Segregation of Resin-Bound Peptides During Solid-Phase Synthesis

I. M. Krishna Kumar, Beena Mathew

School of Chemical Sciences, Mahatma Gandhi University, Priyadarshini Hills, P.O. Kottayam-686 560, Kerala, India

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ABSTRACT: To compare the segregation ability of 1,4-butanediol dimethacrylate-crosslinked polystyrene (BDDMA-PS) and divinylbenzene-crosslinked polystyrene (DVB-PS), a set of difficult sequence peptides characterized by high-arithmetic-average β-sheet stabilizing potential (SP_β) and low-stepwise arithmetic average random coil conformational parameter (P_c*) were synthesized on both supports ($\sim$ 2 mmol Cl g^{-1}) under identical conditions. The yield and purity of the peptides obtained from BDDMS-PS resin were higher than from DVB-PS resin. The synthetic efficiency of the new support was found to be its ability to suppress the aggregation of growing peptide chains by β-sheet formation. © 2002 Wiley Periodicals, Inc. J Appl Polym Sci 86: 1717–1723, 2002

Key words: polystyrene; supports; incompatibility

INTRODUCTION

The improvements and developments in the methodology of solid-phase peptide synthesis (SPPS) are made in such a way that it is now possible to use this method for the synthesis of even large-sized peptides. Still, many problems of stepwise solid-phase peptide synthesis remain unresolved.[1–5] The most serious difficulty often encountered in SPPS is the sudden decrease of reactivity of the resin-bound substrates caused by the steric occlusion of peptide chains within the polymeric network.[2,3] This is due to the interplay of several factors such as the physicochemical incompatibility of the hydrophilic peptide chains and hydrophobic polymeric backbone, inter- and intramolecular hydrogen bonding contributed by the pendent peptide chains, presence of sterically hindered amino acids, and secondary structure-favoring sequences.[4] Among the hydrogen-bonded interactions, interchain interactions due to the formation of definite secondary structures, especially β-sheet aggregates, are chronic problems in both solution-phase (insolubility of peptide intermediates) and solid-phase (repetitive incomplete amino acylation and deprotection) peptide synthesis.[1–3] Stepwise assembly of such peptides (so-called difficult sequences) is found to be very difficult on both Merrifield type of polystyrene and Sheppard type of polyamide resins, currently available commercially.[6] The intrasite interactions that are known to be dependent on the local density of peptide chains[7] may also contribute to coupling difficulty.[4] Early reports from our laboratory[8,9] have shown that hydrophilic flexible crosslinked polystyrene supports possess the suitable chemical nature and topographical structure necessary for the rapid and successful synthesis of peptides even at high capacity. In the present study, we compare the segregation ability of newly developed flexible and hydrophilic 1,4-butanediol dimethacrylate (BDDMA, 2mol %)-crosslinked polystyrene and rigid and hydrophobic divinylbenzene (DVB, 2mol %)-crosslinked polystyrene support bound peptides during solid-phase synthesis. A set of hydrophobic peptides with high SP_β values[10] and low stepwise P_c* values,[2] typical of difficult sequences, were selected and built on both resins under identical conditions. The peptides used in the present investigation are given below.[11] Solid-state Fourier transform infrared (FTIR) spectroscopy was used to monitor the conformational behavior of resin-bound peptides.

1. VAVI
2. KVAVI
3. NKVAVI
4. ANKVAVI
5. VAVAAG
6. VQELG
7. QVGVQELG

EXPERIMENTAL

General

Trifluoroacetic acid (TFA), BDDMA, and styrene were purchased from Aldrich Chemical Co., USA. Protected amino acids, dicyclohexylcarbodiimide (DCC), diisopropylethylamine (DIEA), thioanisole, and 1-hydroxy

Correspondence to: B. Mathew (mgu@md2.vsnl.net.in).

