Mimicry of bioactive peptides via non-natural, sequence-specific peptidomimetic oligomers

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Non-natural, sequence-specific peptidomimetic oligomers are being designed to mimic bioactive peptides, with potential therapeutic application. Cationic, facially amphipathic helical β-peptide oligomers have been developed as magainin mimetics. Non-natural mimics of HIV-Tat protein, lung surfactant proteins, collagen, and somatostatin are also being developed. Pseudo-tertiary structure in β-peptides and peptoids may herald the creation of entirely artificial proteins.

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Abbreviation
TAR *trans*-activation responsive region

Introduction

Peptides are short, sequence- and length-specific oligomers composed of amino acids. These familiar biomolecules are ubiquitous in living cells and assume myriad roles, including cell receptor ligand, endogenous antibiotic, and even components of pulmonary surfactant. Each role assumed by a bioactive peptide will typically correspond to a unique three-dimensional structure. In this way, nature has exquisitely refined bioactive peptide sequences and activities through evolution and, naturally, there has been significant interest in exploiting these molecules as pharmaceutical lead compounds. Unfortunately, peptides themselves provide inferior drug candidates because of their low oral bioavailability, potential immunogenicity and poor metabolic stability *in vivo* [1].

Recent efforts to ameliorate disadvantageous peptide characteristics, and thus generate viable pharmaceutical therapies, have focused on the creation of non-natural peptide mimics. These 'peptidomimetics' can be based on any oligomer that mimics peptide primary structure through use of amide bond isosteres and/or modification of the native peptide backbone, including chain extension or heteroatom incorporation. Peptidomimetic oligomers are often protease-resistant, and may have reduced immunogenicity and improved bioavailability relative to peptide analogues. In addition to primary structural mimicry, a select subset of the sequence-specific peptidomimetic oligomers, the so-called 'foldamers' [2], exhibits well-defined secondary structural elements such as helices, turns and small, sheet-like structures. When peptide bioactivity is contingent upon a precise 3-D structure, the capacity of a biomimetic oligomer to fold can be indispensable. Examples of simple peptidomimetics (Figure 1) include azapeptides, oligocarbamates and oligoureas, and common foldamer examples include β-peptides, γ-peptides, oligo(phenylene ethynylene)s, vinylogous sulfonopeptides and poly-*N*-substituted glycines (peptoids) [2–4].

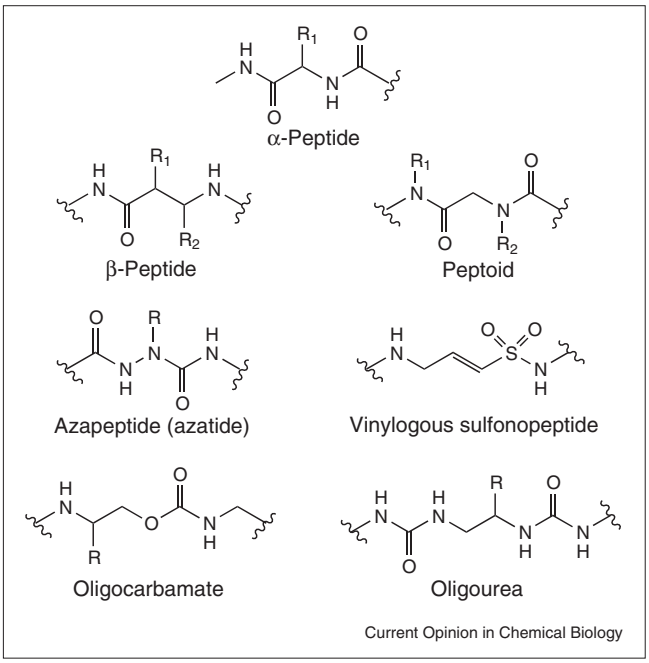
Sequence-specific peptidomimetics are beginning to find important application in the mimicry and optimization of natural peptide products for therapeutic use. This review showcases several of the more significant and recent developments, including HIV-Tat biomimicry, with an emphasis on the expanding field of antibacterial peptide mimicry. Finally, the significance of recent reports suggesting the accessibility of tertiary structure in β-peptides and peptoids is discussed in the context of bioactive peptide mimicry.

