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COMMENTARY

Engineered Biosynthesis of Peptide Antibiotics

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ABSTRACT. In certain bacteria and filamentous fungi, a wide variety of bioactive peptides are produced non-ribosomally on large protein templates, called peptide synthetases. Recently, significant progress has been made towards understanding the modular arrangement of these complex multifunctional enzymes and the mechanisms by which they generate their corresponding peptide products. It has now been established that the synthesis of bioactive peptides and the specification of their sequence are brought about by a protein template that contains the appropriate number and the correct order of activating units (domains). These advances have enabled the development of a technique that permits the construction of hybrid genes encoding peptide synthetases with specifically altered substrate specificities. A programmed alteration within the primary structure of a peptide antibiotic is achieved by the substitution of an amino acid-activating domain in the corresponding protein template at the genetic level by a two-step recombination method. It utilizes successive gene disruption and reconstitution and demonstrates, for the first time, the potential of genetic engineering in the biosynthesis of novel peptide antibiotics. Many organisms, for instance those that cause diseases like tuberculosis and pneumonia, have evolved potent mechanisms of drug resistance. Therefore, the targeted engineering of peptide antibiotics could be one potential strategy for the development of novel drugs that overcome this resistance. BIOCHEM PHARMACOL 52;2:177–186, 1996.

KEY WORDS. non-ribosomal peptide biosynthesis; peptide synthetases; domain structure, peptide antibiotics; genetic engineering; drug design

In biological systems, two different strategies are known for the catalysis of peptide bond formation during peptide biosynthesis: the very common ribosomal machinery and the multienzymatic non-ribosomal system (Fig. 1). The majority of cellular peptides and all proteins are of ribosomal origin. A total of twenty proteinogenic amino acids (twenty-one with regard to selenocysteine [1, 2]) is limited by the amount of tRNA ligases that recognize, carboxyadenylate, and esterify the constituent amino acids to their cognate tRNAs. For example, the bioactive L antibiotics represent highly stable multicyclic structures, resulting from the processing of gene-encoded precursors that have undergone an extensive post-translational modification [3].

However, many microorganisms produce an abundance of bioactive peptides non-ribosomally using large protein templates called peptide synthetases [3–6]. It is this group that contributes to the remarkable structural diversity of low molecular weight peptides. These secondary metabolites are composed of linear, cyclic, and/or branched peptide chains and may be modified by acylation or glycosylation. More than 300 different compounds are known to be incorporated into these enzymatically synthesized peptides including non-proteinogenic, D-, hydroxy-, and *N*-methylated amino acids [3–6]. They exhibit valuable activities and have found widespread applications in medical, biotechnological, and agricultural settings [3]. Here, they are used as toxins, antibiotics, enzyme inhibitors, antiviral and antitumor agents, immunosuppressants, as well as biosurfactants (see Table 1).

Common to that latter group of non-ribosomal peptides is their mode of synthesis by multifunctional enzymes that employ the multicarrier thiotemplate mechanism [3–7] and possess a high diversity in substrate specificity and size. Their molecular masses range from 123 kDa for gramicidin S synthetase 1 (GrsA [8]; Fig. 2), which activates and racemizes a single amino acid, to 1689 kDa for the massive cyclosporin synthetase (SimA [9]; Fig. 2) that activates, modifies, and condenses eleven constituent carboxy acid compounds (Table 1 and Fig. 2).

In this commentary, we have focused on the rational design of novel peptide antibiotics with specifically altered amino acid sequences. In principle, this aim can be achieved by manipulating the genes encoding peptide synthetases. As a model, our recent dissection studies on the peptide synthetase GrsA revealed the modular arrangement of such multifunctional enzymes [10]. Based on these studies, we developed a ubiquitous technique for the targeted replacement of bacterial and fungal domains [11]. This approach permits the construction of hybrid genes encoding peptide synthetases with altered amino acid specificities. After introducing the hybrid genes into the target site of