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benzotriazole (HOBt) were obtained from Sigma Chemical Co., USA. Chloromethyl methyl ether (CMME, a dreadful carcinogen) was prepared by the method of Marvel and Porter.[12] All solvents were doubly distilled and dried before use. FTIR spectra were recorded on KBr disks by using a Bruker IFS 55 spectrophotometer. The KBr disks of peptidyl resins were prepared by grinding resin and KBr in a ratio of 1 : 100 in an agate mortar for 15 min under constant shear stress. Solid-state ^{13}C-CP-MAS-NMR spectrum of the BDDMA-PS resin was recorded on a DSX-300 instrument. UV measurements were carried out on a Shimadzu 160A spectrophotometer. HPLC analyses were performed on a Pharmacia semipreparative system by using reverse-phase C-18 column and binary gradient elution. Solvent system used was 0.1% TFA/H_2O (A) and 0.1% TFA/CH_3CN (B). The flow rate was 1.5 mL/min and detection was done at 214 nm. Amino acid analysis was performed on an LKB alpha plus 4151 amino acid analyzer, using *o*-phthaladehyde, after hydrolyzing with 6*N* HCl at 110°C for 22 h. The peptidyl resin was hydrolyzed using 6*N* HCl : propionic acid (1 : 1 v/v) mixture at 120°C for 24 h. Elemental analysis was done on a Carlo Erba 1108 elemental analyzer.

Preparation of BDDMA-PS

A three-necked polymerization vessel equipped with a Teflon stirrer, water condenser, and nitrogen inlet was used for polymerization. Measures of 11.2 mL styrene, 0.44 mL BDDMA, and 500 mg benzoyl peroxide were mixed and dissolved in 20 mL toluene. The reaction mixture was added to 1% poly(vinyl alcohol) solution (200 mL) and kept under stirring at 600 rpm and 85°C. After 15 h, precipitated copolymer was filtered, washed with hot water, and with toluene, acetone, dichloromethane, and methanol. The copolymer was dried under vacuum at 40°C. Yield: 88%.

Swelling studies

Solvent uptake of the various resins was checked by centrifuge method. A sample of the resin was taken in a sintered glass stick (porosity of 3) and immersed in the solvent for 24 h. The stick was then transferred to a centrifuge tube where it was held in position. Excess solvent was then removed. The stick and the contents were weighed. Immersion in the solvent was continued for 5 min and excess solvent was removed by centrifuge method. These operations were continued until a constant weight was obtained. Finally, a similar blank experiment was conducted by using an empty sintered stick. The data are expressed as the amount of solvent absorbed per unit weight of the dry resin (mL/g).

Synthesis of peptides 1–7 on BDDMA-PS and DVB-PS resins

The peptides were assembled on chloromethylated resin. C-terminal amino acid was attached to the resin by cesium salt method.[13] The amino acylation level was determined by picric acid titration method[14] and amino acid analysis. Syntheses were performed manually on a silanized glass reaction vessel using butyloxycarbonyl (Boc) chemistry. An amount of 2.5 mmol excess HOBt active esters of Boc amino acids was used for each coupling. *N*-methyl pyrrolidone (NMP) containing 20% v/v of DMSO was selected as the coupling medium. A standard time of 1 h was given to each coupling and the reaction was monitored by the Kaiser test.[15] Deprotection of the amino-protecting Boc group was achieved with TFA/DCM (30% v/v) for 30 min, followed by neutralization with 5% DIEA/NMP and 5% DIEA/DCM for 5 min. The completed peptide was cleaved from the support using acidolysis and transesterification methods. For acidolysis, 100 mg of the peptidyl resin was treated with neat TFA containing thioanisole and 1,2-ethanedithiol (10 : 1 : 1) and kept at 35°C for 16 h. Resin was filtered and TFA was evaporated off. The peptide was then precipitated with cool and dry diethyl ether, collected by centrifugation, and lyophilized. Yield of the peptides (%), based on the first amino acid substitution level, obtained from BDDMA-PS and DVB-PS (in parentheses) is as follows: Peptide **1,** 98 (90); Peptide **2,** 98 (82); Peptide **3,** 94 (80); Peptide **4,** 92 (72); Peptide **5,** 91 (70); Peptide **6,** 95 (79); Peptide **7,** 89 (60). The transesterification was performed by adding 30 mL dry methanol and 4 mL triethylamine to 100 mg of the peptidyl resin at 50°C for 10 h. The resin was filtered and the filtrate was evaporated to get the peptide methyl ester, which was washed with diethyl ether and lyophilized. Yield of the peptides (%), based on the first amino acid substitution level, obtained from BDDMA-PS and DVB-PS (in parentheses) is as follows: Peptide **1,** 91 (76); Peptide **2,** 89 (74); Peptide **3,** 84 (67); Peptide **4,** 80 (61); Peptide **5,** 88 (63); Peptide **6,** 94 (68); Peptide **7,** 86 (55).

