Merrifield Resin and 1,6-Hexanediol Diacrylate-Crosslinked Polystyrene Resin for Solid-Phase Peptide Synthesis: A Comparative Study

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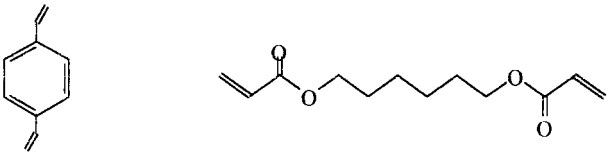
ABSTRACT: Three short peptides were synthesized on Merrifield resin (PS–DVB) and on polystyrene crosslinked with 1,6-hexanediol diacrylate resin (PS–HDODA) to compare their efficiencies in solid-phase peptide synthesis (SPPS). A 2% crosslinked polymeric system having almost equal capacity was used in both cases. The peptides were synthesized using standard solid-phase methodology. In the case of the PS–HDODA resin, all the couplings were completed by the first coupling and the peptides were obtained in $>$ 90% yield and $>$ 95% purity. But in the case of the PS–DVB resin, most of the attachments require two to three couplings and the peptides were obtained in about a 65% yield. © 1999 John Wiley & Sons, Inc. J Appl Polym Sci 71: 1933–1939, 1999

Key words: Merrifield resin; PS–HDODA resin; comparison; SPPS

INTRODUCTION

In the stepwise synthesis of peptides on crosslinked macromolecular supports, it was generally believed that the support would act only as an inert, passive solid carrier. However, many investigations during the last two decades dealing with the quantitative aspects of polymer-supported reactions have shown that the insoluble support does have a significant dynamic influence on the bound substrates. An efficient polymeric support for peptide synthesis should have an optimum hydrophobic–hydrophilic balance compatible with the peptide being synthesized. Moreover, the conformational changes in the growing peptide after each amino acid attachment are also to be considered since the changing conformations can have a dynamic influence on the physical and chemical properties of the growing peptide. The success of solid-phase synthesis depends on the swelling characteristics of the polymer and the solvation of the peptidyl resin in different solvents.[1] Systematic studies on polymer-support reactions have shown that the use of a flexible polymer support enhances the reactivity due to its enhanced solvation characteristics. Based on this idea, polystyrene (PS) crosslinked with 1,6-hexanediol diacrylate (HDODA), with a hydrophobic–hydrophilic balance optimized based on the extent of crosslinking, was developed in our laboratory for the synthesis of peptides.[2] From systematic investigations on swelling and reactivity studies of this support, a 2% crosslinked system was found to be suitable for the synthesis of peptides.[3]

Structural Comparison Between DVB and HDODA



DVB HDODA

1. HDODA is flexible due to the six-carbon chain. A flexible support enhances the re-

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Table I Swelling Capacity (mL/g of Resin) of DVB–PS and HDODA–PS Resins in Solvents Used for Peptide Synthesis

Solvent	DVB–PS	HDODA–PS
DCM	5.2	10.0
DMF	3.5	7.2
MeOH	0.95	2.0
NMP	4.0	9.1

activity due to its enhanced solvation characteristics.

2. HDODA is slightly hydrophilic due to two acrylate linkages.

Comparing Swelling Behavior of DVB–PS and HDODA–PS Resins

From Table I, evidently, the PS–HDODA resin has almost double the value of the swelling capacity in the all the solvents used for peptide synthesis. A high value of the swelling capacity means easy accessibility of functional groups by reagents and chemicals and that leads to high reactivity.