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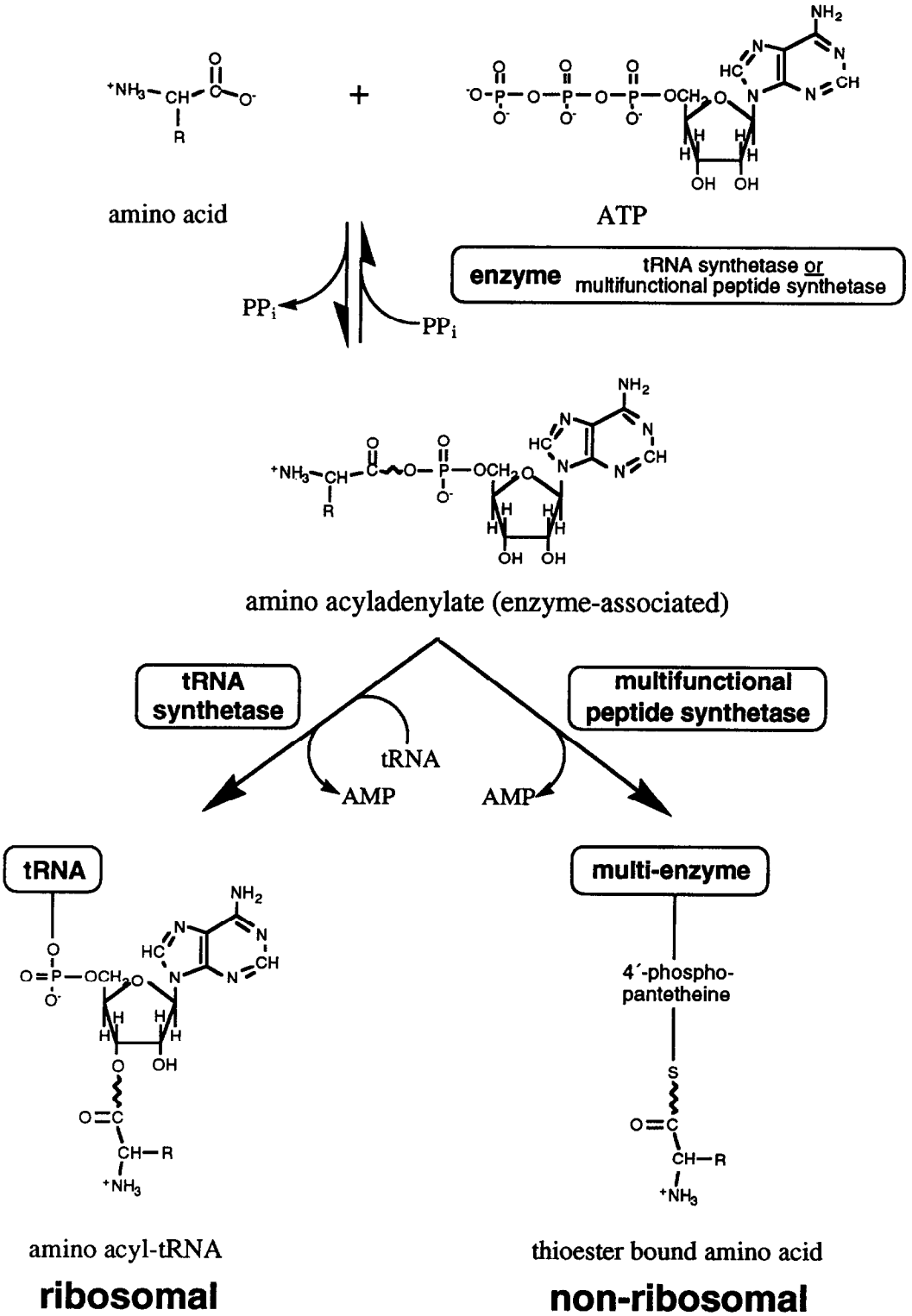

FIG. 1. Amino acid activation in peptide synthesis. Both the ribosomal machinery and the multienzymatic non-ribosomal system activate their cognate amino acids under ATP hydrolysis as amino acyladenylates. These relatively unstable intermediates are stabilized by (thio)esterification on a tRNA or a prosthetic 4'-phosphopantetheine group of a peptide synthetase, respectively. Enzymes and tRNAs involved in these processes are boxed.

