Oligopeptide Foldamers: From Structure to Function

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Dedicated to Prof. Giorgio Modena, former editor of this journal, on the occasion of his 80th birthday

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Peptides may constitute important scaffolds for the construction of supramolecules. They can also provide critical elements that impart peculiar conformational properties to the molecule that can be exploited for carrying out specific functions, including catalysis. This microreview highlights recent advances in the field.

Introduction

Nature performs its chemical tasks, such as catalysis, molecular recognition, or light-energy conversion, by using large polymeric structures, mostly proteins. The tertiary structure of proteins is determined by conformationally well-defined secondary structures such as α-helices, β-sheets, coiled-coils, etc. These locally structured units give order to the overall system by positioning functional groups precisely in three-dimensional space, thus creating an active site where catalysis occurs or recognition takes place. Although amino-acid-based superstructures are almost the rule in the biological world, the number of synthetic supermolecules based on oligopeptides is relatively small. The

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Giulia Licini studied chemistry at the University of Padova (Italy) where she obtained her PhD in 1989 under the supervision of Prof. Giorgio Modena. She spent one year as a postdoctoral researcher with Albert I. Meyers at the Colorado State University, Fort Collins. Back to the University of Padova she got a position as Researcher and, in 2000, she became Associate Professor. Her scientific interests are in catalytic stereoselective synthesis, in particular in chiral catalyst design, and in the study of reaction mechanisms, especially in oxygen-transfer processes.

Leonard Prins studied chemical technology at the University of Twente (Netherlands) and obtained his PhD degree in 2001 at the same university working with Prof. D. N. Reinhoudt. He worked as a postdoctoral researcher with Prof. P. B. Dervan at the California Institute of Technology, Pasadena, and with Prof. G. Licini at the University of Padova, Italy. In January 2004 he took up a position as a researcher at the same university associated to the group of Prof. P. Scrimin. His current research interest is the development of oligopeptide multidentate catalysts.

Paolo Scrimin, after obtaining his Doctor of Chemistry degree at the University of Padova with Prof. Modena in 1976, did postdoctoral work in the same group and then got a lecturer position at the University of Ferrara in 1979. In 1983 he returned to Padova where he is now a full Professor. He has been the Head of the Department of Organic Chemistry from 1999 to 2003. Prof. Scrimin has been a visiting Fulbright Scholar at Rutgers University (1985/86) and at the University of California, Santa Barbara (1992/93). His scientific interests are in the chemistry of aggregates and of supramolecules, with particular emphasis on catalysis of hydrolytic processes by transition-metal complexes. Peptide-based supramolecules represent one of the most recent developments of the research of his group. He is a member of the Editorial Board of the European Journal of Organic Chemistry.

MICROREVIEWS: This feature introduces the readers to the authors' research through a concise overview of the selected topic. Reference to important work from others in the field is included.

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main obstacles are the difficulty to predict and control the conformation of amino acid sequences and, in the meantime, introduce (un)natural functional groups in appropriate positions where they can perform recognition or catalytic tasks. Early examples in this field were extensively reviewed by Voyer a few years ago.[1] More recently, scientists have started to focus on the development of new foldamers[2–4] by introducing key structural elements in an artificial sequence that impose a conformational restriction on the oligopeptide. In this context, a foldamer is defined as an oligomer with a characteristic tendency to fold into a specific structure in solution that is stabilized by non-covalent interactions between non-adjacent subunits.

This microreview will focus on recent work from our laboratory, as well as others, aimed at the synthesis and study of oligopeptide foldamers that are able to recognize and transform selected molecular targets.

Functional Oligopeptides Based on Natural Amino Acids

In recent years great progress has been made in the de novo design of short peptide sequences (<100 residues) that adopt a well-defined conformation. Several reviews have recently appeared that summarize the achievements in this field.[5] Recent accomplishments include metal-peptide models that control the placement of metals within designed secondary structures with remarkable success.[6,7] In this microreview, however, we will only highlight some selected examples where the designed system (peptide or template-peptide conjugate) is able to perform a function, in particular catalysis.

