ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 9, Issue, 05, pp.50303-50306, May, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

COST EFFECTIVE SYNTHESIS OF ANGIOTENSIN II AND ANGIOTENSIN IV ON A HYDROPHILIC POLYMER SUPPORT

***,[1]Rohini, K. C., [2]Selva, R., [1]Dr. V. D. John and [2]Dr. K. P. Subhashchandran**

[1]Department of Chemistry, Christ College, Irinjalakuda, India
[2]Department of Chemistry, Sri Vyasa NSS College, Thrissur, India

ARTICLE INFO

Article History:
Received 11th February, 2017
Received in revised form
07th March, 2017
Accepted 14th April, 2017
Published online 23rd May, 2017

Key words:

Solid phase synthesis,
GDMA-4VP resin,
Suspension polymerisation,
Angiotensin.

ABSTRACT

One of the important factors for a successful solid phase synthesis is the swelling and solvation of peptide bound resin in the reaction medium.Here a novel GDMA-4VP resin support for solid phase synthesis is prepared by suspension polymerization of 4-vinyl pyridine with glycerol dimethacrylate. The hydrophilicity of the cross-linker provides flexibility and polarity to the support. Swelling studies and stability studies were performed. Resin was functionalized and the resin was found to be quite stable even after vigorous conditions of functionalisation. IR spectrum and SEM were used for the characterization of the resin. The efficiency of the peptide was demonstrated by synthesizing biologically active Angiotensin II and Angiotensin IV peptides. Modified Fmoc chemistry is used for the stepwise addition of amino acids in the peptide chain. The procedure of deprotection, coupling, washing is followed. The reagents used were maximum evacuated to reduce the repeated washings. The peptides obtained were with high yield, minimum usage of solvents and almost 99% purity which was checked by HPLC.

Citation: Rohini, K. C., Selva, R., Dr. V. D. John and Dr. K. P. Subhashchandran, 2017. "Cost effective synthesis of angiotensin II and angiotensin iv on a hydrophilic polymer support", *International Journal of Current Research*, 9, (05), 50303-50306.

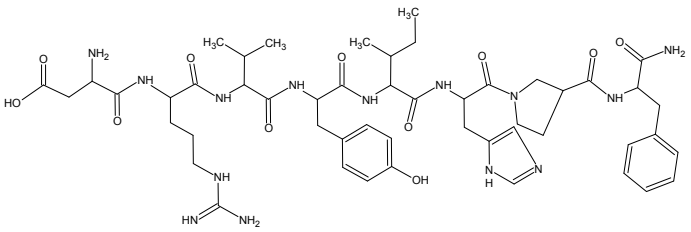
INTRODUCTION

Synthesis of products with low cost, less pollution, least time and lot of yield is always the aim of chemical industry. Researches are going on all the time for this aim in the synthesis of peptides also. Synthetic peptides are widely used to verify the structure of naturally occurring peptides as determined by degradation techniques, to study the relationship between structure and activity of biologically active protein and peptides and establish their molecular mechanisms, and to develop new peptide-based immunogens, hormones, vaccines, etc. (www.peptideguide.com/peptides-Application.html). The solid phase synthesis is widely accepted method for the chemical synthesis of peptides. The basic technique in this method is the assemblence of amino acids in to a peptide sequence keeping one end of the chain anchored to an insoluble support (JayaT.Varkey, 1997). Repeated cycles of coupling-washing-deprotection-washing is followed. The success of solid phase technique depends on the properties of solid support (Hodge and Sherrington, 1998; Medal, 1997). Various structural parameters of polymeric support such as polarity, nature and extent of crosslinking and the solvation of the support and resin bound species in the solvent medium determines the reactivity of functional groups attached to the polymeric backbone. Peptide synthesis using the classical PS-DVB resin meets some drawbacks because of the rigidity, hydrophobicity and physico-chemical incompatibility of the polymer with the growing peptide chain (Zalipzky *et al*., 1994; Hellerman *et al*., 1983). The strong hydrophobic, macromolecular environment of polymer can persuade the growing peptide chain to adopt unfavourable conformation that lead to low yield of purity of target peptides (Sheppered, Peptides 1971). Peptide chemistry utilises different classes of hydrophilic polymers as support for chemical reactions. The solubility and diffusivity of hydrophilic polymers in water facilitates their biomedical applications. In this work, the Influence of Glyceroldimethacrylate cross-linker in the 4-Vinyl Pyridine support for peptide synthesis was studied by synthesising a biologically active Angiotensin II and Angiotensin IV. Angiotensin was independently isolated in Indian apolis and Argentina in the late 1930s (as 'angiotonin' and 'hypertensin', respectively) and subsequently characterised and synthesized by groups at the Cleveland Clinic and Ciba laboratories in Basel, Switzerland. Angiotensin is a peptide hormone that causes vasoconstriction and a subsequent increase in blood pressure. It is part of the renin-angiotensin system, which is a major target for drugs that lower blood pressure. Angiotensin also stimulates the release of aldosterone, another hormone, from the adrenal cortex. Aldosterone promotes sodium retention in the distal nephron,

