A Novel Chemically and Mechanically Stable Glycerol-Based Crosslinked Polystyrene Support for Polypeptide Synthesis: A Comparative Study with Merrifield Resin

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ABSTRACT: A novel chemically and mechanically stable polymer support for solid-phase peptide synthesis with excellent swelling performance in various organic solvents is described. The crosslinked polymer in its bead form was prepared by the aqueous suspension polymerization of the styrene and tri(propylene glycol) glycerolate diacrylate. The support is unique because the hydroxyl group is introduced into the polymer support in the polymerization step itself, which makes it highly cost effective. The hydroxyl functionality of the crosslinker in the polymer was used as a growth site for peptide synthesis, thus minimizing the incompatibility arising because of the styrene core as compared to styrene-based polymer supports currently used in polypeptide synthesis. The utility of the resin for solid-phase peptide synthesis was tested by the synthesis of a 19-residue peptide amide of the envelope region of hepatitis C viral polyprotein and was compared with Merrifield resin by the 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluoro phosphate/1-hydroxy benzatriazole method with a Rink amide linker. The molecular character of the polymer and the extent of crosslinking density on the dependence of the reactivity of the attached amino groups were investigated by a reactivity study of the amide bond formation of a model Val-Ala dipeptide. Enhanced swelling, the increased rate of aminoacylation, and the high purity of the peptides synthesized on the novel support highlighted the influence of the polarity of the solid support in the solid-phase synthesis.
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Key words: resins; hydroxyl supports; peptides; swelling; crosslinking

INTRODUCTION

The selection of polymer support is the key factor that determines the purity and homogeneity of biomolecules such as polypeptide and oligonucleotides synthesized on it. Since R. B. Merrifield introduced this technique, this approach has been improved and generalized for the synthesis of high-purity polypeptides, oligonucleotides, oligosaccharides, and combinatorial peptide libraries.[1–5] The efficiency of this method depends mainly on the physicochemical properties of the polymeric support. For an effective solid-phase reaction, the polarity of the support has to be compatible with those of the reagents and the solvents used as well as those with the resin-bound peptide chains. In the refinement of solid-phase peptide synthesis, much attention has been focused on the development of new polymeric supports with improved polarity to that of the original Merrifield resin, which include polyamide-based supports, poly(ethylene glycol)–polystyrene (PS) graft resins, poly(ethylene glycol)-poly(acrylamide) (PEGA, polyoxyethylene-polyoxypropylene (POEPOP), polyoxyethylene-polystyrene (POEPS), crosslinked ethoxylate acrylate resin (CLEAR), superpermeable organic combinatorial chemistry resin (SPOCC), hydroxy- and amine-functionalized resin (HYDRA), JandaJel, and crosslinked polystyrene-ethylene glycol acrylate resin (CLPSER).[6–16] As an alternate route, a series of supports were developed in our laboratories by the replacement of the rigid hydrophobic divinylbenzene crosslinker by flexible hydrophilic crosslinkers.[17–19]

This article describes the synthesis of a novel tri(propylene glycol) glycerolate diacrylate crosslinked polystyrene support (PS–TRPGGDA), its utility in solid-phase peptide synthesis, and the influence of a hydrophilic crosslinker in solid-phase reactions. The polymer was tailor made in such a way that the crosslinker imparted the optimum hydrophilic–hydrophobic balance and the PS core imparted the necessary mechanical stability. The functional group was introduced in the polymerization step itself, which prevented the initial functionalization on the resin, and excellent control in the functionalization was achieved. In the new support, the functional hydroxyl groups were present on the hydrophilic crosslinker in contrast to the functional moieties being attached on

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the PS part in the usual PS-based supports. Although the attachment of linkers in the polystyrene–divinylbenzene (PS–DVB) resin (Merrifield resin) is able to modify the local environment around the reactive sites of these resins, they do not have a significant effect on the intrinsic hydrophobicity of PS-based resins, whereas in the new support, the excessive hydrophobicity is minimized by the incorporation of a hydrophilic crosslinker directly into the PS core. The synthesis of a 19-residue peptide amide of the envelope region of hepatitis C viral polyprotein (HCV) was carried out from the secondary hydroxyl group present in the crosslinker of the polymer by N^{α}-9-fluorenylmethyloxycarbonyl (Fmoc) chemistry. The high reactivity and excellent purity of the peptide amide synthesized on PS–TRPPGDA resin compared to PS–DVB resin highlighted the role of the polymer backbone in solid-phase peptide synthesis.

