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MEMBRANES AS SOLID SUPPORTS FOR PEPTIDE SYNTHESIS

Scott B. Daniels*, Michael S. Bernatowicz, James M. Coull, and Hubert Köster

MilliGen/Biosearch Division of Millipore Corporation
186 Middlesex Turnpike, Burlington, MA 01803

Summary: A hydroxypropylacrylate coated polypropylene membrane was used as a solid support for the stepwise synthesis of peptides. This novel support can either be used for the preparative synthesis of peptides by first attaching an acid cleavable racemization free Fmoc-amino acid-linker to the membrane or for the assembly of membrane-bound peptides for direct use in a variety of biochemical and analytical applications.

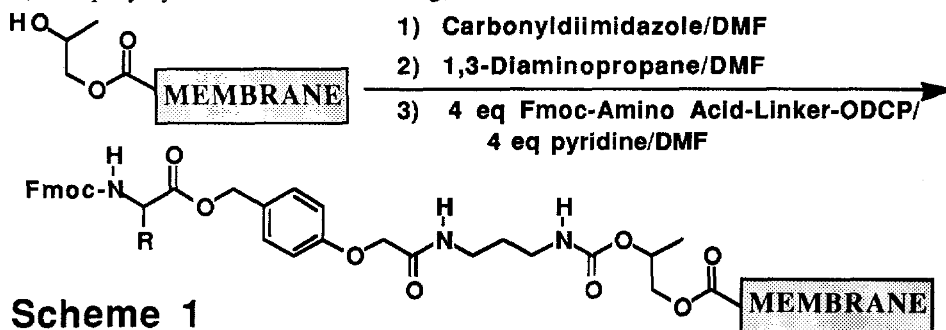
Since the advent of solid phase peptide synthesis chemistry much of the work in the field has been concentrated on protection and activation chemistries and cleavable linkages to the solid support while relatively little attention has been focused on the nature and configuration of the polymeric support[1]. An ideal solid support would allow functionalization of the surface for synthesis and cleavage of the final peptide product as well as the possibility of leaving the peptide covalently attached to the support in order to allow affinity purification of biomolecules[2], new methods of epitope mapping[3], diagnostic testing[4], and covalent sequence analysis[5]. A solid support suitable for all of these applications must necessarily be stable toward peptide assembly and cleavage conditions and be wetted by aqueous solutions. Ideally such a support would be substantially less expensive than currently used materials to reduce the cost of synthesis.

A polypropylene membrane coated with crosslinked polyhydroxypropylacrylate has the potential to fulfill the above requirements and therefore was chosen as a candidate for evaluation as a solid phase synthesis support[6]. Polymeric membranes have additional properties that might make them more favorable than beaded supports. These include mechanical stability, good flow characteristics in a continuous flow system, controlled porosity, high internal surface area to weight ratio, and ease of handling. Unlike the conventional beaded supports, a sheet of a porous contiguous polymer also lends itself more easily to rapid simultaneous synthesis of large numbers of peptides, miniturization of automated devices, and novel reactor devices.

The usefullness and performance of this novel support was tested by the assembly of four target peptides, prothrombin 1-9, acyl carrier protein 54-65, neurotensin, and FOS oncogene protein 147-162[7], using Fmoc chemistry on a flow through synthesizer. These peptides varying in length (9-25 residues) and synthetic difficulty were

assembled on the polypropylene membrane and two beaded supports, PepSyn KA™ (polydimethylacrylamide supported on a kieselguhr matrix)[8], and aminomethyl polystyrene[9]. Each of these beaded supports has been routinely used to successfully prepare many different peptides and serve as a yardstick for comparing products assembled on membranes[1].

A cleavable linker was introduced between the peptide and solid support in order to remove the peptide at the end of the synthesis. Pepsyn KA™ is available with the C-terminal amino acid already attached and has a trifluoroacetic acid (TFA) cleavable linker connecting the first amino acid to the resin[8]. A similar linkage was introduced onto the membrane surface via Scheme 1. Derivatization of hydroxyl groups with 1,1'-carbonyldiimidazole gave the acyl imidazole which was subsequently displaced by 1,3-diaminopropane to give an amino handle on the membrane. The appropriate Fmoc-amino acyl-oxymethylphenoxyacetic acid 2,4-dichlorophenyl ester was then coupled to the amino membrane (or to aminomethyl polystyrene) in DMF with one equivalent of pyridine to provide a racemization-free amino acid linkage to the support[10]. The loading of Fmoc amino acid on PepSyn KA™ was 0.1 mmol/gram of resin, on polystyrene it is 0.5 mmol/g, and on the membrane it was 0.1 mmol/g.



