Proc. Indian Acad. Sci. (Chem. Sci.), Vol. 106, No. 5, October 1994, pp. 1123–1138.

Tetraethyleneglycol diacrylate (TTEGDA)–crosslinked polystyrene resin as an efficient solid support for gel phase peptide synthesis

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Abstract. Various applications of the newly developed tetraethyleneglycol diacrylate (TTEGDA)–crosslinked polystyrene resin are illustrated by the synthesis of model peptides, fully protected peptides, peptide amides and biologically important sequences. PS–TTEGDA resin was prepared by suspension polymerization of styrene and TTEGDA and functionalized with chloromethyl, 4-cholromethyl-3-nitro, aminomethyl, α-bromopropionyl, α-aminopropionyl, 4-bromomethyl 3-nitrobenzamido, 4-aminomethyl-3-nitrobenzamido groups. Peptide synthesis was carried out using these modified resins by standard solid phase methodology. Coupling and deprotection in this synthetic strategy went to near completion showing the positive role of hydrophilic and flexible crosslinking agent TTEGDA in facilitating gel-phase reactions. The peptides were removed from the support by photolysis, trifluoroacetic-acid (TFA) treatment, *trans*-esterification or ammonolysis in high purity and yield. The crude peptides were purified by column chromatography/FPLC and characterized by aminoacid analysis, sequencing or ^{1}H-NMR.

Keywords. High-capacity resin; solid phase peptide synthesis; photolabile.

1. Introduction

The physicochemical incompatibility of the growing peptide chain and the insoluble crosslinked polymeric support has been one of the major problems associated with the polymer-supported method of peptide synthesis (Pillai and Mutter 1982; Sheppard 1986; Bayer 1991). Development of macromolecular supports which swell in both polar and non-polar solvents facilitating the different types of organic reactions employed in repetitive stepwise peptide synthesis has therefore been a challenge to organic and polymer chemists for the past two decades (Sheppard 1973; Hellermann *et al* 1983; Small and Sherrington 1989; Meldal 1992). The concept of optimum hydrophobic–hydrophilic balance serves as a guideline for the development of effective supports for peptide synthesis. Structure–reactivity and structure–property correlations in crosslinked polymeric systems can be made use of in the design of such supports with optimum reactivity characteristics, mechanical stability and other essential requirements of a polymeric support useful for the stepwise synthesis involving a multitude of synthetic operations under widely varying conditions. This paper describes investigations towards the development of tetraethyleneglycol diacrylate–crosslinked polystyrene support as a versatile support for the synthesis of

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peptides. The preparation of this class of polymer supports functionalization with different anchoring groups, and illustration of the applications of these resins for the syntheses of model peptides and biologically active peptide sequences are described in this work.

Experimental procedure

Chemicals: Styrene, TFA and TTEGDA were obtained from Aldrich Chemical Company, USA and thioanisole from Fluka. Sidechain protected aminoacids were obtained from Peninsula Laboratories, USA. Dicyclohexylcarbodiimide (DCC) and diisopropylethylamine (DIEA) were purchased from Sigma Chemical Company, USA. Other Boc-amino acids were synthesised according to reported procedures (Schnabel 1967). Chloromethylmethylether (CMME) was prepared using literature procedure (Marvel and Porter 1967). All solvents were distilled prior to use.

Methods: For aminoacid analysis, peptide resins were hydrolysed using 12 M HCl/propionic acid (1:1) at 110°C for 48 h and peptides were hydrolysed using 6 N HCl, 6 N HCl–TFA (1:1) at 110°C for 24 and 15 h respectively. Aminoacid analysis were performed on a LKB 4151 Alpha Plus analyzer. For HPLC analysis a Shimadzu Model 6A liquid chromatograph equipped with a SPD 6A UV spectrophotometric detector and a C–R6A chromatopac electronic plotter were employed. Fast protein liquid chromatography (FPLC) was done on a Pharmacia instrument on a C-18 reverse phase, semi-prep FPLC column, using solvent system A, 0·1% TFA in water and B, 0·1% TFA in CH_3CN and detection was at 214 nm. The sequence analysis of the peptides was done on an Applied Biosystem 473 A sequencer by Edman degradation.

The photochemical irradiation was carried out with Philips HPK, 125 W-mercury lamp housed in a water-cooled immersion type vessel. IR spectra were recorded on a Shimadzu IR 470 spectrometer in KBr pellets. ^{13}C–CP–MAS solid state NMR measurements were conducted on a Bruker 300 MSL instrument and ^1H NMR spectra were recorded on a Bruker WL 270 NMR instrument. CD spectrum was recorded on a Jasco J 500 A-spectropolarimeter attached with a Jasco DP-501 N data processor.

TTEGDA–crosslinked polystyrene copolymer

In a typical experiment a four-necked reaction vessel equipped with a thermostat, teflon stirrer, water condenser and nitrogen inlet was used. Polyvinyl alcohol (0·5 g) dissolved in double distilled water (200 ml), calcium sulphate (5 mg) and calcium phosphate (10 mg) were added to the vessel. A mixture of styrene (25·50 g) tetraethyleneglycol diacrylate (1·36 g) and benzoyl peroxide (0·5 g) dissolved in benzene (20 ml) was added to the vessel by stirring the aqueous solution at 400 rpm. The temperature was maintained at 80°C using a thermostated water bath. The entire reaction was carried out under a slow stream of nitrogen. After 20 h the solvent-embedded copolymer beads were washed free of stabilizer and the unreacted monomers by treating with distilled water, acetone, chloroform and methanol. The copolymer was further purified by refluxing for 6 h with trifluoroacetic acid to remove any impurity. The polymer beads were filtered, washed with CH_2Cl_2 and CH_3OH, dried under vacuum at 40°C for 10 h to yield 25 g of dry beads (92·5%).

General procedure for the solid-phase assembly of peptides

Solid phase peptide synthesis (SPPS) was carried out manually in a silanized glass reaction vessel clamped to a mechanical shaker. The Boc amino acids, were coupled to the resin by the symmetrical anhydride procedure. In a typical procedure Boc amino acid (3 eq.) was dissolved in CH_2Cl_2 and the solution was cooled to 0°C. A solution of DCC (1·5 eq.) in CH_2Cl_2 was added and stirred for 1 h at 0°C. The white precipitate of DCU was removed by filtration and the symmetric anhydride solution was added to the resin suspended in CH_2Cl_2. The coupling time was 1 h for most of the cases and a second coupling was performed when necessary. The extent of coupling was monitored by the ninhydrin test (Kaiser *et al* 1970). Boc group was deprotected using 4N–HCl–dioxane or 30% TFA–CH_2Cl_2 for 30 min. The neutralization was effected by treatment with 5% DIEA in CH_2Cl_2 or 10% TEA/CH_2Cl_2. In the case of photocleavable resin, *in-situ* neutralization was conducted for the first two cycles.