EXPERIMENTAL

Materials and methods

Styrene, tri(propylene glycol) glycerolate diacrylate (TRPGGDA), poly(vinyl alcohol) (PVA; $M_r \approx 75{,}000$), potassium acetate, hydrazine hydrate, thioanisole, ethanedithiol, piperidine, diisopropylethylamine (DIEA), trifluoroacetic acid (TFA), and *N*-methylimidazole were purchased from Sigma-Aldrich (Milwaukee, WI). Solvents (HPLC grade) were purchased from E. Merck (Mumbai, India) and BDH (Mumbai, India). *p*-{(R,S)-α[1-(9H-fluorene-9-yl)methoxy foramido]-2,4-dimethoxy benzyl}phenoxyacetic acid (Rink amide handle), 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluoro phosphate (HBTU), 1-hydroxy benzatriazole (HOBt), and 1-(2-mesitylenesulfonyl)-3-nitro-1H-1,2,4-triazole (MSNT) were purchased from Nova Biochem, Ltd. (Nottingham, UK). Fourier transform infrared (FTIR) spectra were recorded on a Bomem MB-series spectrometer (Quebec, Canada) with KBr pellets. Scanning electron microscopy (SEM) of the polymer was performed on a Hitachi S-2400 instrument (San Jose, CA). The cross-polarity/magic-angle spinning (CP-MAS) ^{13}C-NMR measurements were conducted on a Varian Unity 400 CP-MAS instrument (Palo Alto, CA) operating at 100 MHz. HPLC was done on a Pharmacia Akta purifier instrument with C-18 Sephasil peptide reverse-phase semiprep column (Amersham Biosciences, Uppsala, Sweden). The amino acid analysis was carried out on an LKB 4151 Alpha plus instrument. Mass spectra were recorded on a Kratos matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (Manchester, UK).

Synthesis of PS–TRPGGDA polymer

We removed the inhibitors from styrene and TRPGGDA by washing them with a 1% NaOH solution and distilled water. A four-necked reaction vessel equipped with a thermostat, a Teflon stirrer, a water condenser, and a nitrogen inlet was used for the polymerization. A net volume of 1% solution of PVA (~75,000) was prepared by the dissolution of PVA (1.1 g) in double distilled water (110 mL) and was added to the reaction vessel. The solution was deoxygenated with bubbling N_2 gas. In a typical polymerization for 4% PS–TRPGGDA, the monomers, styrene (9.9 g), TRPGGDA (1.94 g), and the radical initiator benzoyl peroxide (0.5 g) were mixed with toluene (8 mL) and added to the reaction vessel. The biphasic solution was stirred at a constant rate of 1500 rpm with a mechanical stirrer. The system was kept under a continuous flow of N_2 gas. The temperature of the reaction mixture was maintained at 80°C with a thermostatic oil bath, and the reaction was allowed to continue for 6 h. The copolymer was obtained as beads 100–200 mesh in size. The beads were washed thoroughly with hot water (to remove the stabilizer), acetone (3 × 50 mL), benzene (3 × 50 mL), toluene (3 × 50 mL), and methanol (MeOH; 3 × 50 mL). The copolymer was further purified by Soxhlet extraction with toluene, dichloromethane (DCM), and MeOH and was dried in a vacuum oven (P_2O_5, 50°C) for 10 h to yield 11.2 g of dry resin. The hydroxyl-group capacity of the resin was 0.20 mmol/g as determined by the MSNT method.[20]

Preparation of hydroxymethyl PS–DVB resin

Chloromethylated PS–DVB resin (1 g, 0.23 mmol) was allowed to swell in excess methyl cellosolve for 1 h. The resin was filtered, potassium acetate (0.22 g, 2.3 mmol) in methyl cellosolve (25 mL) was added, and the reaction mixture was stirred at 110°C for 48 h. The resin was collected and washed with *N*-methylpyrrolidone (NMP; 3 × 15 mL), DCM (3 × 15 mL), ethanol (3 × 15 mL), MeOH (3 × 15 mL), and ether (3 × 15 mL) and was dried *in vacuo*. The dried resin was suspended in ethanol (20 mL), hydrazine hydrate (0.11 mL, 2.3 mmol) was added, and the mixture was refluxed at 80°C for 48 h. The resin was collected by filtration and washed with DCM (3 × 15 mL), ethanol (3 × 15 mL), MeOH (3 × 15 mL), and ether (3 × 15 mL) and was dried *in vacuo*. The hydroxyl capacity of the resin was 0.20 mmol/g as estimated by the MSNT method.