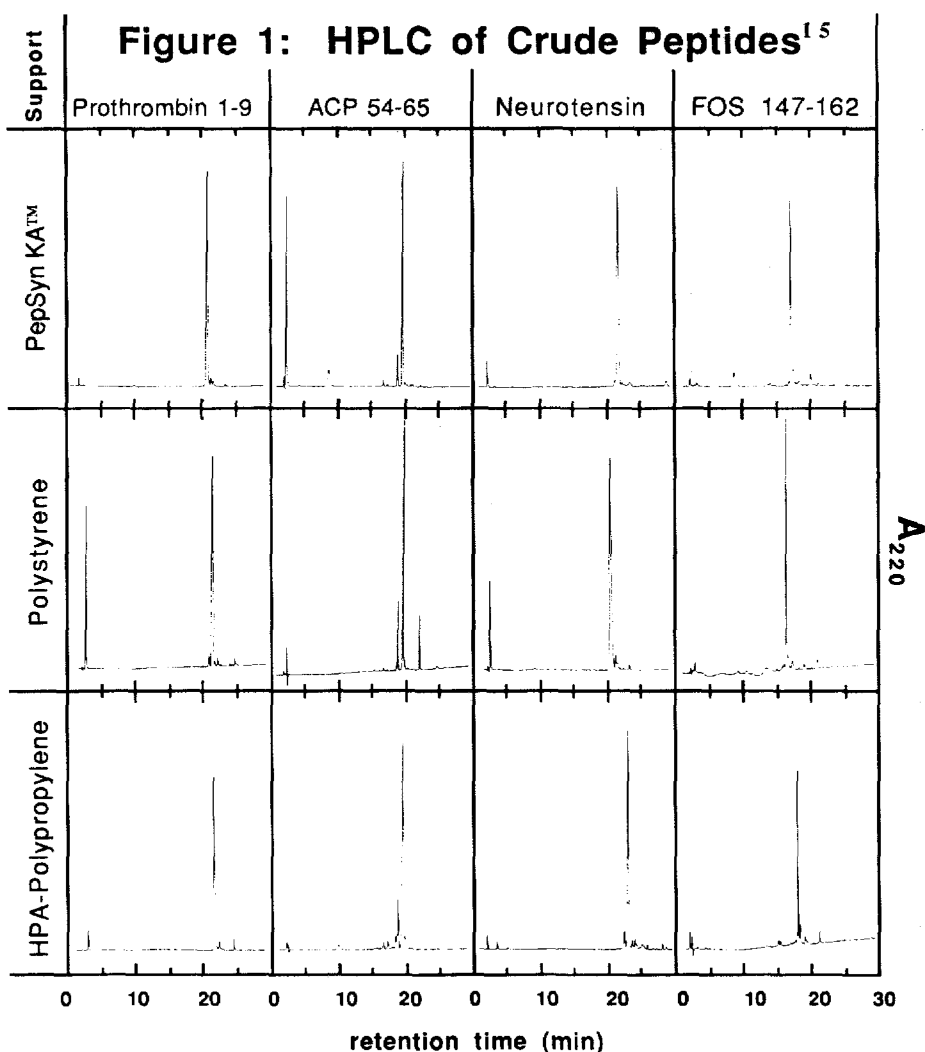
Scheme 1

The PepSyn KA™ and polystyrene resin were packed in columns[11] and the membrane was packed in a disposable polypropylene cartridge[12]. The same synthesis protocols were used for each support examined. The syntheses were performed using a continuous flow MilliGen/Biosearch 9050 PepSynthesizer with standard protocols and standard scale (a 0.2 mmol theoretical yield of crude peptide) at a flow rate of 5 ml/min. The cycle for the addition of an amino acid consisted of a 7 min wash of the solid support with 20% piperidine in DMF to remove the N^{α}-Fmoc-protecting group, a 12 min DMF wash, a 30 min acylation reaction with 4 equivalents of an Fmoc-amino acid active ester[13], and an 8 min DMF wash for a total cycle time of about 1 hour.

