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CYCLIC PEPTIDES AS THERAPEUTIC AGENTS AND BIOCHEMICAL TOOLS

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ABSTRACT

There are many cyclic peptides with diverse biological activities, such as anti-bacterial activity, immune-suppressive activity, and anti-tumor activity, and so on. Encouraged by natural cyclic peptides with biological activity, efforts have been made to develop cyclic peptides with both genetic and synthetic methods. The genetic methods include phage display, intein-based cyclic peptides, and mRNA display. The synthetic methods involve individual synthesis, parallel synthesis, as well as split-and-pool synthesis. Recent development of cyclic peptide library based on split-and-pool synthesis allows on-bead screening, in-solution screening, and microarray screening of cyclic peptides for biological activity. Cyclic peptides will be useful as receptor agonist/antagonist, RNA=binding molecule, enzyme inhibitor and so on. New cyclic peptides will emerge as therapeutic agents and biochemical tools.

Keywords: Intein-based, Peptide, mRNA, microarray, split-and-pool.

INTRODUCTION

Cyclic peptides are polypeptide chains taking cyclic ring structure. The ring structure can be formed by linking one end of the peptide and the other with an amide bond, or other chemically stable bonds such as lactone, ether, thioether, disulfide, and so on[1-5]. N-to-C (or head-to-tail) cyclization is amide bond formation between amino and carboxyl termini, and many biologically active cyclic peptides are formed this way. Several cyclic peptides found in nature are used in clinic. The examples are gramicidin and tyrocidine with bactericidal activity, cyclosporin A with immune-suppressive activity, and vancomycin with anti-bacterial activity, and so on. While peptides have been generally considered to be poor drug molecules, there are some advantages of peptide drugs. The weakness of peptides and the strength will be discussed afterward. First, per oral absorption is poor for peptide drugs. In most cases, the route of administration is injection as peptides are not well absorbed in the gastrointestinal

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tract. Second, peptides are rapidly metabolized, even after successful absorption, by proteolytic enzymes[6-7]. Third, peptides usually do not cross cell membrane as some small molecules do. If the target of a peptide drug is in the cytoplasm, the peptide may not even reach the target. In spite of these limitations, peptides can be good alternatives to small synthetic molecules because of following advantages. Compared to small synthetic molecules, peptides possess less toxicity and they would not accumulate in organs. Even the fact that peptides get degraded rapidly can be a good thing.[8] Peptide drugs can be less harmful, after acting on target molecules, as they will disappear rapidly by proteolytic degradation. The degradation products are simply amino acids and would not have toxicity. Peptides can work on their targets very selectively, as the interaction with the targets is very specific compared to small molecules. Considering these strengths, it is not surprising that there are many peptide drugs available in the market.[52] These peptide drugs include receptor agonists and antagonists, peptide hormones and analogs, HIV protease inhibitors, and so on. In addition to the merits of peptides as drug molecules, cyclic peptides could make even better peptide drugs.[53]

Usually, cyclic peptides show better biological activity compared to their linear counterparts due to the conformational structures. The rigidity of cyclic peptides decreases the entropy term of the Gibbs free energy, therefore allowing the enhanced binding toward target molecules, or receptor selectivity. Another benefit from cyclic structure is the resistance to hydrolysis by exo-peptidases due to the lack of both amino and carboxyl termini. Cyclic peptides can be resistant even to endo-peptidases, as the structure is less flexible than linear peptides. Some cyclic peptides, though not all, can cross the cell membrane. Cyclosporin A is a good example of the membrane- permeable cyclic peptides. Until recently, it has been suggested that cyclic peptides cross the membrane better than the linear counterparts. To test this, a group of peptides have been synthesized, and their cell permeability was compared between cyclic and linear peptides. The results indicated that a peptide does not cross the membrane better simply because it is cyclized. If a certain cyclic peptide is membrane-permeable, it is because there are structural features allowing the molecule to cross the cell membrane. For example, cyclosporin A has several intra-molecular hydrogen bonds keeping hydrophilic groups from the surface of the molecule. Overall, structural rigidity, receptor selectivity, biochemical stability are general features of cyclic peptides and some cyclic peptides can be membrane permeable. These features allow cyclic peptides to be good therapeutic agents or biochemical tools, and efforts have been made to develop synthetic cyclic peptide with biological activity. In this review, the role of cyclic peptides in therapeutics and biochemistry will be described, as well as the approaches to develop cyclic peptide compounds for such purposes.[56]