Antibacterial peptide mimicry

Antibacterial peptides are found in many organisms, ranging from bacteria through mammals [5,6]. Most antibacterial peptides share a simple structural motif; they are most commonly short (< 40 residues) linear, cationic, amphipathic α-helices [7••]. They exert antibacterial activity by cell membrane permeabilization and lysis, although the precise lytic mechanism has not been conclusively determined [8]. Selectivity for the bacterial cell appears to be mediated by favourable electrostatic interaction between positively charged peptides and the negatively charged bacterial cell surface [9,10]. Mammalian cells, typified by a largely zwitterionic (and net-neutral) cell surface, are less favourable targets. However, because an excessively hydrophobic peptide can bind indiscriminately to any cell membrane [11], antibacterial peptide selectivity is contingent upon a precise balance of peptide hydrophobicity and electrostatic charge. In general, studies suggest that the overall physico-chemical parameters of antibacterial peptides, rather than any specific receptor–ligand interactions, are responsible for antibacterial activity [11]. As a result, antibacterial peptides are attractive targets for biomimicry and peptidomimetic lead development, as reproduction of critical peptide biophysical characteristics in an unnatural, sequence-specific oligomer should presumably be sufficient to endow antibacterial efficacy, while circumventing the limitations associated with peptide pharmaceuticals [1].

Recent studies in which β-peptides were designed to mimic the magainin antibacterial peptides [12] have helped to illustrate which physical characteristics are critical for ideal antibacterial efficacy and biocompatibility in non-natural oligomers. β-Peptides have more conformational freedom than α-peptides, because of an additional methylene unit

Figure 1



Representative sequence-specific peptidomimetic oligomers.

Figure 2

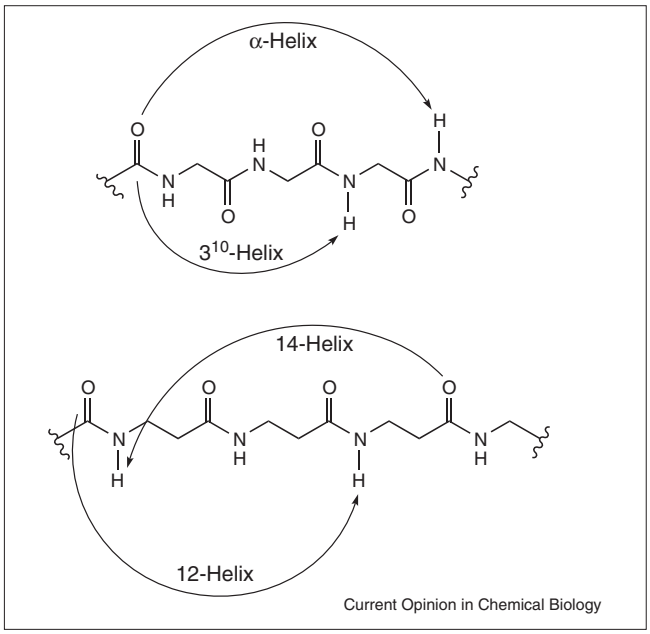


Illustration of hydrogen bonding in α-peptide and β-peptide helices.

present in the polymer backbone. Consequently, whereas α-peptide helices most commonly adopt the α-helix conformation, β-peptide sequences have been shown to adopt several distinct helical conformations, the choice of which depends largely upon the substitution pattern at backbone C_α and C_β atoms [13•,14,15]. Of these, the β-peptide 12-helix and the 14-helix have been successfully employed as magainin mimics (Figure 2). The terms '12-helix' and '14-helix' correspond to the number of atoms participating in a ring created by intrachain hydrogen bonds.