RESULTS AND DISCUSSION

Preparation of 2 mol % crosslinked BDDMA-PS

The copolymer, BDDMA-PS, was obtained in the bead form by the free-radical-initiated suspension polymerization of styrene with BDDMA in 85–90% yield. The copolymer beads were characterized by infrared spectroscopy (IR) and ^{13}C-CP-MAS-NMR spectroscopy. IR spectrum showed a sharp peak at 1720 cm^{-1} corresponding to the ester carbonyl group of the crosslinking agent. Solid-state ^{13}C-CP-MAS-NMR spectrum showed an intense peak at 130.4 ppm corresponding to the aromatic carbons and a small peak at 148.2 ppm

TABLE I
Solvent Uptake Capacities of the DVB-PS and BDDMA-PS in mL/g

Solvent	DVB-PS	BDDMA-PS
Chloroform	4.0	7.0
Dioxane	3.2	6.8
Dichloromethane	4.1	10.3
N,N-Dimethylformamide	2.6	7.0
Methanol	1.6	3.8
Toluene	4.5	7.4
N-Methyl pyrrolidone	4.3	9.1
Tetrahydrofuran	5.0	8.6
Pyridine	3.9	6.9

due to the para carbon of the styrene. Peak due to the methylene carbons of the crosslinking agent appeared at 64 ppm. and polymer backbone methylenes gave a singlet peak at 42.7 ppm.

Swelling studies

From the swelling studies of BDDMA-PS resins in solvents commonly used for SPPS, it was found that 1 and 2% crosslinked resins swell maximum in polar aprotic solvents. The crosslink density and polarity of crosslinks can change the solvation properties of the macromolecular network.[16] As the percentage of crosslinking increases, solvent content of the polymer network decreases. Maximum solvent uptake should be favored in solid-phase reactions for the easy permeation of the reagents into the polymer networks. Considering both the high solvent content and the increased mechanical stability, 2 mol % crosslinked resins were selected for the present study. A comparison of the solvent-imbibing characteristics of the 2 mol % crosslinked systems showed that BDDMA-crosslinked resin swell better than DVB-PS resin (Table I). The presence of four methylene carbons and two ester linkages of BDDMA make it more flexible and polar than the rigid and hydrophobic DVB-PS (Fig. 1), which contribute a more open network of the polymer chains in BDDMA-PS. This may be the reason for the better uptake of both polar and nonpolar solvents.

Chloromethylation of BDDMA-PS and DVB-PS resins

BDDMA-PS and DVB-PS resins were functionalized by chloromethylation by using CMME and $ZnCl_2$.[17] On keeping the reaction for 3.5 h, BDDMA-PS gave a chlorine capacity of 1.97 mmol/g. Under the same conditions, DVB-PS resin was kept for 8 h to get a capacity of 2.07 mmol/g. The high reactivity of BDDMA-PS resin may be due to the greater uptake of dichloromethane (DCM) in which the reaction was carried out. A crosslinked polymer becomes a highly swollen gel when it comes in contact with good solvents,[18] and hence, the reagents diffuse well into the polymer network. IR (KBr): 1420 and 670 cm^{-1} (C—Cl *str*.).

Stability of BDDMA-PS resin

For a polymer support, certain mechanical and chemical stabilities are required because the resin has to be subjected to vigorous shaking, filtration, and drastic acid-base treatment during the peptide synthesis and various polymer analogous reactions. Therefore, a temperature of 130°C for 24 h was applied to 2 mol % BDDMA-PS resins without affecting the physical or morphological parameters. Acid stability of the resin was checked by keeping 500 mg resin in neat TFA for 24 h and at 6*N* HCl at 110°C for 48 h. Another sample of the resin was kept in concentrated KOH solution at 80°C for 48 h. IR spectrum of these posttreated resins showed that acid–base treatments have not made any change in the physicochemical nature of the polymer. The greater stability of the alkyl ester linkage of the crosslinker in BDDMA-PS resin may be due to the fact