In the DCC coupling procedure, the following protocol was employed. (i) CH_2Cl_2 4 × 0·5 min (ii) 30% TFA in CH_2Cl_2 1 × 1 min, 1 × 30 min (iii) CH_2Cl_2 3 × 0·5 min,

Table 1. Aminoacid analysis of protected peptides synthesized using modified PS–TTEGDA supports†.

Peptide	Gly	Phe	Ala	Leu	Val	Met	Glu	Tyr
1	—	—	0·95 (1)	1·01 (1)	—	0·93 (1)	—	—
2	1·01 (1)	—	1·05 (1)	—	0·98 (1)	—	—	—
3	—	1·01 (1)	—	1·2 (1)	—	0·95 (1)	—	—
4	1·01 (1)	—	—	—	0·99 (1)	—	—	—
5	1·05 (1)	0·98 (1)	—	—	—	—	—	—
6	1·00 (1)	—	1·05 (1)	1·09 (1)	1·01 (1)	—	—	—
7	—	—	1·04 (1)	2·04 (2)	0·98 (1)	—	0·99 (1)	1·05 (1)
8	—	1·1 (1)	—	1·01 (1)	—	0·95 (1)	—	—
9	1·02 (1)	—	0·95 (1)	1·00 (1)	0·97 (1)	—	—	—
10	0·98 (1)	1·92 (2)	—	1·01 (1)	—	1·1 (1)	—	—
11	—	—	2·05 (8)	0·98 (4)	—	—	—	—

†Theoretical values given in parentheses.

Table 2. Aminoacid analysis of biologically important peptides synthesized using high capacity chloromethyl PS–TTEGDA supports†.

Peptides	Gly	Phe	Ala	Leu	Val	Asp	Glu	Ser	Thr	Cys	Arg	Tyr	Pro	Ile	Lys
12	0·98 (1)	0·98 (1)	1·01 (1)	—	2·1 (2)	1·1 (1)	—	—	—	—	—	1·14 (1)	—	—	—
13	2·01 (2)	—	—	—	—	1·00 (1)	—	0·75 (1)	—	—	0·93 (1)	—	1·1 (1)	—	—
14	2·01 (2)	—	—	—	—	—	—	0·98 (1)	0·73 (1)	—	1·01 (1)	—	—	—	—
15	—	—	—	—	1·1 (1)	—	—	0·74 (1)	0·63 (1)	na	0·96 (1)	—	—	2·1 (2)	3·95 (4)
16	2·02 (2)	—	2·01 (2)	3·01 (3)	0·99 (1)	—	3·12 (3)	4·16 (5)	0·60 (1)	—	—	—	1·02 (1)	—	—
17	2·07 (2)	3·02 (3)	2·87 (3)	4·91 (5)	0·97 (1)	—	—	3·51 (4)	0·90 (1)	—	—	—	2·10 (2)	2·35 (3)	2·00 (2)

†Theoretical values given in parentheses. Values of Ser and Thr are uncorrected, Cys is not determined. Ile values are slightly low due to partial hydrolysis.

(iv) 5% DIEA/CH_2Cl_2 3 × 0·5 min, (v) CH_2Cl_2 4 × 0·5 min, (vi) Boc-amino acid (2·5 eq.) in CH_2Cl_2 after 2 min add DCC (2·5 eq.) in CH_2Cl_2 (total volume 15 ml/g resin) stand 45 min at room temperature with occasional agitation, (vii) 33% EtOH in CH_2Cl_2 4 × 0·5 min, (viii) CH_2Cl_2 4 × 0·5 min. The qualititative ninhydrin test (Kaiser *et al* 1970) was used to monitor the synthesis. If the test was positive the protocol was repeated from step 4.

Incorporation of first amino acid: First amino acid was attached to the resin by DCC coupling procedure, cesium salt method or in presence of triethylamine (Stewart and Young 1984). The extent of substitution was estimated by picric acid method/ amino acid analysis (Stewart and Young 1984). Photolabile resins with a substitution of 0·3–0·7 mmol/gm were used for synthesis of peptide. Chloromethyl resin of substitution 1·5–2 mmol/gm was used in the synthesis of peptides.

Detachment of final peptides from the resins

The following methods were used depending upon the solid support for the cleavage of final peptide.

1. Photolysis in 30% EtOH/CH_2Cl_2
2. *Trans*-esterfication (MeOH)
3. TFA: thioanisole: *m*-cresol (10:1:1) treatment at 37°C under anhydrous conditions
4. Ammonolysis in methanolic ammonia.

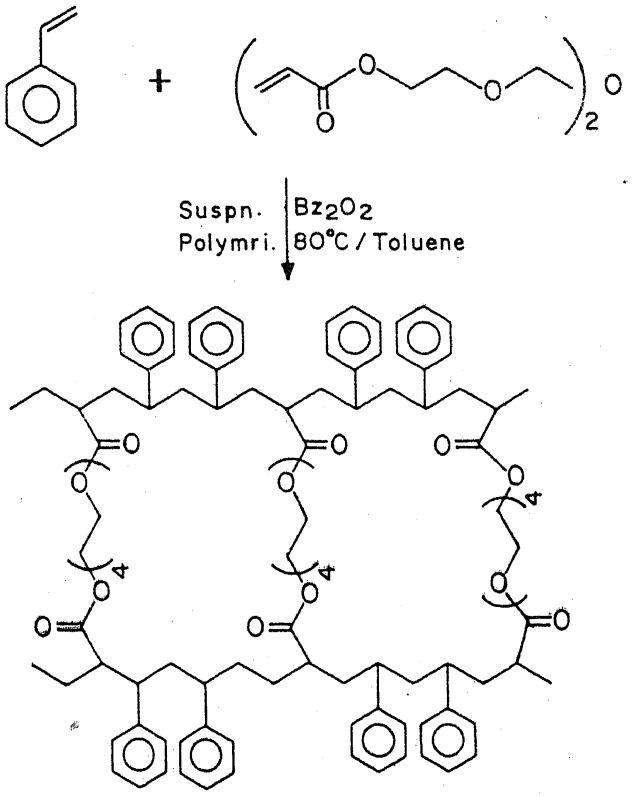
Characterization: All the peptides were characterized by aminoacid analysis (tables 1 and 2) after purification