In the present article, it was proposed to synthesize three short peptides using these two supports in order to compare their efficiency in solid-phase peptide synthesis. A 2% crosslinked polymeric system was used in both cases. The following peptides were synthesized on both resins to compare their efficiency in the synthesis:

1. Leu–Thr–Val–Ala–Lys–Leu.
2. Pro–Lys–Tyr–Ile–Gly.
3. Ala–Thr–Lys–Val.

These peptides are some of the partial sequences of thioredoxin (T). Thioredoxin is a naturally occurring sulfur-reducing protein containing 108 amino acid residues.[4] Most of the sequences are conformationally important regions.[5] All these peptides were synthesized on 2% PS–HDODA and PS–DVB resins using a standard solid-phase technique. The homogeneity of the peptides was checked by fast protein liquid chromatography (FPLC) and the peptidyl resins were subjected to amino acid analysis.

EXPERIMENTAL

Materials

Styrene, HDODA, protected amino acids, dicyclohexylcarbodiimide (DCC), trifluoroacetic acid (TFA), and thioanisol were purchased from Sigma Chemical Co. (St. Louis, MO). Simple Boc–amino acids were prepared according to reported procedures.[6,7] Chloromethyl methyl ether (CMME) was prepared using a literature procedure.[8] Peptide homogeneity was demonstrated by FPLC. Amino acid analyses were performed on a Pharmacia LKB Alpha Plus amino acid analyzer after hydrolyzing the samples.

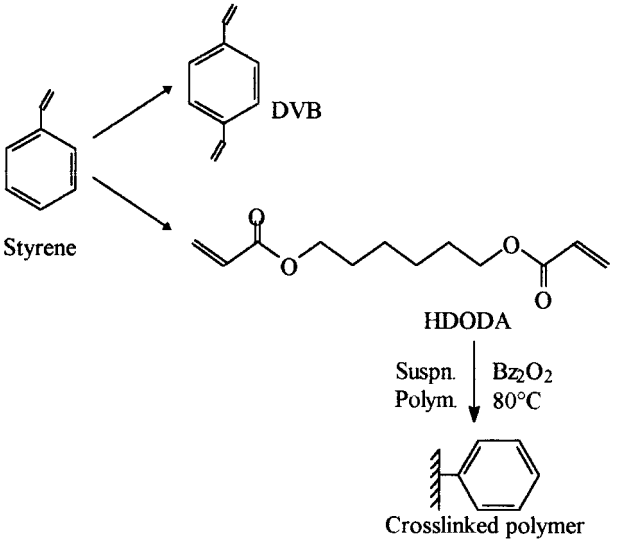
Polymer Synthesis

Preparation of HDODA-Crosslinked PS

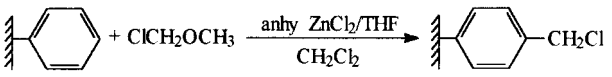
Both polymers were synthesized by the suspension polymerization method. A mixture of styrene, HDODA or DVB, toluene as an inert diluent, and benzoyl peroxide were suspended in a solution of poly(vinyl alcohol) (MW 75,000) dissolved in water. The reaction mixture was kept mechanically stirred at 80°C. The polymerization was completed within 6 h. The beaded product was filtered, washed with hot water, acetone, and methanol and dried. The resin was purified by Soxhlet extraction using acetone. The dried beads were sieved into different mesh sizes (Scheme 1). Beads of 200–400 mesh sizes were used for all the syntheses.

Functionalization of the Supports with Chloromethyl Groups[9]

Chloromethyl functional groups were introduced into the resin by CMME in the presence of anhy-



Scheme 1 Preparation of crosslinked polymers by suspension polymerization.



Scheme 2 Functionalization of the supports.

drous $ZnCl_2$ as a catalyst (Scheme 2). The chlorine capacity was determined by the pyridine fusion method.[10]

