Review Article

J Integral Sci, 2023, Volume 6, Issue 1, 27-35

Journal of Integral Sciences [JIS]

[An International Open Access Journal]

Available at www.jisciences.com ISSN: 2581-5679

A Review on Peptide Synthesis: Therapeutic agents, stabilizing agents and its applications

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Received: 12 Jan 2022 Revised: 22 Feb 2023 Accepted: 19 Mar 2023

Abstract

Peptides are required as a part of health through their extended activity. Peptides are also purified because of their high target affinity. The synthesis includes both the conventional method and the microwave method among which microwave procedure is considered superior due to less reaction time and higher yeilds. The peptides were assessed for the inhibition activity of Urokinase plasminogen (UpA), coagulation factor XII, and also for the cytotoxicity and anti-microbial activity.

Key words: Peptides, Urokinase plasminogen, cytotoxicity, coagulation factor XI, purification.

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DOI: https://doi.org/10.37022/jis.v6i1.53

Produced and Published by

South Asian Academic Publications

Introduction

Peptides are applied in different fields including pharmaceutical, cosmeceutical industries. Pharmaceutical peptides such as antibodies, antimicrobials, and peptide vaccines are the main subclasses of peptide molecules. Because peptides have high target affinity, low toxicity, target wide range of molecules they are advantageous. Whereas, low oral bioavailibility, costly production, poor membrane permeability and metabolic liability are the limitations. Earlier, peptides were obtained from natural sources such as skin of frogs, animal venoms and not used directly for therapeutic usages; thus advances in peptide engineering, analytical evaluations, structural bioinformatics sequencing technologies, rational design has opened its way in making efficient peptides [1]. Biologically active peptides may be natural compounds of food or part of protein that are inactive in the precursor molecule. Because peptides are synthesized by focusing on developing general condensation agents, ensuring low risk of racemization issues like health hazards, waste generation and atom economy have abonded they are being extensively exploited in pharmaceuticals and cosmetics, by repeating a condensation-agent-mediated amidation followed by deprotection the peptides are synthesised [2]. After enzymatic cleavage with selective heat-induced precipitation of (Glutathione-S-transferase) GST-affinity tag the peptides are purified. Because of the limitaions like coupling efficiency, steric hindrance of protective groups or larger side chains and the tendency of peptides to aggregate results in low yields [3]. The peptides synthesised by conventional method requires more time and thus an alternative implement like automated microwave technology offers advantages like higher yeilds and less reaction time.

There are three ways for the synthesis of peptides: Solid phase, liquid phase and recombinant technology. Bioactive peptides play an important role in health through broad spectrum of bioactivity like an antioxidant, antimicrobial, anti-inflammatory, immunomodulatory, anti-proliferative activities also used as food preservatives because of antimicrobial and antioxidant activities [4]. In the transference of information to and from the central nervous system the peptides play a vital role. Based on the availibility of genome and gene expression in the tissues, the secretory peptides that are expressed has the ability to provide the information from specific tissue and organ back to the brain. As unique information is encoded not only in

frequency and amplitude modulation, but also with their primary amino acid sequences and subsequent secondary and tertiary structures peptides are appropriate for the transfer of information. Prominent examples of cyclic peptide research reagents are phalloidin, a bicyclic peptide that binds filamentous actin and is used for staining the cytoskeleton in cell microscopy and the cyclic Arginylglycylaspartic acid(RGD) peptides that bind integrin receptors and are used for interfering with the interactions of cells and the extracellular matrix. Extensive lists of these commercially available cyclic peptides are found in the product catalogs of common distributors such as Bachem, IRIS and Sigma. The approximately 40 approved cyclic peptide drugs are used for a wide range of diseases, including the treatment of cancer, infections and Cardiovascular diseases [5].

APPLICATIONS:

According to Kaycie et.al, a trypsin like serine protease Urokinase type plasminogen activator (uPA), which is overexposed in some tumors and thus regarded as a possible target for the tumor growth are inhibited and invaded by bicyclic peptide inhibitors. These inhibitors were assessed in a human tumor xenograft mouse model to evaluate the inhibition activity of uPA that affects the tumor growth. In this experiment the bicyclic peptides were tested in vivo, UK18 an uPA inhibitor incubated in blood plasma revealed that a part of bicyclic peptide remained as such while monocylic analogs were degraded. When the two rings of bicyclic peptide UK18 were separated they were considered less stable than together, this indicates that the two rings in bicyclic peptides stabilized each other, by hindering the access of proteases sterically or by noncovalent interactions that decreases the conformational flexibility. The administration of UK18 to mice by intravenous route showed that in invivo the peptide was stable but was filtered by the kidney with a half-life of 30 min. The bicyclic peptides were distributed to all the regions of the tumor tissues and this was examined by microscopic studies. The bicyclic peptides extracted from tumor tissue samples were analyzed and showed that 90% of the peptide remained functional, though the inhibition of uPA in mice which is having a solid MDA-MB-231 xenograft tumor did not reduce the growth compared to the controls [5].

