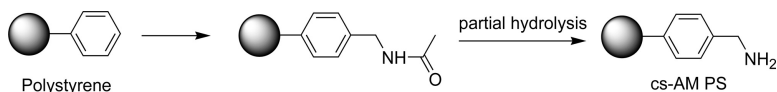## Report

Preparation of Core–Shell-Type Aminomethyl Polystyrene Resin and Characterization of Its Functional Group Distribution

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Reports

Preparation of Core−Shell-Type Aminomethyl Polystyrene Resin and Characterization of Its Functional Group Distribution

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Introduction

Since Merrifield introduced the concept of solid-phase synthesis,[1] various polymer supports have been developed to improve the synthetic efficiency of peptides, nucleotides, or small organic compound libraries. As an ideal polymer support, such properties as the mechanical/chemical stability and the facility of functionalization should be considered. For the diffusion of reagents into the polymer support, good swelling or compatibility is also needed. Among the various supports, 1% cross-linked PS/DVB resin beads have proven to be the most suitable and, so far, have been widely used.

In addition to the above factors, the distribution of the functional groups within the resin beads is another factor, in association with the accessibility of the reagents and the reaction kinetics, which are partly controlled by diffusion to the reaction sites. In the early days, autoradiography studies were performed to determine whether the reaction sites were distributed uniformly throughout the polymeric resin beads.[2] More recently, confocal microscopy has been used for the visual cross sectioning of the fluorophore-labeled beads. With this technique, it was demonstrated that there was a uniform distribution of reactive sites in ArgoPore (poly(ethylene glycol)-grafted polystyrene (PS-g-PEG)) resin but that the distribution in aminomethyl polystyrene (AM PS) resin was nonuniform.[3] In fact, no fluorescence was detected at the interior of the optical slices, due to the problems associated with the quenching and reabsorption of the fluorescence.[4,5] However, confocal microscopic analysis of the physically sliced beads demonstrated the homogeneity of the functional groups in the AM PS resin, in contrast to the results obtained from the optically sliced beads.[4] Furthermore, confocal Raman spectroscopy revealed that the

functional groups were uniformly distributed throughout the entire bead volume.[5,6]

On the other hand, we previously demonstrated that CutiCore resin, which was prepared by the copolymerization of styrene and PEG macromer, possessed a core−shell structure.[7] The CutiCore resin, poly(ethylene glycol)-surface grafted polystyrene (PS-sg-PEG) resin, was more efficient than PS or TentaGel (PS-g-PEG) resin in the coupling of amino acids during the early stages of solid-phase peptide synthesis (SPPS) and the photocleavage of peptides from the resin.[8] Other types of the core−shell-type resin were prepared from TentaGel resin by derivatizing only the outer layer of the bead using a biphasic solvent environment[9] or from AM PS resin by cross-linking it with 2,4,6-trichloro-1,3,5-triazine (cyanuric chloride) and grafting further with diamino PEG.[10] However, until now, no core−shell-type resin has ever been constructed from the conventional PS resin. In this paper, we report a novel method of preparing this core−shell-type resin starting from the PS resin.

Results and Discussion

Aminomethyl polystyrene (AM PS) resin, which is widely used for coupling a variety of linker/spacers in solid-phase synthesis, has been prepared by various methods, including phthalimidomethylation-dephthaloylation,[11,12] chloromethylation−ammonia substitution,[13] amidomethylation−deacylation,[14] and suspension copolymerization of phthalimide monomer−dephthaloylation,[15] among which the first method has been the most widely utilized. However, all of these methods suffer from various drawbacks, such as byproduct removal/long reaction time or undesired cross-linking during functionalization, especially in the case of the first and second methods.

Amidomethylation, which has been used to introduce the aminomethyl moiety to aryl groups,[16] can also be used for AM PS resin preparation. The acetamidomethyl group, a precursor of the aminomethyl group, can be introduced to PS resin using *N*-(hydroxymethyl)acetamide. Subsequent hydrolysis of the acetamidomethyl group with strong acid gives the aminomethyl group.

