods of PEGylation. We found out that sequential attachment of Fmoc-TEG-SA, Fmoc-Lys-OH and Fmoc-TEG-SA via Fmoc chemistry showed the best result. For preparation of this resin, Fmoc-TEG-SA was introduced to ReSure resin (1.01 mmol/g) twice and Fmoc-Lys(Fmoc)-OH was introduced to increase the loading level of resin. After that, Fmoc-TEG-SA was introduced to the resin once again. The final loading level of PEG-ReSure resin was 0.47 mmol/g and core-shell type structure was confirmed by CLSM imaging. Overall, PEG-ReSure resin showed consistent swelling properties to various solvents because of its amphiphilic nature.

For solid-phase peptide synthesis, Rink amide linker was introduced to the PEG-ReSure resin. Then, Leu-enkephalin amide (90 % of yield and 98 % of purity) and SBP amide (73 % of yield and 79 % of purity) were synthesized well. PEG-ReSure showed better yield than HiCore® resin, and with comparable purity. In order to examine the possibility of on-bead bioassay in aqueous conditions, streptavidin coated fluorescent particles were treated to Ac-IQHPQ-PEG-ReSure resin and Ac-PEG-

ReSure resin in water. As expected, stereptavidin coated fluorescent particles were only interacted with Ac-IQHPQ-PEG-ReSure, not with Ac-PEG-ReSure.

For IgG purification, protein A was immobilized onto the PEG-ReSure resin, and it showed almost no leaching in elution condition. Protein A immobilized resin selectively captured IgG without non specific protein binding and cluted in elution buffer.

V. References

(1) Merrifield, R. B. *Journal of the American Chemical Society* **1963**, *85*, 2149.

(2) Booth, S.; Hermkens, P. H.; Ottenheijm, H. C.; Rees, D. C. *Tetrahedron* **1998**, *54*, 15385.

(3) Hermkens, P. H.; Ottenheijm, H. C.; Rees, D. *Tetrahedron* **1996**, *52*, 4527.

(4) Hermkens, P. H.; Ottenheijm, H. C.; Rees, D. C. *Tetrahedron* **1997**, *53*, 5643.

(5) Früchtel, J. S.; Jung, G. *Angewandte Chemie International Edition in English* **1996**, *35*, 17.

(6) Bernard, M.; Ford, W. T. *The Journal of Organic Chemistry* **1983**, *48*, 326.

(7) Crosby, G. A.; Weinshenker, N. M.; Uh, H.-S. *Journal of the American Chemical Society* **1975**, *97*, 2232.

(8) Farrall, M. J.; Frechet, J. M. *The Journal of Organic Chemistry* **1976**, *41*, 3877.

(9) Choi, J.-H.; Lee, T.-K.; Byun, J.-W.; Lee, Y.-S. *Tetrahedron Letters* **2009**, *50*, 4272.

(10) Kim, H.; Cho, J. K.; Chung, W.-J.; Lee, Y.-S. *Organic Letters* **2004**, *6*, 3273.

(11) Cho, H.-J.; Lee, T.-K.; Kim, J. W.; Lee, S.-M.; Lee, Y.-S. *The Journal of Organic Chemistry* **2012**, *77*, 9156.

(12) Lee, T.-K.; Ryoo, S.-J.; Byun, J.-W.; Lee, S.-M.; Lee, Y.-S. *Journal of Combinatorial Chemistry* **2005**, *7*, 170.

(13) Lee, T.-K.; Lee, S.-M.; Ryoo, S.-J.; Byun, J.-W.; Lee, Y.-S. *Tetrahedron Letters* **2005**, *46*, 7135.

(14) Lam, K. S.; Salmon, S. E.; Hersh, E. M.; Hruby, V. J.;

