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Ribosome-mediated synthesis of natural product-like peptides via cell-free translation

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Peptide natural products (PNPs) represent a unique class of compounds known for their fascinating structural motifs with important biological activities. Lately, PNPs have garnered a lot of interest for their application in drug discovery. Nevertheless, lack of diversity oriented synthetic/biosynthetic platforms to generate large natural product-like libraries has limited their development as peptide therapeutics. The promiscuity of cell-free translation has allowed for the synthesis of artificial PNPs having complex structural features. Modified cell-free translation systems coupled with the display technologies have generated diverse natural product-like peptide libraries and led to the discovery of several biologically active molecules. Such technologies have drastically decreased the time to obtain peptide drug leads and therefore, revolutionized the field of peptide drug discovery. In this account, we review recent developments in the synthesis of natural product-like peptides via cell-free translation.

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Introduction

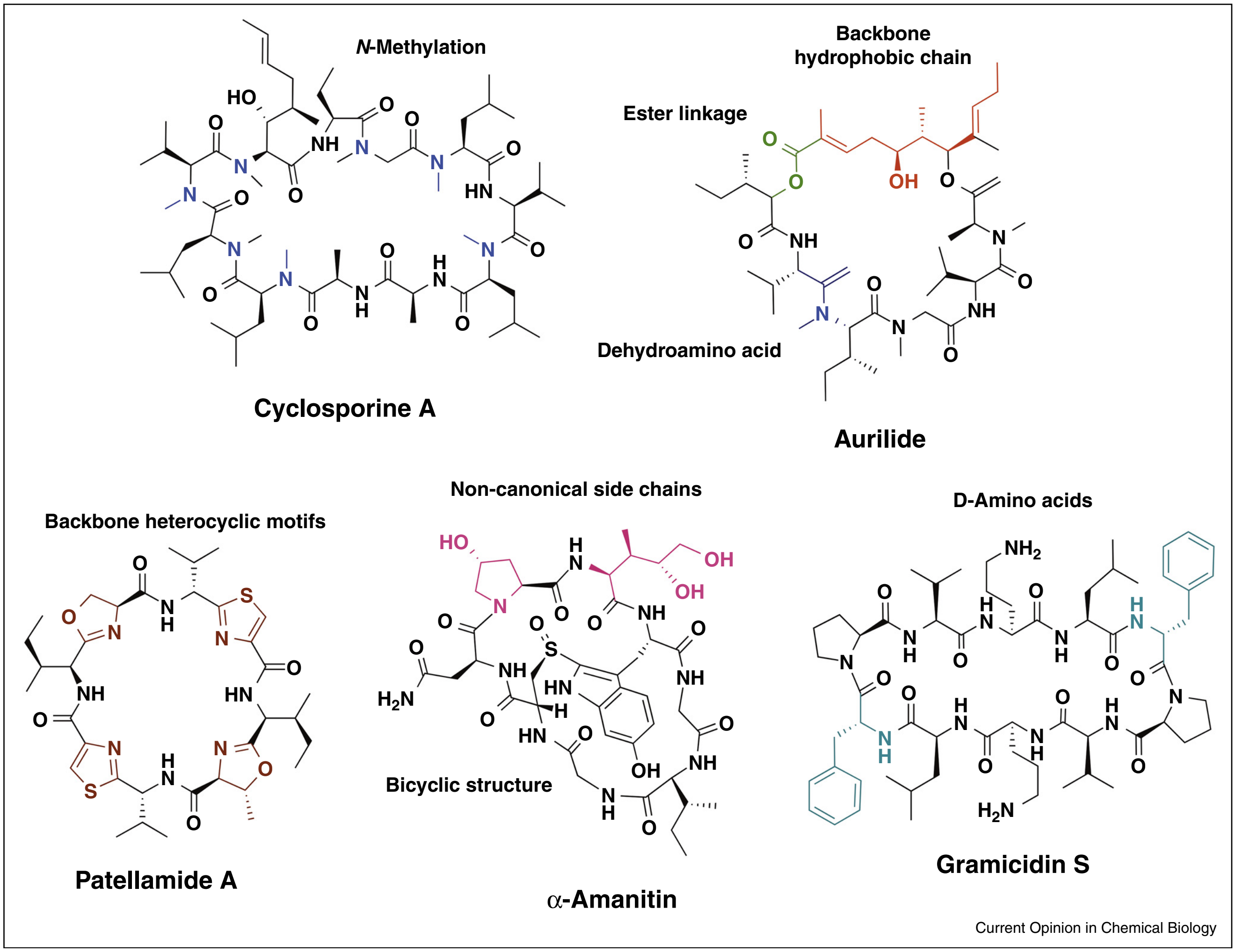
Peptide natural products (PNPs) possess interesting structural features, which make them great therapeutic agents and allow them to modulate difficult ‘undruggable’ targets not easily accessible by small molecules. Some common structural designs observed in PNPs are ring topologies, non-standard amino acids (nsAAs) including *N*-methyl amino acids, D-amino acids, amino acids with non-canonical side chains, β-amino acids/γ-amino acids and backbone heterocyclic motifs such as oxazolines and thiazolines (Figure 1). In the past decade, substantial research efforts were dedicated to generate diverse and large set of peptide libraries having either natural product-like motifs or novel architectures [1]. Genetic code manipulation approaches and chemical post-translational modifications allowed phage and mRNA display technologies to revolutionize the field of peptide therapeutics [1,2]. Cell-free translation system integrated with mRNA display technology [3] has provided several potent bioactive natural product-like peptides with high target-binding affinities [1,2,4]. Moreover, a recently described modified translation/mRNA display format [5•] has significantly decreased the time and efforts spent for the search of bioactive peptide sequences. Moving beyond the drug discovery application, coupled translation-selection systems have generated peptides, which were also useful as co-crystallization ligands [6•], fluorogenic peptide aptamers [7,8] and fluorescent probes [9•]. Clearly, such discoveries were made possible by the ability of cell-free translation to incorporate diverse structural motifs observed in PNPs into peptides. In this review, we will focus on different strategies employed for the ribosomal synthesis of natural product-like peptides using cell-free translation systems and their applications.