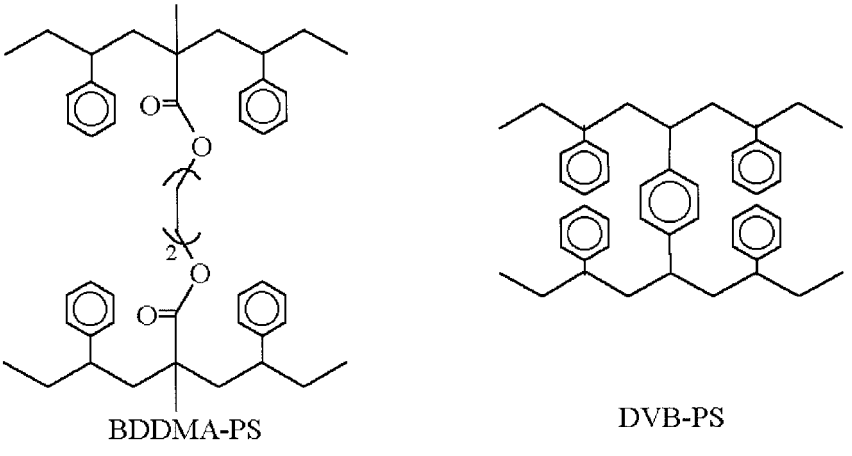

Figure 1 Structure of BDDMA-PS and DVB-PS resins.

that the chance of formation of alkyl cation is very small because it is not as stable as the benzylic cation formed during TFA cleavage of the peptide from the support. Therefore, the peptides can be conveniently cleaved off from the support without affecting the ester linkage of the BDDMA crosslinking in the polymer matrix.

Synthesis of peptides 1–7 on BDDMA-PS and DVB-PS resins

The peptides were synthesized on BDDMA-PS and DVB-PS resins by the stepwise attachment of the amino acids to the C-terminal amino acid, which was already anchored to the polymer support. The reaction time required by the cesium salt of Boc-glycine and Boc-isoleucine to get attached to BDDMA-PS resin (24 h) was considerably less than the time required on DVB-PS resin (56 h). Boc-glycine was attached to BDDMA-PS and DVB-PS resins to a level of 1.92 and 1.87 mmol of NH_2/g and Boc-isoleucine was esterified to 1.85 and 1.79 mmol/g, respectively. The high reactivity of BDDMA-crosslinked polystyrene arises from the increased segmental mobility of the polymer chains owing to the greater flexibility and compatibility of this copolymer under the conditions of esterification. Therefore, the reagents can easily penetrate into the resin networks and all the reactive sites are equally accessible for nearly quantitative coupling. However, in DVB-PS resin, the final stage of the reaction may be suppressed by the steric effect caused by the poor swelling of the rigid polymer backbone.

The remaining amino acids were assembled by using 2.5 mequiv excess of HOBt active ester of Boc-amino acids in NMP, which disrupt β-sheet formation.[19] Dimethyl sulfoxide (DMSO) 20% v/v was also added to the reaction mixture at the end of 30 min to destabilize the β-sheet structure.[20] All the couplings on BDDMA-PS resin were complete within 1 h. However, many instants on DVB-PS resin were incomplete and couplings were repeated until a negative Kaiser was obtained. This was evident from the total acylation time required for the synthesis of each peptide on both support. Reaction time (h) of each peptide on BDDMA-PS and DVB-PS (in parentheses) is as follows: peptide **1**, 3 (3); peptide **2,** 4 (7); peptide **3**,5 (7); peptide **4**, 6 (9); peptide **5**, 5 (8); peptide **6**, 5 (9); peptide **7**, 9 (14). No termination reaction was performed during synthesis. The completed peptides were cleaved from the supports by using both TFA/thioanisole and transesterification (triethylamine and methanol) methods. All peptides were cleaved nearly quantitatively from BDDMA-PS support. The yield of the peptides obtained from DVB-PS resin was low, especially with nucleophilic cleavage. It has been reported that the aggregation of resin-bound peptide chains can interfere with nucleophilic cleavage, which is a method to prepare protected peptide fragments.[21] Intermolecularly hydrogen-bonded aggregation of the pendant peptide chains may be the reason for the difficulty in synthesis and low yield of the peptides from DVB-PS resins. Aggregation of peptide chains produces additional crosslinking in the peptidyl resin network, which would markedly decrease the solvation of polymer and peptide chains. For transesterification, methanol was used as the reaction medium. The swelling of DVB-PS in methanol is already low (1.6 mL/g) as compared to BDDMA-PS (3.8 mL/g).

