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A novel core-shell type polymer support for solid-phase peptide synthesis

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Abstract

Novel core-shell type resins with a highly cross-linked polystyrene (PS) core and a poly(ethylene glycol) (PEG) shell were prepared by suspension polymerization for use in solid-phase synthesis. All the resins were of bead type with a diameter of 50–200 μm. The loading capacities of the amino groups were 0.05–0.2 mmol/g resin and the thickness of the PEG shell was 2–4 μm. Compared with the other resins such as 1% cross-linked PS resin and TentaGel resin, the resins showed excellent performance in photolytic release of peptide product from photolabile linker. © 2000 Elsevier Science Ltd. All rights reserved.

Advances in the chemical reactions on solid supports for high throughput screening (HTS) have aroused an interest in searching for an ideal polymer support. Various polymer supports have been developed since Merrifield introduced the concept of solid-phase peptide synthesis (SPPS).[1] Solid-phase organic syntheses (SPOS) are frequently performed using bead type solid supports, taking mechanical stability and compatibility with a range of solvents into account.[2] Polystyrene (PS) resins cross-linked with 1% divinylbenzene (DVB) are in common use in SPOS. The value of 1% is the best compromise between compatibility and mechanical stability of the resin in the presence of suitable solvents.[3] Higher degrees of cross-linking yield more stable resins, but they swell less and consequently possess lower loading capacity or reactivity. To overcome these limitations, Pepsyn,[4] TentaGel,[5] and PolyHIPE[6] were developed and are now widely used for SPOS. However, in photolytic cleavage of peptide products, all these resins suffer from the same problem—inadequate penetration of UV light into the interior of the resin beads.

In this paper, we report a novel method of preparing a new resin that has a highly cross-linked (>4%) PS core covered with a poly(ethylene glycol) (PEG) shell. To prepare it, we

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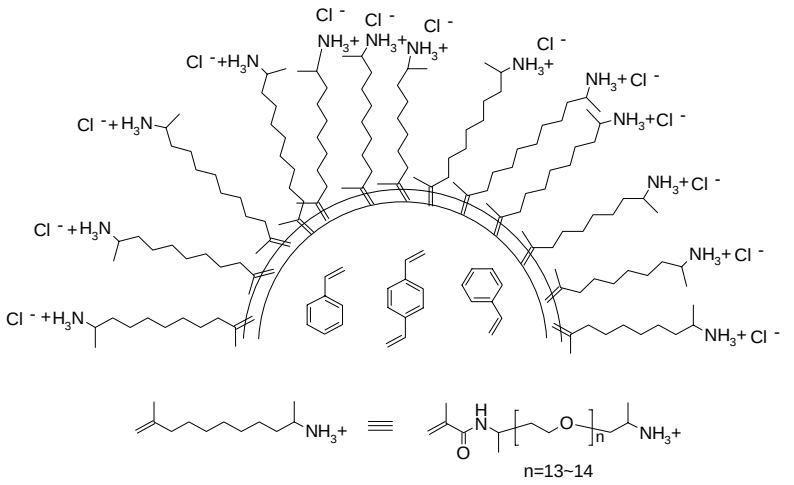
used suspension polymerization of styrene. In addition, a synthetic PEG macromonomer that has both a vinyl group and an amino group at each terminus was added as a co-monomer and a stabilizer of the suspension system (Scheme 1). If we could prepare such a co-polymer with all the functional groups located at the PEG shell, we would expect that reagents need not diffuse into the interior of the bead, so that all the chemical reactions, including photolysis, can proceed effectively.



Scheme 1.

The macromonomer was synthesized by the following procedure. *O*,*O*′-Bis(2-aminopropyl)poly-ethylene glycol 500 (12 ml, 20 mmol, Jeffamine ED-600) and triethylamine (TEA) (3.1 ml, 1.1 equiv.) were dissolved in dry tetrahydrofuran (THF) (100 ml), and methacryloyl chloride (2.2 ml, 1.1 equiv.) in dry THF (10 ml) was added over a period of 30 min with stirring in an ice bath. After the mixture was stirred for another 30 min, the resulting TEA·HCl salt was filtered off and then THF was evaporated under reduced pressure to give oily mixtures. Excess bis-2-methacrylamidoprop-1-yl-PEG_{500} was removed by extracting with peroxide-free diethyl ether (30 ml×4) at a low temperature (−78°C). Core-shell type resins were prepared by utilizing the conventional suspension polymerization conditions with a reactor and an overhead stirrer. An aqueous phase consisting of water (100 ml), sodium chloride (10 g) and the freshly synthesized macromonomer was adjusted to pH 7 with 1N HCl. An organic phase consisting of styrene (6 ml), DVB (0.6 ml, 50% in ethylvinylbenzene), *n*-hexanol (3 ml) and benzoyl peroxide (0.2 g) was added with stirring (250 rpm) to the aqueous phase under a nitrogen atmosphere and then the mixture was polymerized for 20 h at 80°C. The resulting polymer beads were sieved and washed with water, methanol and dichloromethane (DCM). They were finally dried in vacuo overnight. All the resins are of the bead type (diameter of 50–200 μm) with amine loading capacities of 0.05–0.2 mmol/g resin.† The cross-sectioned view of the fluorescence-stained beads[7] showed that all the amino groups are located at the shell; its thickness was 2–4 μm (Fig. 1).[8]