One of the earliest examples of a short (14 amino acids), catalytically active sequence was reported by Benner et al. more than ten years ago.[8] The oligopeptide H_2N-Leu-Ala-Lys-Leu-Leu-Lys-Ala-Leu-Ala-Lys-Leu-Leu-Lys-Lys-$CONH_2$ is not very organized at infinite dilution in aqueous buffer (pH 7), with an α-helical content of only 18%. However, an increase of the concentration induces aggregation into a four-helix bundle characterized by a much higher helical content. The oligopeptide catalyzes the decarboxylation of oxaloacetate via an imine intermediate with a rate acceleration of three orders of magnitude with respect to the uncatalyzed reaction. The catalytic activity is due to two factors: firstly, placement of the reacting amine at the terminus of the helix causes a critical decrease of the pK_a (ΔpK_a = 0.6), and secondly, the anionic transition state is stabilized by electrostatic interactions with the cationic lysine residues present on the peptide surface. As a consequence, imine formation is no longer the rate-determining step, which is at variance with the natural enzyme oxaloacetate decarboxylase.

The group of Baltzer has systematically modified specific residues in key positions of an oligopeptide in order to catalyze the hydrolysis and transesterification reactions of *p*-nitrophenyl esters by exploiting the conformational preference of designed 42 amino acid sequences for a helix-loop-helix conformation (Figure 1).[9,10] Imidazole-functionalized peptides obtained by introducing several histidines in the sequence were found to provide substrate recognition and accelerations exceeding three orders of magnitude compared to *N*-methylimidazole. For example the sequence depicted in Figure 1 hydrolyzes 2,4-dinitrophenyl acetate with a second-order rate constant of 0.18 M^{-1} s^{-1} at pH 3.1 compared with a value of 9.9×10^{-5} M^{-1} s^{-1} for *N*-methylimidazole. Interestingly, it was demonstrated that the reaction mechanism takes advantage of the cooperativity of two adjacent histidines, one of which acts as the nucleophile and the other as a general base, as shown in Figure 2.

Figure 1. The helix-loop-helix-forming 42-mer peptide motif used by Baltzer for the design of catalysts for the cleavage of carboxylate esters (the dimeric form is shown here)

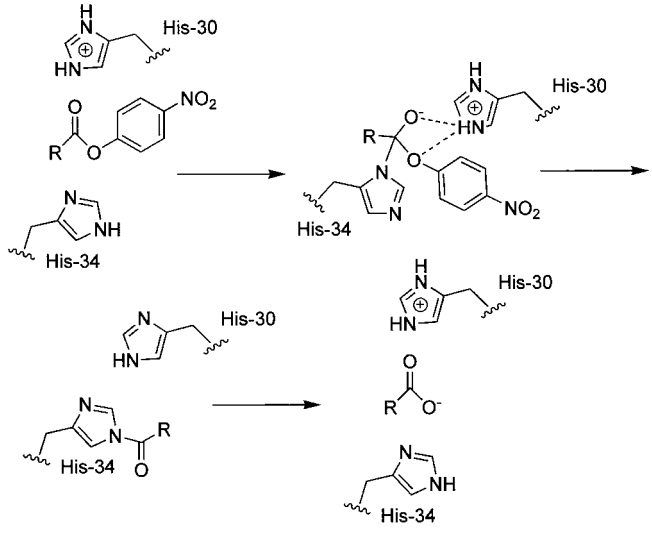

Figure 2. Cooperative mechanism involving two His in the cleavage of a carboxylate ester reported by Baltzer

In the case of flanking His-Lys sequences the unprotonated form of the histidine attacks the ester in the rate-determining step of the process followed by subsequent transacylation of the lysine. If several lysine residues are present, only those that flank the His are acylated at low pH. This leads to site-selective incorporation of an acyl residue in a natural sequence.[11] Direct alkylation of lysines occurs in sequences devoid of histidines. In this case the nucleophilicity of the lysines could be controlled by site-selective pK_a depression.[12] As stated by the authors, the elucidation of

the principles that control the reactivity in such elementary processes is the first step toward the construction of biomimetic catalysts that accelerate reactions for which naturally occurring enzymes do not exist.