THE ROLE OF CYCLIC PEPTIDES IN THERAPEUTIC

Bactericidal activity of Tyrocidine and Gramicidin S

Tyrocidine is a cyclodecapeptide with anti-bacterial activity. It was found from a culture extract of a soil bacillus, *Bacillus brevis*, as bactericidal agent as early as 1939 (Fig. 1 left top). Initially, this compound was characterized as a peptide lacking the free amino terminus, and therefore was proposed to have cyclic structure where amino terminus and carboxyl terminus are linked with an amide bond. While gramicidin S is a cyclic peptide, gramicidin refers to the mixture of linear pentadecapeptides with anti-bacterial activity. Tyrothricin, the mixture

of gramicidin and tyrocidine, was the first commercialized antibiotic and it is still used in clinic today. Gramicidin S, or Soviet gramicidin, is a cyclodecapeptide similar to tyrocidine, and was discovered in early 1940's for its anti-bacterial activity. Looking at the structure of these cyclic peptides, there can be two b-chains linked by proline residues and four intra-molecular hydrogen bonds. This model was proposed in 1950s and later confirmed by X-ray crystallography. As there are four intra-molecular hydrogen bonds, the peptide has a very rigid structure, a characteristic of cyclic peptides. In addition to the structural rigidity, these cyclic peptides are amphipathic. One side of molecule is hydrophobic while the other side is cationic. It appears that the cationic face interacts with lipid head groups of cell membrane which is negatively charged. This initial interaction is followed by interaction between hydrophobic portions of cyclic peptide and membrane lipid, resulting in the rupture of bacterial cell membrane[9-11]. The use of tyrocidine A is, however, limited to topical use as the membranolysis can happen even to the mammalian cells.

Cyclic Peptides Found in Natural Peptide Hormones

We can find several cyclic peptides from natural peptide hormones such as calcitonin, oxytocin, somatostatin, vasopressin, and so on. These peptides form rigid structure by forming disulfide bond connecting two Cysteine residues in the peptide[12-16].

APPROACHES TO DEVELOP CYCLIC PEPTIDE COMPOUNDS

As described above, there are many cyclic peptides used in clinic, and most of these originate from the natural cyclic peptides[17-18]. As several features make cyclic peptides attractive, lead compounds for drug development as well as nice tools for biochemical research, scientists made diverse efforts to develop biologically active cyclic peptide compounds. Peptides can be prepared by either genetic or synthetic method[19-21]. The genetic method, as described below, is usually limited to ribosomal 20 amino acids, whereas the sequence determination of hit compounds is straightforward[22-28]. The synthetic method can provide more versatile cyclic peptide compounds as the repertoire of amino acids and the way of forming cyclic peptides is diverse. Solid-phase peptide synthesis combined with split-and-pool synthesis can prepare fairly large libraries. However, sequence determination is challenging after screening of these libraries[29-30]. Conventional Edman degradation cannot be used for cyclic peptides once the free N-terminus disappears after cyclic peptide formation by N-to-C cyclization.[31-32] While tandem mass spectrometry (MS) can be used to analyze peptide sequences, the analysis of cyclic peptide sequence is more difficult than the analysis of linear peptide sequence. The fragmentation pattern is very complex for cyclic peptides. For a hypothetical cyclic peptide containing only 3 amino acids, namely cyclo (ABC), the fragments formed from tandem MS would be ABC, BCA, CAB, AB, BC, CA, A, B, and C (total 9), while the linear peptide ABC would yield ABC, AB, BC, A, B, and C only (total 6). The fragmentation pattern gets more complex as the number of amino acid increases. Therefore, sequence analysis of cyclic peptides by tandem MS is not practical where the quantity of peptide from each micro-bead of split-and-pool synthesis library can be as little as about 100 pmol for -90 mm beads[33].

In the following paragraphs, both genetic and synthetic approaches to develop cyclic peptide compounds will be discussed.