Swelling behavior

The resin (1 g) was placed in a syringe fitted with a sintered Teflon filter, and the solvent was poured from the top of the syringe. A solvent flow was generated by the application of a constant suction at the syringe outlet. The suction was regulated to obtain a flow rate of 1 mL/min. The solvent was allowed to flow

through the resin for 30 min. The syringe outlet was closed, and the resin was suspended in the solvent for 1 h. The swollen resin was compressed with the piston of the syringe, and the pressure was slowly released. The volume of the resin at this point was noted and was related to the sample weight to determine the swelling abilities of the resin. The same experiment was used for the hydroxymethyl PS–DVB resin and the PS–DVB resin. The weight increases of the solvent-swollen resin beads were also noted and compared with the dry resin.

Preparation of PS–TRPGGDA–Rink amide resin

PS–TRPGGDA resin (1 g, 0.20 mmol) was swelled in dry DCM. After 1 h, the excess DCM was removed. Rink amide handle (377 mg, 0.54 mmol) and *N*-methyl imidazole (58 μL, 0.40 mmol) were dissolved in dry DCM (5 mL) and few drops of dry THF and were shaken with the resin in a septum-stoppered flask attached to a N_2 balloon. MSNT (207 mg, 0.54 mmol) was dissolved in dry DCM (5 mL) and injected to the reaction mixture. After 1 h, the resin was washed with dry DCM (5 $\times$ 15 mL) and ether (5 $\times$ 15 mL) and was dried *in vacuo*. The dried resin (10 mg) was mixed with 3 mL of 20% piperidine in dimethylformamide (DMF) for 30 min. The percentage incorporation of Rink handle was estimated by the measurement of the ultraviolet absorbance of the previous solution containing dibenzofulvene-piperidine adducts at 290 nm. The amino capacity of the resin was 0.194 mmol/g. PS–DVB Rink amide resin was also synthesized by the same procedure with PS–DVB–OH resin (1 g, 0.20 mmol/g), as described previously. The amino capacity of the resin was 0.186 mmol/g, as estimated spectrophotometrically.

C-terminal Fmoc–ala incorporation into the PS–TRPGGDA–Rink amide resin

PS–TRPGGDA–Rink amide resin (500 mg, 0.097 mmol) was swelled in DMF. After 1 h, the excess DMF was filtered off. Fmoc-protection of the resin was removed by 20% piperidine in DMF, and the resin was washed thoroughly with DMF (5 $\times$ 15 mL). Fmoc–Ala (105 mg, 0.34 mmol), HOBt (46 g, 0.34 mmol), HBTU (129 g, 0.34 mmol), and a DIEA (59 μL, 0.34 mmol) mixture in DMF (3 mL) were added to the swollen resin and kept for coupling for 1 h. The resin was filtered, washed with DMF (3 $\times$ 15 mL) and ether (3 $\times$ 15 mL) and dried *in vacuo*. The resin was negative to the sensitive Kaiser test.[21] The amino capacity of the resin was 0.187 mmol/g as estimated by the spectrophotometric method. PS–DVB Rink amide resin (500 mg, 0.093 mmol) was also anchored with Fmoc–Ala with the same procedure as described previously. The amino capacity of the resin was 0.173 mmol/g.