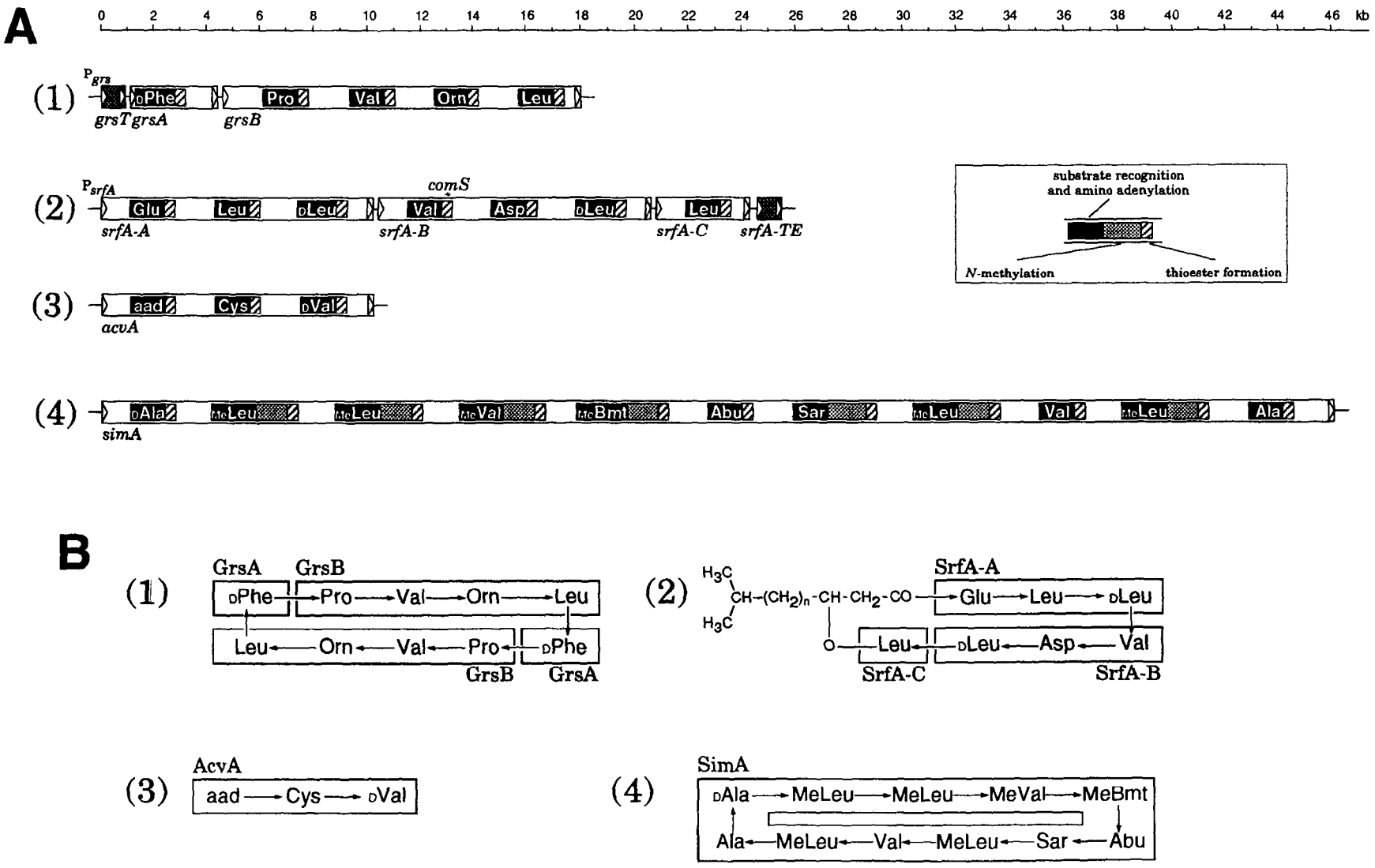

FIG. 2. Multidomain structure of peptide synthetases (A) Schematic diagram of the conserved modular structure of peptide synthetases encoded by the bacterial operons grs (row 1) [22] and srfA (row 2) [31], as well as the fungal genes acvA (row 3; aad, δ-(L-α-aminoadipyl)) [12] and simA (row 4) [9]. Homologous type I domains are about 650 aa in length and contain individual modules involved in amino acid adenylation (black boxes) and thioester formation (striped boxes) [6, 10]. Type II domains shown in simA (row 4) contain an additional insertion of about 430 aa required for substrate N-methylation (shaded boxes) [6, 9, 21]. White areas represent the low-conserved interdomain regions that bear the putative elongation modules necessary for acyltransferation of the growing peptide chains [6, 41]. The locations of promoters (P) and genes associated with antibiotic production (gsp/sfp) [23, 53], grsT/srfA-TE [8, 31], and comS [62–64] are also shown. (B) Primary structures of the synthesized peptides gramicidin S (row 1), surfactin (row 2; n = 7–9), δ-(L-α-aminoadipyl)-cysteinyl-D-valine (ACV; row 3), and cyclosporin A (row 4). The amino acid sequences and the enzymes that catalyze peptide synthesis are shown (see Table 1).

TABLE 1. Non-ribosomally synthesized peptide antibiotics: Producing organisms, structures, and properties

Antibiotic	Organism(s)	Structure	Properties	Ref.
Albomycin	*Streptomyces griseus*	Modified cyclic peptide (5n)*	Iron binding, membrane acting, clinically used	13
Amphomycin	*Streptomyces canus*	Branched acylpeptide (11n)	Antibacterial, feed additive, inhibition of cell wall synthesis	14
Bacitracin†	*Bacillus licheniformis*	Branched cyclic peptide (12n)	Antibacterial, topical antibiotic, metal ion binding, membrane acting, inhibition of cell wall synthesis	15,16
Bialaphos†	*Streptomyces hygroscopicus*, *Streptomyces viridochromogenes*	Modified peptide (3n)	Antifungal, antibacterial	17
β-Lactams†	*Penicillium chrysogenum*, *Aspergillus nidulans*, *Streptomyces clavuligerus*, *Norcardia lactamdurans*, *Flavobacterium SC12*	Modified peptide (3n)	Precursor of penicillins and cephalosporins (clinically used, inhibition of cell wall synthesis)	12, 18–20
Cyclosporin A†	*Tolypocladium niveum*	Modified and *N*-methylated cyclic peptide (11n)	Immunosuppressant, clinically used	9
Enniatin†	*Fusarium scirpi*	Cyclic depsipeptide (6n)	Antifungal	21
Gramicidin S†	*Bacillus brevis*	Cyclic peptide (10n)	Antibacterial, biosurfactant, nucleotide binding	8, 22,23
HC-toxin†	*Cochliobolus carbonum*	Modified cyclic peptide (4n)	Antifungal, phytotoxic	24,25
Iturin†	*Bacillus subtilis*	Cyclic lipopeptide (7n)	Antifungal, clinically used	26
Micrococcin	*Micrococcus* spp., *Bacillus pumilis*	Modified and branched cyclic peptide (12n)	Antibacterial, antimycobacterial, fibosome binding	27
Mycosubtilin	*Bacillus subtilis*	Cyclic peptide (9n)	Antifungal	28
Octapeptin	*Bacillus circulans*	Branched cyclic acylpeptide (12n)	Antibacterial, antimycobacterial, antifungal, antiprotozoal	29
Saframycin†	*Myxococcus xanthus*, *Streptomyces lavendulae*	Modified and branched cyclic peptide (4n)	Inhibition of DNA/RNA synthesis, antimicrobial, antitumor	30
Surfactin†	*Bacillus subtilis*	Cyclic lipopeptide (7n)	Antimycobacterial, biosurfactant, membrane acting, hemolytic	31,32
Syringomycin†	*Pseudomonas syringae*	Cyclic lipodepsipeptide (9n)	Phytotoxic	33
Tyrocidine†	*Bacillus brevis*	Cyclic peptide (10n)	Antibacterial, topical antibiotic, hemolytic	34–36
Tolaasin	*Pseudomonas tolaasii*	Lipodepsipeptide (18n)	Antifungal	37