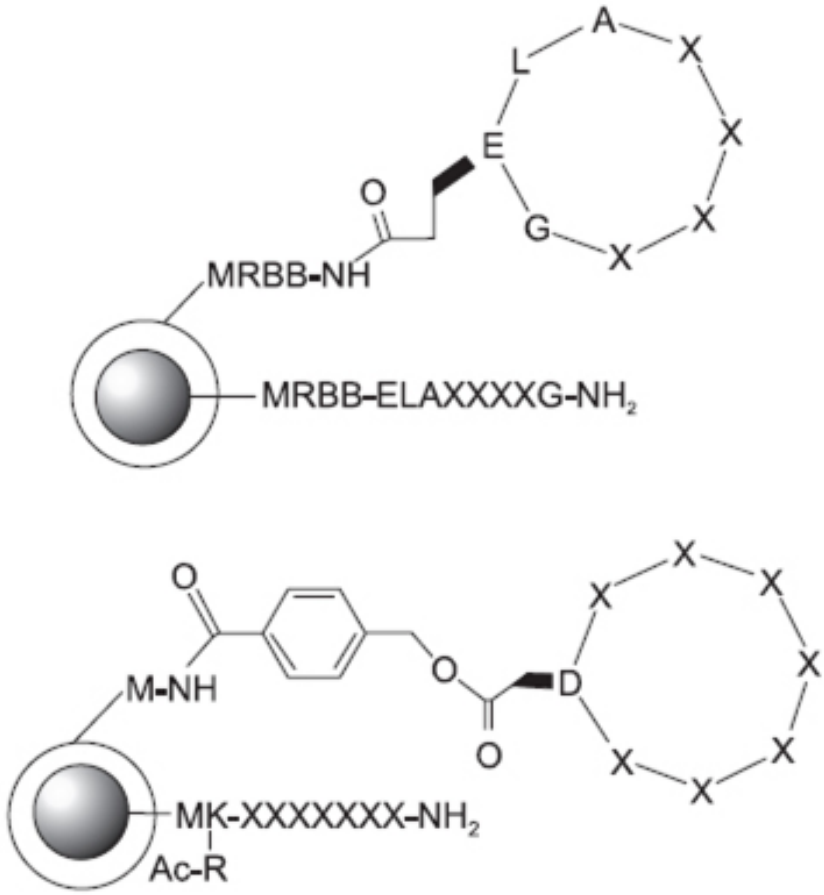


Fig 1: Structures of cyclic peptide libraries based on split-and-pool synthesis. Top: Cyclic peptide for on-bead screening. Bottom: Cyclic peptide for in-solution screening. Cyclic peptides can be released from the bead by hydrolysis of ester linkage, and then the remaining linear peptides are used for sequence determination.

THE CHEMICAL SYNTHESIS OF PEPTIDES

Peptides are the long molecular chains that make up proteins. Synthetic peptides are used either as drugs (as these are biologically active) or in the diagnosis of disease. Peptides are difficult to make as the synthetic chemist must ensure that the amino acids that make up the chain are added in the correct order and that they don't undergo any other reactions. This involves adding one amino acid, washing away any unreacted acid then adding the next and so on.[34-37] As can be imagined, this is very time consuming and only gives very low yields.

A technique that has been relatively recently developed involves attaching one end of the peptide to a solid polymer, meaning that the peptide cannot get washed away alongwith the excess acid. This is much quicker than classical synthesis, and leads to dramatically improved yields. The process consists of five steps carried out in a cyclic fashion[38].

Step 1 : Attaching an amino acid to the polymer

The amino acid is reacted with a molecule known as a "linkage agent" that enables it to attach to a solid polymer, and the other end of the linkage agent is reacted with the polymer support.[40]

Step 2: Protection

An amino acid is an acid with a basic group at one end and an acid group at the other. To prevent an amino acid from reacting with itself, one of these groups is reacted with something else to make it unreactive.

Step 3: Coupling

The protected amino acid is then reacted with the amino acid attached to the polymer to begin building the peptide chain.

Step 4 :Deprotection

The protection group is now removed from the acid at the end of the chain so it can react with the next acid to be added on. The new acid is then protected (**Step 2**) and the cycle continues until a chain of the required length has been synthesised.

Step 5: Polymer removal

Once the desired peptide has been made, the bond between the first amino acid and the linkage agent is broken to give the free peptide.

A peptide is a chain of special acids called amino acids linked together by bonds known as amide bonds. A protein consists of one peptide folded in a particular way, or several peptides folded together. Such peptides are synthesised very rapidly within living cells, but until recently could only be artificially synthesised in very long, slow processes that had poor yields and gave impure products. Recently a new technique known as solid phase peptide synthesis (SPPS) has been developed. SPPS results in high yields of pure products and works more quickly than classical synthesis, although still much more slowly than among living cells.

USES OF SYNTHETIC PEPTIDES:

Synthetic peptides have two main uses: as peptide drugs and as peptides for diagnostic purposes.

Peptide drugs

Peptide drugs are either naturally-occurring peptides or altered natural peptides. There are many naturally-occurring peptides that are biologically active. If a patient does not naturally produce a peptide that they need, this peptide can be synthesised and given to them. In addition, the amino acids in an active peptide can be altered to make *analogues* of the original peptide. If the analogue is more biochemically active than the original peptide it is known as an *agonist* and if it has the reverse effect is known as an *antagonist.*[41-44] Contraceptives have been made by synthesising the antagonists of fertility peptides.

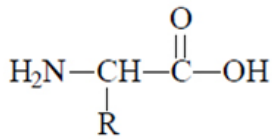
Diagnostic peptides

Peptides can be designed that change colour under certain conditions, and these can be used for diagnostic purposes. For example, a chromogenic peptide substrate can readily detect the presence, absence and varying blood levels of enzymes that control blood pressure and blood clotting ability.