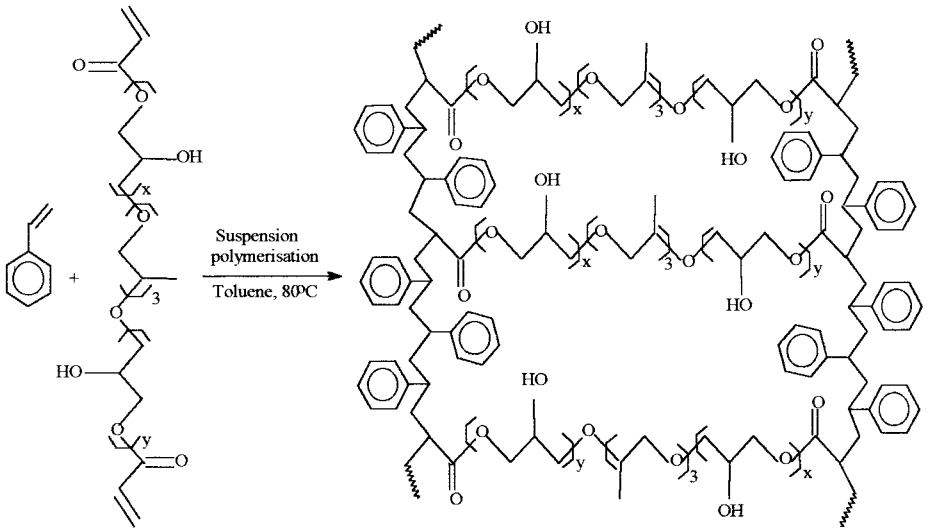
Reactivity comparison of amide bond formation

The influence of the solid support and the effect of crosslinking density in polymer supported reaction was studied with various crosslinking densities of PS–TRPGGDA resin and compared with Merrifield resin. 2% PS–TRPGGDA–Ala–NH_2 (825 mg, 0.08 mmol), 4% PS–TRPGGDA–Ala–NH_2 (410 mg, 0.08 mmol), 8% PS–TRPGGDA–Ala–NH_2 (180 mg, 0.08 mmol), and 1% PS–DVB–Ala–NH_2 (432 mg, 0.08 mmol) resin beads were used for the reactivity studies. The Fmoc–Ala attached resins were deprotected with 20% piperidine in DMF. The resins were washed with DMF (6 $\times$ 20 mL). Fmoc–Val (95 mg, 0.28 mmol) coupling was carried out with HOBt (38 mg, 0.28 mmol) and HBTU (106 mg, 0.28 mmol) as coupling reagents in the presence of DIEA (48 μL, 0.28 mmol). About 8 mg of the resin was withdrawn from the reaction mixture every 5 min up to 90 min. The resin was washed with DMF (5 $\times$ 6 mL), MeOH (5 $\times$ 6 mL), and ether (5 $\times$ 6 mL) and was dried. The percentage of coupling was calculated by measurement of the Fmoc release at regular intervals of accurately weighed resin by spectrophotometric monitoring of the absorbance of dibenzofulvene-piperidine adducts at 290 nm.

Comparative synthesis of the 19-residue peptide amide of the envelope region of the HCV

Thr-Pro-Gly-Cys-Val-Pro-Cys-Val-Arg-Glu-Gly-Asn-Ser-Ser-Arg-Cys-Trp-Val-Ala-NH_2

To demonstrate the utility of the new support in peptide synthesis, a 19-residue peptide amide of envelope region of HCV was synthesized. Fmoc–Ala anchored on 4% PS–TRPGGDA–Rink amide (270 mg, 0.05 mmol) and 1% PS–DVB–Rink amide resin (290 mg, 0.05 mmol) was used for the comparative study. Fmoc protection of these amino acid bound resins were removed by 20% piperidine in DMF, and the resins were washed thoroughly with DMF (5 $\times$ 15 mL). All of the amide bond formation was carried out in neat DMF in the presence of 3.5 equiv of excess (with respective to Fmoc–Ala Load) of the remaining Fmoc–amino acid (0.175 mmol), HBTU (66 mg, 0.175 mmol), HOBt (24 mg, 0.175 mmol), and DIEA (30 μL, 0.175 mmol). The coupling was allowed to continue for 50 min at room temperature. After the synthesis, the peptide was removed from the individual supports under same cleavage conditions with Reagent K [TFA/thioanisole/ethanedithiol/phenol/water (82.5:5:2.5:5:5)] for 4 h at room temperature.[22] The solution was filtered, and the filtrate was concentrated under pressure. The peptide was precipitated by the addition of ice-cold ether, and the precipitate was washed with ether until the scavengers were removed and was dried *in vacuo*. HPLC analysis of the peptide was carried out by the injection of a small amount of peptide dissolved in