To synthesize the core−shell-type resin beads with most of the amino groups at the shell layer of the resin, we took advantage of the resistance of the amide bond to hydrolysis and the inaccessibility of polar solvents to the hydrophobic polystyrene residue. The acetamidomethyl group was loaded onto the PS resin with *N*-(hydroxymethyl)acetamide in the presence of trifluoroacetic acid (TFA) to give the acetamidomethyl PS resin (**1**) (1.4 mmol/g resin) (Scheme 1). In this reaction, the active species is the *N*-acylmethylene−

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Scheme 1. Preparation of Acetamidomethyl PS Resin (**1**), and cs-AM PS Resin (**2**) by Partial Hydrolysis[a]

Polystyrene   **1**   **2**
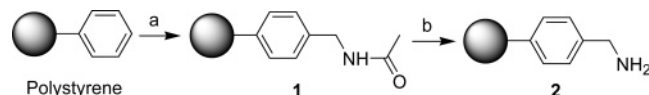
[a] (a) *N*-(hydroxymethyl)acetamide, TFA, 1,2-dichloroethane, reflux, 20 h. (b) 35% HCl, ethylene glycol, 110°C, 22 h; 1.0 N NaOH washing.

immonium ion[16] formed by the dehydration of *N*-(hydroxymethyl)acetamide catalyzed by TFA. The presence of the acetamidomethyl group was confirmed by IR (amide carbonyl band: 1649 cm^{-1}, Figure 1a) and elemental analysis. Then, the acetyl groups were removed from the resin with concentrated HCl. Even under harsh conditions (35% HCl/110 °C/overnight), the hydrolysis did not proceed to completion, and a portion of the acetamidomethyl groups inside the resin beads still remained. The resulting resin beads contained both aminomethyl and acetamidomethyl groups. The existence of acetyl groups in the partially hydrolyzed AM PS resin (**2**) was confirmed by the observation of the amide carbonyl band at 1649 cm^{-1} (Figure 1b). Compared with that of the acetamidomethyl PS resin (**1**), the intensity of the carbonyl band of the partially hydrolyzed AM PS resin (**2**) was weakened. After coupling with Fmoc-Gly-OH and removing the Fmoc group, the loading of free amino groups in the resin (**2**) was determined to be 0.92 mmol/g resin.

To investigate the distribution of the functional groups within the resin, the partially hydrolyzed AM PS resin (**2**)

was coupled with fluorescein isothiocyanate (FITC) and visualized by confocal microscopy.[3] As shown in the confocal image of the cs-AM PS resin (**2**) (Figure 2a1), the fluorescence was detected only at the surface, not in the interior of the optical slices, which means that most of the functional groups are located at the surface layer. In this case, the possibility of incomplete coupling of FITC to the amino groups located in the interior of the resin can be excluded, because the amino groups in the AM PS resin (**2**) were coupled in high yield with FITC (see the Experimental Section).

Recently, the Jung group[4] and the Bradley group[5] reported that the results from fluorescence microscopy might be misleading and that it would be dangerous to draw any firm conclusions purely on the basis of confocal microscopy (optical slicing) data, because the fluorescence originating from the resin core cannot be fully detected due to self-quenching or reabsorption.

To check the difference between the core−shell structure and non-core−shell structure using another method, the AM PS resins were coupled with FITC, cross sectioned, and viewed through an optical microscope. In the case of the partially hydrolyzed cs-AM PS resin (**2**), the stained area was concentrated at the shell layer, which demonstrates that most of the amino groups were located at the skin layer of the resin beads (Figure 2b1). Since the PS resin beads swell poorly in an aqueous solvent system, only the acetyl groups
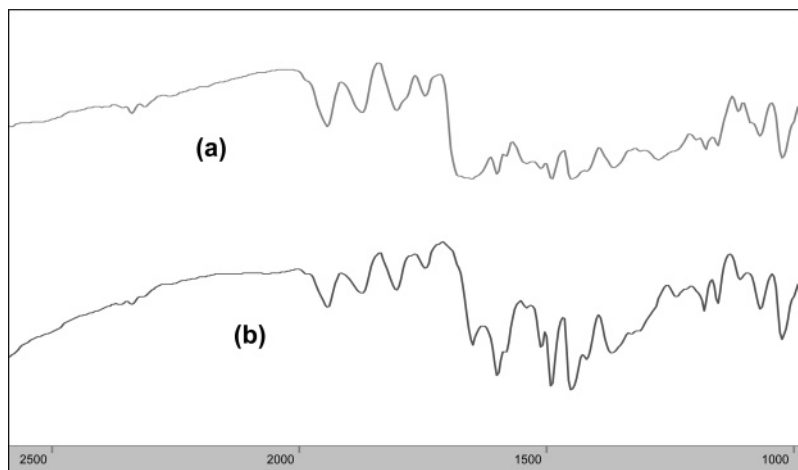