† The loading capacity of amino groups was determined by the picric acid titration.[9]

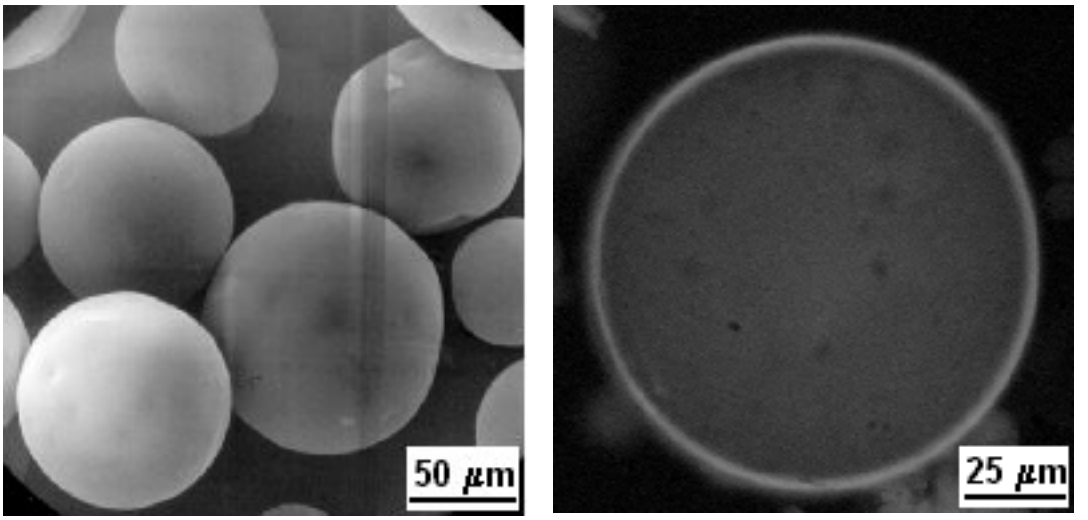


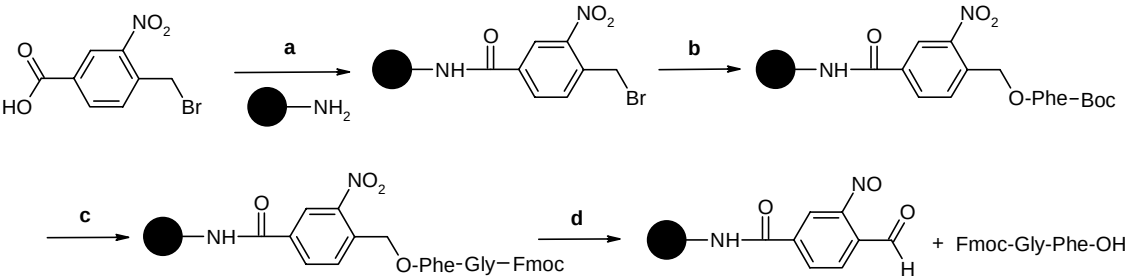
Figure 1. (a) Scanning electron micrograph showing the spherical beads prepared by suspension polymerization and (b) cross-sectioned view of core-shell type PS-*co*-PEG resins through fluorescence microscope

It swells less than TentaGel resin does in water and most organic solvents. With a hydrophobic solvent such as DCM, it swells less than PS resins. This is due to the high cross-linking at the core of resin bead. The amount of the initial loading level remained unchanged even in severe reaction conditions[10] and no changes in functional group patterns occurred in the FT-IR spectra.

Although photolyses have been frequently used to release the protected peptides from polymer supports under conditions that do not cleave acid- or base-labile protecting groups, the problem of light penetration into the core of the resin bead still remained. To test the performance of the new resin in SPPS, a photolabile linker, the *o*-nitrobenzyl group, was introduced to the resins.[11] After a model dipeptide, Fmoc-Gly-Phe-OH, was subsequently synthesized on the resins,[‡] the peptide was released from the linker by the photolysis reaction (350 nm)[12] and the product was analyzed quantitatively with HPLC[13] by using an internal standard, benzophenone (Scheme 2). The core-shell type PS-*co*-PEG resin (0.13 mmol NH_2/g) and the resins of amplified loading capacities such as the PS-*co*-PEG-Lys resin (0.21 mmol NH_2/g) and the PS-*co*-PEG-Lys-$(Lys)_2$ resin (0.30 mmol NH_2/g) were employed for this test.[14] For comparison, aminoisopropyl polystyrene resins (1.22 mmol NH_2/g)[15] and the TentaGel resin (0.26 mmol NH_2/g) were tested under identical reaction conditions.