After the peptide synthesis was complete, the final Fmoc- protecting group was cleaved with the piperidine solution and the support washed with methylene chloride. The peptide was cleaved from the solid support with 95% TFA containing 5% scavengers[14]. The TFA was evaporated and the peptide precipitated and washed

with anhydrous diethyl ether to remove the scavengers. The peptide was dried dissolved in 0.1% TFA and analyzed by HPLC (Figure 1)[15].

All four target peptides were obtained in crude yields >85% as the major product of their respective syntheses regardless of the solid support used. Amino acid analyses and mass spectral data were used to confirm the identity of the products. As can be seen from the HPLC data, the peptides assembled equally as well on the membrane as on the beaded supports. The data presented clearly indicates the feasibility of using membrane-based solid supports for continuous flow peptide synthesis by the Fmoc strategy. Studies are currently underway to assess the feasibility of using membrane-bound peptides in biochemical applications.



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6. The polypropylene membrane was manufactured and coated with hydroxypropylacrylate by Millipore Corporation and has a pore size of 0.22 μm.
7. The sequence of Prothrombin 1-9 is ANKGFLEEV-OH, Acyl carrier protein 54-65 is VQAAIDYING-OH, Neurotensin is <ELYENKPRRPYIL-OH (<E is pyroglutamic acid), FOS oncogene protein 147-162 CVEQKSPEEEEKRRIRRERNKNAAA-OH.
8. Dryland, A. and Sheppard, R. C. (1986) *J. Chem. Soc., Perkin Trans.*, **1**, 125-137. PepSyn K™ resins are available from MilliGen/Biosearch.
9. A 1% divinylbenzene crosslinked polystyrene (200-400 mesh) derivatized to an aminomethylation level of ~0.7 mmol/g. Purchased from Peninsula Laboratories.
10. Bernatowicz, M. S. et al. Submitted to *Tet. Lett.*
11. The Fmoc-amino acid PepSyn KA™ resin was swelled in DMF, the fines decanted, and slurry packed into a 1x10 cm glass column with porous fritted endpieces. One part aminomethyl polystyrene beads (150-200 μm) were mixed with 4 parts of glass beads (150-212 μm from Sigma) and dry packed into 1x10 cm glass column. After the resin was swelled with DMF, the endpieces were adjusted so that a 1 cm space remained above the resin.
12. The membrane was wrapped around a central rod and sealed into a cartridge so that the entire surface was efficiently wetted by the reagents and solvents.
13. The t-butyl group was used for side chain protection of Asp, Glu, Ser, and Tyr, the t-butyloxycarbonyl group for Lys, the methoxytrimethylbenzenesulfonyl (Mtr) group for Arg, and the trityl group for Cys. The Fmoc-amino acid active esters (pentafluorophenyl esters except for Ser which was the dihydrooxobenzotriazine ester) were dissolved in a solution of 0.3 M 1-hydroxybenzotriazole in DMF.
14. The peptides were cleaved from the resin with 95% TFA containing 5% phenol for prothrombin 1-9, acyl carrier protein 54-65, and neurotensin and 2.5% phenol/2.5% ethanedithiol for FOS oncogene protein 147-162. The peptides were cleaved from the resin in 2 hours but neurotensin and FOS oncogene protein 147-162 were treated with the cleavage reagent for an additional 24 hours to ensure complete removal of the Mtr side chain protecting group from arginine.
15. HPLC conditions- Column: Delta-Pak C18, 100 Å, 5μm (0.39 x 15 cm); Flow rate: 1.0 ml/min; Temperature: 30°C; Wavelength: 220 nm; Eluent A: 0.1% trifluoroacetic acid; Eluent B: 0.1% trifluoroacetic acid in 95% acetonitrile; Gradient: 6% to 62% eluent B in 30 min linearly. The samples were dissolved in 1 N acetic acid or 6% eluent B.

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