Figure 1: Urokinase; Plasminogen activator

Bicyclic peptides an inhibitor of the coagulation factor XII, which acts as a target to prevent pathological thrombosis, to inhibit the contact activation in extracorporeal circulation. And the swelling disorder hereditary angioedema. Vanessa et.al, Coagulation factor XI has appeared as a favourable target for the development of anticoagulation drugs that are safe and also with low risk of severe and critical bleeding. The anticoagulation effects were achieved by the cyclic peptides and compared with the standard heparin at a therapeutic dose (0.3-0.7 IU/ml in plasma) with substantially broader estimated therapeutic range. They extended the plasma half-life of the peptide through PEGylation and revealed effective inhibition of FXIa over prolonged periods in vivo. The anticoagulant effects of the PEGylated inhibitor in an ex vivo hemodialysis model with human blood were validated by them. Their work showed that FXI selectively targeted with the peptides and provided a promising candidate for the development of a safe anticoagulation therapy.

Peptides specified by three conserved disulfide bonds like defensins a large family of cationic antimicrobial peptides that form an essential element of innate immunity are microbicidal and shows cytotoxic activity by the formation of multimeric pores in the membrane of microbes, the microbicidal activity of defensins is limited by physiological concentrations of mono and divalent cations and also by components of plasma or serum. Defensins also shows the immunoregulatory actions6.Administration of peptides through nasal or oral mucosa, reported by David A Lovejoy et.al., where the two sensory systems differ in which the olfactory organ is predisposed to sample volatile chemical signals, and the vomeronasal is sensitive to non soluble and non volatile compounds. Both the organs are linked with the vascular network associated with the CNS. Vomeronasal epithelium (VNE) is the embryonic tissue source of gonadotropin releasing hormone (GnRH) neurons developed early in the fetal lifethat migrate into the

telecephalon to eventually regulate the neuroendocrine aspects of the reproductive system. The sensory aspects of the VNE degrades due to mutation of genes specific to Vomeronasal organ (VNO) function. The function of VNO with respect to peptide uptake is unclear, the olfactory epithelium of the sensory neurons extend their dendrites into the nasal cavity and allows these sensory neurons to come into contact directly with the external environment. Thus, the olfactory signals directly interact with the CNS. Transport of the peptides across the oral mucosa occurs by passive diffusion and avoids GI degradation via bypass of the initial hepatic metabolic processes [7].

Peptides acts on cancerous cells by membranolytic mechanism or mitochondrial disruption. Teerasak E et.al, reported the antimicrobial and anticancer activities of mucous glycoproteins extracted from African snail. The mucous fractions showed in vitro cytotoxicity against the breast cancer cell line Michigan cancer foundation-7 (MCF-7) and normal epithelium cell line8.Neghadaripour et.al, reported that CPP's are regarded as encouraging drug delivery tools for a variety of diseases such as infections, inflammatory and neoplastic diseases could all be targeted. Chemotherapeutic drugs like doxorubicin and methotrexate are used in this study. CPP's selectively delivered cyclosporine A and Paclitaxel. The provision of chemotherapeutic agents delivered via CPP's improved their pharmacokinetic properties and overcame drug resistance. CPP-Dox(The CPP attached to the drug) demonstrated resistance in comparison to the bare drug (Doxorubicin). There is compelling evidence that various types of enzymes can be delivered by as a result of CPP, thus diverse conditions such as oxidative stress or acute lymphocytic leukeamia were under control.

Figure 2: Doxorubicin

Figure 3: Methotrexate

PROCEDURE FOR PEPTIDE SYNTHESIS CONVENTIONAL METHOD [9]:

The general procedure for the synthesis of peptides by Ford et.al, Fluorenylmethoxycarbonyl protecting group (Fmoc-Spps). Rink amide resin was treated with 40% vol piperidine in Dimethyl formamide for 4 min, drained, and then treated with 20% vol piperidine in DMF for 12 min, drained and washed with DMF. The resin was then treated with an Fmoc-Xaa-OH and Oxyma solution in DMF followed by solution of N, N'-Diisopropylcarbodiimide in DMF and shaken at 75°C for 30min. Then the resin was drained and washed with DMF before being treated with a solution of 5% vol acetic anhydride and 10% vol N, N-Diisopropylethylamine in DMF for 5min room tempreature, drained, washed with DMF and drained again. These deprotection and coupling steps were repeated for additional amino acids until the target polypeptide was assembled.