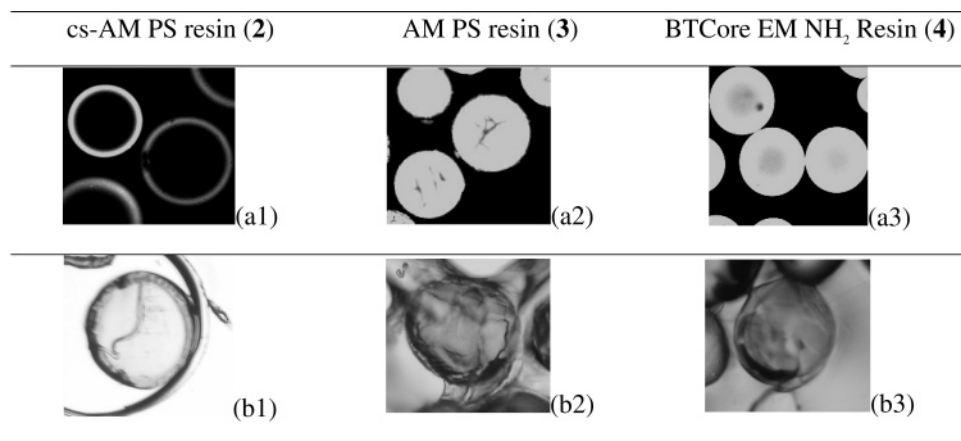**Figure 1.** FT-IR spectra of (a) acetamidomethyl PS resin (**1**), and (b) cs-AM PS resin (**2**).

Figure 2. Microscopic images of FITC-coupled resins: (a) confocal fluorescence images and (b) optical images of cross sectioned beads.
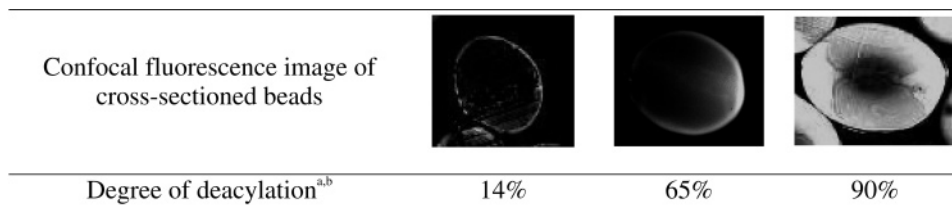
Figure 3. Confocal fluorescence images of cross sectioned cs-AM PS resins with different degree of deacylation, after fluorescence-staining with FITC. [a]Degree of deacylation (%) = (the loading level of cs-AM PS resin/the loading level of acetamidomethyl PS resin) × 100. [b]The degree of deacylation of 14, 65, and 90% correspond to the reaction time of 1 h, 22 h and 4 day, respectively.

at or near the shell layer are accessible to the strong acidic reagent and are, therefore, able to be removed, thus resulting in the core−shell-type structure.

In contrast to the above core−shell AM PS resin (**2**) and despite the incomplete coupling of FITC (see the Experimental Section), the other amino resins (commercially available AM PS resin (**3**)[17] and BTCore EM NH$_2$ resin[18] (2-aminoisopropyl PS) (**4**)) showed uniform distributions of the functional groups, in both the core and shell of the resins, which was confirmed by the confocal image and cross-sectional view of the FITC-coupled resins (Figure 2a2,b2,a3,b3).

On the other hand, the acetyl groups were removed from the acetamidomethyl PS resin (**1**) under the same condition as previously described (35% HCl/ethylene glycol, 110 °C) with varying the reaction time (1 h, 22 h and 4 day, respectively), and the functional group distributions in the cross-sectioned beads was analyzed as the degree of deacylation varies (Figure 3). We can clearly observe that as the deacylation proceeded, the FITC-stained area was increased. When the deacylation proceeded to 90%, the stained layer covered almost all of the inner area of the resin; however, the resin with a low degree of deacylation showed a very thin stained layer. These results confirm that the acetamidomethyl groups were functionalized uniformly within the resin beads, and the deacylation proceeded from the outer layer to the inner part of the resin beads.