Results and discussion

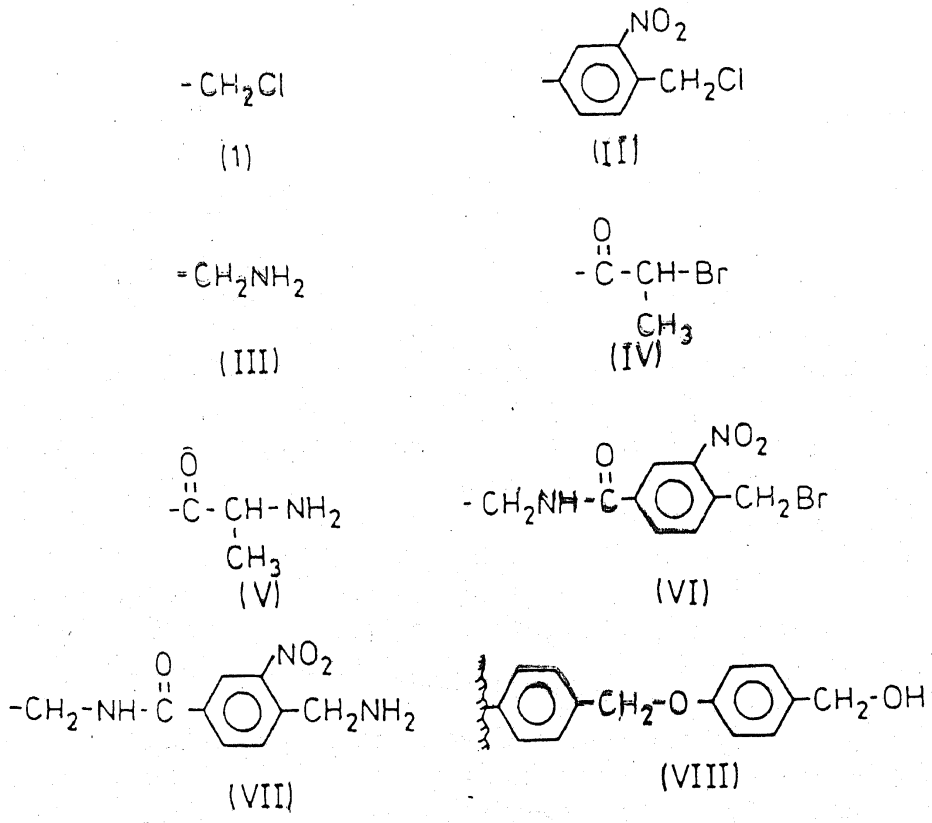
Polymer preparation, characterization and functionalization

The synthesis of tailor-made polymer supports with optimum hydrophilic–hydrophobic balance for facilitating quantitative coupling and deprotection reactions during stepwise synthesis was the target of our work (Renil *et al* 1994). This was achieved during the development of functionalized TTEGDA–crosslinked polystyrene supports for SPPS of fully protected model peptides, peptide amides and naturally occurring peptides. Hydrophobic styrene and hydrophilic tetraethyleneglycol diacrylate (TTEDGA) were copolymerized by suspension polymerization to yield beaded polymer of uniform size and shape (scheme 1). The crosslinked polymers were functionalized with chloromethyl (I), aminomethyl (II), 4-chloromethyl-3-nitro (III), 4-bromomethyl-3-nitro-benzamido (IV), 4-aminomethyl-3-nitrobenzamido (V), α-methylphenacyl (VI), α-methylphenacylamido (VII) and 4-(hydroxymethyl)-phenoxymethyl (VIII) anchoring groups for synthesis of peptides under different attachment and cleavage conditions (scheme 2).

These polymeric supports were characterized using IR, solid state ^{13}C–CP–MAS–NMR and chemical methods. The IR spectrum of PS–TTEGDA resin showed a sharp band at 1720 cm^{-1} corresponding to the ester carbonyl and a broad band at



Scheme 1. Suspension polymerization of styrene and TTEGDA.



Scheme 2. Different types of functional groups introduced on PS–TTEGDA resin.

1150 cm^{-1} characteristic of ether linkages of the crosslinking agent. In addition to these spectral features, IR spectrum of the resin (VI) showed characteristic bands at 1350 and 1540 cm^{-1} (NO_2) and at 1650 and 3400 cm^{-1} (NH–CO). The IR spectrum of the resin (IV) showed a strong carbonyl absorption at 1685 cm^{-1}, and that of resin (V) showed absorption bands at 3400–3500 cm^{-1} (NH_2) and 1685 cm^{-1} (CO). Solid state ^{13}C CP–MAS NMR spectra exhibited an intense peak at 127·9 ppm corresponding to aromatic polystyrene carbons and a small peak at 145·6 ppm arising from C-3 carbon of the styrene. The backbone methylene carbon of the polymer gave a singlet at 40·4 ppm and the methylenes of ether linkages of the crosslinking agent TTEGDA appeared as a small peak at 70·6 ppm.

Aminomethyl TTEGDA–crosslinked polystyrene resin was prepared from chloromethyl resin by Gabriel's phthalimide method and was coupled with 4-bromomethyl-3-nitrobenzoic acid in the presence of dicyclohexylcarbodiimide (DCC) to give the photolabile 4-bromomethyl-3-nitrobenzamidomethyl TTEGDA–crosslinked polystyrene (VI) support (Haridasan and Pillai 1991). In this reaction only single coupling in the presence of pyridine was needed for complete reaction as compared to the double coupling and acetylation to block the remaining amino group in the case of DVB–crosslinked polystyrene resin. This suggests increased reactivity of the functional groups in the case of TTEGDA–crosslinked polystyrene resin. 4-Bromomethyl-3-nitrobenzamidomethyl TTEGDA–crosslinked polystyrene resin was converted to 4-aminomethyl-3-nitrobenzamidomethyl resin by hexamine method as described earlier (Devaky and Pillai 1988). The resin swells very well in dichloromethane, DMF and other solvents used in SPPS.