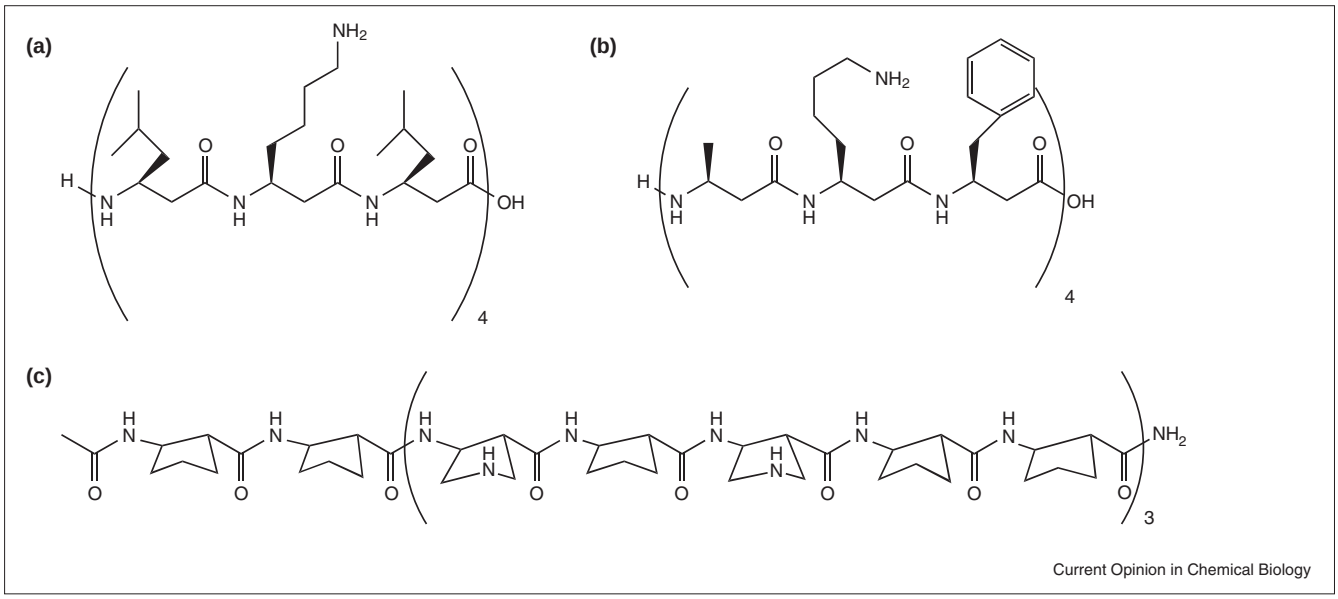
DeGrado and co-workers [16] first reported the *de novo* design of amphipathic, cationic, monosubstituted β-peptide 14-helices as antibacterial compounds against the Gram-negative bacterium K91 *Escherichia coli*. Although potent antibiotics, these β-peptides displayed poor selectivity for bacteria, as evidenced by extensive mammalian red blood cell lysis (haemolysis). Assuming that excessive side-chain hydrophobicity was responsible for the poor selectivity observed, DeGrado and colleagues [17] modified their original 14-helix designs to substitute a valine-like (β-HVal) residue with a less hydrophobic alanine-like (β-HAla) residue. In support of their original hypothesis, this modification abolished haemolytic activity, while retaining good antibacterial efficacy in both 12- and 15-residue oligomers.

Simultaneously and independently, Seebach's group [18] synthesized quite similar mono-substituted β-peptides, also designed to adopt the β-peptide 14-helix. For a hydrophobic moiety, Seebach selected a phenylalanine-like residue (β-HPhe) *in lieu* of the β-HVal residue employed by DeGrado. Interestingly, although haemolytic activity was comparable, this sequence difference alone resulted in a one order of magnitude reduction in antibacterial activity relative to DeGrado's refined oligomers.