While the above examples concern the recognition and subsequent transformation of small substrates, Ghadiri[13] and Chmielewski[14] have reported short sequences based on the coiled-coil folding motif[15,16] that act as templates for the recognition of shorter sequences and, eventually, for their ligation. When the two short peptides each constitute complementary pairs of the templating unit, the systems are examples of self-replicating peptides.[13c,13d,14] For instance a 33-residue polypeptide reported by Ghadiri[13] (Figure 3) accelerates the ligation of a 16- and 17-amino acid sequence using the coupling chemistry introduced by Kent et al.[17] Initial rate accelerations up to 4100-times that of a poorly matching system were observed. In terms of catalytic efficiency this gives a value of $(k_{cat}/K_m)/k_{uncat}$ of 7×10^5, which is larger than that found for catalytic antibodies.[18] The source of the catalytic acceleration derives from the specific recognition of the two substrates by the templating peptide, which places the reactive termini in close proximity. The recognition selectivity was further exploited for the chiroselective amplification of homochiral peptide fragments from a mixture of racemic substrates. The general limitation of the approach is the fact that it is marred by product inhibition, contrary to what is found with the antibody-based catalyst.[18] However, by destabilizing the coiled-coil structure Chmielewski was able to enhance the catalytic efficiency of the system[14b] and obtained a self-replicating peptide approaching exponential growth.[19]

The examples described above have in common that the peptide catalysts have been prepared based on a rational design. In sharp contrast are the recent reports by Miller et al.[20c,20d] which describe the synthesis of a 39-member library of five-amino-acid peptides in a combinatorial way. Screening for catalytic activity in the conversion of *myo*-inositol into D-*myo*-inositol-1-phosphate revealed one of the members to be an extremely efficient kinase mimic (Figure 4). Delivery of a phosphoryl group exclusively to one of the three hydroxyl sites of the substrate was observed with an *ee* of more than 98%. Further focusing of the library led to even more remarkable results. Although previous work by the same group using conformationally restrained short sequences in asymmetric transacylation reactions[21] led to promising results with high stereoselectivity factors ($s > 50$) the present peptide appears not to be organized at all. Clearly more research is needed to understand the formation and structure of the catalyst–substrate complexes in order to propose a rationale for these exciting results.

Although devoid of catalytic properties, the cyclic peptides reported by Ghadiri et al. are an excellent example of conformationally restricted oligopeptides.[22] These peptides are composed of an even number of alternating D- and L-amino acids and are designed in such a way that a flat conformation of the sequence is energetically favored. In this conformation the amide functionalities of the backbone lie perpendicular to the plane of the ring structure, which consequently allows stacking of the cycles into β-sheet-like tubular aggregates (Figure 5). It was shown that the peptides also aggregate in the lipid membrane of bacteria and, by increasing its permeability, exert antibacterial activity.[23]