Chloroacetic acid coupling: The completely elongated resin-bound peptide was shaken in a chloroacetic acid solution, N, N-Diisopropylcarbodiimide (DIC) and oxyma in Dimethylformamide (DMF) for 15min at 75°C. The coupling solution was dispended, the resin was washed with DMF and Dichloromethane.

Peptide cleavage from resin: The peptide bound to resin was shaken in a cleavage solution of trifluoroacetic acid for 2 hours at room temperature. The crude product was then dispended, and with the cleavage cocktail was rinsed with resin. The solutions were combined and concentrated to crude peptide was dried under a nitrogen flow and then re-dissolved in acetonitrile in water for cyclization.

Linear peptide cyclization: To a solution containing linear peptide, an acetonitrilesolution in water, Hunig's base was added and the abover reaction was shaken for 2 hours at room temperature. The cyclic peptide solution was then reacidified with TFA and filtered preparation for purification.

MICROWAVE PROCEDURE [10]

Microwave assisted parallel synthesis method is used for both α and β peptides, to accomplish the solid phase

β peptide syntheis:

(a) The activated ester of a β-amino acid residue with the free amine of a resin bound linker or growing oligopeptide chain should be coupled.

(b) The base labile 9-fluorenylmethoxycarbonyl (Fmoc) group from the main chain nitrogen should be removed by treating with piperidine.

The above two steps are repeated until the oligomer of desired length is obtained.

(c) From the solid support the peptide was cleaved and at the same time the side chain was deprotected by treating it with Trifluoroacetic acid (TFA). The same coupling, Fmoc removal and cleavage reactions occur even in Parallel synthesis of peptides, except the addition of different protected amino acid to each well during the coupling steps which results in each well containing a peptide with a unique sequence.

Procedure

Plan Library Synthesis

Once the library is designed, planned the library synthesis by preparing a map that divides the wells of the plate into different sectors for each reaction step. The plate is subdivided into sectors which facilitates reagent delivery using a multichannel pipette, and starting with the plate map prevents the confusion during the synthesis.

Prepare Reagents

Calculated and weighed the proper amount of Fmoc-aa into 20ml scintillation vials separately. If the two separate vials are prepared if the same amino acids are incorporated at the two positions of the library.

Array the solid support into each well of the filter plate

A slight excess of Polystyrene(PS) or PEG-PS resin was weighed, into a 50ml polypropylene centrifuge tube.The resin is suspended in 50 ml Dichloromethane/Dimethylformamide. Stir bar was added to the centrifuge tube and while stirring a 500ml aliquot into each well of the 2 ml deep sealed polypropylene filter plate. The homogeneous resin suspension produced by the DCM/DMF, allowing the distribution of resin in equal amount throughout the plate by dispensing the slurry of constant volume into each well.

A small magnetic stir bar was placed inside each well. 'PAUSE POINT setup and steps 1-5 performed the day before the actual synthesis. The reagent solutions, dry amino acid and arrayed resin are stored overnight at room temperature before starting to step 6.

The bottom sealing matte was removed from the plate.The plate was transferred quikly to the vaccum filtration manifold.The solution was drained from the plate by applying vaccum.The vacuum was closed using a squirt bottle, DMF was added to each well rapidly.The steps 8 and 9 were repeated four times and drained the solution for a final time.The bottom sealing matte was reaffixed to the plate.Fmoc-removal: Fill a pre-made deprotection solution of piperidine in DMF into a Polypropylene trough.A 12-channel multipipette, was used to dispense 250ml deprotection solution into each well of the plate.The plate was placed on the microtiter plate turnable inside the multimode microwave cavity of the CEM MARS a batch acid digestion microwave system.

Figure 4: CEM MARS.

The temperature probe was positioned in the center of the plate using the arm attached to the turntable, ensuring the tip of the probe in contact with the bottom of the well.