For the preparation of protected peptides and peptide amides by photolytic cleavage, α-bromopropionyl and α-aminopropionyl anchoring groups were introduced into TTEGDA–crosslinked polystyrene resin by the polymer–analogous reaction. The capacity of the resin could be varied from 0·9 mmol of Br/g to 2·64 mmol of Br/g by adjusting the amount of the reagent and duration of reaction. The use of α-methylphenacylamido linkage has been reported recently for the SPPS of peptide amides (Ajayaghosh and Pillai 1988).

Synthesis of protected peptides using modified PS–TTEGDA supports

A 12-residue hydrophobic peptide, Boc–Ala–Leu–Ala–Ala–Leu–Ala–Ala–Leu–Ala–Ala–Leu–Ala–OMe (11), was synthesised on a high-capacity chloromethyl TTEGDA–crosslinked polystyrene support using N–Boc protecting groups. There was 2·5 fold weight increase in the case of the peptide resin after the synthesis. This agrees approximately with the molecular weight of the target peptide. The high capacity resin has the advantage of obtaining peptides in relatively large amount as in the solution phase method. The monitoring of the reaction by ninhydrin test is much easier in the case of these high capacity and highly swelling resins. Final cleavage of the completely protected peptide was achieved in high yield by *trans*-esterification procedure (Reddy and Nagaraj 1986). The liberated product was found to be insoluble due to the extremely hydrophobic nature of the peptide sequence, defying purification by usual chromatographic techniques. Since the crude product was partially soluble in warm DMSO, purification by reprecipitation in DMSO/ether mixture and then washing off the soluble impurities gave the final product in 85% yield as a single spot on tlc. It was identified as the target peptide by amino acid analysis and by ^1H NMR. (figure 1).

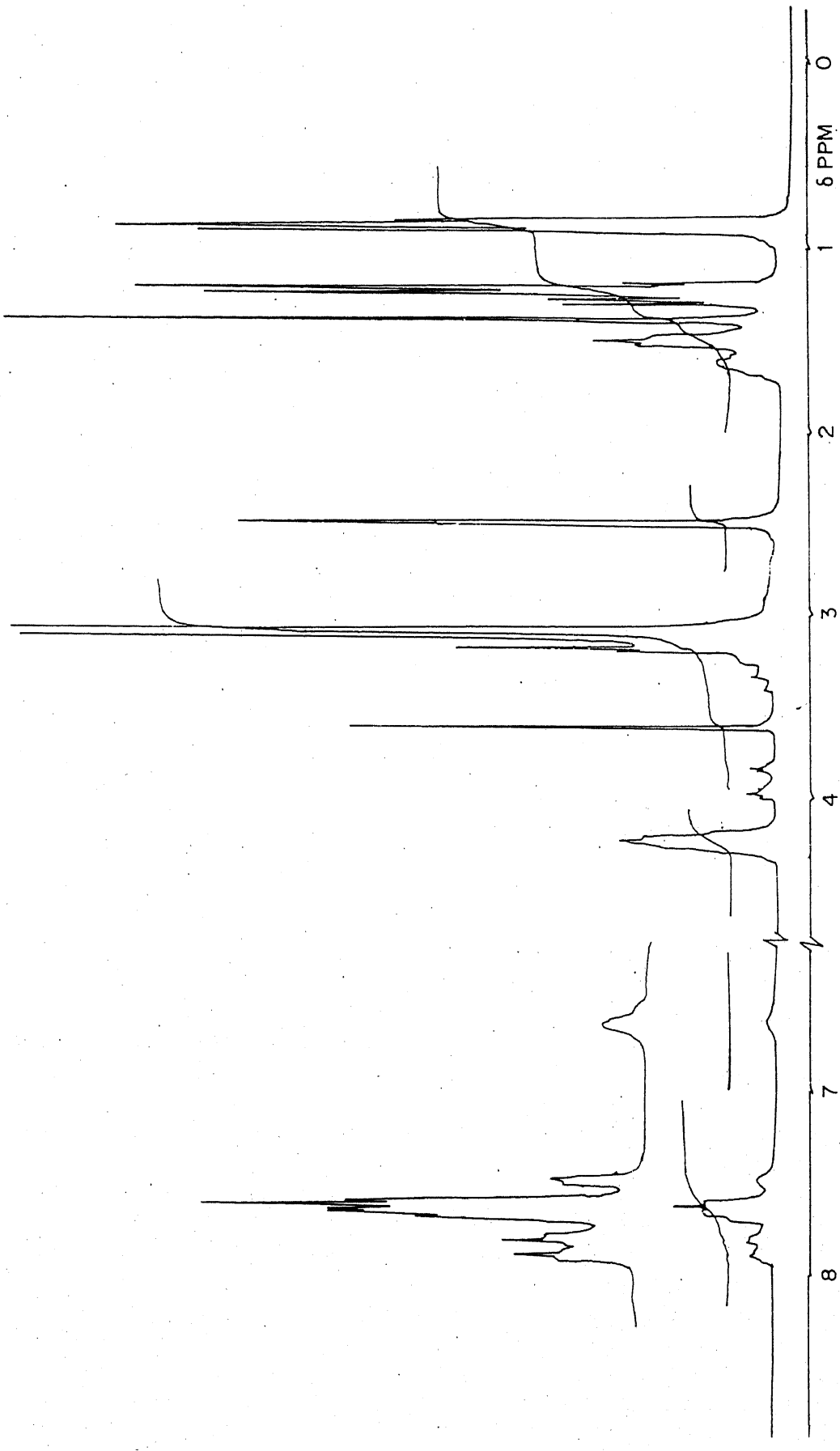
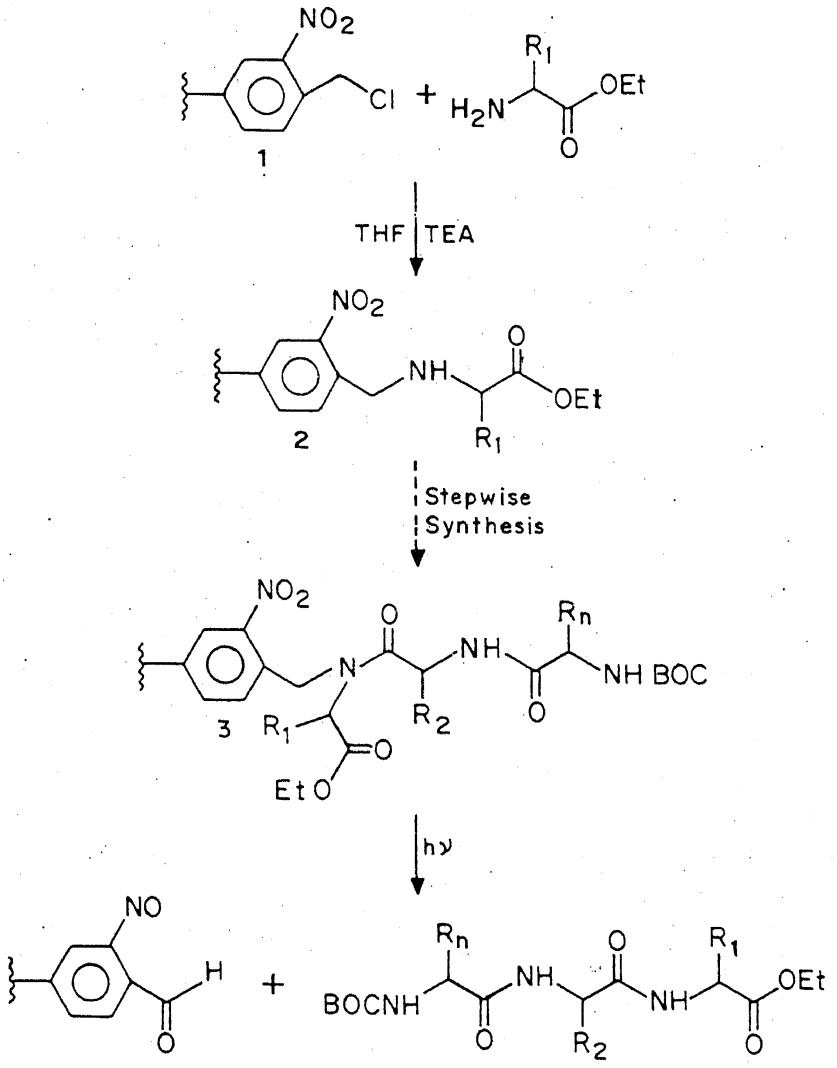