In conclusion, through the partial hydrolysis of the acetamidomethyl groups, we prepared a new type of core−shell aminomethyl polystyrene (cs-AM PS) resin in which all of the amino groups were located at the skin layer, in contrast to the existing AM PS resin, in which the amino groups were distributed in both the core and shell parts of the resin. Since the core−shell-type resin is expected to be less influenced by the diffusion of reagents, its application to solid-phase synthesis should be promising, and investigations in this area are currently under way. The results will be reported elsewhere in due course.

Experimental Section

Preparation of Acetamidomethyl PS Resin (1). To a suspension of polystyrene resin (20.0 g, 1% DVB cross-linked, 100−200 mesh, BeadTech Inc.) in 1,2-dichloroethane (200 mL) were added N-(hydroxymethyl)acetamide (10.5 g, 115 mmol) and trifluoroacetic acid (50 mL), and the resulting resin mixture was heated at reflux for 20 h. After cooling to room temperature, the resin was collected by filtration; washed several times with THF × 2, DMF/water (3:1) × 2,

DCM × 2, and MeOH × 2; and dried in vacuo to give 21.5 g of acetamidomethyl PS resin **1** (loading level: 1.4 mmol/g). The loading of the acetamidomethyl group was determined by measuring the nitrogen content using elementary analysis, and the IR spectra of the resin were acquired after palletizing the resin (~10 mg) with 300 mg of KBr.

Preparation of Core−Shell-Type AM PS (cs-AM PS, Partially Hydrolyzed AM PS) Resin (2). After suspending the acetamidomethyl PS resin **1** (20 g) in ethylene glycol (150 mL), 35% HCl (150 mL) was added, and the mixture was stirred at 110 °C for 22 h. The resin was washed with THF/1 N NaOH (3:1) × 3, THF/water (3:1) × 3, THF × 3, and MeOH × 2 and dried in vacuo, yielding 18.5 g of cs-AM PS resin **2**.

For the resins with low and high degrees of deacylation, another batch of acetamidomethyl PS resin (20 g, 0.97 mmol/g) was reacted under the same condition. The resin samples were taken from the reaction mixture after 1 h and 4 days, respectively, and washed in the same way. Fmoc titration of the resin after coupling with Fmoc-Gly−OH gave 0.14 mmol/g (reaction time, 1 h) and 0.87 mmol/g (reaction time, 4 day), respectively.

Procedure for the Preparation of the FITC-Coupled Amino Resin. The amino resins were swollen in NMP, and 3 equiv of Fluorescein isothiocyanate (FITC, Aldrich) and 6 equiv of N,N-diisopropylethylamine were added. After 15 h, the resin was washed with NMP × 2, DCM × 2, MeOH × 2, NMP × 2, DCM × 2, and MeOH × 2 and dried in vacuo. The FITC-coupled resins were viewed through a fluorescence microscope (LSM 5 Pascal Zeiss). The FITC coupling yields were calculated by the elementary analysis of the FITC-coupled amino resins and the results are as follows: cs-AM PS resin (**2**), 0.92 mmol/g, 2.41% (0.60 mmol/g), 90%; AM PS resin (**3**), 1.1 mmol/g, 1.76% (0.30 mmol/g), 35%; BTCore EM NH$_2$ resin (**4**), 1.4 mmol/g, 2.35% (0.61 mmol/g), 67% (initial loading of the amino group, nitrogen content of the FITC-coupled amino resin (FITC loading), FITC-coupling yield, respectively).

Procedure for the Visualization of the Resin Cross Section. The FITC-coupled resin was suspended in 2-hydroxyethyl methacrylate (2.0 mL, Aldrich), and 2,2′-azobisisobutyronitrile (20 mg, Aldrich) was added. The mixture was maintained at 70 °C for 24 h, and the resin was fixed. The fixed FITC-coupled resin was sliced off using a microtome. The cross sectioned beads were viewed through a microscope (Olympus BX51-33MU).

Note Added after ASAP Publication. This paper was originally published ASAP on January 12, 2005 in "Article" format, rather than "Report" format. The corrected version was posted March 2, 2005.

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