Corresponding author:* **Rohini, K. C.
Department of Chemistry, Christ College, Irinjalakuda, India.

in the kidney, which also drives blood pressure up. Angiotensin II acts on the adrenal cortex, causing it to release aldosterone, a hormone that causes the kidneys to retain sodium and lose potassium. Elevated plasma angiotensin II levels are responsible for the elevated aldosterone levels present during the luteal phase of the menstrual cycle (Boulpaep and Boron, 2005; Basso and Terragno, 2001). In this work we concerned purity (hygienic) which should applicable for biological research and yield for further development. The yield and purity of the peptides reveals the advantage of the resin. The chemical structure of AngiotensinII and Angiotensin IV peptide were shown in Figure 1 & 2.

(NH_2)Asp-Arg-Val-Tyr-Ile-His-Pro-Phe($CONH_2$)



Scheme 1. Chemical composition and structure of Angiotensin II contains 8 amino acid residues (m.wt:1046 g/mol)

(NH_2)Val-Tyr-Ile-His-Pro-Phe($CONH_2$)

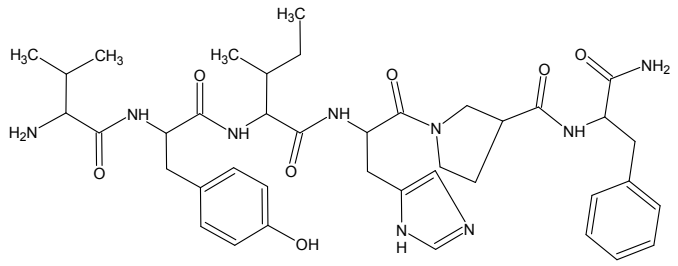


Fig.2. Chemical composition and structure of Angiotensin IV contains 6 amino acid residues (m.wt: 775 g/mol)

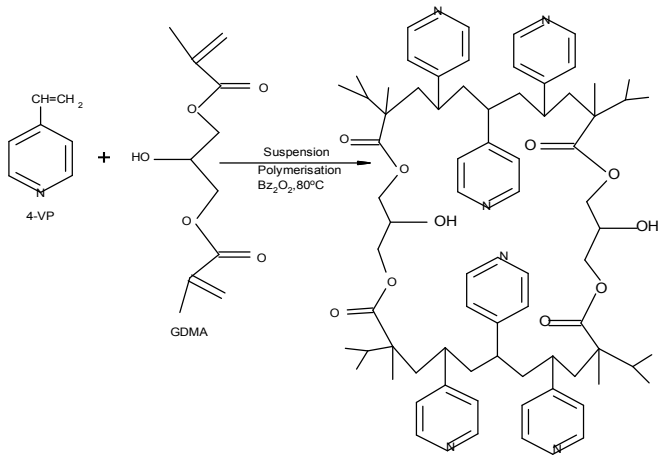
MATERIALS AND METHODS

Materials

Glyceroldimethacrylate, Polyvinyl alcohol, Benzoylperoxide, 4-Vinylpyridine, toluene, sodiumhydroxide, dimethyl formamide, dichloromethane, methanol and chloroform were purchased from Merkmillipore (India). Fmoc- Amino acids were purchased from Sigma-Aldrich, Switzerland and Alfa-aesar England. 4-Dimethylaminopyridine (DMAP), Hydroxzybenzotiazole (HOBt), Diisopropylethylamine (DIPEA), Diisopropylcarbodiimide(DIC), Trifluroaceticacid (TFA),Triisopropylsilane (TIS), Pyridine and Pipyridine reagents was purchased from Sigma-Aldrich, Germany and china,Alfa-asear England and Merkmillipore(India).Other solvents were purchased from Merkmillipore(India) and lobachemi Mumbai.