Figure 1. ^{1}H NMR spectrum of Boc(Ala–Leu–Ala)$_4$–OMe NMR (DMSO d_6, 270 MHz) δ 1·44 (*s*, 9 H, Boc); 0·95 (*t*, 24 H, CδH Leu); 1·37–1·52 (*m*, CγH and Cβ Leu); 1·25 (*d*, 24 H, CβH Ala); 3·6 (3 H, OCH_3); 4·13–4·35 (8 H, CαH, Ala, 4 H, CαH Leu); 7·5–7·92 (8 H, –CONH Leu–Ala); 6·65 (1 H, Boc NH Ala).

Photolabile supports

In solid phase peptide synthesis, the introduction of anchoring group between the solid support and the growing peptide chain is a convenient strategy for the mild non-destructive cleavage of peptides. The anchoring linkage should be stable under the conditions of the various reactions which are repeated and at the same time it should be cleavable finally by mild and selective reaction which does not affect the finished peptide. The use of different types of anchoring groups with varying stability between the polymer support and the first amino acid facilitates the attachment of the first residue and the final cleavage of the peptide in the free carboxyl form or as the peptide amide. The principle of photolytic deprotection of functional groups has been made use of to provide mildly and selectively cleavable anchoring linkages between the first amino acid and the polymer support. Photochemical cleavage from the polymer support permits the preparation of N^{α}-amino and side chain protected peptides which are useful in segment condensation (Williams *et al* 1993).



Scheme 3. Synthesis of fully protected peptide using *o*-nitrochloromethyl PS–TTEGDA support.

The 2-nitrobenzyl ester linkage has found widespread applications as anchoring group in the polymer-supported methods of peptide synthesis (Williams *et al* 1993). This was introduced into the 4% TTEGDA–Crosslinked polystyrene for the stepwise synthesis of fully protected peptide esters (scheme 3). In the present new synthetic strategy, the first C-terminal amino acid ester was attached to the support through the free N-terminal and the peptide is grown from the secondary amino group by stepwise incorporation of N-protected amino acids (Renil and Pillai 1994). Finally, the peptide was cleaved in the fully protected form by photolysis at 350 nm under neutral conditions at room temperature. The applicability of the polymer support was illustrated by synthesis of Boc–Met–Leu–Ala–OMe (1), Boc–Ala–Val–Gly–OEt (2), Boc–Met–Leu–Phe–OMe (3), Boc–Val–Gly–OEt (4) and Boc–Phe–Gly–OEt (5).

4-Bromomethyl-3-nitrobenzamido (VI) and 4-aminomethyl-3-nitrobenzamido (VII) resins were prepared starting from chloromethyl TTEGDA–crosslinked polystyrene resin through a series of polymer–analogous reactions. These resins have the advantage that they possess increased functional group reactivity which permits more effective synthesis. The 4-aminomethyl-3-nitrobenzamidomethyl (VII) resin permits the photolytic cleavage of the peptide in the C-terminal carboxy amide form whereas the 4-bromomethyl-3-nitrobenzamidomethyl (VI) resin releases the free C-terminal peptides. The applicability of these two resins were illustrated by the synthesis of Boc–Gly–Val–Ala–Leu (6) and Boc–Val–Glu(OBzl)–Ala–Leu–Tyr(Bzl)–Leu–NH_2 (7) according to the general procedure. Cleavage of the protected peptides/amides were accomplished by irridation of the suspension of the peptide polymer at 350 nm. The crude peptide was purified by silica gel column chromatography and characterized by aminoacid analysis (table 1).

TTEGDA–crosslinked polystyrene support containing the photocleavable α-methylphenacyl (III) and α-methylphenacylamido (IV) groups were used for the SPPS of peptides. The photolysis of the peptide resin at 320–340 nm resulted in the cleavage of the peptides in the free acid and α-carboxamide form. Boc–Met–Phe–Leu (8), Boc–Leu–Ala–Gly–Val–NH_2 (9) and substance P (7–11) Boc–Phe–Phe–Gly–Leu–Met–NH_2 (10) were synthesised in high purity and yield using this procedure.