* n refers to the number of amino acids in the corresponding peptide molecule.
† Indicates secondary metabolites, whose biosynthesis genes are (partially) cloned and sequenced.

the desired biosynthetic operon using homologous recombination, the engineered gene products derive programmed alterations in the primary structure of the corresponding peptide. Therefore, this approach represents a valuable method for the specific engineering of novel and potentially useful secondary metabolites.

MULTICARRIER THIOTEMPLATE MODEL

The biochemistry of non-ribosomal peptide synthesis has been investigated extensively over the last 35 years. Most of what is known about that mechanism was discovered by studies on the cyclic bacterial antibiotics gramicidin S and tyrocidine [3, 5] as well as by work that focused on the synthesis of the tripeptide δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine (ACV) [12], a common precursor of penicillin and cephalosporin (Fig. 2). Many other peptide antibiotics are produced by manifold bacteria and lower eucaryotes, including a number of pathogens, that inhabit soil and aquatic environments [3]. Table 1 summarizes the producing organisms, structures, and properties of some non-ribosomally synthesized secondary metabolites, particularly those whose corresponding biosynthesis genes have been characterized.

In spite of their structural diversity (Table 1), most of the metabolites share a common mode of synthesis [3–7]. According to this model, amino acid activation occurs in two steps: ATP-dependent amino acyladenylation and subsequent thioesterification on the prosthetic 4′-phosphopantetheine group, a cofactor that is covalently bound to the multienzyme (Fig. 1). Distinct domains, each about 1000 aa* in length, were found to represent the functional building units of these multienzymes required for such sub-

* *Abbreviations:* aa, amino acids; and PCR, polymerase chain reaction.

strate activation. They also catalyze the modification of the substrate (e.g. racemization and *N*-methylation) as well as the peptide bond formation [6]. It has been demonstrated that each of these domains is equipped with a separate cofactor essential for covalent binding of the cognate amino acid and the elongation of the growing peptide chain [38–40].

Originally, it was proposed that, in analogy to fatty acid synthases, specific peripheral cysteine residues represent the thiotemplate sites, and a single intrinsic 4′-phosphopantetheine cofactor interacts with these thioesterified substrates [3–5, 7]. With the recently suggested multicarrier model, which is supported by the finding that each amino acid activating domain bears an individual 4′-phosphopantetheine cofactor, an easier description of the biosynthetic process is possible: the growing peptide chain is transferred from one domain-linked cofactor to the next [6, 38–41]. Current investigations have suggested the existence of three specific positions for the interaction of the prosthetic group [38, 39, 41]: a charging site for the covalent binding of the cognate amino acid (thioester formation), a peptidyl acceptor site (interaction with preceding domain and elongation module), and a peptidyl donor position (interaction with consecutive domain and elongation module). Basically, this mode of transpeptidation resembles the ribosomal peptidyl transfer from the A (aminoacyl) to the P (peptidyl) site. With regard to the initial ATP-dependent substrate activation (Fig. 1), this represents an additional similarity between ribosomal and non-ribosomal peptide biosynthesis. In conclusion, the cofactors facilitate the ordered shift of the carboxy thioester-activated substrates between the domains that constitute the peptide synthetases, resulting in the formation of a defined peptide. The termination of the enzyme-catalyzed peptide synthesis is induced by the release of the thioester-bound peptide product by hydrolysis, cyclization, or specific transfer to a functional group.