Whereas DeGrado and Seebach have focused on the 14-helix, Gellman's group has reported a 17-residue β-peptide 12-helix, which they call 'β-17', that possesses potent antibacterial activity against a variety of Gram-positive and Gram-negative bacteria and exhibits minimal haemolysis [19••] (Figure 3). Unlike DeGrado's monosubstituted 14-helices, β-17 is disubstituted at C_α and C_β to form intra-residue five-membered rings and consequently possesses significantly less conformational freedom than either DeGrado's or Seebach's oligomers. Therefore, conformational rigidity does not appear to adversely affect activity or selectivity. In fact, Gellman's group has recently described a β-peptide 12-helix with both mono- and di-substituted residues, with activity comparable to β-17 and magainin [20].

Although oligomer hydrophobicity was implicated as perhaps the primary factor responsible for antimicrobial selectivity, precisely which molecular characteristics are responsible for β-peptide antibacterial action remained ambiguous. This is well-illustrated by the large and intriguing difference in efficacy between DeGrado's and Seebach's similar oligomers. Thus, to better define precisely which molecular characteristics do in fact affect antibacterial activity and selectivity in non-natural oligomers such as β-17, Gellman's group synthesized a series of β-17 (12-helix) analogues in which molecular characteristics such as amphipathicity and the ratio of cationic to hydrophobic residues were systematically varied [21•]. They conclusively demonstrated that

Figure 3



Chemical structures of select antibacterial β-peptides. **(a)** DeGrado [16]. **(b**) Seebach [18]. **(c)** Gellman β-17 [19••].

facial amphipathicity is requisite for bactericidal activity in 12-helix β-peptide magainin mimics, while a ratio of 40% cationic to hydrophobic residues provides optimal selectivity. More recently, Gellman and co-workers [22] have begun to examine 14-helical antibacterial β-peptides, and the relationship between their folded structure and activity. Such studies hopefully will facilitate oligomer design by providing a clear correlation between physico-chemical properties and the biophysics of the antibacterial mechanism.