Scheme 1 Synthesis of the PS-TRPGGDA polymer by free-radical suspension polymerization (x, $y \approx$ 2–3 glycolerate/tripropylene glycol).

water to C-18 reverse phase column (RPC) and elution with a gradient of solvent A (nanopure water containing 0.5% TFA) and solvent B (80% acetonitrile in nanopure water containing 0.5% TFA). The yield of crude peptide from PS–TRPGGDA resin was 95.7 mg (95%). The amino acid analysis results follow: Ala, 1.06 (1); Gly, 2.03 (2); Val, 3.13 (3). Cys, 3.14 (3); Arg, 2.05 (2); Pro, 1.43 (2); Ser, 1.58 (2); Asp, 1.02 (1); Thr, 0.62 (1); and Glu, 0.92 (1). Tryptophan was destroyed during hydrolysis. The low values for serine and threonine were due to partial degradation during hydrolysis, and the high value for aspartic acid was due to the hydrolysis of asparagine to aspartic acid.

Matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy (MALDI-TOF-MS) m/z = 2020.41 $(M + H)^+$; C_{83} H_{134} O_{25} N_{28} S_3 required a M^+ value of 2020.36. Yield of crude peptide from PS–DVB resin = 83.6 mg (83%).

RESULTS AND DISCUSSION

PS–TRPGGDA was synthesized by the free-radical aqueous suspension polymerization of the respective monomers with toluene as diluent and benzoyl peroxide as the initiator (Scheme 1). The mechanical agitation of this mixture resulted in the formation of small uniform droplets of the dispersed monomer mixture. Resins with varying crosslinking densities were prepared by the adjustment of the comonomer feed ratio. The beaded polymer was obtained in 96% yield when the impeller speed was kept at a constant rate of 1500 rpm. At very high speeds, the polymer yield was reduced drastically, and this resulted in the formation of emulsion particles. When the speed increased beyond 3000 rpm, the polymer yield decreased, perhaps because of excessive shearing of the polymer bead. In every round of polymer synthesis, by the fine-tuning of the stirring speed, the quantity of the stabilizer, and the geometry of the vessel and the stirrer, the insoluble polymer could be reproduced as spherical beads.

The morphological characteristics of the polymer were determined by SEM analysis (Fig. 1). The polymer surface was smooth and spherical, as evidenced by SEM. The polymer was characterized by a gel-phase CP-MAS ^{13}C-NMR spectrum. The spectrum of the resin in $CDCl_3$ showed an intense peak at 127.79 ppm corresponding to aromatic PS carbons and a peak at 145.034 ppm for the C-3 carbon of the styrene. The peak at 75.012 ppm corresponded to the methine carbon of the crosslinker with secondary hydroxyl groups. The peaks at 40.221 and 30.443 ppm corresponded to the backbone methylene carbon of the polymer.

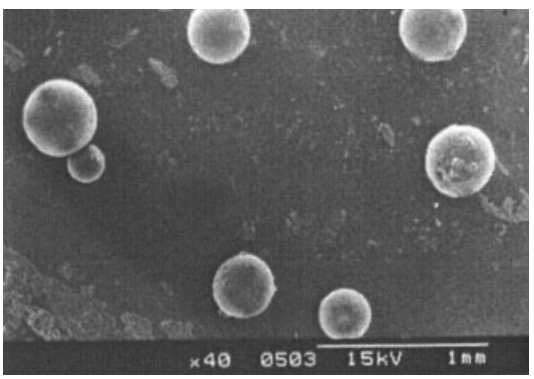


Figure 1 Scanning electron micrograph of a few beads of the PS-TRPGGDA.