Preparation of GDMA cross-linked 4-VP support

The polymerisation was carried out in a conventional suspension polymerisation reactor. Glyceroldimethacrylate and 4- Vinylpyrine were destabilised using 1%NaOH. 1 g PVA was dissolved in 100 ml distilled water at 80^oC to prepare a 1% aqueous solution. A mixture of GDMA, 4VP and benzoyl peroxide were added to the PVA solution keeping the solution stirred mechanically at 500 rpm. The temperature of the system was maintained at 80^oC and the reaction was continued to 5 hrs. Resin beads began to appear on the wall of the vessel. The system was kept overnight as such. The beaded resin was then filtered and washed with hot water to remove PVA. The unreacted monomers were washed off and the resin beads were dried under vacuum. The polymer was soxheletted with acetone followed by methanol to remove all linear polymers. The beads were meshed to 100-200 range. The solubility and the swelling of resin in various solvents used for solid phase synthesis were conducted. The structure of the resin is given in Fig.1.



Scheme 1. Suspension polymerisation of glyceroldimethacrylate and 4-VP

Synthesis of Angiotensin II and Angiotensin IV

Sequence:

Angiotensin II - NH2 -Asp-Arg-Val-Tyr-Ile-His-Pro-Phe – COOH

Angiotensin IV-NH2-Val-Tyr-Ile-His-Pro-Phe-COOH

The peptide was synthesised on GDMA-4-VP resin (250mg resin, 0.65mmol/g capacity) using F-moc chemistry. Each coupling was performed with 2 equivalents of HOBt with respect to resin capacity and equivalence of amino acid. The first amino acid Fmoc- Phe - OH was anchored by esterification to the resin using the following procedure. The resin was transferred to a clean, dry, sililated peptide synthesiser, added sufficient amount of NMP and kept for an hour for swelling and the excess NMP was removed. Fmoc-Phe-OH (3 eq.), DMAP (0.1 eq.) and DIC (3 eq.) were added to the swollen resin and shaken for 60 minutes. Washing of the resin was performed with NMP (2 times), DMF (2 times), MeOH (1 time). The above esterification reaction was repeated to enhance the reaction. The reaction was monitored by thin layer chromatography. Second amino acid Fmoc-Pro-OH was coupled to the sequence as follows.

Coupling: Fmocprotection was removed from the resin bound amino acid. Fmoc- Pro-OH dissolved in minimum quantity of NMP in well closed 25ml RB flask to that HOBt were added and dissolved and DIC were added and sake it well for 3mins and immediately the content was transferred in to the resin

with moisture free atmosphere and sake it well for 5mins, to that DIPEA was added and shaken well for 45mins. Reaction progress was monitored by TLC. Small pinch of the resin were taken and washed, ninhydrin were tested in case positive means same amino acid coupling was repeated, in case negative means washed, deprotected, and remaining amino acids coupling were done by above method. The detailed synthetic strategy, time duration of reaction process and conditions are given in Table 1.

Table 1. Synthesis of Angiotensin-2 (NH_2) Asp-Arg-Val-Tyr-Ile-His-Pro-Phe ($CONH_2$)

Aminoacid (Fmoc-)	Coupling(mins) 1st	2nd	3rd	Ninhydrin	Washing	Deprotection 15mins(×2)	Washing
Fmoc-Phe-OH	45	40	-	-ve	Done	Done	Done
Fmoc-Pro-OH	30	35	-	-ve	Done	Done	Done
Fmoc-His(trt)-OH	30	30	-	-ve	Done	Done	Done
Fmoc-Ile-OH	30	30	45	-ve	Done	Done	Done
Fmoc-Tyr(tbu)-OH	30	40	-	-ve	Done	Done	Done
Fmoc-Val-OH	30	35	40	+ve*	Done	Done	Done
Fmoc-Arg(pbf)-OH	30	35	-	-ve	Done	Done	Done
Boc-Asp(tbu)-OH	30	40	-	-ve	Done	-	-