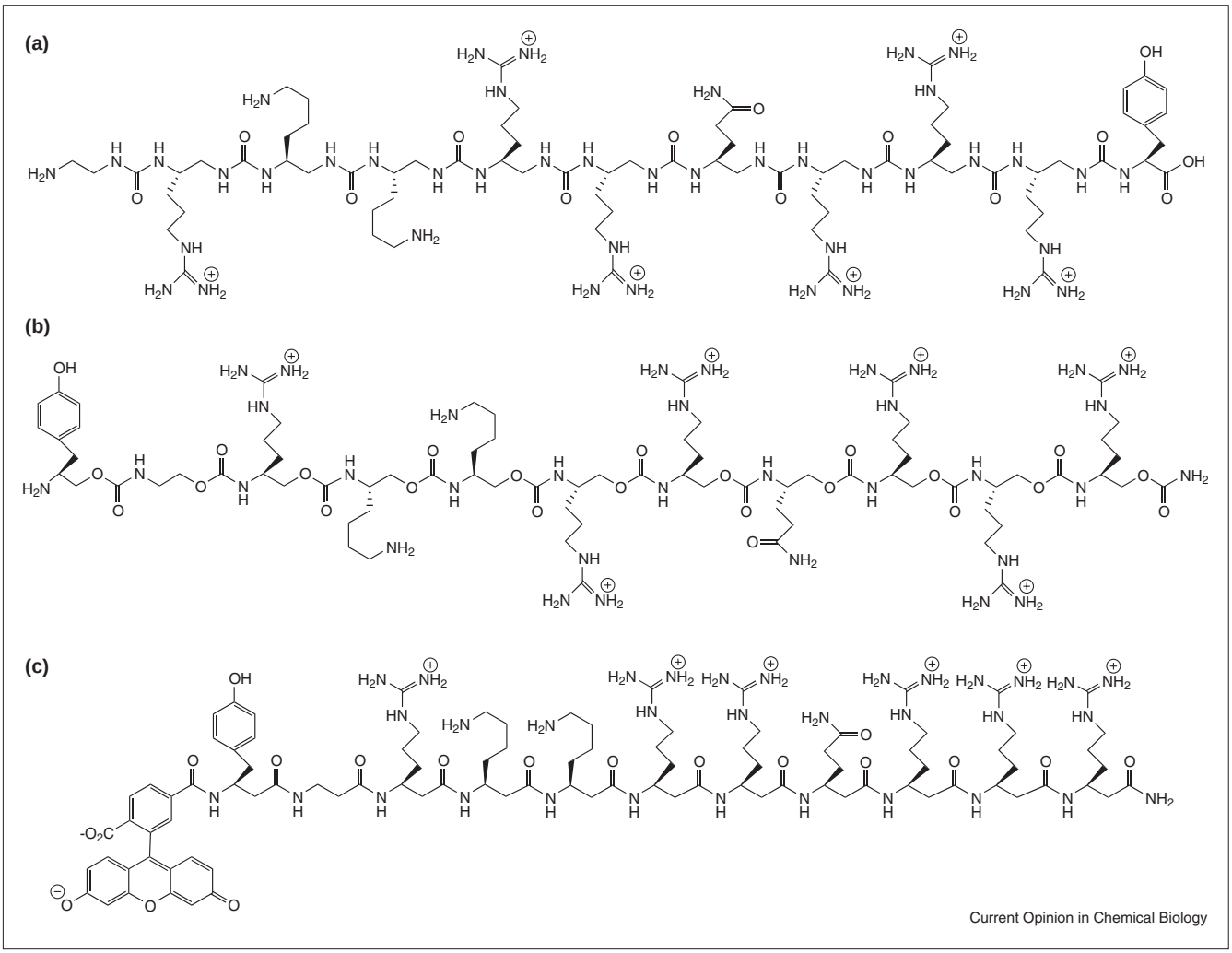
As a result of this work, it might seem that any cationic and facially amphiphilic helical oligomer with the appropriate balance of charge to hydrophobicity would be potentially capable of antibacterial activity and selectivity. Correspondingly, several groups have recently reported such peptidomimetics, perhaps with the intent of future antibiotic development. For instance, Arnt and Tew [23] reported membrane-active oligo(phenylene ethynylene)s with cationic, facially amphipathic structure. Although these oligomers may prove capable of bacterial membrane lysis, their water insolubility and very hydrophobic nature will probably require modification before they can serve as a viable, selective antibiotic. Based on the β-peptides, water-soluble hydrazino peptides are predicted on the basis of molecular mechanics to have stable, helical structure [24], and may be good candidates for development. Poly-*N*-substituted glycines, or peptoids [25], with certain organo-soluble [26,27] and facially amphipathic, cationic, water-soluble [28•] sequences have also been shown to form very stable helices. Since peptoids are easily synthesized and highly customizable [25,29], molecular characteristics such as hydrophobicity and charge are readily modified. In tandem with demonstrated protease resistance [30], peptoids are particularly amenable to development as biomimetic antibiotics.

Regardless of the particular oligomer identity, it is clear that the repertoire of peptidomimetic antibiotics will soon expand beyond the exclusive realm of β-peptides. DeGrado's group has already developed an easily and inexpensively synthesized class of water-soluble polyarylamides with facially amphiphilic structure (as predicted by computational studies) [31•]. Polymers of eight monomeric units had effectiveness comparable to that of β-peptide antibiotics against Gram-positive and Gram-negative bacteria. Although these polymers are haemolytic, reduction of the molecular hydrophobicity in subsequent redesigns should not be difficult. Other examples of magainin-mimetic, sequence-specific polymers such as these should be forthcoming.

HIV-Tat mimics

The mimicry of peptides that interact with nucleic acids has important implications for molecular biology and medicine. DNA- and RNA-binding peptidomimetics can affect gene transcription and replication in a predictable way for therapeutic benefit. In a prime example, HIV-1 replication is contingent upon the interaction of HIV-Tat protein with a 59-base RNA stem-loop structure, termed the *trans*-activation responsive region (TAR), located at the 5′ end of all HIV-1 mRNA molecules [32]. Disruption of this interaction would have obvious impact on AIDS therapy and, thus, research has been directed at the development of small-molecule Tat protein mimics that are capable of competitive inhibition of the Tat–TAR interaction (Figure 4). For instance, Rana and co-workers have developed both oligourea and oligocarbamate mimics of the

Figure 4



HIV-Tat$^{47-57}$ analogues. **(a)** Oligourea [33,35•]. **(b)** Oligocarbamate [34,35•]. **(c)** β-peptide [39•].