MODULAR ARRANGEMENT OF PEPTIDE SYNTHETASES

As expected from the multicarrier thiotemplate model, the multifunctional peptide synthetases should possess a highly conserved and ordered structure of semi-autonomous domains. Each of those has to activate and covalently bind an amino acid specifically by the two-step reaction shown in Fig. 1. Previous protein chemical studies have identified proteolytic fragments, with molecular masses of about 120 kDa, which are able to activate individual amino acids [42–46]. These fragments seemed to correspond with the activating spots proposed by Lipmann [7].

Significant progress concerning the comprehension of the structure–function relationship of peptide synthetases has been achieved with the elucidation of their primary structure. Sequence comparisons of an increasing number of peptide synthetases revealed the presence of homologous domains whose occurrence and specific linkage order have been shown to dictate the sequence of the synthesized peptide (Fig. 2) [9, 21, 22, 25, 31, 32]. Thorough analysis revealed the existence of a superfamily of adenylate-forming enzymes, including all peptide synthetases as well as several adenylating enzymes like luciferase, enterobactin synthetases EntE and EntF, 4-coumarate CoA ligase, and long chain fatty acid and CoA synthetases [4, 6, 22]. Shared among this group is an ATP-dependent carboxy acid activation and a homologous region of about 550 aa that shows homology in a range of 30 to 70%. Such a domain contains the highly conserved core sequences 1–5, shown in Fig. 3 [22]. Numerous studies, including site-directed mutagenesis and photoaffinity labelling with ATP analogs, suggested the involvement of core sequences 2–5 in ATP binding and hydrolysis, whereas the function of core 1 remains unclear [38–40, 47–50]. These studies also indicated the association of core 6 in covalent binding to both the cofactor and the substrate. This latter motif (LGGHSL) represents an integral part of a C-terminal extension (100 aa) of the above-mentioned adenylation domain [6, 10]. Such an additional thioester module was only found in peptide synthetase domains (type I), which catalyze the amino acid adenylation and thioester formation (Figs. 1 and 3) [6, 10]. In type II domains, both the adenylating and the thioester forming modules are separated by a 430-aa insertion that bears a common glycine-rich *S*-adenosylmethione binding motif (VLE/DxGxGxG) [6, 9, 21]. This insertion catalyzes the *N*-methylation of the cognate amino acid and, therefore, can be only detected in *N*-methyl peptide synthetases (e.g. SimA [9]; Fig. 2).

Between two amino acid-activating domains, there is an interdomain segment (Fig. 2; white areas), approximately 500 aa in length, which shows low conservation [6, 22, 41]. Such spacer regions contain a conserved His-motif (Fig. 3; HHxxxDG) that is located asymmetrically between two adjacent domains but not at the N-terminus upstream of domains that initiate peptide synthesis (e.g. GrsA and TycA) nor in the C-terminal region downstream of domains that catalyze the incorporation of the terminal amino acid. Therefore, it is possible that those motifs facilitate module interactions and are most probably involved in peptide elongation (Fig. 3) [6, 41].

Within interdomain regions, downstream of domains that catalyze the activation of D-amino acids (e.g. GrsA, TycA, AcvA-II, SrfA-A-III, and SrfA-B-III), Zuber and co-workers have also identified a putative racemization module that spans a region of about 130 aa and contains four highly conserved and ordered core sequences (Fig. 3) [32]. Recently, biochemical studies on GrsA as a model peptide synthetase clearly pointed out the location of individual modules involved in substrate recognition and adenylation, thioester formation, and racemization (Fig. 3) [10]. These investigations enabled the amplification of specific domain-coding regions from a diverse group of bacterial and fungal genes encoding peptide synthetases. In turn, these modules were used for targeted domain substitutions and for the rational design of novel peptide antibiotics [11].