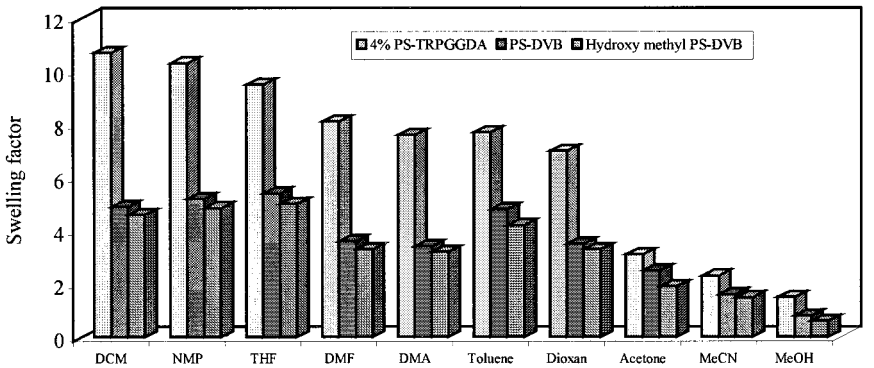


Figure 2 Swelling comparison between 4% PS-TRPGGDA, PS-DVB, and hydroxymethylated PS-DVB resin in various solvents.

The major advantage of the new support was that the drastic initial functionalization of the phenyl ring that is necessary for PS-based supports could be prevented in the new PS–TRPGGDA support because of the secondary hydroxyl group of the crosslinker. Excellent control in the degree of functionalization could be achieved because these functional groups were introduced in the polymerization step itself. This property makes the new support highly cost effective. A resin with a hydroxyl content of 0.1–0.45 mmol/g was synthesized by the variation of the crosslinking density of the TRPGGDA monomer. The hydroxyl capacities of the support were determined by esterification of the resin with Fmoc–Gly by the MSNT method. In the swollen state, the highly flexible nature of the crosslinker had a positive impact on the reaction because the growth point was easily available to perform various chemistries. The presence of sufficient secondary hydroxyl functional sites and the hydrophilicity of the crosslinker due to its oxypropylene chains, ester functionalities, and hydroxyl groups were the primary factors in the selection of TRPGGDA as the comonomer for the polymer synthesis. The percentage of crosslinker in the polymer support was chosen for each synthesis based on the nature of peptide sequence and the quantity of peptide required. A comparative synthesis of the 19-residue peptide amide of the envelope region of the HCV was carried out on a 4% PS–TRPGGDA support and Merrifield support (1% PS–DVB resin). The direct incorporation of the C-terminal Fmoc–amino acid to the secondary hydroxyl functionality of the crosslinker in the polymer could be used for the synthesis of polypeptide. However, in this method, the rate of the final cleavage of the peptide from the support by acid hydrolysis is slow. In this study, a rink amide linker was incorporated between the resin and the growing peptide chain. This helped the cleavage of the peptide from the support under preferred conditions and also as a peptide amide.

Success of a solid-phase synthesis depends on the solvation degree of the polymer matrix.[23] For an effective solid phase peptide synthesis (SPPS), the accessibility of the immobilized substrate to low-molecular-weight reagents and solvents is very important. The reactive functional group in the resin will have maximum accessibility toward the reactants only when polymer matrix swells extensively in the solvating medium. The high swelling character of PS–TRPGGDA resin in a broad range of solvents revealed the hydrophilic nature of the crosslinker, which conferred an optimum hydrophobic/hydrophilic balance to the resin. Comparative swelling studies with PS–DVB resin showed that the swelling characteristics were mainly dependent the chemical nature of the resin (Fig. 2). The swelling of the new resin was almost twice that of the PS–DVB resin in DCM, NMP, THF, dioxane, DMF, and DMA. The chemical stability of the resin was tested under various reaction conditions of polypeptide synthesis such as TFA, 30% TFA in DCM, piperidine, 20% piperidine in DMF, NH_2OH, and liquor ammonia. After 48 h, the FTIR spectrum of the resin showed no change from the pretreated resin, suggesting that the crosslinks were stable enough to withstand the various conditions used in solid-phase peptide synthesis.

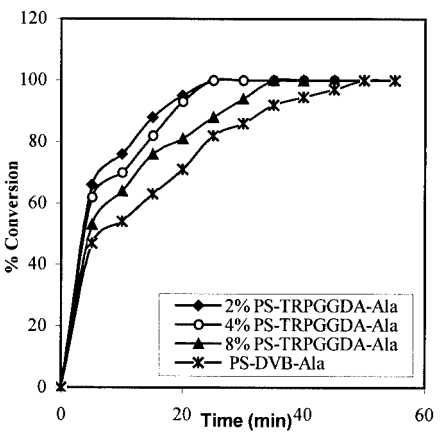


Figure 3 Reactivity comparison of amide bond formation with PS-DVB resin.

