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An Efficient Support for Solid-phase Peptide Synthesis

KEYWORDS: Introduction, Objectives and Conclusion.

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ABSTRACT *This research paper illustrates the successful and efficient solid phase assembly of hydrophobic difficult sequence peptides following both t-Boc and Fmoc chemistry. The peptides were synthesized on an optimized 1, 4-butanediol dimethacrylate-crosslinked polystyrene support (BDDMA-PS). Four difficult sequence test peptides, VAVAG, VIVIG, QVGQVELG and VQAAIDYING, were synthesized in relatively good yield and purity without any aggregation problems. The peptides were assembled on chloromethylated and 4-hydroxymethyl phenoxymethyl (HMP) BDDMA-PS resins. The peptides were fabricated using Boc amino acid 1-hydroxybenzotriazolyl and Fmoc amino acid pentafluorophenyl active esters in coupling reactions. The peptides after synthesis were cleaved from the polymeric support by exposing the peptidyl resin to 90% trifluroacetic acid/5% thioanisole/5% EDT mixture. The HPLC and MALDI TOF MS studies of the peptides revealed the high homogeneity of the synthesized peptides. Chloromethylated resin having a functional group loading of 1.14 mmol Cl/g was used for the synthesis. The yield and homogeneity of these peptides synthesized using the new supports were high when compared with the conventional DVB-PS resin.*

Introduction

Among the many biomaterials, synthetic hydrogels has recently been gaining a lot of attention for their resemblance to living tissues. Hydrogels are macro-molecular networks that swell, but do not dissolve, in water. Synthetic hydrogel networks are useful for applications that require a material, which has good compatibility with aqueous fluids, yet will not dissolve. Such applications include biomaterials, controlled release devices and electrophoresis gels. Many properties of hydrogels make them suitable for biomedical applications that require contact with living tissue.

The ability to absorb and retain aqueous media not only gives hydrogels a strong superficial resemblance to living tissue, but also makes them permeable to small molecules such as oxygen, nutrients and metabolites [1]. That is why hydrogels are better as implants, as long as their mechanical properties are acceptable, when compared to other materials such as ceramic and metals [2].

Hydrogels can be synthesized by the c-irradiation technique [3]. A large number of papers have been published on the characterization of the hydrogels prepared by crosslinking of a homo- or copolymer in solution in the presence of crosslinkers with c-irradiation. It is argued that more homogeneous network structures can be synthesized, if crosslinking is accomplished with c-irradiation in the absence of an initiator and a crosslinking agent. The structural homogeneity of the network affects the swelling behavior and mechanical properties.

The water content of the hydrogels at equilibrium is one of their basic properties. A hydrogel with higher water content is generally more advantageous in increasing permeability and biocompatibility. A lot of work was dedicated to various aspects such as the swelling and shrinking of non-ionic and ionic gels in aqueous solution, phase transition in gels, the elastic properties of gels and the structure of solvents inside a gel [4, 5].

Particularly, hydrogels based on polyacrylamide gels have been investigated by many research groups [6]. Polyacrylamide hydrogels exhibit a very high capability to absorb water, are permeable to oxygen and possess good biocompatibility.

In our previous works, radiation synthesized acrylamide based copolymeric hydrogels have been studied in the adsorption of protein [7, 8], biocompatibility with human sera [9, 10], the influence of amino acids and simulated body fluids to the swelling behavior [11 – 13] and in vivo biocompatibility with subcutaneous tissues of rats [14]. The objective of this work is the influence of simulated physiological body fluids on the swelling behavior of radiation synthesized polyacrylamide (AAm) hydrogels containing different types and amounts of crosslinkers.

Proteins are natural polymers derived from 20 L-a-amino acids under nucleic acid control. An amide or peptide bond links these various building blocks leading to a very complex structure. They are present in abundance and diversity in every living organism on earth and mediate all the molecular programmes in biology with precision and efficiency. Majority of the biological functional elements such as enzymes, hormones, antigens, antibodies, neurotransmitters, toxins, agonists, antagonists, enzyme inhibitors, oxygen carriers, electron transport systems, etc. are essentially proteins or peptides. Even DNA, which controls all fundamental functions of life including the protein synthesis, is highly organized in compact association with the basic proteins called histones. But it is surprising that out of the astronomical number of possible of amino acid sequences, only a small proportion of proteins occur in nature. That is, peptides and proteins are the optimized systems for performing biological functions. The term peptide was derived by Emil Fischer1 for molecules containing less than 50 amino acid residues and they form a large group of biologically active biopolymers. Since they influence the endocrine, neurobiological, immune and enzymic processes with high specificity and prodigious potency, they have varied applications in the field of medicine both in diagnosis and therapy2. The drastic variations in potency and activity profile of biologically active peptides even with slight structural changes make them rich source of new pharmacophores. In fact, many of the naturally occurring peptides are available as drugs and many as focal points for further developments as drugs3. Cancer therapy, connective tissue diseases, cardiovascular problems, digestive disorders, fertility regulation, growth simulation, diabetes and mental illness are the some areas where peptide drugs find significance4. In spite of the numerous advantages associated with peptide drugs, their dosage cost, instability to proteases, poor drug delivery, poor selectivity of action and poor membranes permeation limit their potential. This hurdle can be circumvented only through the understanding of the biological, biochemical and biophysical features of peptides which in turn is possible only with the ready availability of peptides and their