The homogeneity of the synthesized peptides was checked by thin layer chromatography (TLC), HPLC, and amino acid analysis of peptidyl resins. It was observed from TLC that all the peptides obtained from BDDMA-PS resin were purer. The purity of the crude peptides was further ascertained by HPLC analysis. From the profiles, it is clear that the peptide obtained from BDDMA-PS was highly homogenous (R_t values are given in Table II). As an example, HPLC profile of the crude peptide **7** synthesized on both the supports are given in Figure 2. This was confirmed by the amino acid analysis of peptidyl resins, which is an indication of the homogeneity of the growing peptide chains. The values of the peptides grown on BDDMA-PS resins correlate well with the actual value, whereas the amino acid analysis data of peptides bound to DVB-PS indicate the presence of impure peptides (Table III). The homogeneous peptides obtained from BDDMA-PS support were characterized by elemental analysis and mass spectroscopy.

The reason for the formation of deletion peptides in the case of DVB-PS resin may be attributed to the steric occlusion of the growing peptide chains in the polymer matrix. Moreover, the solvation of DVB-PS resins in methanol is less (1.6 mL/g), in which the ninhydrin solution (Kaiser test) was prepared. So, the reactive amino groups buried under the polymer network may not be available for reaction, which may lead to the wrong conclusion in the Kaiser test. The additional crosslinking via β-sheet formation in the peptide-resin network further reduces the solvent uptake of resins. However, the flexible and polar nature of the crosslinking agent, BDDMA, increases the solvation and the mobility of the functional sites attached to the polymer backbone.

FTIR investigation of resin-bound peptides

To gain insight into the conformational behavior of peptides when bound to flexible BDDMA-PS and rigid DVB-PS resin, a set of difficult sequence peptides characterized by high SP_β values[10] (> 5) and low P_c* values[2] (< 0.9) were selected and built on both the supports. These peptides have preferential β-sheet propensity as evidenced from their average conformational parameters (Table IV).[22] According to Narita et

TABLE II
Synthetic Results of the Peptides 1–7

Peptides	CHN analysis (%)[a]			R_t (min)	Mol. wt.[b]
	C	H	N		
$C_{25}H_{46}N_4O_7$	57.98	8.89	10.88	14.6	514.1
	(58.34)	(9.01)	(10.95)		(514.6)
$C_{39}H_{62}N_6O_{10}Cl$	57.06	7.08	10.18	14.3	810.9
	(57.72)	(7.83)	(10.36)		(811.4)
$C_{43}H_{69}N_8O_{12}Cl$	55.21	7.93	12.94	13.8	925.5
	(55.80)	(7.52)	(12.11)		(925.3)
$C_{46}H_{74}N_9O_{13}Cl$	54.90	7.11	13.04	14.1	996.1
	(55.44)	(7.48)	(12.65)		(996.6)
$C_{27}H_{48}N_6O_9$	53.84	8.87	13.68	12.1	600.3
	(53.98)	(8.05)	(13.98)		(600.1)
$C_{35}H_{54}N_6O_{11}$	57.49	7.09	11.14	16.7	656.2
	(57.21)	(7.41)	(11.43)		(657.7)
$C_{47}H_{74}N_{10}O_{15}$	54.97	6.91	13.01	14.6	942.1
	(55.39)	(7.32)	(13.74)		(940.9)

[a] Theoretical values are given in parentheses.
[b] Molecular weight is determined by electrospray ionisation mass spectroscopy. Calculated values are given in parentheses.

al., peptides with SP_β values > 5.0 will be highly stabilized in β-sheet structure and hence difficult to synthesize.[10] FTIR investigation of peptidyl resins was carried out, as described by Narita et al.[23] Although an isolated planar amide bond (—CO—NH—) can have nine bands of stretching frequencies in the range 200-3500 cm^{-1}, the most significant regions, namely, amide A (3500–3200 cm^{-1}) and amide I (1600–1700 cm^{-1}), were selected for the conformational assignments of the resin-bound peptides. Both BDDMA-PS and DVB-PS resins have no characteristic absorptions in this region. The amide V region (600–750 cm^{-1}) was not selected because strong bands due to the aromatic rings of the polymer support may appear in this region. The absorption band observed at 1603 cm^{-1} was taken as the standard and was normalized to unity for each relative intensity.