General Procedure for Solid-Phase Peptide Synthesis

Solid-phase peptide synthesis (SPPS) was carried out manually in a glass reaction vessel. Peptides were synthesized using this polymer support by following the conventional Boc–benzyl ester strategy of Merrifield.[11] The first amino acid was attached to the chloromethyl resin by Gisin's[12] cesium salt method and the amino capacity was determined by the picric acid method.[13] Subsequent amino acids were assembled in a stepwise manner to form the desired sequence by the DCC method.[14] The Boc group was deprotected by using 30% TFA in dichloromethane (DCM) and neutralization was carried out by using 5% TEA. *N*-Methyl 2-pyrrolidone (NMP) was used as the solvent and the coupling time was 1 h. The same procedure was adopted for the coupling of all the remaining amino acids. The progress of the coupling was monitored at every stage by the Kaiser test.[15] In all the couplings, a threefold molar excess of Boc–amino acid was used and the precipitated DCU was removed by washing with 33% MeOH in DCM. Final cleavage of the peptide from the support was effected by TFA/thioanisol method.[16] Benzyl ester cleavage is catalytically favored by thioanisol. The benzyl side-chain protecting groups were removed by hydrogenation of the crude peptide using activated palladium charcoal in methanol under hydrogen for 24 h.[17]

Purity of the Peptides

Purity of the crude peptides were checked by FPLC. Conditions: Solvent A, water containing 0.1% TFA; solvent B, acetonitrile containing 0.1% TFA; flow rate 0.5 mL/min; detection 214 nm.

Amino Acid Analysis

The peptidyl resin was hydrolyzed using a mixture of propionic acid and concentrated HCl (1:1 v/v) and heating to 120°C for 6 h. The resin was removed by filtration and the solution was quantitatively transferred to a standard flask with distilled water. This solution was then diluted with buffer and applied to the amino acid analyzer.

RESULTS AND DISCUSSION

Synthesis of Leu–Thr(OBzl)–Val–Ala–Lys(2ClZ)–Leu (T53–58)

Synthesis of this hexapeptide was carried out on a 2% PS–HDODA resin (2.01 mmol/g). Boc–Leu was attached to this by the cesium salt method and the substitution level (1.75 mmol/g) was determined by the picric acid method. The remaining amino acids were coupled to the Boc–Leu resin by the standard solid-phase strategy. The final peptide was cleaved from the resin by TFA. The crude peptide was obtained in a $>$ 90% yield and purity was checked by FPLC [Fig. 1(a)]. The single peak obtained is a clear indication of the high purity of the crude peptide ($>$ 95% purity).

Amino acid analysis of the peptidyl resin gave the following results:

Leu 2.10 (2.00), Thr* 0.60 (1.00), Val 1.00 (1.00), Ala 0.89 (1.00), Lys 0.92 (1.00).

The above peptide was synthesized also on the 2% PS–DVB resin (2 mmol/g). Boc–Leu was attached to this and the amino capacity was found to be 1.65 mmol/g. The remaining amino acids were attached to the resin by DCC coupling. Here, for all the attachments of amino acids, double coupling was required for the completion of the reaction. The crude peptide was obtained in about a 65% yield and the purity was checked by FPLC [Fig. 1(b)].

Amino acid analysis of the peptidyl resin gave the following results:

Leu 1.80 (2.00), Thr* 0.31 (1.00), Val 0.85 (1.00), Ala 0.75 (1.00), Lys 0.80 (1.00).

Synthesis of Pro–Lys(2ClZ)–Tyr(Z)–Ile–Gly on PS–HDODA Resin (T68–72)

Boc–Gly was attached to the 2% PS–HDODA resin by the cesium salt method (1.82 mmol/g).

*Thr was lost during hydrolysis.