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analogues, cost effectively. Currently there is a great interest in peptide antibiotics5, peptides vaccines and various structural and functional analogues7-12 with more stability and potency. But the tremendous molecular diversity, complexity in structure and dynamic conformations of peptides point towards the need of a basic understanding of the structural features inherent in their biological activity. This is the main goal to be achieved by the bioorganic chemists who are trying to establish structure-activity relationships of peptides. A combinatorial search algorithm which allows the parallel synthesis of diverse molecular structures exponentially, was a really break through for the bottleneck of synthesizing and evaluating the candidate molecules one at a time.

Objectives:
The present work involves the development of a new flexible crosslinked polymeric support for the solid-phase synthesis of peptides. The success of hydrophilic and flexible crosslinked polymers as support material over the rigid and hydrophobic support was the driving force behind the present work.

The main objectives of the present work are:
- To prepare a new flexible and amphiphilic support for the solid-phase peptide synthesis.
- To optimize the conditions required for the incorporation of various functional groups relevant in peptide synthesis.
- To compare the efficiency of the newly developed BDDMA-PS support with the conventional Merrifield resin, divinylbenzene-crosslinked polystyrene.
- To investigate the role of the support upon the conformation of resin bound peptides and hence to correlate between nature of the support and coupling difficulty.
- To demonstrate the utility of this support for the synthesis of biologically relevant peptides especially hydrophobic and difficult sequences.

Conclusion:
This article illustrates the successful and efficient solid phase assembly of hydrophobic difficult sequence peptides following both t-Boc and Fmoc chemistry. The peptides were synthesized on an optimized, 4-butanediol dimethacrylate-crosslinked polystyrene support (BDDMA-PS). Four difficult sequence test peptides, VAVAG, VIVIG, QVGQVELG and VQAAIDYING, were synthesized in relatively good yield and purity without any aggregation problems. The peptides were assembled on chloromethylated and 4-hydroxymethylphenoxymethyl (HMP) BDDMA-PS resins. The peptides were fabricated using Boc amino acid 1-hydroxybenzotriazolyl and Fmoc amino acid pentafluorophenyl active esters in coupling reactions. The peptides after synthesis were cleaved from the polymeric support by exposing the peptidyl resin to 90% trifluroacetic acid/5% thioanisole/5% EDT mixture. The HPLC and MALDI TOF MS studies of the peptides revealed the high homogeneity of the synthesized peptides. Chloromethylated resins having a functional group loading of 1.14 mmol Cl/g was used for the synthesis. The yield and homogeneity of these peptides synthesized using the new supports were high when compared with the conventional DVB-PS resin.

In this work a new class of functional composites was prepared based on the organically modificated montmorillonite and methacrylic acid. In the first step, the native montmorillonite was mixed with poly (ethylenimine) monomer to promote the intercalation process. In the second step, the methacrylic acid and initiator (potassium persulfate) were added and polymerization was carried out at 800 C for 2 hours. Characterization of the obtained composite material was performed by Fourier transform infrared spectroscopy. The change in interlamellar spacing of montmorillonite after polymer composite preparation was determined by X-ray diffraction. In prepared composite samples the existence of two crystalline phases was estimated by X-ray measurements. In the first, the poly (methacrylic acid) chains are intercalated within the montmorillonite layers, and in the second, they are dispersed in the polymer matrix. The influence of montmorillonite on the thermal properties of the obtained composites was measured using the combined thermogravimetry and differential scanning calorimetry method. The porosity of the prepared samples was determined by isotherm adsorption and desorption of nitrogen (Brunauer-Emmett-Teller method). It was assessed that a certain amount of polymer is deposited on the montmorillonite and thus covering of the micropores and partially covering of the mesopores was achieved.

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