TAR-binding portion of the Tat protein (^{48}GRKKRRQRRR^{57}), which compete with Tat for TAR binding. Both oligourea and oligocarbamate mimics have dissociation constants comparable to that of a synthetic Tat$^{48-57}$ [33,34], binding quite tightly to HIV-1 TAR. Rana and co-workers [35•] have also demonstrated that both oligocarbamate and oligourea versions of Tat$^{48-57}$ are in fact capable of suppressing HIV-1 gene expression *in vivo*.

Apart from its critical role in HIV-1 replication, Tat protein has been shown to rapidly cross cellular membranes, aggregating in the nucleus [36]. Membrane translocation activity appears to be dependent upon small motifs within the Tat protein, particularly the basic sequence contained in residues 48–60 [37]. Wender *et al.* [38] have designed an oligoguanidine peptoid sequence, derived from the cationic Tat$^{49-57}$ motif, that exhibits membrane translocation activity superior to that of Tat$^{49-57}$ itself. Gellman and colleagues [39•] have synthesized a β-peptide sequence mimic of Tat$^{47-57}$ but with activity merely comparable to that of the native peptide. Conceivably, these sequences may soon be incorporated into other non-natural peptidomimetic oligomers to facilitate cellular uptake, providing crucial access to the intracellular environment.

Other applications

Researchers are currently developing many different non-natural oligomers for use in the mimicry of diverse bioactive peptides. Non-natural azapeptide [40] and peptoid ligands [41] for MHC-II are being developed to help modulate immune system response, and possibly provide therapy for auto-immune disorders. Novel somatostatin analogues incorporating peptoid [42] and β-peptide [43–45] residues have been reported. Helical peptoid mimics of lung surfactant protein C are the first non-natural synthetic replacements reported [46,47]. Even peptoid-based collagen mimics have been generated [48,49].

Tertiary structure

Although simple secondary structural motifs have so far been adequately mimicked by a variety of peptidomimetic oligomers, the more complex tertiary structural elements have remained outside the reach of biomimetics. While true tertiary structure has not yet been observed, recent developments suggest that such non-natural, compact conformations may be accessible. In a series of analytical centrifugation experiments, Gellman and colleagues [50••] have reported the formation of tetrameric or hexameric bundles (dependent upon buffer choice) of amphipathic β-peptide 14-helices in aqueous solution. Similarly, after synthesizing and screening a 3400-member combinatorial library, Burkoth *et al.* [51••] discovered an amphiphilic 15-mer peptoid that appears to form tetrameric aggregates capable of binding a fluorescent dye. This intermolecular aggregation of hydrophobic portions of amphipathic β-peptide and peptoid helices seems analogous to the intramolecular hydrophobic collapse in proteins that leads to tertiary structure. Presumably, non-natural tertiary structure could be obtained by intramolecular incorporation of several adjacent amphipathic helices, spaced with appropriate linkers to allow hydrophobic collapse. Such peptidomimetics, rendered capable of forming compact tertiary structure, would transcend mere peptide mimicry to become truly 'protein-mimetic' oligomers. Needless to say, the potential therapeutic applications of entirely artificial proteins are boundless.

Conclusions

The extensive work that has been done to determine the preferred three-dimensional conformations of foldamers, especially of the β-peptides, has begun to prove immensely useful in bioactive peptide mimicry. The knowledge linking primary sequence to secondary structure in peptidomimetic oligomers is being exploited to create mimics of many diverse natural products. Experience gained from the synthesis of these relatively simple mimics has revealed the possibility of tertiary structure in non-natural oligomers, which in turn raises the exciting prospect of entirely artificial proteins. In short, these successful attempts at relatively facile projects in peptide mimicry illustrate that as the field of biomimicry matures, so does our understanding of the intricate relationship between sequence, folded structure and function, not only in non-natural systems, but also in peptides and proteins.

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