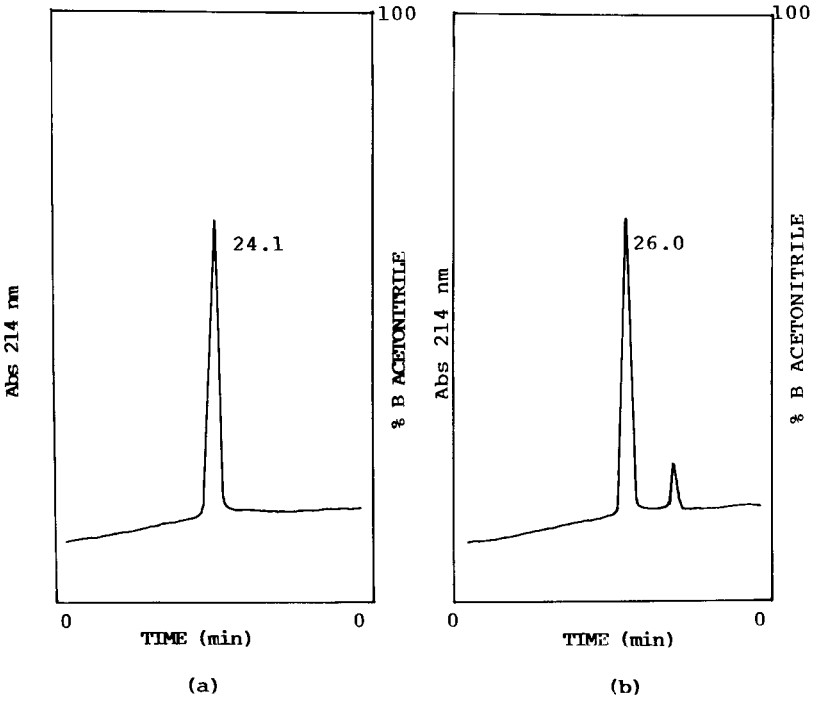


Figure 1 FPLC profiles of crude peptide Leu–Thr–Val–Ala–Lys–Leu on (a) PS–HDODA resin and (b) PS–DVB resin. Conditions: Solvent A, water containing 0.1% TFA; solvent B, acetonitrile containing 0.1% TFA; flow rate 0.5 mL/min; detection 214 nm.

The amino capacity of the first loading was determined by the picric acid method (1.71 mmol/g). The same procedure of deprotection, neutralization, and coupling was carried out subsequently. All the couplings were completed by the first coupling itself, except in the case of Tyr where a second coupling was required for the completion. The crude peptide was obtained in $> 90\%$ yield.

The FPLC profile of the crude peptide showed only one major peak, indicating that the peptide is $> 95\%$ pure [Fig. 2(a)]. This was characterized by amino acid analysis by hydrolyzing the peptidyl resin.

The amino acid analysis was as follows:

Pro 0.98 (1.00), Lys 0.95 (1.00), Tyr† 0.3 (1.00), Ile 0.89 (1.00), Gly 1.01 (1.00).

The values shown in parentheses are theoretical values to which we are comparing the experimental values.

This peptide was also synthesized on a 2% PS–DVB resin. Boc–Gly was anchored to the resin (1.8 mmol/g) and the amino capacity was found to be 1.62 mmol/g. The remaining amino acids were attached by DCC coupling. Here, for all the amino acids, a second coupling was required, and for Tyr, a third coupling was required for the completion of the coupling. The finished peptide was cleaved from the support by TFA. Crude peptide was obtained in about a 60% yield. The purity of the crude peptide was checked by FPLC [Fig. 2(b)]. The peptidyl resin was subjected to amino acid analysis:

Pro 0.80 (1.00), Lys 0.85 (1.00), Ile 0.80 (1.00) Gly 0.90 (1.00).

Tyr was lost during hydrolysis.

Synthesis of Ala–Thr(OBzl)–Lys(2ClZ)–Val on PS–HDODA Resin (T88–91)

This tetrapetpide was synthesized on the 2% PS–HDODA resin. Boc–Val was attached to the chloromethyl resin (2.01 mmol/g capacity) by the cesium salt method. The substitution level of Boc–Val was determined by the picric acid method

† Tyr undergoes degradation during hydrolysis.