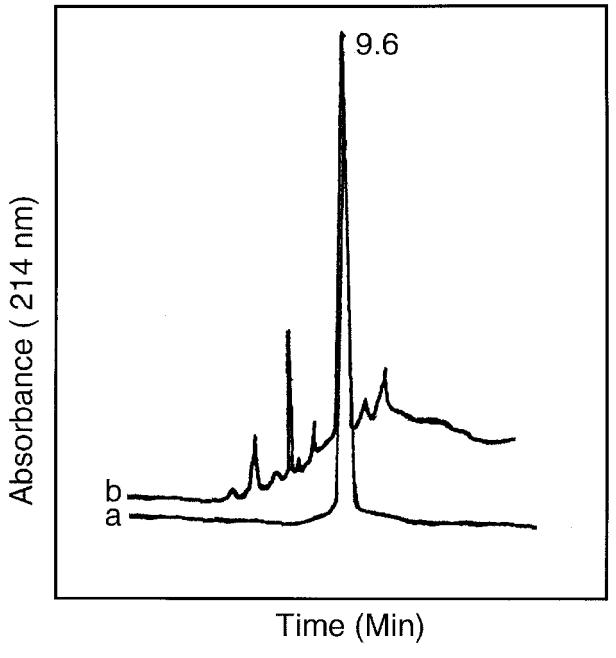

Figure 2 HPLC profiles of peptide **7** synthesized on (a) BDDMA-PS and (b) DVB-PS.

Boc-deprotected peptides **1–7** showed a weak and broad peak around 3280–3285 cm^{-1} (amide A) and 1635–1645 cm^{-1} (amide I) when bound to DVB-PS resins. This was assigned to β-sheet structure.[24] The same peptides appeared when bound to BDDMA-PS resin; the intense peaks appeared at 3430 cm^{-1} (amide A) and 1665 cm^{-1} (amide I), in addition to the peaks corresponding to β-sheet structure. These peaks were assigned to a random coil conformation.[24] Therefore, β-sheet formation is largely suppressed on BDDMA-PS support. The correct position of the absorption bands for each peptide bound to both BDDMA-PS and DVB-PS resins are given in Table IV. Thus, it is clear that a β-sheet to random coil conformational transition occurs when the support was changed from DVB-PS to BDDMA-PS, although the β-sheet structure was not completely disrupted. Thus, the pendant peptide chains can easily interact with one another by intermolecular hydrogen bonding when they were bound to DVB-PS resins. Therefore, β-sheet formation may be the reason for the coupling difficulty with DVB-PS support. Because random coil conformation is more prone to solvation, aminoacylation reaction on BDDMA-PS support is fairly easy.

An interaction between the growing peptide chain and the polymer backbone was noticed from the FTIR spectrum of the BDDMA-PS support carrying peptide chains. The characteristic peak observed at 1720 cm^{-1} for BDDMA-PS resin was shifted to a broad peak at 1705 cm^{-1} when peptide chains are growing on it. As an example, the spectra of peptidyl resin **1** was given