As expected, the core-shell type PS-*co*-PEG resins were found to be more efficient than PS resin, even the TentaGel resin, in coupling Boc-Phe-O^-Cs^+ to the *o*-nitrobenzyl bromide linker[17] and the photolytic cleavage rate of the peptide product on the core-shell type PS-*co*-PEG resins was much faster than on the PS resin or the TentaGel resin. The rates became rather rapid when the initial loading level was amplified by coupling with Boc-Lys(Boc)-OH. Moreover, the core-shell type PS-*co*-PEG resins released the product in quantitative yield within 5 h. On the other hand, the PS resin released only 29% (similar to previously reported results[18]) and the TentaGel resin released only 59% of the products, even after 15 h (Fig. 2).

[‡] The coupling step was monitored for completion using the ninhydrin method.[20]



Scheme 2. (a) *N*,*N*′-Diisopropylcarbodiimide (DIPCDI) (3 equiv.), DCM, 1 h; (b) Boc-Phe-$O^{-}Cs^{+}$ (3 equiv.), *N*,*N*-dimethylformamide (DMF), 50°C, 6 h; (c) i. 25% trifluoroacetic acid (TFA)/DCM, 2 min and repeated for another 20 min; ii. 5% TEA/DCM, 2×5 min; iii. Fmoc-Gly-OH (3 equiv.), benzotriazoyl-*N*-oxytris-(dimethylamino)phosphonium hexafluorophosphate (BOP) (3 equiv.), 1-hydroxybenzotriazole (HOBt) (3 equiv.), diisopropylamine (DIPEA) (3 equiv.), 1 h; (d) *hv* (350 nm), MeOH

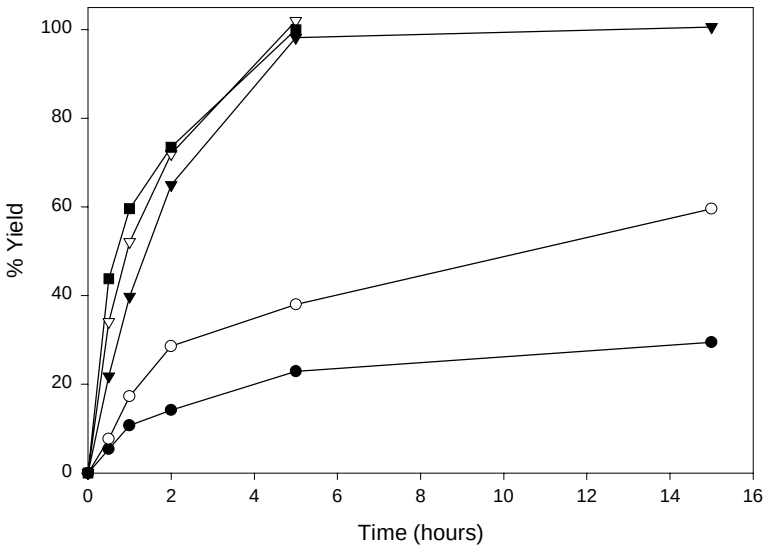


Figure 2. The kinetic profiles of photolysis for each resin: PS (●), TentaGel (○), PS-*co*-PEG-NH_2 (▼), PS-*co*-PEG-NH-Lys (▽), PS-*co*-PEG-NH-Lys-$(Lys)_2$ (■). Yield based on loading after coupling Phe (determined by picric acid titration)

From the results we have found that the core-shell type PS-*co*-PEG resins were free from the filtering effect caused by the resulting nitroso group during the photolysis.[19] The lower yield on the PS resin appeared reasonable because it did not swell enough in a hydrophilic solvent such as methanol.

In conclusion, we have developed a new resin consisting of a high cross-linked PS core and a PEG shell. The resin exhibited a rapid reaction rate in photolysis reactions, affording a high yield in SPPS. Since the resin has no diffusion problems for reagents, it can be utilized as an ideal support for SPOS. Further results will appear elsewhere soon.

Acknowledgements

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7. The cross-section was visualized by the following procedures: fluorescamine was attached to the amino groups of the resin and the resins were fixed by 2-HEMA. The stained beads were viewed through a fluorescence microscope after being sliced off by a microtome.
8. HERTEL & REUSS kassel CN-hF microscope was used for scanning electron microscopy (SEM) and cross-sections of stained beads were visualized using Olympus IX 70 fluorescence microscope and Sanyo VCB3524 1/2" CCD B&W camera.
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12. Photolyses were performed with 5 ~ 10 mg resins in a 300 μL degassed solvent containing 0.05 μmol of internal standard in 1.5 ml polypropylene Eppendorf tubes under Electro-Lite BONDW and UV Lamp at a measured intensity of 25 mW/cm^2.
13. HPLC was performed using Waters μBondapak C18 125 Å 10 μm 3.9×300 mm reverse-phase column. A flow rate of 2 ml/min and a 10 min gradient of 30–40% CH_3CN in water/0.1% TFA, a 10 min gradient of 40–60% CH_3CN in water/0.1% TFA followed by a 10 min constant flow of 60% CH_3CN in water/0.1% TFA was used. Absorbance was measured at 254 nm.
14. The loading capacity of the amino group was amplified by coupling Boc-Lys(Boc)-OH.
15. It was transformed from hydroxyisopropyl PS resin.[16]
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