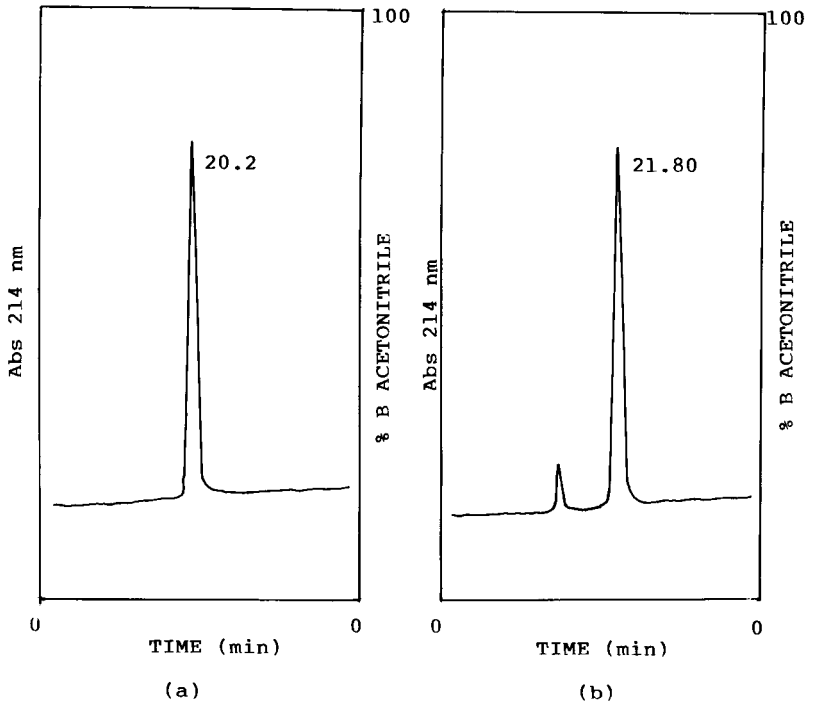


Figure 2 FPLC profiles of crude peptide Pro–Lys–Tyr–Ile–Gly on (a) PS–HDODA resin and (b) PS–DVB resin. Conditions: Solvent A, water containing 0.1% TFA; solvent B, acetonitrile containing 0.1% TFA; flow rate 0.5 mL/min; detection 214 nm.

(1.91 mmol/g). The Boc group was removed by treatment with 30% TFA/CH_2Cl_2 and the resulting amine salt was neutralized with 5% TEA. The peptide chain was built by sequentially extending it toward the amino terminus by stepwise addition of Boc–amino acids. Here, all the four couplings were completed by the first coupling itself. But in all cases, a second coupling was also carried out to ensure a complete reaction. The final peptide was cleaved from the resin by TFA in the presence of scavengers. All the side-chain protecting groups except the benzyl groups were removed by TFA. The benzyl side-chain protecting group was removed by hydrogenation. Crude peptide was obtained in 95% yield. Homogeneity of the peptide was checked by FPLC and had only one major peak [Fig. 3(a)]. The crude peptide itself was 100% pure. The peptidyl resin was subjected to amino acid analysis:

Ala 0.90(1.00), Thr* 0.62(1.00),
Lys 0.85(1.00), Val 0.91 (1.00).

The same peptide was also synthesized on the PS–DVB resin. Here, also, a 2% crosslinked system having a chlorine capacity (2 mmol/g) was used. Boc–Val was attached to this resin by the cesium salt method and the substitution level was determined by the picric acid method (1.85 mmol/g). The synthesis procedure used for the PS–HDODA resin was exactly followed here.

Here, in the case of Thr and Lys, a second coupling was required for the completion of the coupling. The crude peptide was obtained in about 70% yield. The purity of the crude peptide was checked by FPLC and had only one major peak [Fig. 3(b)]. The peptidyl resin was subjected to amino acid analysis:

Ala 0.85 (1.00), Thr* 0.60 (1.00),
Lys 0.81 (1.00) Val 0.90 (1.00).