Crictical Step

Microwave reactor will not accurately detect the increasing temperature of the solution if the fiber-optic probe is not positioned inside the well and in the solution. As the air temperature being sensed by the probe does not increase, the reactor will continue to irradiate at full power throughout the experiment, overheating the samples. The sample was irradiated.The plate was removed from the microwave reactor, and the resin was washed as in steps [6-11].

Amino Acid Coupling

A pre-made solutions of HBTU, DMF, iPr2EtN,HOBt aaded to the vial containing pre-weighed solid Fmoc-aa from step 2. To the solid Fmoc added 2-(1H-benzotriazol-1-yl)-1, 1, 3, 3-tetramethyluronium hexafluorophosphate, Hexaflurophosphate benzotriazole tetramethyl uronium) HBTU in DMF, and DMF, Hydroxybenzotriazole (HOBt) in DMF, finally

Diisopropylethylamine iPr2EtN in DMF. Until the solid AA is dissolved the mixture was vortexed, and allowed to stand for 60-90s.The mixture was poured into a polypropylene trough. The solution was transferred to each well of plate as per the plate map prepared in first step. At the particular position of the library, if there is more than one diversity element then the different AA will be coupled one at a time. Once the coupling solution is added to the appropriate wells, then transfer the DMF to all the wells in the sector of the plate that do not contain AA coupling solution. On the microtiter plate turntable inside the multimode microwave cavity of the CEM MARS, the plate was placed. CRITICAL STEP: The fiber optic probe was positioned in the center of the sector containing AA coupling solution. Ensured that the tip of the probe is in contact with the bottom of the well using the arm attached to the turntable. Overheating the samples, if the microwave reactor will continue to irradiate at full power throughout the experiment if the fiber-optic probe is not positioned inside the well and coupling solution. The sample irradiated. The plate was removed from the microwave reactor, the resin was washed like in steps 6-11. The steps 18-24 were repeated untill the diversity elements have been incorporated for that position. PAUSE POINT: After a coupling reaction, the washed resin can be stored at room temp foe several hours or overnight.Steps 12-25 were repeated, added one monomer per cycle until the growing peptide chains reached the desired oligomer length.The Fmoc protecting groups were removed from the N-terminal residue by repeating steps [12-17].The resin was washed five times like in steps 6-11, but use DCM instead of DMF. In a new vial prepared the acetylation cocktail by mixing DCM, triethylamine and acetic anhydride. The solution was poured into a polypropylene trough. The acetylated cocktail was transferred into the each well in the plate using a multichannel pipette.The plate was allowed to stand for 5-15min in a hood (if desired the plate may be genty agitated on an orbital shaker). The resin was washed five times as in steps 6-11, use Dichloromethane (DCM) instead of DMF. PAUSE POINT: The washed resin can be stored at room temp for several hours or overnight. Used polypropylene trough and a multichannel pipette to transfer triethylsilane and water to each well of the plate in the hood.Used a small polypropylene squirt bottle to add Trifluoroacetate (TFA) slowly to each well of the plate in a well ventilated hood. The plate was covered with aluminium foil, allowed to stand for 2 hours in the hood. AWARENESS: TFA is corrosive. So wear eye protection, a lab coat and gloves. The aluminium foil was removed from the plate, the botttom sealing matte was removed carefully and quickly and placed the synthesis plate in a bottom plate. The cleavage solution was allowed to be transferred to the solidbottom plate by gravity filtration. AWARENESS: TFA is much less viscous than the DMF used in earlier steps of synthesis. The bottom sealin matte was removed and transferred to the solid-bottom plate and performed quickly to avoid drips and carefully to prevent spills. The cleavage solution was concentrated by rotary evaporation in a rotary concentrator. The samples were concentrated uusing steam of nitrogen by placing the plate under an inverted funnel attached to a nitrogen line. AWARENESS: TFA is corrosive, take care the nitrogen flow rate is low toprevent spattering. If TFA and triethylsilane have been removed,Dimethylsulfoxide (DMSO) added to dissolve the peptide product mixtures in each well. The purity of the library members was assessed by analyzing the peptide product mixtures on an analytical Reverse phase high performance liquid chromatography (RP-HPLC) and collected the fractions based on an Ultravoilet absorbance threshold. The peptide mass of the collected fractions were measured using matrix assisted laser desorption ionization-time of flight mass spectrometry.

PHARMACOLOGICAL ACTIONS

A. Transport of proteins and peptides across the oral mucosa occurs primarily by passive diffusion. Avoids GI degradation via bypass of the initial hepatic metabolic processes. The previous studies of peptide transit across the various BBBs indicate several essential attributes of these barriers.Most importantly, this barrier evolved to ensure interactive transit between the peripheral tissues and the brain to ensure homeostatic communication among all tissues and organs of the organism.Secondarily, the specificity of compounds that could transit the BBB was likely based on the suite of soluble proteinaceous and metabolic compounds as defined by the genomes of the species [11].