Synthesis of biologically important peptides using high-capacity chloromethyl PS–TTEGDA support

Fmoc–solid phase chemistry (Sheppard 1986) was extended to the TTEGDA–crosslinked polystyrene system in the synthesis of 7-residue deltorphin, Tyr–D–Ala–Phe–Asp–Val–Val–Gly–NH_2 (12). The first amino acid, Boc–Gly was anchored to chloromethyl resin by the triethylamine method in high capacity (1·65 mmol/g). The Boc-group was removed by treatment with 30% TFA/CH_2Cl_2 and the resulting amine salt was neutralized with triethylamine. The peptide chain was built by sequentially extending it towards the amino terminus by stepwise addition of Fmoc amino acid (3 eq.), DCC (3 eq.) and HOBt (1 eq.). The N-terminal amino acid Tyr was incorporated as benzyloxycarbonyl (Z)–Tyr–(Bzl)–OTCP (trichlorophenylester) (3 eq.) in the presence of HOBt (1 eq.). The protected heptapeptide amide was cleaved from the support by ammonolysis in 85% yield. The benzyl group from Tyr(Bzl) and Asp (OBzl) residues and terminal Z group were removed by catalytic transfer hydrogenation (Pd black/85% HCOOH at room temperature). The resulting free peptide amide was purified by gel filtration on sephadex G-15.

Two 7-residue peptides, Gly–Arg–Gly–Asp–Ser–Pro (13) and Gly–Arg–Gly–Glu–Ser–Pro (14) which are biologically important in cytoadhesive studies of fibronectin (Pierchbacher and Ruoslahti 1984) were synthesised on high capacity chloromethyl TTEGDA–crosslinked polystyrene support. Boc–Pro was quantitatively attached to the chloromethyl resin by the cesium salt method (1·7 mmol/g). N–Boc protection was used throughout the synthesis with mesitylene sulphonic acid (Mts) side chain protection for Arg and benzylester side chain protection for Glu/Asp. DCC double coupling procedure was followed throughout the synthesis with HOBt coupling for Boc–Arg(Mts). After the synthesis, the peptide was cleaved from the resin by treatment with TFA. The deprotected peptide was obtained in 95% yield as evidenced by amino acid analysis of the residual resin after cleavage of the peptide. The peptide was purified by reprecipitation from methanol/ether mixture. The same procedure was adopted for the synthesis of reference peptide Gly–Arg–Gly–Glu–Ser–Pro. The peptide was cleaved in 95% yield and purified by recrystallisation from methanol/ether. The FPLC profile of the purified peptide is shown in figure 2. Biological studies were in perfect agreement with the previously reported results. Cell adhesion experiments were conducted according to reported procedures using AK-5 tumor cells (Khar 1986) and it was found that Gly–Arg–Gly–Asp–Ser–Pro (GRGDSP) synthetic peptide inhibit the cell adhesion while the control peptide Gly–Arg–Gly–Glu–Ser–Pro (GRGESP) does not. It was also found that the synthetic peptides were not toxic on AK-5 cells and they can be directly used for biological studies.

Another biologically important 11-residue nuclear signal sequence Ser–Thr–Pro–Pro–Lys–Lys–Lys–Arg–Lys–Val–Cys(Acm) (15) was synthesised on chloromethyl TTEGDA–crosslinked polystyrene support using a similar procedure. N–Boc

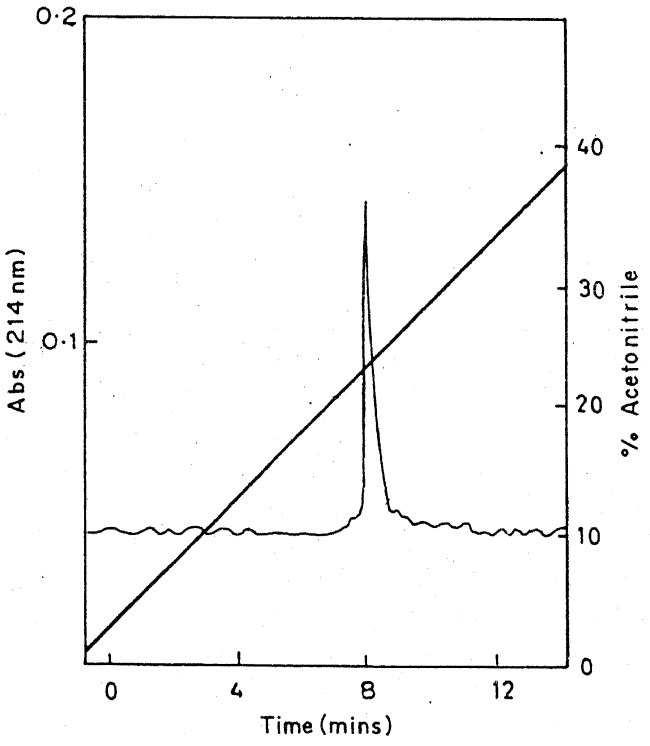


Figure 2. FPLC purified Gly–Arg–Gly–Asp–Ser–Pro on a Pharmacia C-18 column using 0·1% TFA in water (A), 0·1% TFA in acetonitrile (B) solvent system. Flow rate 0·5 ml/min.

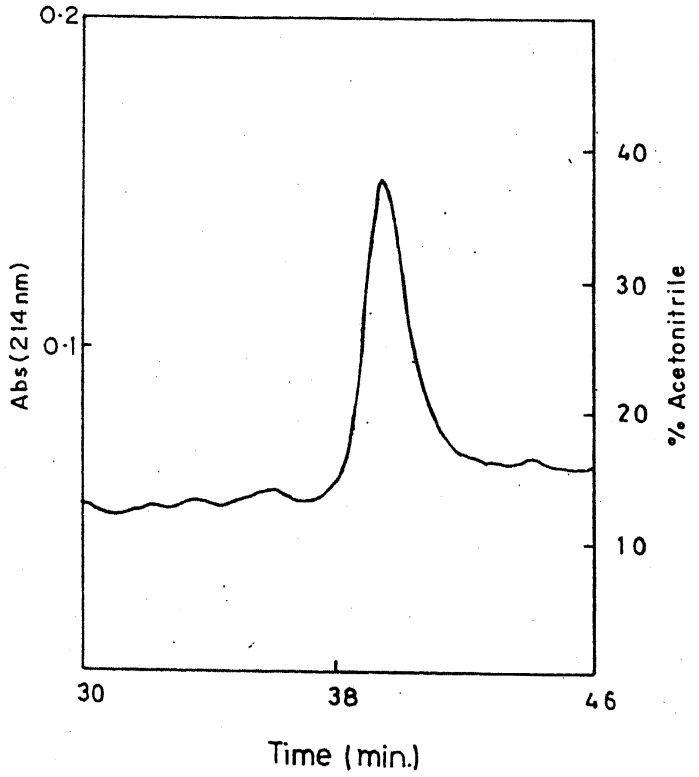


Figure 3. FPLC of Ser–Thr–Pro–Pro–Lys–Lys–Lys–Arg–Lys–Val–Cys(Acm)–OH on a Pharmacia prep. C-18 column using 0·1% TFA in water (A), 0·1% TFA in acetonitrile (B) solvent system. Flow rate 2 ml/min.

protection was used for the synthesis of the 11-residue signal sequence. A resin of 0·33 mmol Boc–Cys(Acm)/g capacity was employed and upto four amino acid residues could be attached per day by the DCC mediated double coupling procedure. The duration of HOBt coupling in the case of Boc–Arg(Mts) was increased from 45 to 120 min. The final peptide was cleaved from the support using TFA in 90% yield. The purification was achieved using a reverse phase C-18 column on a FPLC (figure 3), and gave amino acid analysis expected for the target peptide (table 2).