Cleavage of crude peptide from resin: After synthesis the resin were washed with hexane, DCM, $CHCl_3$ and MeOH and dried. The cleavage were done with 95% TFA:2.5% TIS:2% water:0.5%m-crosal at 3hours under nitrogen atmosphere and the resin were washed 4times with TFA, the filtrate were collected in 50ml RB-Flash and all the traces of TFA was evaporated by using Rota vacuum evaporator (Atherton and Shepard, 1984; Bodanszky, 1984). The peptide were isolated with excess of peroxide free pure cold diethylether and the peptide were washed 7times with diethylether and centrifuged. The clear white powder form of peptide was collected in a small tubes and sealed (Merrifield, 1986).

RESULTS AND DISCUSSION

GDMA-4VP RESIN support was prepared by suspension polymerisation using benzoyl peroxide as initiator. The insoluble polymer support was obtained as spherical uniform beads. The resin has excellent swelling properties and stability and satisfies all conditions of solid phase peptide synthesis. It was found to be quite stable even after vigorous conditions of functionalization. The scanning electron micrograph image of the resin is given in Fig.1.

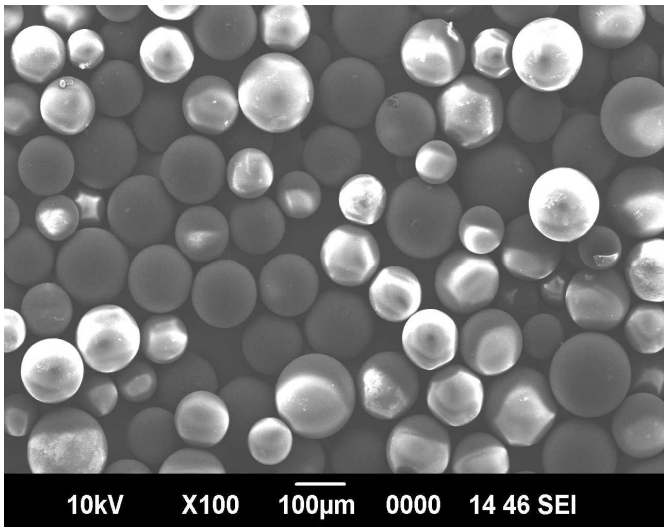


Fig.3. Scanning electron micrograph of GDMA-4VP

The IR spectrum of GDMA-4VP resin gave a sharp and intense peak at 1716 cm^{-1}corresponding to the ester carbonyl group of the cross linker. The peak at 3430 cm^{-1} corresponding to hydroxyl group of the cross linker in addition to those of monomers. The accuracy of HPLC assay method was assessed by standard method (the known peptide sample purity were recorded and compared with standard reference). The purity of both crude peptides was shown in Figure 5 and 6.