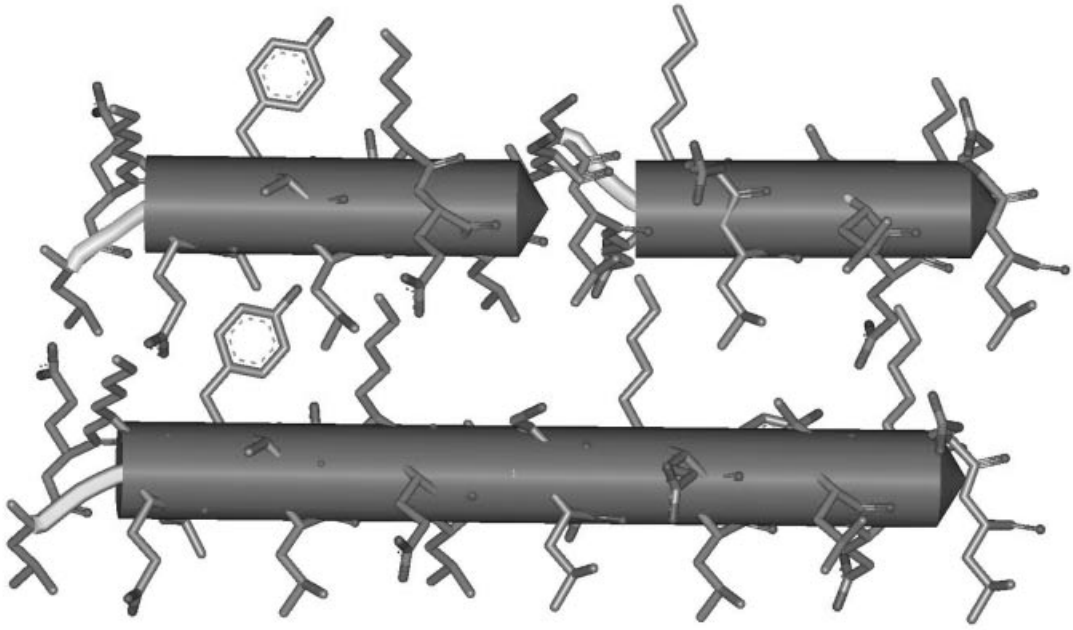

Figure 3. A self-replicating peptide reported by Ghadiri; peptide **1** (bottom) (AcLEKELYALEKELACLEKELYALEKEL-$CONH_2$) binds the two halves of **1** (actually a coiled-coil trimer, $\mathbf{1}_3$) by acting as a template and placing **1a** (top left) (AcLEKELYALEKELA-COSR) and **1b** (top right) (CLEKELYALEKEL-$CONH_2$) in the correct position for the occurrence of the ligation reaction

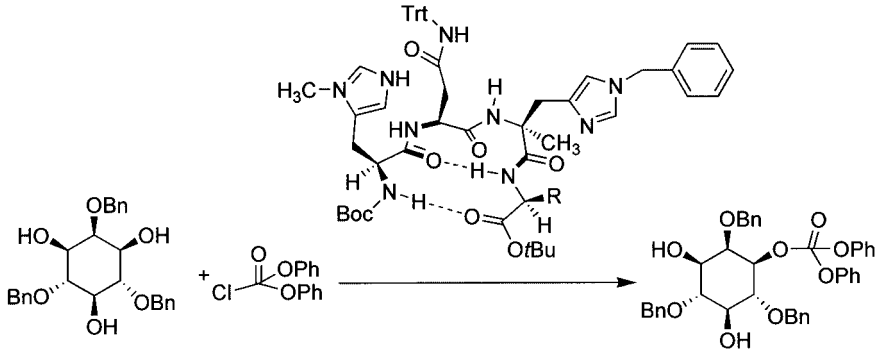

Figure 4. Miller's peptide used to catalytically convert *myo*-inositol into D-*myo*-inositol-1-phosphate

Furthermore it was demonstrated that they could mediate transmembrane transport of glutamic acid.[24]