Pardaxin is a 33-residue peptide toxin isolated from the secretion of Red Sea Moses sol fish (Thompson *et al* 1986). The 26-residue hydrophobic N-terminal segment (1–26) of pardaxin Gly–Phe–Phe–Ala–Leu–Ile–Pro–Lys–Ile–Ile–Ser–Ser–Pro–Leu–Phe–Lys–Thr–Leu–Leu–Ser–Ala–Val–Gly–Ser–Ala–Leu (17) was synthesized on high-capacity chloromethyl resin (2·1 mmol Cl/g) starting from Boc–Leu attachment by the cesium salt procedure. Boc-group was deprotected using 30% TFA–CH_2Cl_2 and stepwise synthesis was carried out using double coupling procedure with 2·5-fold molar exess of Boc-amino acid. HOBt coupling in DMF was found effective for Boc–Ile. Coupling yields averaging > 99·8% were observed in each coupling. After the synthesis, the resin showed a five-fold weight increase which is in agreement with the molecular weight of the 26-residue peptide.

Cleavage of the completely deprotected 26-residue peptide was accomplished by TFA treatment. The yield of acidolytic cleavage was about 95% which was determined by the measurement of the remaining peptide still bound to the resin. The crude product was purified using reverse phase C-18 FPLC semi prep column to obtain

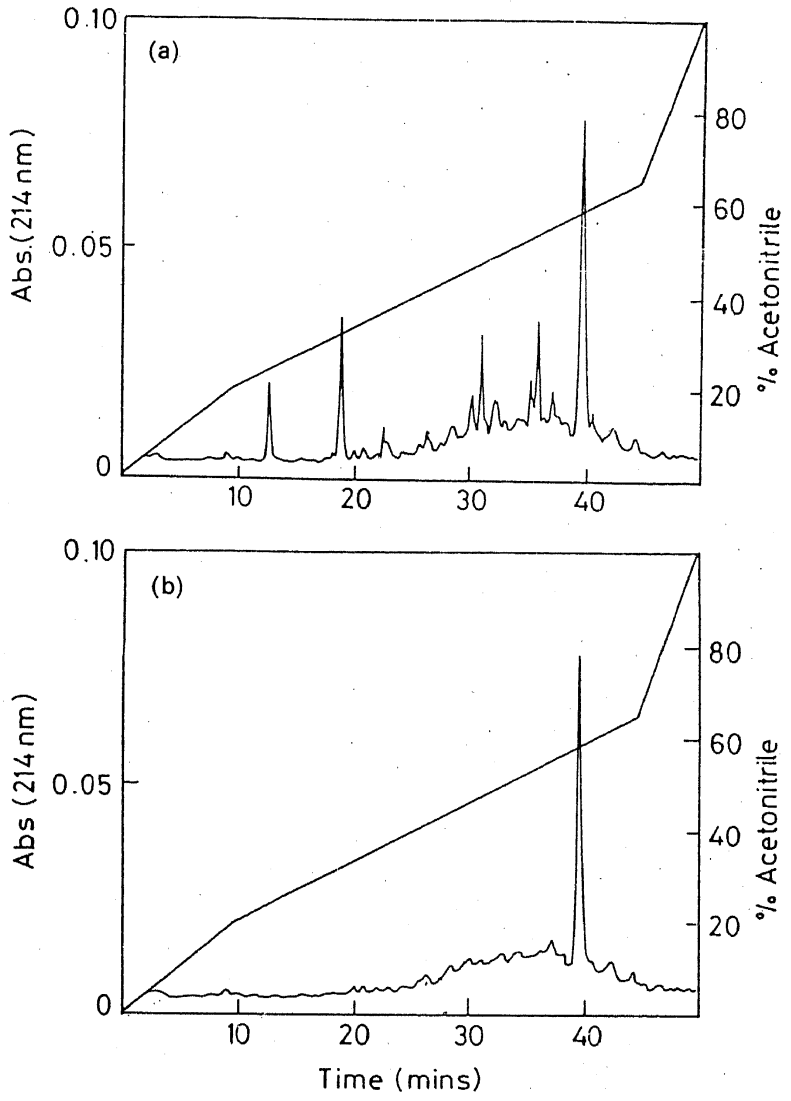


Figure 4. FPLC of total crude (**a**) and purified (**b**) 1–26 pardaxin peptide sequence. A Pharmacia semi prep C-18 column was used. Elution was carried out with a gradient of 0·1% TFA in water (A) and 0·1% TFA in acetonitrile (B); flow rate: 0·5 ml/min.

26-residue pure peptide corresponding to an overall yield of 20% based on the starting Leu–polymer (figure 4). In the amino acid analysis the values of Ile is slightly low due to partial cleavage of Ile–Ile bond under the hydrolytic conditions and Ser content was low partial degradation during hydrolysis. The peptide was characterised through sequencing by Edman degradation on an Applied Biosystem gas phase sequencer. The free peptide in methanol showed a circular dichroism curve with a strong positive peak at 195 nm and two strong negative bands at 209 nm and 222 nm confirming α-helical structure.

The 18-residue C-terminal pardaxin peptide (16–33) Lys–Thr–Leu–Leu–Ser–Ala–Val–Gly–Ser–Ala–Leu–Ser–Ser–Ser–Gly–Glu–Gln–Glu (16), was synthesized on high capacity chloromethyl resin using N–Boc protection and final TFA cleavage done. The peptide was purified on a reverse phase FPLC column and was obtained as a single peak [overall yield 67%] (figure 5).