B. Robert M.H. et.al reported that:MK571[5-(3-(2-(7-Chloroquinolin-2-yl) ethenyl) phenyl)-8-dimethylcarbamyl-4, 6-dithiaoctanoic acid] (30nM-30μM) concentration-dependently inhibited hCASMC [Human coronary artery smooth muscle cell] proliferation compared to control:

Figure 4: MK571

MK571 completely arrested cell growth but also changed cell morphology and lowered viability. Based on the above data, a sub-threshold concentration of MK571 was selected to study effects of MRP inhibition on the antiproliferative effects of NO and ANP. Deta-NO [Diethylenetriamine-NONOate] and ANP [Atrial natriuretic peptide] were chosen due to their ability to elicit sub-maximal inhibition of human Vascular smoooth muscle cell (VSMC) proliferation. Deta-NO and ANP alone did not significantly alter hCASMC proliferation, although a tendency for both to reduce mean fold-change in cell number was observed. However, when the sub-threshold concentration of MK571 was combined with either Deta-NO or ANP a significantly enhanced inhibitory effect on hCASMC proliferation was observed compared to control. Moreover, MK571 combined with ANP significantly inhibited hCASMC growth compared to ANP alone, although a similar potentiation was not seen with MK571 plus Deta-NO12.

Extended AMPs Translocate across the Membrane via Short-Lived Pores: Licui Chen et.al reported that, The Indolicidin40, 41 and HIV-1 Tat (residues 37−47 of HIV-1 Tat protein) peptides containing high proportions of Arg and Trp residues do not fold into regular secondary structures and are in extended conformations. They found that these highly charged small peptides also exhibit antimicrobial activities by inducing pores and translocation across the membrane. Indolicidin adsorbs onto the membrane surface and inserts into the membrane in a manner similar to that of the β-sheet AMPs [Antimicrobial peptide] Indolicidin itself also has the potential to translocate across the membrane via the pores. Following this movement, another peptide previously located near the mouth of the pore on the upper leaflet may also enter the pore. During this process, the pore does not open much. Some experiments indicated that Indolicidin can induce discrete channels but does not lyse bacterial cells showing that HIV-1 Tat peptides can translocate through the plasma membrane via transient pores and accumulate in the cell nucleus [13].

PURIFICATION OF PEPTIDES

Kasper K et.al demonstrated that reversed-phase high-performance flash chromatography (HPFC) is an alternative technique for rapidly and efficiently purifying large laboratory-scale quantities of crude synthetic peptides and insulins with high recoveries and purities. HPFC was used either as the sole method of peptide purification or as part of a multistep process to initially remove most of the undesired impurities by HPFC prior to a final purification step by RP-HPLC. HPFC allows more peptide to be processed in a single injection, and significant amounts of impurities can be removed that would require more time and effort to purify by RP-HPLC alone, thereby increasing the speed and throughput of peptide purification, whilst reducing the cost [14].

According to Chiara et.al, if a peptide is produced through hydrolysis, for instance, it can be separated by means of ultrafiltration from the enzyme employed during the process and from other protein residues with higher molecular mass. Generally, in this case, the membranes of choice have a low molecular mass cut-off and the size of their pores depends on the molecular weight of the desired peptide. Anyway, the main disadvantage of this technique is the poor selectivity of the membrane. A technique particularly useful in case of separation of charged peptides and proteins is IsoElectroFocusing (IEF), which is based on the same separation principles as electrophoresis. The sample is injected in a chamber where an electric field is applied, in presence of a pH gradient. Acidic species move towards the anode and basic ones towards the cathode. When a species reaches a zone with pH identical to its isoelectric point, it stops migrating. Then it can be moved to a detection windows to be identified. Therefore, IEF separates analytes depending on their isoelectric point. Several modes of IEF have been developed, some of which can be used on analytical while others on preparative scale, which is the case of IEF in solution or in a cellulose-based separation medium [15].