CONCLUSIONS

Three partial sequences of thioredoxin were synthesized on PS–HDODA and PS–DVB resins to compare their efficiency in the solid-phase peptide synthesis. Both resins were prepared by the suspension polymerization method and functionalized to obtain almost equal chlorine-capacity values. The peptides were synthesized using the

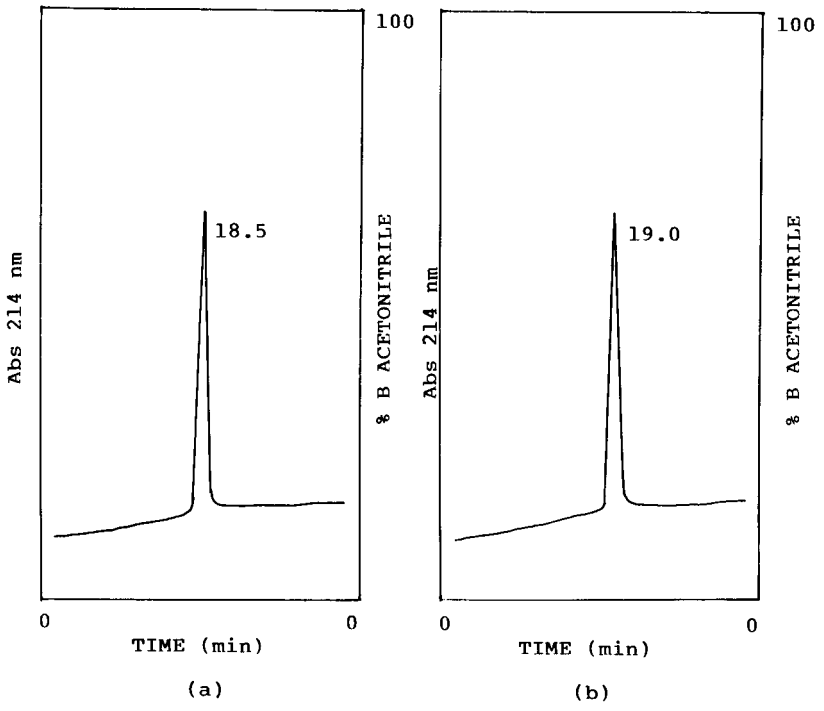


Figure 3 FPLC profiles of crude peptide Ala–Thr–Lys–Val on (a) PS–HDODA resin and (b) PS–DVB resin. Conditions: Solvent A, water containing 0.1% TFA; solvent B, acetonitrile containing 0.1% TFA; flow rate 0.5 mL/min; detection 214 nm.

standard solid-phase method using both resins. The first amino acid was attached to both resins by the cesium salt method and adjusted to obtain an almost equal amino capacity. The peptides were synthesized in both the cases using the Boc strategy, and for coupling, the DCC method was used. In the case of the PS–HDODA resin, most of the couplings were completed by the first coupling itself. But in the case of the PS–DVB resin, most of the couplings required double coupling for the completion of the reaction. All the peptides were obtained in $> 90\%$ yield on the PS–HDODA resin. But on the PS–DVB resin, the peptides were obtained in about a 60–65% yield. The crude peptides were $> 95\%$ pure on the PS–HDODA resin as evident from the FPLC profiles. In the case of the PS–DVB resin, the purity of the crude peptides obtained were $< 90\%$.[‡]

From these studies, it is evident that the PS–HDODA resin is more suitable for peptides synthesis than is the Merrifield resin. In the case of the PS–HDODA resin, the favorable solvation and swelling characteristics of the support facilitated effective synthesis and the flexibility of the support enhances its reactivity. From the high yield and purity of the peptides obtained, it is evident that the PS–HDODA resin is an ideal support for peptide synthesis compared to the Merrifield resin.

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[‡] We have compared the efficiencies of the PS–HDODA and PS–DVB resins by solid-phase peptide synthesis by synthesizing three peptides using both resins under identical conditions. From the high yield and the purity of the peptides obtained, it is evident that the PS–HDODA resin is better than is the PS–DVB resin for solid-phase synthesis.

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