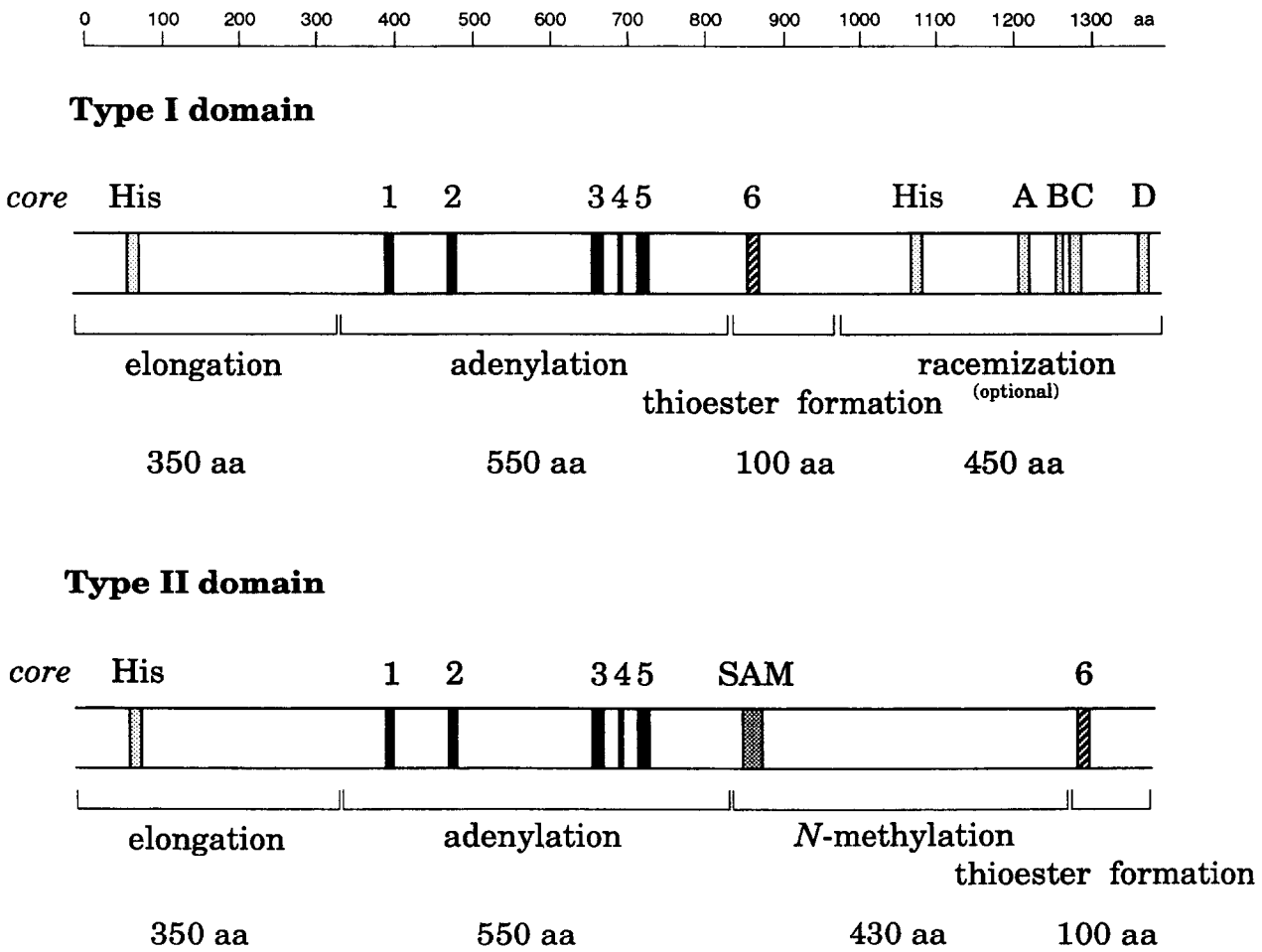

FIG. 3. Two types of amino acid activating domains in peptide synthetases. The schematic diagram shows the relative location, extension (size in aa residues), and organization of specialized modules (adenylation, thioester formation, elongation, *N*-methylation). Core sequences involved in acyladenylation (1, LKAGGAYVPID; 2, YSGTTGxPKGV; 3, GELCIGGxGxARGYL; 4, YxTGD; and 5, VKIRGxRIELGEIE) are indicated by black boxes. The conserved core motifs of modules required for thioester formation (striped; 6, DNFYxLGGHSL), *N*-methylation (shaded; VLE/DxGxGxG), peptide elongation (pointed; His, HHILxDGW), and optional peptide racemization (pointed; His, HHILxDGW; A, AYxTExNDILLTAxG; B, EGHGRExIIE; C, RTVGWFTSMYPxxLD; and D, FNYLGQFD) are also shown [6, 10, 22, 41].

ENGINEERED BIOSYNTHESIS OF NOVEL PEPTIDE ANTIBIOTICS

The reprogramming of peptide synthetase genes was accomplished by gene disruption and gene replacement, as monitored by a selectable marker (Fig. 4). The recombination method comprises two successive steps: marking the chromosomal target site with a selectable gene (e.g. for drug resistance) by double crossing-over and, subsequently , delivering an engineered hybrid gene that encodes a peptide synthetase with an altered amino acid specificity [11]. The advantage of this molecular genetic technique over an *in trans* approach is that wild-type mechanisms are used now for gene regulation, expression, enzyme folding, assembly, and localization of a recombinant hybrid synthetase gene within the chromosomally encoded (single-copy) biosynthetic system.

The scheme shown in Fig. 4 depicts the gene disruption and replacement method [11]. An *Escherichia coli* expression vector was constructed that contains the PCR-amplified domain-flanking regions (I and III). This integration vector was used for the insertion of a selectable marker (DR) into the chromosomal target site through a double recombination event, as directed by the specific linker fragments (I and III). Gene disruption was accomplished by selection for drug resistance and screening for an antibiotic-deficient phenotype. For the engineering of hybrid genes encoding peptide synthetases with the altered amino acid specificities of the substituted domains, PCR-amplified heterologous domains of bacterial and fungal origin (IV) were inserted *in frame* between the linker fragments (I and III) of the integration vector. These replacement plasmids were used to substitute the selectable marker (DR, drug resistance) and to reconstitute the interrupted biosynthesis gene(s) within the chromosome.