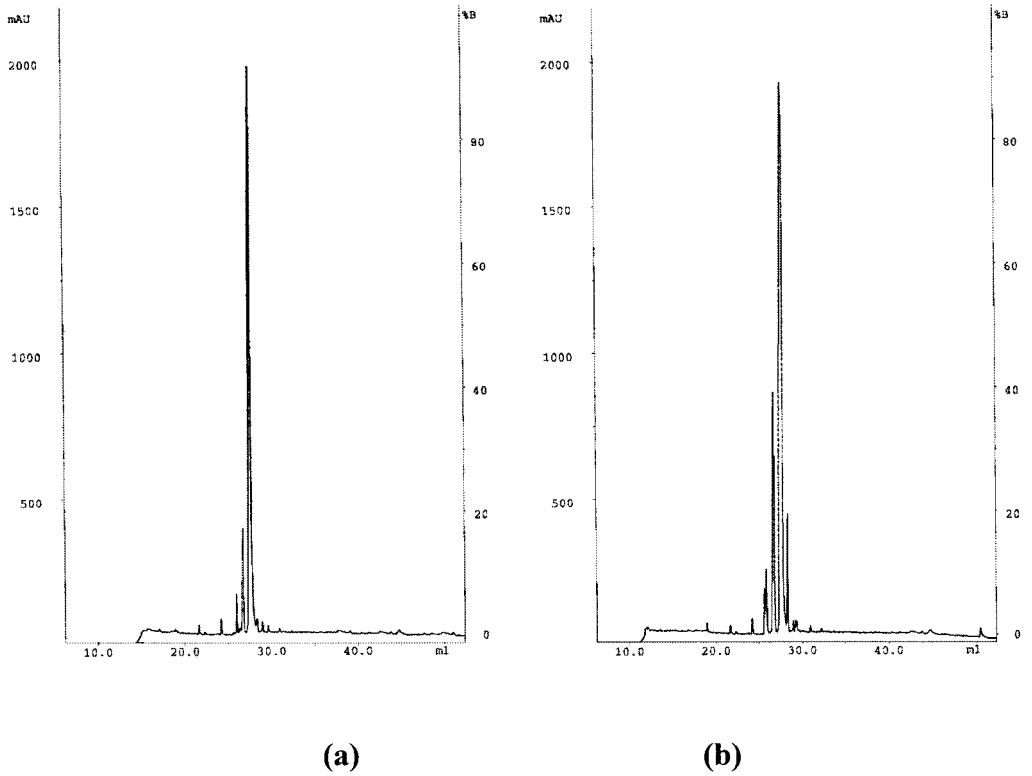


Figure 4 HPLC profile of the 19-residue peptide of the envelope region of the HCV synthesized on (a) PS-TRPGGDA-Rink amide resin and (b) Merrifield–Rink amide resin. Eluting buffer A was composed of 0.5 mL of TFA in 100 mL of water. Eluting buffer B was composed of 0.5 mL of TFA in 100 mL of 30% water/acetonitrile. Samples were eluted with a linear gradient at 1 mL/min.