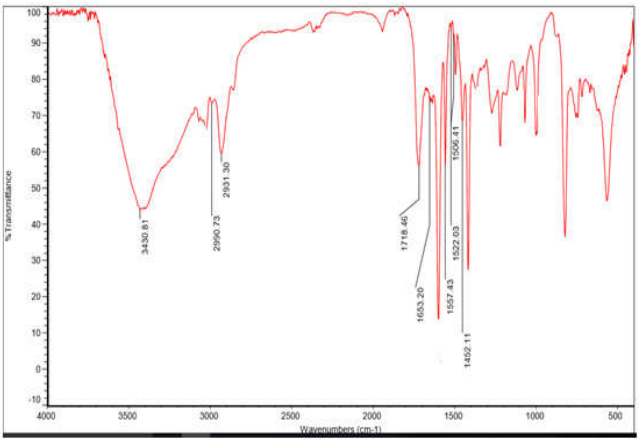


Fig.4. FT-IR spectral analysis of GDMA-4VP

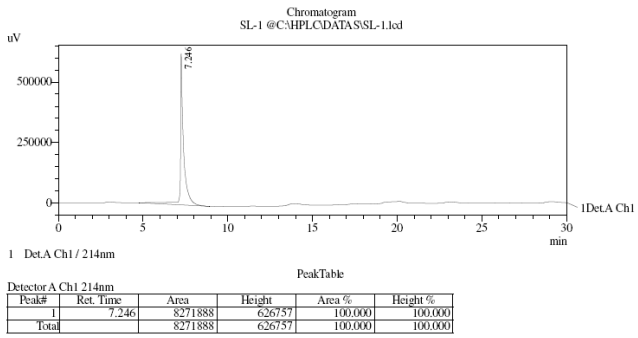


Fig.5. HPLC of Angiotensin-2 -NH_2Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-COOH

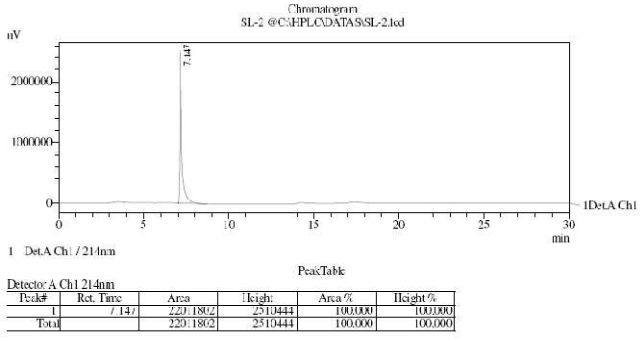


Fig.6. HPLC of NH2.Val-Tyr-Ile-His-Pro-Phe-COOH, the single sharp peak at ret.time 7.14mins shows our target peptide purity. Crude peptide yield: 85.78%

The single sharp peak at ret.time 7.24mins shows our target peptide purity.

Crude peptide yield: 83.67%

The given HPLC analysis report gives clear evidence for the purity of the crude Angiotensin II and Angiotensin IV peptide.

Conclusion

The hydrophilic, flexible support GDMA-4VP for solid phase organic synthesis developed, shows extra ordinary swelling and stability in the solvents used for solid phase peptide synthesis besides the ease of preparation and functionalization. So the synthesis can be done in a cost effective way by minimising the quantity of solvents used. The biologically active peptide fragment prepared by improved F-moc solid phase peptide strategy shows hygienic purity and good yield. So the aim of peptide synthesis with low cost, less pollution, maximum yield and purity is achieved. The coupling method is also relatively fast as compared with previously reported procedures. It will helpful for further research and improvement.

REFERENCES

Atherton, E. and Shepard, R.C. 1984. "Solid Phase Peptide Synthesis- A Practical Approachusing the Fmoc-Polyamide Techinique" IRL Press, Oxford.

Basso N. and Terragno NA December 2001. "History about the discovery of the renin-angiotensin system". *Hypertension*, 38 (6):1246–9.

Bodanszky, M.1984. "Principles of Peptide Synthesis"; Springer Verlag: New York.

Boulpaep EL. and Boron WF 2005. Medical Physiology: a Cellular and Molecular Approach. St. Louis, Mo: Elsevier Saunders. pp.771.

Hellerman, H., H.W.Lucas., J.maul, V.N.R.pillai and M.Mutter, 1983. Ethylene glycol)s grafted onto cross linked polystyrenes, 2 multi detachably anchored polymer systems for the synthesis of solubilized peptides *Makromol.Chem*., 184, 2603.

Hodge PC and Sherrington DC. 1998. Polymer supported reactions in Organic Chemistry, Wiley: Chichester.

JayaT.Varkey., Synthesis of thioredoxin partial sequences on a hydrophilic cross-linked polymeric support

Medal, M. 1997. Solid phase peptide synthesis; Fields G.Ed, Academi press: New York, 83-104.

Merrifield, B. 1986. Solid phase synthesis, *Science, 232*, 341-347.

Sheppered, Peptides R.C. 1971. Nesvadba, H.,(ed); North Holland, Amsterdam 1973.

www.peptideguide.com/peptides-Application.html

Zalipzky, S., J.L.Chang, F.Albercio and G. Barany, 1994. *React.Polym*., 22, 243.

****