TABLE III
Amino Acid Analysis of Peptidyl Resins

Peptide	Systems used	Amino acid analysis[a,b] G	A	L	V	I	K	D	E
	BDDMA-PS	—	1.04	—	1.98	1.0	—	—	—
VAVI	DVB-PS	—	0.86	—	1.88	1.0	—	—	—
			(1.0)		(2.0)	(1.0)			
	BDDMA-PS	—	1.01	—	2.11	1.0	1.01	—	—
KVAVI	DVB-PS	—	0.88	—	1.73	1.0	1.01	—	—
			(1.0)		(2.0)	(1.0)	(1.0)		
	BDDMA-PS	—	0.97	—	2.08	1.0	1.06	0.82	—
NKVAVI	DVB-PS	—	0.73	—	1.81	1.0	1.18	0.41	—
			(1.0)		(2.0)	(1.0)	(1.0)	(1.0)	
	BDDMA-PS	—	2.13	—	1.92	1.0	0.96	1.0	—
ANKVAVI	DVB-PS	—	1.72	—	1.88	1.0	1.22	1.0	—
			(2.0)		(2.0)	(1.0)	(1.0)	(1.0)	
	BDDMA-PS	1.0	3.09	—	1.91	—	—	—	—
VAVAAG	DVB-PS	1.0	2.53	—	1.61	—	—	—	—
		(1.0)	(3.0)		(2.0)				
	BDDMA-PS	1.0	—	0.89	1.11	—	—	—	—
VQELG	DVB-PS	1.0	—	0.68	0.81	—	—	—	—
		(1.0)		(1.0)	(1.0)				
	BDDMA-PS	2.02	—	0.91	1.86	—	—	—	2.61
QVGVQELG	DVB-PS	1.62	—	0.81	1.58	—	—	—	2.20
		(2.0)		(1.0)	(2.0)				(3.0)

[a] Theoretical values are given in parentheses.
[b] N and Q are converted to D and E during hydrolysis.

along with the spectrum BDDMA-PS resin in Figure 3. A similar observation was reported on 1,6-hexanediol diacrylate-crosslinked polystyrene also.[9] The flexibility of the support offers a significant possibility of intermolecular hydrogen bonding between the carbonyl group of the crosslinking agent, BDDMA, and the amide hydrogen of the growing peptide chain. The peptide–polymer interaction may destabilize the regular intermolecular hydrogen bonding of pendant peptide chains which causes the suppression of resin-bound β-sheet structure. Therefore, the synthesis of difficult sequences on this support becomes easy. This is again the reason for the nearly quantitative cleavage yield of peptides even with nucleophilic reagents and the homogeneity of the products. In the case of DVB-PS resins, the β-sheet once formed cannot be disrupted and solvated even with DMSO in NMP.

CONCLUSION

The present investigations clearly demonstrate the efficiency of hydrophilic and flexible BDDMA-PS in synthesizing hydrophobic and difficult sequence peptides, over the conventional DVB-PS resin. This copolymer can be easily synthesized in bead forms and easily functionalized. The resin possesses the required physicochemical properties such as mechanical strength, chemical stability, and greater swelling capacity in polar and nonpolar solvents, which facilitates the rapid and successful synthesis of both hydrophobic and hydrophilic peptides. The ability of the resin to suppress the aggregation of resin-bound β-sheet structure during chain assembly renders the synthesis of even difficult sequence peptides relatively easy. At this point, BDDMA-PS would seem to be a considerable aid for the synthesis of difficult se-

TABLE IV
Conformational Parameters and IR Absorptions of the Resin Bound Peptides in Amide A and Amide I Regions

Peptides	Conformational parameters $<P_\alpha>$	$<P_\beta>$	$<P_c^*>$	$<SP_\beta>$	Amide A (3200–3500 cm^{-1}) DVB-PS	BDDMA-PS	Amide I (1600–1700 cm^{-1}) DVB-PS	BDDMA-PS
1	1.18	1.46	0.69	5.5	3284	3425	1642	1658
2	1.16	1.32	0.76	5.0	3282	3428	1646	1666
3	1.08	1.21	0.86	5.3	3281	3420	1646	1664
4	1.14	1.17	0.83	5.1	3278	3418	1640	1662
5	1.19	1.17	0.78	5.3	3285	3415	1639	1666
6	1.14	1.03	0.88	4.4	3282	3420	1641	1660
7	1.06	1.10	0.91	4.7	3280	3422	1643	1658

Figure 3 FTIR spectra of (a) BDDMA-PS and (b) BDDMA-PS resin carrying peptide **1**.

quences because it effectively reduces the total reaction time by improving the extent of amino acylations and deprotections. Because this resin can be used successfully in high loading levels, it has a very important advantage of production-scale preparation of peptides, making use of minimum reagents and solvents. A detailed study for the better understanding of the kinetics of chain assembly, the effect of peptide chain length on reactivity, and morphology are in progress in our laboratory.

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