Comparative reactivity study of amide bond formation

To gain the full advantage of solid-phase peptide synthesis, the reaction performed on the resin should go to completion, preferably at high rate. This ensures the high purity of the end product. A better understanding of the reactivity of the attached groups enables one to optimize the reaction conditions in SPPS, where multiple operations are used repeatedly. The influence of chemical nature and the effect of the crosslinking density of the polymer support in solid-phase peptide synthesis were studied with the amide bond formation of a Val-Ala model peptide. PS–TRPGGDA–Ala resins of identical functional-group capacity were used for this investigation, and a comparative study of the same reaction was carried out with the PS–DVB resin. The effects of various crosslinking densities of the resin on the reaction rate of the PS–TRPGGDA resin supported synthesis were also investigated by selection of resins with 2, 4, and 8% crosslinking densities. The time-dependent aminolysis studies revealed that when PS–TRPGGDA resin was used in 2 and 4% crosslinked resins, 95% of the amino acid coupling reaction took place within 20 min, whereas in PS–DVB, only 71% of the reaction was completed under identical conditions (Fig. 3). The slow reaction rate of PS–DVB at the initial stage was due to the slow swelling rate. The amine groups that resided inside the matrix were available for reactions only when the resin swelled extensively. In the case of the new resin, the solvent uptake was very fast, and the swelling characteristics were higher than those of the PS–DVB resin. The differences in the reactivity among 2 and 4% PS–TRPGGDA resin were marginal. Even 8% crosslinked PS–TRPGGDA showed a higher rate of aminolysis compared to the PS–DVB resin. The reactions on the PS–DVB resin tended to be affected by the hydrophobicity of the polymer matrix, which resulted in a reduction in the reactivity involving polar species. The enhanced reactivity in the PS–TRPGGDA support was primarily due to the maximum solvation of the polymer matrix and the positioning of the functional group on the crosslinker, which reduced the steric hindrance caused by the PS core.

Comparative synthesis of the 19-residue peptide amide of the envelope region of the HCV

To demonstrate the synthetic efficiency of the novel glycerol-based support in solid-phase synthesis, a 19-residue peptide amide of the envelope region of the HCV was synthesized and compared with the Merri-

field resin. Rink amide handle attached 4% PS–TRPGGDA and 1% PS–DVB resins were used for the comparative study. The Rink amide handle was incorporated into the resin hydroxyl functionality (0.2 mmol/g) before respective Fmoc–amino acid incorporation by the MSNT method. The C-terminal Fmoc–Ala was anchored to the linker-attached supports with the HBTU/HOBt/DIEA method. Fmoc–Ala (94%) was incorporated into the PS–TRPGGDA resin within 15 min of reaction, whereas under identical conditions, the attachment was 61% for the Merrifield resin. The spacer effect was the same in the two supports because both were derivatized with Rink amide linker; the PS–TRPGGDA resin had a higher reactivity because the reactions in the new support were less affected by the hydrophobic PS core, as the reactive site was located on a flexible crosslinker, which was separated away from the PS backbone. The sequential incorporation of the remaining Fmoc–amino acids was performed by a standard Fmoc protocol with HOBt, HBTU, and DIEA. DMF was used as the solvent throughout the synthesis. Temporary amino protection was removed with 20% piperidine in DMF. After the synthesis, the peptide was removed from the corresponding resins under the same cleavage conditions with Reagent K. The yields of the crude peptide amide obtained after 4 h cleavage were 95.7 and 83.6 mg from PS–TRPGGDA and PS–DVB, respectively. The HPLC profile of the crude peptide obtained from PS–TRPGGDA showed a sharp single major peak, which indicated the homogeneity of the peptide synthesized (Fig. 4), whereas the PS–DVB resin showed additional peaks along with the major peak corresponding to the deletion sequences. Amino acid analysis and MALDI-TOF-MS confirmed the identity of the target peptide sequence. The enhanced purity and yield of the peptide in the case of the PS–TRPGGDA resin could be attributed to the flexible nature of the crosslinks, which provided optimal swelling and compatibility of the growing peptidyl resin, leading to successful peptide chain assembly. The comparative study confirmed that PS–TRPGGDA resins could be used successfully in the solid-phase synthesis of polypeptides.

CONCLUSIONS

This study illustrated the synthesis of a novel crosslinked polymer support by the combination of the hydrophilic feature of TRPGGDA crosslinker and the hydrophobicity of the PS core. The new support showed excellent physicochemical properties, thereby providing a range of solvent compatibility for solid-phase peptide synthesis. The increased reactivity of PS–TRPGGDA support in the comparative aminolysis studies revealed that the nature of the crosslinking agent in the copolymer had a striking influence on the reactivity of the attached amino groups. The utility of the polymer in SPPS was demonstrated by the synthesis of a 19-residue peptide amide of the envelope region of the HCV. The enhanced reactivity, high yield, and purity of peptide synthesized on PS–TRPGGDA resin confirmed that the polarity of the macromolecular matrix, high degree of swelling–solvation, optimum hydrophobic–hydrophilic balance, and mechanical and chemical stability are the important criteria for an ideal polymeric support.

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