Since 1973, the SPPS laboratory at Massey University has supplied peptides for research purposes to universities, CRIs, research institutes and private industry. These peptides have been used for medical research into areas such as heart disease, leprosy and tuberculosis. The laboratory itself is involved in research into and development of synthetic methods and peptide production[45].

INTRODUCTION TO PROTEIN CHEMISTRY

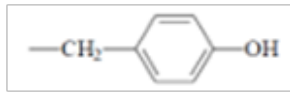
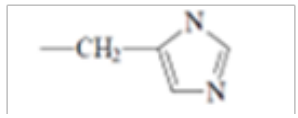
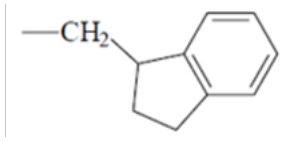
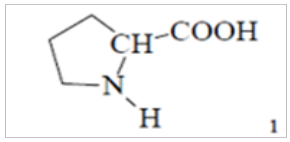
Peptides are polymers of amino acids made using anything from two to hundreds of amino acids. They are all based on the α-amino acid structure



There are twenty amino acids that commonly occur in nature (**Table 1**) and many others have been synthesized.

Table 1: Side chains of the Common Naturally Occurring Amino Acids

Name	Side chain	Protection
Glycine	-H	Never necessary
Alanine (ala)	$-CH_3$	Never necessary
Valine (val)	$-CH(CH_3)_2$	Never necessary
Leucine (Leu)	$-CH_2-CH(CH_3)_2$	Never necessary
Isoleucine(ile)	$-CH(CH_3)-CH_2-CH_3$	Never necessary
Lysine (lys)	$-CH_2-CH_2-CH_2-CH_2-NH_2$	Protecting group is tBOC
Arginine (arg)	$CH_2-CH_2-CH_2-NHC(NH_2)(NH)$	Protecting group is PMC
Aspartic acid (asp)	$-CH_2-COOH$	Protecting group is tBu
Asparagine (asn)	$-CH_2-CONH_2$	Protecting group is tBu
Glutamic acid (glu)	$-CH_2-CH_2-COOH$	Protecting group is tBu
Glutamine (gln)	$-CH_2-CH_2-CONH_2$	Protecting group is Trt
Theronine (thr)	$-CH(OH)(CH_3)$	Protecting group is tBu
Methionine (meth)	$-CH_2-CH_2-S-CH_3$	Protecting group is S=O
Cysteine (cys)	$-CH_2-SH$	Protecting group is Trt and tBu

Serine (ser)	-CH_2-OH	Protecting group is tBu
Phenylalanine (phy)	-CH2-C_6H_6	Never necessary
Tyrosine (tyr)		Protecting group is tBu
Histidine (his)		Protecting group is Trt
Tryptophan (trp)		Protecting group is tBOC
Proline (pro)		Never necessary

These peptides (or combinations of them) fold in characteristic ways to give proteins. Among mammals, all optically active amino acids are in the L form, so one change that can be made to peptides is to substitute a D amino acid for one of the amino acids in the chain.

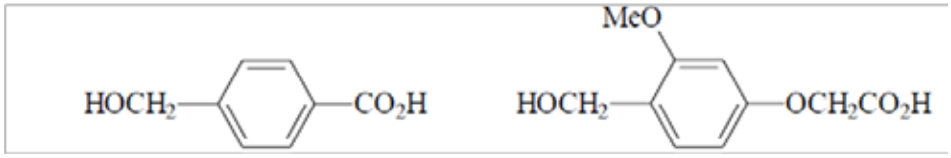
SOLID PHASE PEPTIDE SYNTHESIS

Peptide synthesis is much more complicated than simply forming amide bonds by mixing the desired amino acids together in a test tube.[47-48] With twenty natural amino acids and a number of unnatural ones as well the possible combinations formed with this technique are numerous. This complexity makes the synthesis of peptides both fascinating and challenging.