Initially, our recombination method was set up for the reprogramming of the *srfA* operon in *Bacillus subtilis* (Fig. 2), which encodes the template for the biosynthesis of the cyclic lipopeptide antibiotic surfactin (Table 1) [11, 31]. As

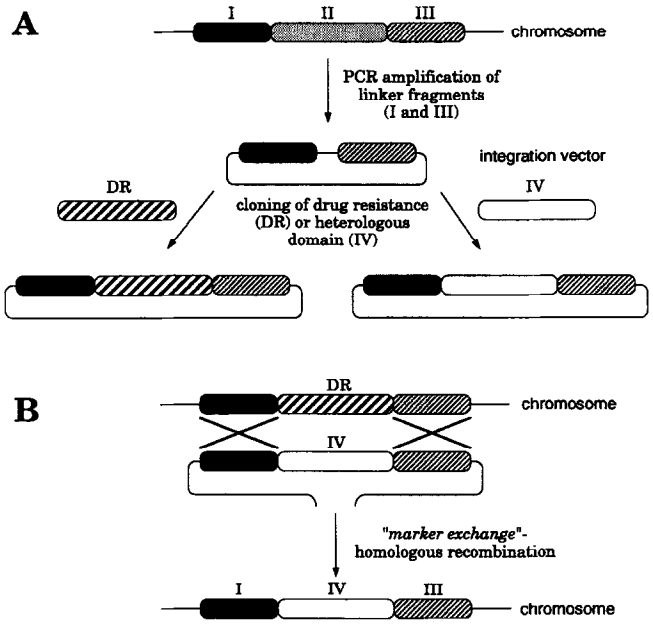

FIG. 4. Simplified scheme for the realization of targeted domain replacements [11]. (A) Construction of hybrid genes encoding peptide synthetases with altered amino acid specificities based on the definition of the minimal size of an amino acid activating domain. An integration vector was constructed, containing the flanking regions (I and III) of the functional domain (II), which is intended to be substituted. This was performed by PCR amplification of these fragments. Cloning of a selectable marker (DR, drug resistance) between the linkers led to the construction of a gene disruption plasmid. Otherwise, the introduction of domain-coding regions of bacterial and fungal origin resulted in the ability to distinguish domain substitution plasmids. In any case, PCR-amplified heterologous domains were inserted *in frame* between the linkers, resulting in different hybrid genes encoding peptide synthetases with the altered acid specificities of the substituted domains. (B) The integrative plasmids constructed were used for the targeted gene disruption and replacement in the chromosome of the producing strain, as directed by the specific linker fragments. Two successive recombination events are required: the disruption plasmid was used for marking the chromosomal target site with a selectable gene (DR) by double crossing-over, whereas, subsequently, the replacement plasmid delivers an engineered hybrid gene into the marked chromosome by marker exchange reaction.

a selectable marker, we used the *cat* gene, which confers chloramphenicol resistance. To avoid a negative selection on drug-sensitive transformants during the reconstitution of the biosynthetic operon, the plasmid pNEXT33A was co-transformed in congression with the substitution plasmids [51]. pNEXT33A is able to integrate in the *metD* gene (unlinked locus to *srfA*) of *B. subtilis* and confers neomycin resistance upon the transformed cells [52]. Such neomycin-resistant cells were selected and screened for chloramphenicol-sensitive colonies. Approximately 0.1 to 1% of the transformants carried the postulated phenotype and were further checked for the correct integration of the hybrid genes into the genetically marked chromosome. Southern blots determined that the derived clones were double-recombinants in which the heterologous domain-coding regions had been inserted into the *srfA* operon in place of the *cat* gene (Fig. 4). The targeted replacements of the leucine-activating domain of *srfA-C* was carried out successfully for several bacterial (*grs:* phe, orn, leu) and fungal (*acvA:* cys, val) domains (Fig. 2) [11].

Surfactin derivatives produced by the engineered *Bacillus* strains were extracted from the cultured broth and analyzed by infrared spectroscopy. As concluded from the infrared spectra, all chimeric strains produce peptide antibiotics similar in structure to the wild-type surfactin. To determine the differences between the various isoforms, we further analyzed the products using mass spectrometry. These studies confirmed the identity of five novel lipopeptides derived by targeted domain replacement within the *srfA-C* gene [11]. The wild-type surfactin is a powerful biosurfactant, and therefore it has many potential industrial applications [53–55]. To investigate the influence of amino acid replacements on hemolytic activity, the extracted derivatives of surfactin were investigated for their ability to lyse erythrocytes [53, 54]. It was found that the consequence of disrupting *srfA-C* was a complete loss of biological activity, whereas the engineered biosurfactants restored that activity [11].

LIMITATIONS AND FEASIBILITIES OF PEPTIDE ANTIBIOTIC ENGINEERING

With the demonstration of the engineered production of novel surfactin derivatives using hybrid genes, the stage for the rational design of a versatile class of bioactive peptides is no longer a mirage. The method described represents a general comprehensive approach for reaching this goal. Nevertheless, there are a few limitations to this promising technique that one has to recognize, and further investigations have to conquer.