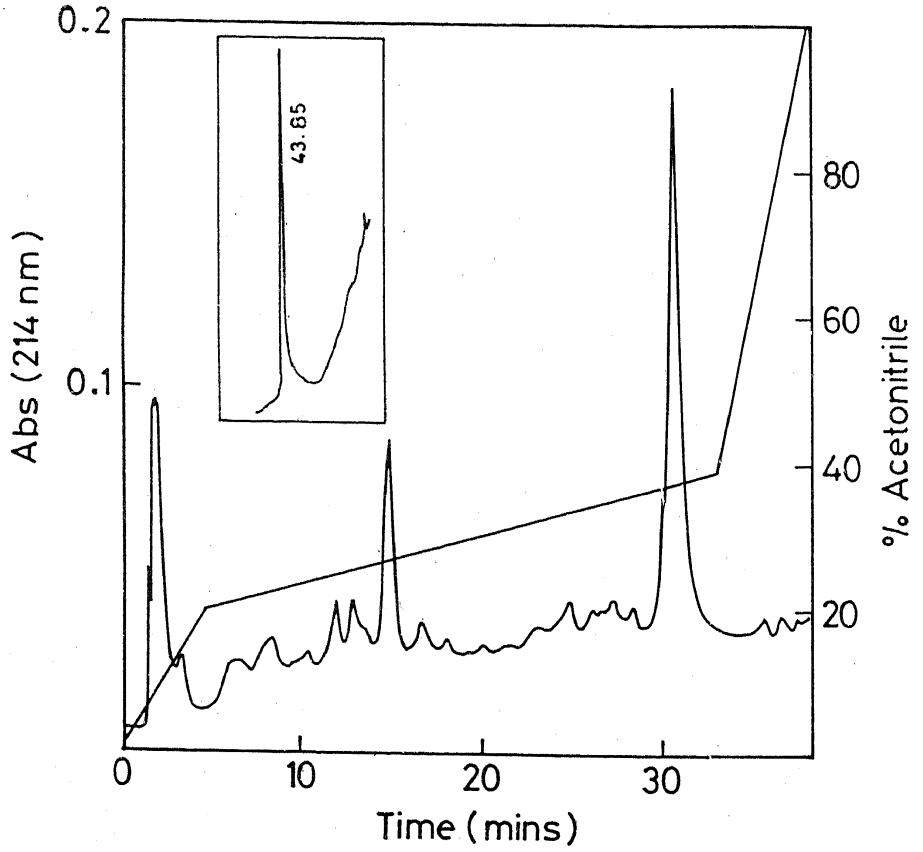


Figure 5. FPLC of unpurified 18-residue pardaxin sequence after TFA cleavage: Gradient used 0 time 0% B; 20% B in 15 min; 39% B in 30 min; 100% B in 5 min; a flow rate of 0·5 ml/min. (Inset) HPLC profile of the purified peptide: Gradient used: 0 time 5% B; 40% B in 35 min; 40% for 5 min; 95% B in 25 min; flow rate 0·8 ml/min.

Advantages of PS–TTEGDA support

A new resin has been developed for peptide synthesis based on a systematic analysis of solvation, swelling and reactivity characteristics of functionalized crosslinked polymeric supports and the existing problems in SPPS. This resin, which could be conveniently prepared by suspension polymerization of styrene and tetraethyleneglycol diacrylate, serves as a new class of versatile polymer support for peptide synthesis. The main advantage of this class of supports are compatibility with the growing peptide chain, optimum hydrophilicity–hydrophobicity balance and increased functional group reactivity. Compared to the classical solid phase synthesis at conventional loading these high capacity resins have a number of advantages, which stems from the much more efficient use of available volume within each gel particle. These advantages include enhanced coupling rates during peptide bond formation, major saving in cost due to the more effective use of reagents and of reaction and washing solvents, greatly improved sensitivity in the monitoring of the coupling reaction to effect peptide bond formation and the synthesis of peptides in quantities approaching those obtainable by solution phase method.

The resin was found to be stable under all the conditions of functionalization and peptide synthetic operations. The diacrylate crosslinks withstand all the conditions

which are commonly employed in SPPS like TFA treatment. It is possible that the ester linkages in the interior of the crosslinked matrix resist the hydrolysis due to the relative inaccessibility for hydrolytic reagent. The other attractive feature of the resin is that it could be easily functionalized with chloromethyl, 4-chloromethyl-3-nitro, aminomethyl, 4-bromomethyl-3-nitrobenzamidomethyl, 4-aminomethyl-3-nitrobenzamido, α-bromopropionyl, α-aminopropionyl and 4-(hydroxymethyl) phenoxymethyl groups which permit cleavage under varying sets of reaction conditions. The resin swells efficiently in a variety of solvents used in peptide synthesis. Application of the resin in SPPS was illustrated by the synthesis of various model peptides, peptide amides and biologically important peptides in high purity and yield. The synthesized peptides were purified by FPLC, silica gel chromatography and/or gel filtration chromatography. The purity was further checked by HPLC, amino acid analysis or tlc. The structures were confirmed by automatic sequencing or ^{1}H-NMR. The optical purity was illustrated in a number of cases by the CD spectral measurements. These investigations clearly indicate that the requirements of an ideal polymeric support for solid phase synthesis of peptides can be attained in this class of resins.

The synthetic investigations using the newly developed TTEGDA–crosslinked polystyrene supports described in this paper could be extended and can be subjected to refinements in a number of ways. The crosslinked polymeric system is amenable to immense structural variations and such possibilities could be made use of in introducing orthogonal handles for the final cleavage of peptides in modified forms. The hydrophilicity–hydrophobicity balance can also be adjusted similarly without affecting the overall course of the reaction of the attached substrate. The nature of the crosslinking agent can be changed from oligoethyleneglycol diacrylate to amino oligoethyleneglycol bisacrylamides in order to increase the compatability of the support with growing peptide chain. A comparative study of the chain mobility of the attached functional moieties on differently crosslinked polystyrene supports by ^{13}C-NMR time relaxation techniques could lead to more insight into the topography of the resin support. Detailed kinetic analysis of the heterogeneous gel phase reaction is a problem worthy of extensive investigation. Further synthetic studies and proper refinements of these basic results would contribute to the development of more different types of resins for solid phase peptide synthesis.

Acknowledgements

We thank Prof P Balaram, Indian Institute of Science, Bangalore and Dr R Nagaraj, Centre for Cellular & Molecular Biology, Hyderabad for providing analytical facilities. MR thanks Dr K N Ganesh, National Chemical Laboratory, Pune for his critical appraisal of the manuscript.

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