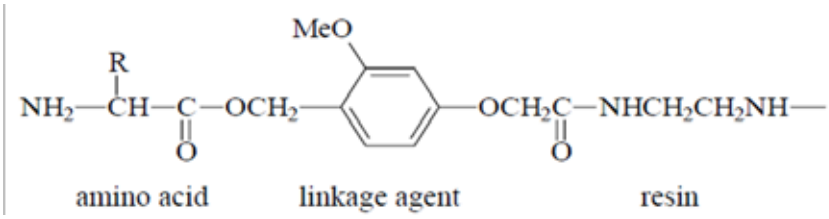
If solutions containing two amino acids are mixed together, four different dipeptides (as well as other longer peptides) will be formed. For example, for a mixture of glycine and alanine the four dipeptides would be glygly, glyala, alagly, alaala.[49-50] In this representation of peptides, the free amino group or N-terminus is on the left hand amino acid and the free carboxylic acid group, the C-terminus is at the right hand end. To ensure that only the desired dipeptide is formed, the basic group of one amino acid and the acidic group of the other must both be made unable to react. This 'deactivation' is known as the *protection* of reactive groups, and a group that is unable to react is spoken of as a protected group.[51-52] In classical organic synthesis, the acids are protected, allowed to react and deprotected, then one end of the dipeptide is protected and reacted with a new protected acid and so on. In SPPS, the amino acid that will be at one end of the peptide is attached to a water-insoluble polymer and remains protected throughout the formation of the peptide, meaning both that fewer protection/ deprotection steps are necessary and that the reagents can easily be rinsed away without losing any of the peptide.[53-55]

Step 1: Attaching an amino acid to the polymer

Peptide chains have two ends, known respectively as the N-terminus and the C-terminus, and what end is attached to the polymer depends on the polymer used. This article assumes that polyamide beads are used wherein the C-terminus of the peptide is attached to the polymer. The attachment is done by reacting the amino acid with a linkage agent and then reacting the other end of the linkage agent with the polymer. This means that a peptide polyamide link can be formed that will not be hydrolysed during the subsequent peptide-forming reactions. Common linkage agents are di- and tri-substituted benzenes such as those shown below:

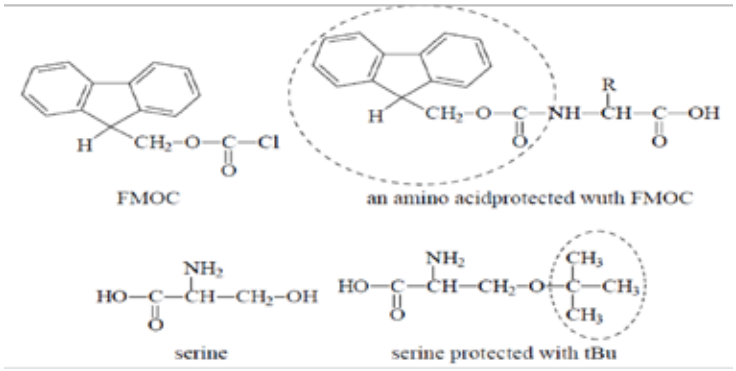


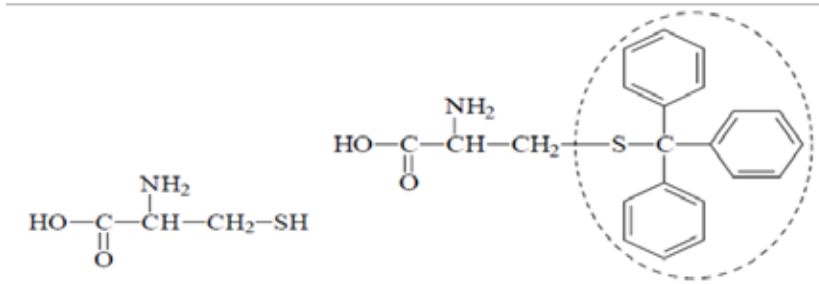
These then join the C-terminus amino acid and resin together as follows:

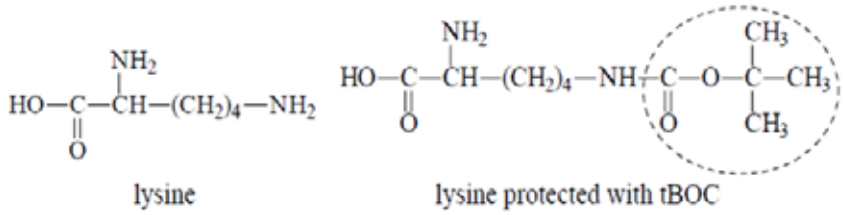


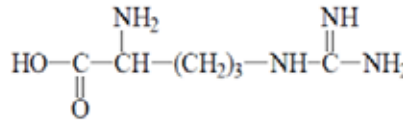
Step 2: Protection

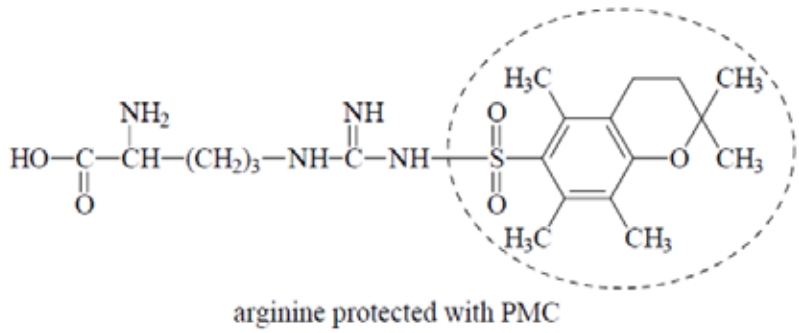
Amino acid also needs to have its amino group protected to prevent the acids reacting with each other. This is done by protecting it with FMOC (9-fluorenylmethoxy-carbonyl). In addition, any amino acid side chains that are aromatic acid, basic or highly polar are likely to be reactive (see **Table 1**). These must also be protected to prevent unwanted branched chains from forming. There are four main groups used in this way: tBu (a tertiary butyl group), Trt (a triphenylmethyl group), tBOC (a tertiary butyloxycarbonyl group) and PMC (a 2, 2, 5, 7, 8-pentamethylchroman-6-sufonyl group). Examples of a carboxyl group protected with FMOC and examples of the different types of side chain protection are given below.









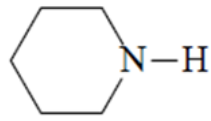


Step 3 : Coupling

The FMOC protected amino acid is then reacted with the last amino acid attached to the polyamide. The reaction is catalysed by DCC (1, 3 dicyclohexylcarbodiimide), which is itself reduced to DCU (1,3-dicyclohexylurea).

Step 4 : Deprotection

Excess DCC is washed off the insoluble polymer with water, then the FMOC group removed with piperidine (a cyclic secondary amine). This is a trans-amidification reaction.

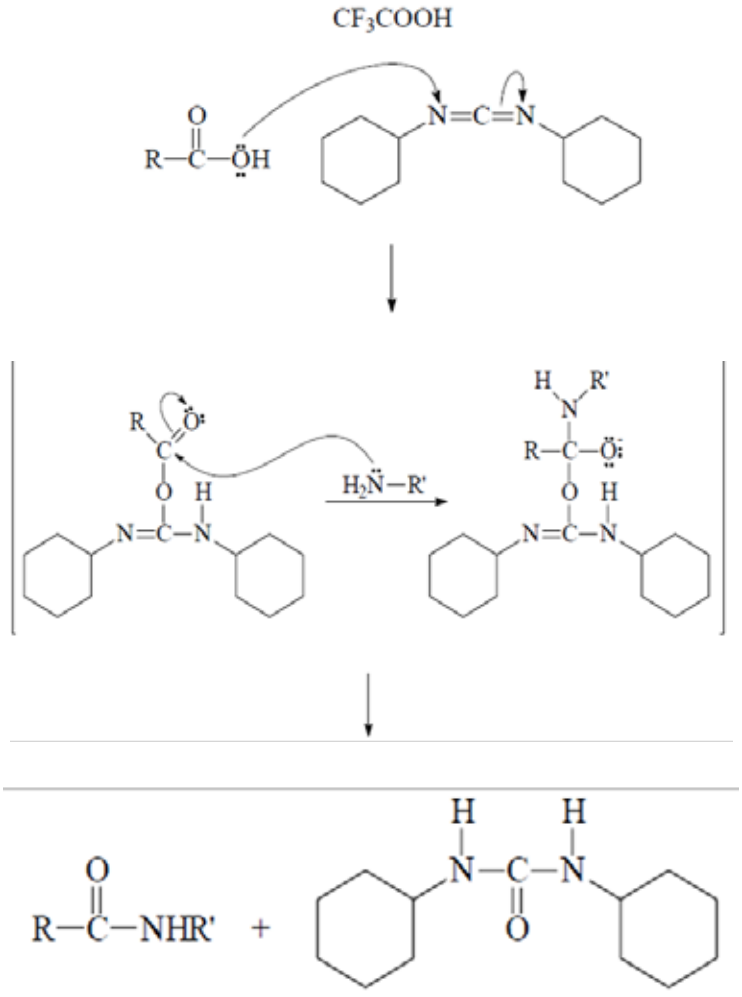


piperidine

Steps 2 to **4** are repeated as each new amino acid is added onto the chain until the desired peptide has been formed.

Step 5 : Polymer Removal

Once the peptide is complete, it must be removed from the polyamide. This is done by cleaving the polyamide - peptide bond with a 95% solution of trifluoro acetic acid (TFA). The side-chain protecting groups are also removed at this stage.



Reaction scheme of amide bond formation

PRACTICAL AND FINANCIAL ADVANTAGES OF SPPS

The primary advantage of SPPS is its high yield. As peptides consist of several amino acids, if the yield for each amino acid addition is much less than 100%, overall peptide yields are negligible. For example, if each amino acid addition has a 90% yield then the overall yield of a 50 amino acid peptide is only 0.5%. Modern SPPS instrumentation pushes coupling and deprotection yields to greater than 99.99%, giving an overall yield of greater than 99% for a 50 amino acid peptide.