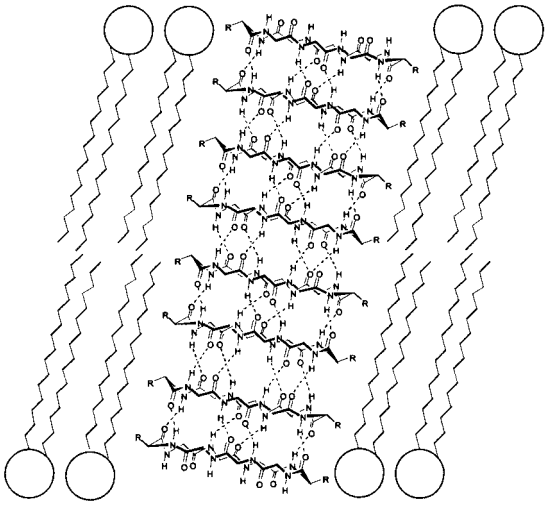

Figure 5. Ghadiri's cyclic peptides form tubular stacks upon binding to biological membranes, thereby altering their permeability

Functional Oligopeptides Based on Non-Natural Amino Acids

An alternative approach to the engineering of functional peptides is the introduction of synthetic unnatural amino acids in the sequence. The scope can be twofold: synthetic amino acids can be used to limit the conformational freedom of the oligomer or, alternatively, to introduce functional groups not available in the biological world.[25] Illustrative examples of both approaches will be discussed next, starting with the latter.

Introduction of Artificial Functionalities

The group of Imperiali has focused on the incorporation of unnatural amino acids bearing ligands for metal cations in their lateral chain and has obtained very effective peptide-based metal-ion sensors.[26] For instance, a heptapeptide[26c] that adopts a reverse-turn conformation[27] in aqueous solution induced by the internal -Val-Pro-DSer-Phe- sequence proved to selectively bind Zn^{II} ions at submicromolar concentrations in the presence of competing ions like Mg^{II}, Ca^{II}, or Mn^{II}. Alternatively, sensing may be provided by an appropriate fluorescent reporter in the presence of a metal-ion-binding sequence.[28] Even more striking is the use of the metal ion itself as fluorescent reporter of a specific sequence. The development of fluorescent proteins as molecular tags may allow complex biochemical processes to be correlated with the functioning in living cells. The work by Imperiali was based on previous studies by other laboratories that had focused on sequences based on specific loops of calcium-binding proteins for the similarities in ionic radii and coordination preferences between Ca^{2+} and Ln^{3+} ions. This previous work[29] had pinpointed hot positions relevant both for binding and fluorescence enhancement in 14-mer peptides. Imperiali's screening[30a] of a large (up to 500 000 member) library of peptides of the general sequence Ac-Gly-Xaa-Zaa-Xaa-Zaa-Xaa-Gly-Trp-Zaa-Glu-Zaa-Zaa-Glu-Leu, where Xaa was varied between the potential metal-binding residues Asp, Asn, Ser, or Glu, and Zaa was a hydrophobic amino acid, led to the discovery of a peptide with a K_D for Tb^{3+} ions as low as 0.22 μM. They suggested that these lanthanide binding tags (LBTs) may constitute a new alternative for expressing fluorescent fusion proteins by routine molecular biological techniques. The idea was successfully tested by appending the most promising sequences to ubiquitin (Figure 6).

In a conceptually similar approach, a pyridoxamine coenzyme amino acid chimera was introduced into designed ββα motif peptides (23-amino-acid sequences) to obtain a transamination catalyst.[31] The uncatalyzed transamination of α-keto acids to α-amino acids is a difficult reaction that is catalyzed efficiently by the coenzymes pyridoxamine phosphate and pyridoxal phosphate. A series of 18 peptides was synthesized in order to create different microenvironments for the introduced pyridoxamine functionality. This led, in the best case, not only to acceleration of the transamination rate, but also to promising enantioselectivity in the production of L-alanine (up to 27% *ee*).