Macrocyclization

A prevalent structural motif among PNPs is a ring topology (macrocyclic structures), which decreases the number of conformations available for a peptide. Such a structural rigidity decreases the entropic cost to adopt the bioactive conformation and thus, improves target-binding affinity [10,11]. Moreover, macrocyclization distorts the alignment of residues around an amide bond, which in turn decreases the chance to be recognized by peptidases and therefore, potentially increases resistance against proteolysis. In addition, some studies have shown that cyclization could improve passive membrane permeability by promoting intra-molecular interactions [12,13]. Because of these advantages, methodologies for peptide cyclization have been extensively explored [4,14,15].

Cysteine-mediated macrocyclization strategies, where the nucleophilic reactivity of sulfhydryl group is exploited, are frequently applied. Inspired by the post-translational modifications and cyclization in lantipeptide biosynthesis [16], Szostak and co-workers developed a cyclization method to produce lantipeptide analogues (Figure 2a) [17]. Using *in vitro* translation, 4-selenolysine (**1**, Figure 3a) and 4-selenoisoleucine amino acids were incorporated into a ribosomal peptide and subsequently oxidized by hydrogen peroxide to generate dehydroalanine (Dha) and dehydrobutyrine (Dhb), respectively. Upon deprotection of a cysteine, a selective nucleophilic

Figure 1



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Representative structures of peptide-natural products (PNPs).

attack on a more reactive Dha over Dhb residue afforded desired macrocyclization (lanthioine formation). Afterwards, this strategy was combined with mRNA display to generate a library of functional lantipeptides (10^{11} membered library) and a low micromolar (3 μM) binder of Sortase A was obtained from the selection [18]. Eventually, Suga and co-workers devised a more versatile strategy involving a reaction between cysteine and the electrophilic carbon of chloroacetyl moiety on the N-terminus of a peptide (Figure 2b) [19]. In this work, a custom-made reconstituted cell-free translation system called *F*lexible *I*n vitro *T*ranslation (FIT) system [3] was used. Central to the FIT system is a ribozyme-mediated aminoacylation of tRNAs (called flexizyme technology) [20,21] and genetic code reprogramming [22,23] for the incorporation of non-canonical amino acids [24]. Methionine residue was removed from the FIT system and initiation event was reprogrammed to accept N^{α}-(2-chloroacetyl)-Trp (**2**, Figure 3a). After translation, spontaneous cyclization occurred between C-terminal cysteine side-chain and N-terminal head of the tryptophan residue (Figure 2b). Later on, this strategy was integrated into mRNA display format and was called the *Ra*ndom non-standard *P*eptide *I*ntegrated *D*iscovery (RaPID) system [3]. The FIT/RaPID system have been successfully utilized for the construction and screening of large macrocyclic peptide libraries (10^{13}) to afford active natural product-like molecules, which gave remarkable results as enzymes inhibitors [25,26], modulators of protein–protein interactions [27,28] stable hormone analogue [29], protein co-crystallization ligands [30] and fluorescent imaging probe [9•].

Bicyclic structures are not common among PNPs. However, larger size bicyclic peptides may give rise to more

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