SPPS is also much quicker than conventional step-by-step solution synthesis. With SPPS, a 20 amino acid peptide can be synthesized in a 24-hour period and longer ones in less than a week. With the advent of automated synthesizers and sophisticated analytical and purification equipment the peptide chemist can now make peptides in the range of 20-50 amino acids in length and in amounts from 20-100 milligrams. This is often more than enough for biochemists and biologists to carry out extensive pilot studies and as they often only look at a particular peptide once this speed is particularly useful. If very large amounts of peptide are required (e.g. for the industrial production of peptide drugs) ,then this speed is sacrificed for purity. However, production rates are still high, and hundreds of grams of peptide can be produced on kilograms of polymer every year. As an often-only milligram of polymer is needed per dose, this represents hundreds of thousands of doses.[57]

ENVIRONMENTAL IMPLICATIONS

SPPS, like much of organic chemistry, makes use of organic solvents which are hazardous to the environment. The SPPS group at Massey University is currently researching ways of producing peptides in aqueous or partially aqueous (e.g. water/ethanol mixtures) solutions to avoid the use of organic solvents. The cyclotides are a family of plant-derived proteins that occur in plants from the *Violaceae(violet), Rubiaceae (coffee)* and *Cucurbitaceae (cucurbit*) families and have a diverse range of biological activities, including uterotonic, anti-HIV, anti-microbial, and insecticidal activities; the latter suggests their natural function lies in plant defence. Individual plants express suites of 10–100 cyclotides. Cyclotides comprise w30 amino acids, contain a head-to-tail cyclised backbone, and incorporate three disulfide bonds arranged in a cystine knot topology. The combination of a knotted and strongly braced structure with a circular backbone renders the cyclotides impervious to enzymatic breakdown and makes them exceptionally stable. The cyclotides are the largest of several groups of naturally occurring circular proteins that have been discovered in bacteria, plants and animals over recent years.[58] Next section describes the discovery of the cyclotides in plants, their structural characterisation, evolutionary relationships and their applications in drug design.[59-60]

Cyclotides: Structures and Activities

Cyclotides are small disulfide-rich proteins found in plants of the Rubiaceae, Violaceae and Cucurbitaceae families, with recent studies suggesting that they might also be found in other families. Individual plants express suites of 10–100 cyclotides, distributed in a wide range of tissues, including leaves, stems, flowers and roots, as highlighted for a selection of

cyclotides. A full list of cyclotide sequences is available on Cybase. They were originally discovered in indigenous medicinal applications, where women in Africa used a tea made from the Rubiaceae plant *Oldenlandia affinis* to accelerate childbirth. Gran subsequently discovered that the active uterotonic agent was a peptide of around 30 amino acids, which was named kalata B1.[67] Many years later, it was established that it was a head-to-tail macrocyclic peptide, which contained a cystine knot motif. Subsequently, a large number of similar macrocyclic peptides from plants have been discovered and these are now known as the cyclotides, the term being defined in our 1999 paper in the *Journal of Molecular Biology*. Kalata B1 has now been discovered in five plant species, but in general different plants express different suites of cyclotides. Cyclotides are distinguished from many other proteins in that they are exceptionally stable. In particular, they are highly resistant to proteases, are thermally stable, and are resistant to chemical chaotropic. All of the early studies on the structural characterization of cyclotides involved NMR spectroscopy as the primary characterisation technique shows the consensus NMR structure of cyclotides and highlights a number of important features, including the cystine knot, a small b-sheet structure and a series of loops and turns that project from the molecular core. Recently, we reported the crystal structure of a cyclotide and these structural features were verified using this technique. Fig.1 also highlights the combinatorial nature of cyclotides, which tolerate a wide range of sequence substitutions at all non-Cys positions, apart from a few conserved residues in their backbone, notably,a highly conserved Glu residue in loop 1 and an Asn or Aspresidue in loop 6.