Crown ether functionalized unnatural amino acids have been introduced in peptide sequences by Voyer and coworkers (Figure 7).[32] Incorporation of six crown ether

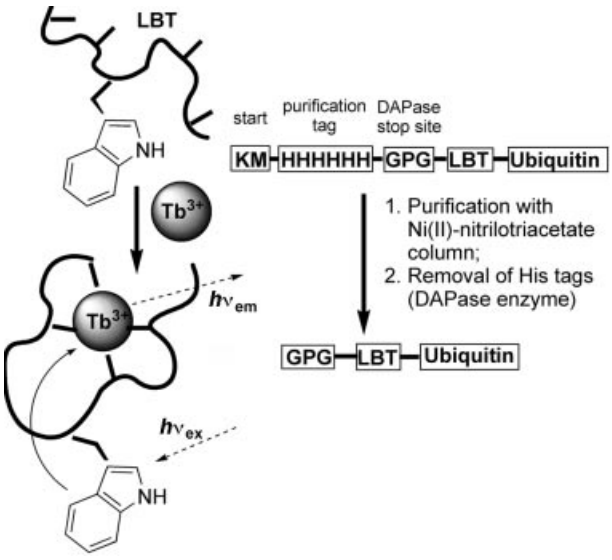

Figure 6. Imperiali's lanthanide binding tag for protein detection

functionalized amino acids in oligopeptides with a high propensity to form an α-helix resulted in the formation of artificial ion-channels.[32a,32d,32e] Small oligopeptides (eight amino acids) containing the crown ether amino acid were shown to enantioselectively recognize aromatic carboxylate and ammonium derivatives.[32d] Similar dipeptides were able to form pseudorotaxanes with thermoregulated optical properties.[32c] In this specific case the peptide binds to the diammonium derivative to form a "threaded" complex. The binding process leads to a conformational change of the oligopeptide from a partial helical to a β-turn structure. As reflected by changes in the CD spectrum, binding, and thus the conformational change in the peptide, is temperature dependent.

Our contribution to this area has been the synthesis of the artificial amino acid ATANP[33] and its incorporation into a wide variety of oligopeptides. ATANP is characterized by the presence of an appending 1,4,7-triazacyclononane, which forms 1:1 complexes with metal ions such as Cu^{II} and Zn^{II} with strong affinities. Since these metals play an essential role in the hydrolysis of DNA and RNA, our aim is to use this amino acid for the development of multinuclear transphosphorylation metallocatalysts. In collaboration with Baltzer's group a 42-mer peptide analogous to the one reported in Figure 1 was synthesized, with the difference that these new sequences incorporate up to four copies of ATANP (Figure 8).[34] These new peptides also form helix-loop-helix motifs and bind Zn^{II} ions with the triazacyclononane subunits present in the lateral arms of ATANP. We observed that metal complexation causes a decrease in the helical content of the peptide. However, even upon partial unfolding of the structure, an acceleration of the cleavage of HPNP (2-hydroxypropyl-*p*-nitrophenyl phosphate, an RNA model substrate) was observed. As will be shown in the next section, the incorporation of ATANP in more-structured peptide sequences controls the three-dimensional positioning of the metals even better and, consequently, significantly improves their catalytic activity.

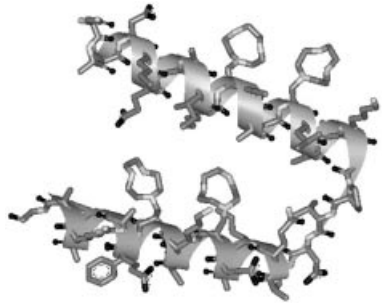

Figure 8. This 43-mer sequence incorporating four ATANP units is effective, as its Zn^{II} complex, in the cleavage of phosphate esters

Induction of a Well-Defined Secondary Structure Using Artificial Subunits

The ability to construct oligopeptides with a well-defined conformation is highly advantageous for the functional implementation of this class of molecules. Next, we will address two approaches, used by us and others, which allow a precise positioning of functional groups essential for molecular recognition and catalysis. The first is based on the use of metal ions that act as allosteric factors and control the organization and activity of multiple oligopeptide chains. Secondly, the use of unnatural C^{α}-tetrasubstituted