Kazmierski, W. M.; Knapp, R. J. *Nature* **1991**, *354*, 82.
(15) Bergström, K.; Holmberg, K. *Biotechnology and Bioengineering* **1991**, *38*, 952.
(16) Hojo, K.; Ichikawa, H.; Fukumori, Y.; Kawasaki, K. *International Journal of Peptide Research and Therapeutics* **2008**, *14*, 373.
(17) Kim, D.-H.; Lee, H. Y.; Kim, H.; Kim, H.; Lee, Y.-S.; Park, S. B. *Journal of Combinatorial Chemistry* **2006**, *8*, 280.
(18) Liang, R.; Yan, L.; Loebach, J.; Ge, M.; Uozumi, Y.; Sekanina, K.; Horan, N.; Gildersleeve, J.; Thompson, C.; Smith, A. *Science* **1996**, *274*, 1520.
(19) Bayer, E. *Angewandte Chemie International Edition in English* **1991**, *30*, 113.
(20) Shin, D.-S.; Kim, Y.-G.; Kim, E.-M.; Kim, M.; Park, H.-Y.; Kim, J.-H.; Lee, B.-S.; Kim, B.-G.; Lee, Y.-S. *Journal of Combinatorial Chemistry* **2007**, *10*, 20.
(21) Kim, Y.-G.; Shin, D.-S.; Yang, Y.-H.; Gil, G.-C.; Park, C.-G.; Mimura, Y.; Cooper, D. K.; Rudd, P. M.; Dwek, R. A.; Lee, Y.-S. *Chemistry & Biology* **2008**, *15*, 215.
(22) Kim, M.; Park, Y.-S.; Shin, D.-S.; Lee, S.; Lee, Y.-S. *Tetrahedron Letters* **2012**, *53*, 4576.
(23) Kaiser, E.; Colescott, R.; Bossinger, C.; Cook, P. *Analytical Biochemistry* **1970**, *34*, 595.
(24) Novabiochem Catalog and Peptide Synthesis Handbook **1997**/**1998**, method 16.
(25) Meienhofer, J.; Waki, M.; Heimre, E. P.; Lambros, T. J.; Makofske, R. C.; Chang, C. D. *International Journal of Peptide and Protein Research* **1979**, *13*, 35.
(26) Aitken, A.; Learmonth, M. In *The Protein Protocols Handbook*; Springer: **1996**, p 3.

(27) Song, A.; Wang, X.; Zhang, J.; Mařík, J.; Lebrilla, C. B.; Lam, K. S. *Bioorganic & Medicinal Chemistry Letters* **2004**, *14*, 161.

Abstract in Korean

본 논문에서는 기존 고체상 합성에서 흔히 사용되는 폴리스티렌 비드의 표면에 친수성을 띄는 폴리에틸렌글리콜을 다양한 조건하에서 결합시키고, 이 고분자 지지체를 이용하여 고체상 펩타이드 합성과 면역글로불린 G (immunoglobulin G)의 정제를 시도하였다.

먼저 친수성이 없고 기능기가 겉과 밖에 골고루 분포된 아미노메틸 폴리스티렌 비드를 물질의 확산 정도를 조절하는 방법을 사용하여 기능기가 표면에만 위치한 코어-쉘 형태로 만들었다. 또한, 이 과정에서 확산시킬 물질의 양을 조절함으로써 기능기가 존재하는 쉘의 두께를 다양하게 조절하였다. 이렇게 제작된 고분자 비드의 기능기에 폴리에틸렌글리콜을 결합시켜 고분자 비드의 친수성을 증가시켰다. 제작된 고분자 비드의 물리적, 화학적 성질을 형광염료 염색, 팽윤 실험 등 다양한 분석 방법을 통해 확인하였다.

이렇게 제작된 고분자 비드를 고체상 펩타이드 합성에 응용하였다. YGGFL-NH_2 와 IQHPQ-NH_2 펩타이드를 합성한 결과, YGGFL-NH_2 를 96%의 수율로, IQHPQ-NH_2 를 95%의 수율로 각각 합성하였다. 이는 기존에 펩타이드 합성에 높은 효율을 가진다고 보고된 HiCore® 비드와 비교하여 높은 수율과 비슷한 순도를 보였다. 또한, 고분자 비드 상에서 펩타이드 리간드와 단백질간의 특이적 상호작용이 가능함을 확인하고자 고분자 비드 위에 스트렙트아비딘과 친화도가 높은 IQHPQ 펩타이드를 합성하였다. 이후 스트렙트아비딘이 고정된 형광 나노 입자가 분산된 완충용액에 고분자 비드를 담지시키고 형광 현미경 분석을 통해 고분자 비드 상에서 펩타이드와 단백질간의 선택적 상호작용이 일어남을 확인하였다.

고분자 비드에 직접 단백질을 고정화하여 다른 단백질과 상호작용을할 수 있는지 알아보기 위해 고분자 비드 상에 단백질 A를 화학적으로 결합시켰다. 단백질 A가 결합된 고분자 비드를 면역글로불린 G가 녹아있는 완충용액에 담지시켜, 면역글로불린 G와 단백질 A의 상호작용을 통해 면역글로불린 G를 고분자 비드의 표면에 결합시켰다. 이 고분자 비드를 충분하게 세척한 후 산성 완충용액에 담지시켜, 면역글로불린 G를 고분자 비드로부터 분리시킴으로써 면역글로불린 G가 단백질 A가 결합된 고분자

비드에 선택적으로 흡착되고, 온화한 조건에서 분리될 수 있음을 확인하였다.

주요어 : 코어-쉘 형태의 고분자 지지체, 친수성 고분자 지지체, 고체상 펩타이드 합성, 지지체상 단백질 분석, 단백질 A, 면역글로불린G 분리

학 번 : 2014-20581