Cyclotides have a range of biological activities, including uterotonic activity, anti-HIV activity, neurotensin antagonism, haemolytic activity, anti-microbial activity, cytotoxic activity, insecticidal activity and anthelmintic activity. Only three of these activities that are of greater relevance to toxicology are discussed here, namely haemolytic, anti-HIV and insecticidal activities. First, haemolytic activity was one of the original activities discovered for the cyclotide Viola peptide 1. Numerous cyclotides have since been tested for this activity and typically most have mild potency, with HD50 values>10 mM; none are as potent as melittin, from bee venom, the gold standard for haemolytic activity of a peptide. Haemolysis is a toxic activity that might well be important for cyclotides as host defence peptides but this specific role has not yet been investigated. Interestingly, synthetic analogues of cyclotides in which the backbone is broken are essentially devoid of haemolytic activity. This trend of activity being lost upon the backbone being rendered acyclic is common not only to haemolytic activity but also to anti-HIV activity of cyclotides. The second activity of cyclotides, i.e., anti-HIV activity, has been reviewed extensively. Typically, cyclotides are effective against virus infected cells with EC50 values of approximately 100 nM and it was this activity, identified in early screening studies, which attracted interest in cyclotides.[61]However, cyclotides are not currently being pursued clinically as anti-HIV agents because their therapeutic index (i.e., the ratio of their therapeutic to toxic effects) is too narrow. Some examples of the anti-HIV activity of cyclotides also emphasises that synthetic linear examples are inactive, confirming the important role of the cyclic backbone in activity.

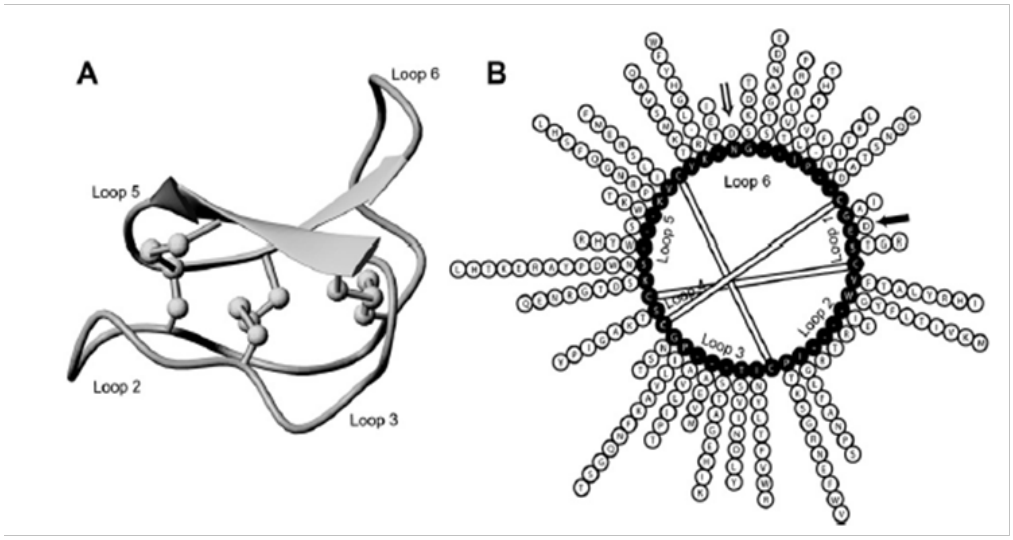


Fig2: Cyclotide structures and sequences. Panel A shows the prototypic cyclotide kalata B1 (PDB ID:1NB1), illustrating the cyclic backbone and cystine knot motif, along with a small b-sheet and loops between successive Cys residues. Panel B shows a diversity wheel representation of sequence variation seen in cyclotides. The inner circle shows the consensus sequence of all cyclotides, with the radiating arms showing residues that are substituted at corresponding positions in currently known cyclotides. At each position amino acids are sorted according to their frequency, the closer to the wheel being the more conserved. There are only a few positions, apart from the completely conserved cystine residues, that are almost invariant. Key positions include an Asn or Asp residue in loop 6 (light arrow) and a Glu, or rarely Asp (dark arrow) in loop 3.

CONCLUSION

Cyclic peptides are naturally occurring mini- protein bioactive molecules with interesting pharmacological and biochemical properties. They are present in several species of plant families such as *Annonaceae, Araliaceae, Asteraceae, Caryophyllaceae, Euphorbiaceae, Fabaceae, Labiatae, Linaceae, Olacaceae, Rhamnaceae, Rubiaceae, Rutaceae, Schizandraceae, Solanaceae*, and *Violaceae*, Basidiomycetes is the group of higher fungi and these cyclopeptides display various biological properties such as protease inhibitory, anti-microbial, insecticidal, cytotoxic, anti-human immune-deficiency virus, cytotoxic, anti-malarial, estrogenic, sedative, nematicidal, immune-suppressive, and enzyme-inhibitory activities. Gramicidin S is an example of naturally occurring cyclic decapeptide extracted from the soil bacterium *Aneurini bacillus*.The biological characteristic features of cyclic peptides are different from linear peptides. There are different ways to cyclize peptides. The linear peptide strand can be cyclized not only from head to tail, connecting the C and N-termini, but also by linking to amine and carboxylic functions in amino acid side chains, giving side-chain-to-head or side-chain-to-tail connections. Side-chain-to-side-chain bridges have also been observed.

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