For the realization of domain substitutions using the two-step recombination method presented, very detailed information (sequence data) about the desired biosynthetic systems is required. Unfortunately, only limited number of peptide synthetases are currently well characterized at this primary level (Table 1). However, advances in the identification and cloning of additional peptide synthetase genes from various organisms have been made recently. Based on the strong conservation of core sequences in the domain structure of peptide synthetases [22], a universal PCR approach for amplifying putative synthetase genes of diverse origin has been developed [56]. An amplified fragment derived by PCR can be utilized as a probe for screening genomic libraries of peptide antibiotic-producing strains. After successful identification of clones bearing parts of a synthetase gene, it is possible to characterize the entire biosynthetic system of the desired secondary metabolite. This could be facilitated by established procedures and has already been used successfully to identify bacterial and fungal peptide synthetase genes from various organisms [56].

A further limitation among the targeted engineering of peptide antibiotics is the profuse amount of knowledge required about structure–activity relationships of the secondary metabolites. The success of rational design of novel peptide antibiotics mainly depends on the biological and pharmacological activities of the engineered drugs. It remains time-consuming to introduce an abundance of desired amino acid substitutions into bioactive peptides. Moreover, it also seems unlikely that random changes would lead to improved activities of these antibiotics. Therefore, to produce more suitable and refined drugs, it is essential to understand how an antibiotic attacks its target site. For example, the well characterized, cyclic undecapeptide cyclosporin (Table 1 and Fig. 2) achieves its immunosuppressive activity by the interaction with cyclophilin. This binary complex binds to calcineurin (protein phosphatase 2B) and inhibits its crucial role during T cell activation [57]. The interaction of the inhibitory complex cyclosporin–cyclophilin with the Ca^{2+}- and calmodulin-dependent phosphatase calcineurin has been investigated extensively by X-ray crystallography, photoaffinity labelling, and chemical cross-linking. These studies revealed, for example, an essential role of leucine side chains in positions 8 and 10 of cyclosporin (Fig. 2) for interaction with calcineurin [57]. Some natural non-immunosuppressive derivatives are modified in these residues, indicating an increased sensitivity of those positions with respect to alterations of the amino acid composition. Otherwise, some of those natural mutants exhibit valuable refined activities, for instance against the human immunodeficiency virus (HIV) [58]. This implies that it is always worthwhile to screen derived hybrid antibiotics not only for improved but also for enhanced bioactivities.

Historically, identification of antibiotics has relied on a sequential trial-and-error approach—testing an abundance of potential bioactive compounds and observing their biological or pharmacological effects. More recently, special computational tools have indicated areas that could increase effectiveness in the design of novel drugs. For example, the major goal of computer-aided drug design (CADD) is to determine whether a molecule will bind to a specific target and to evaluate the characteristics of this interaction [59–61]. This method utilizes crystallographic data and computer procedures to describe a molecule exactly in terms of mutual effects (van der Waal's and electrostatic interaction) with the receptor. Starting with a specific target site, the approach determines what types of molecules would have a desired effect on that target and subsequently constructs molecules that fit these criteria. Basically, the clinical interest in neuropeptides and peptide hormones has stimulated the computational research and development of peptide-based drugs [61]. We strongly suggest that the increased structural and functional diversity of non-ribosomally synthesized peptides could be a more suitable subject for drug engineering. Their emerging capabilities—more than 300 miscellaneous compounds, various modifications, as well as linear, cyclic, or branched structures—have the potential to increase the effectiveness and variability in design of novel bioactive peptides.

In general, current studies and concepts have focused on the modification of natural secondary metabolites that are known to possess bioactivities. In the near future, the field of study may be moving to a complete engineering of a desired biosynthetic system (thiotemplate). With a more enhanced comprehension of the organization of peptide synthetases, engineers will be able to take advantage of the modular nature of these multifunctional enzymes. Creation of new thiotemplates and the approximately unlimited structural feasibilities of non-ribosomally synthesized peptides will allow the generation of drugs with enhanced or improved activities that will fit almost any desired target.

CONCLUSIONS

The purpose of this commentary was to review the latest progress made in the comprehension of structural and mechanistical aspects of the non-ribosomal synthesis of bioactive peptides. Our main intention was the stimulation of thought and studies dealing with this pharmaceutically important class of natural compounds. Many diseases, as well as an increasing number of pathogenic bacteria, viruses, parasites and tumors, are becoming more and more resistant to traditional therapy, necessarily requiring innovative concepts to find novel and pharmaceutically useful drugs.

Because of their enormous structural and functional diversity, the feasibilities of non-ribosomally synthesized peptides to fit these demands should be pointed out and proven. We believe that the rational design of peptide antibiotics, using the two-step recombination method described, represents a powerful and comprehensive advance in medical, biotechnological, and agricultural research. The near future will show how far this approach will go towards the engineering of novel therapeutically useful peptide antibiotics.

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