Sridhar et.al demonstrated that, Purification of bioactive peptides is a vital process to identify the peptide structure and measure individual activity by different assays for producing single bioactive peptide. It involves a series of efficient multistep processes based on the physical and chemical characteristics of peptides, such as molecular size, charge, polarity, solubility and specific covalent or noncovalent interactions Arumugam et al. Several analytical methods are widely used for

purification of bioactive peptides including membrane filtration systems, chromatography techniques (SEC, affinity chromatography, ion-exchange column chromatography (IEC), ultra-high-pressure liquid chromatography (UHPLC), hydrophilic interaction liquid chromatography (HILIC), reversed-phase high-performance liquid chromatography (RP-HPLC) and capillary electrophoresis (CE) Abuine et al., Arumugam et al., De Castro & Sato, Jo et al., Also, both the degree of purification and peptide sequence can be confirmed by mass spectrometry (MS) methods, such as matrix-assisted laser deionization time-of-flight (MALDI-TOF), matrixassisted laser desorption/ionization mass spectrometry (MALDI-MS), electrospray ionization–mass spectrometry (ESI-MS), fast atom bombardment mass spectrometry (FAB-MS) Abuine et al., Jo et al., More recently, the quadrupole-time-of-flight mass spectrometry (Q-TOF MS), MALDI-TOF and ESI-MS have become a vital technique for characterization of bioactive peptides and proteins [16].

De luca et.al, reported that a technique particularly useful in case of separation of charged peptides and proteins is IsoElectroFocusing (IEF), which is based on the same separation principles as electrophoresis. The sample is injected in a chamber where an electric field is applied, in presence of a pH gradient. Acidic species move towards the anode and basic ones towards the cathode. When a species reaches a zone with pH identical to its isoelectric point, it stops migrating. Then it can be moved to a detection windows to be identified. Therefore, IEF separates analytes depending on their isoelectric point [17].

Table No 1: Choice of microwave peptide synthesisers

Manufacturer	Instrument	Reaction vessels(mL)
CEM	Discover Bio	Manual
CEM	Liberty Blue	Automated
Biotage	Initiator+SP Wave	Automated
Biotage	Initiator+Alstra	Automated
AAPPTEC	Infinity 2400	Automated
Protein Technologies	Prelude X	Automated
Protein Technologies	Symphony X	Automated
Protein Technologies	Tribute	Automated

STABILITY OF PEPTIDES:

A report by Sarabandi K et.al, says that the peptides give rise to cluster formation, instability and changes in the structure of the compounds that affects storage, effective properties also the absorption of moisture leads to chemical and microbial degradation. Thus, stabilization is the main aim of Microencapsulation by spray drying. The vital method to stabilize the compounds is the utilization of carrier agents with high glass transition temperature, low adhesion. The compounds were maintained at a relative humidity of 0-84% for five days. The change in the moisture, aggregation were tested while storage. The results reported that, the sample at a relative humidity(>20%) caused increase in the aggregation. Thereby, the reduced aggregation and adherence increased the stability [18].

Wang et.al, studied, by building the cross-links through the side chains or by substituting the hydrogen bonds by strong covalent bonds, also known as hydrogen bond surrogates (HBS) the α-helix structure of peptides can be stabilized. The formation of intramolecular crosslinks can stabilize other dissimilar secondary structures of peptides. Stapled peptides signify a current crosslinking method known to stabilize the α-helix structure using non-natural electrophilic amino acids to substitute remainders at the i and i + 4 or i and i + 7 position and form ligations with nucleophilic cross-links. Also the HBS alteration approach includes substitution of one hydrogen bond of α-helix peptide with a strong covalent bond to organize the helical structure in advance [19].

Conclusion

Based on the findings we suggest that, the determined C-N bond cleavage reaction using lactams as a constituent is an economical design priniciple for peptide synthesis.This method bearing functional groups that can be productively used to avoid racemisation while peptide bond formation. The focus on the isoform and purification of recombinant peptides and small protiens using the GST-affinity tag. Also, because of its large size, the fused small peptide is harmed to proteolytic degradation. Thus it is commonly used affinity tag in molecular biology and it can also be performed under biological conditions.Leukocytes show a broad array of (Antimicrobial proteins and peptides) APP, which selectively bind and damage microbial membranes by a variety of enzymatic and non-enzymatic mechanisms. All these peptides modulate the inflammatory activity of microbial products. We assume

that stable peptides regulate the synaptic plasticity of vicinity of the CNS associated both with energy metabolism and fear based learning behavior, this says that bioactive peptides therefore regulate the energy requirements of the associated neurons. Anticancer property of small peptide contents within the F2 and F5 HPLC separated fractions from A.fulica mucus against the breast cancer cell line MCF-7. Thus, these peptides will be favourable targets for new anticancerdrug development.

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