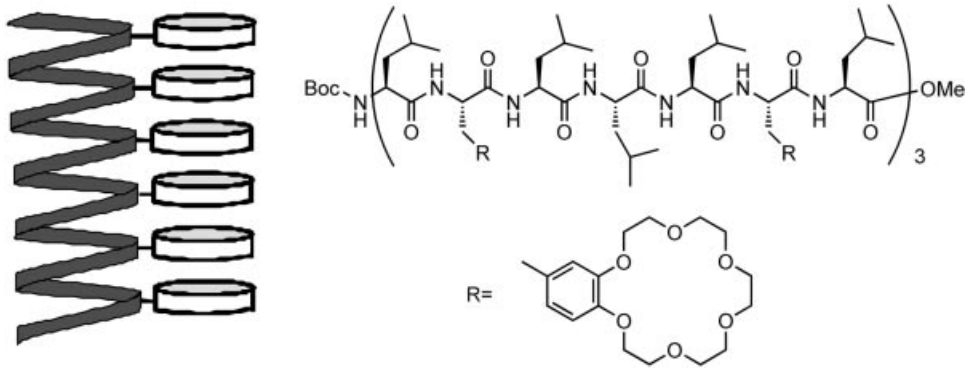

Figure 7. Peptides comprising crown-ether-incorporating amino acids may insert into membranes, thus affecting their permeability (as reported by Voyer)

amino acids to induce a specific conformation in oligopeptides will be discussed. Examples will be given of oligopeptides that contain different kinds of unnatural amino acids both to control the conformation and to introduce metal coordination sites.

The attachment of peptide sequences to conformationally flexible templates results in polypeptidic structures with the separate peptide chains adopting a random orientation with respect to each other. Of special interest are those templates whose conformation can be rigidified by an external stimulus, such as pH or the presence of a metal ion, since this may also induce an organization of the peptide chains. One example of such a template is the tetraamine tris(2-aminoethyl)amine (Tren) whose three arms are aligned upon complexation of a metal ion, such as Zn^{II}, in the tetradentate binding pocket. We have conjugated peptide chains to this template for the obtainment of new molecules with tunable properties. Thus, we have prepared systems that are able to affect the permeability of biological membranes, to induce the inhibition of enzyme activity, or to act as synthetic metallonucleases, and have studied the role of metal ions as allosteric affectors. For instance, three copies of peptide sequences from the peptaibol family, a class of natural antibiotics known to act on cell membranes through channel formation, were coupled to the Tren template. Leakage experiments of trapped fluorescent dyes from unilamellar vesicles showed that a minimum of five amino acid residues per peptide chain was required for the formation of an active species. More important, we observed that the tripodal apopeptide was far more effective than its Zn^{II} complex, and proposed a mechanism in which the Zn^{II} ion causes a change in the conformation from an extended to a globular one (Figure 9). Molecular modeling indicated that, in the extended form, the apopeptide is able to span the lipid bilayer, thus enabling pore formation by clustering. In this example, binding of Zn^{II} has an inhibitory effect on the activity of the peptide.[35]

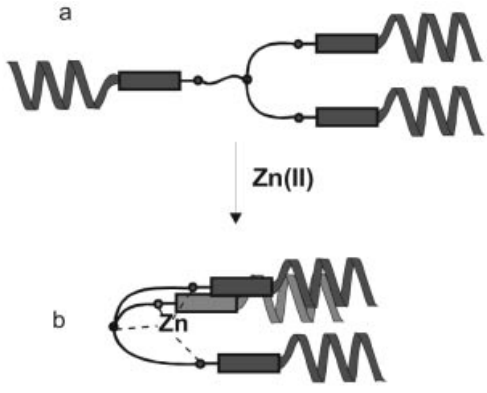

Figure 9. Conformational change of the peptide template upon Zn^{II} binding (a: extended, active; b: globular, inactive)

A completely opposite effect of Zn^{II} ions was observed when we prepared a putative inhibitor of HIV-1 protease.[36] HIV-1 protease[37] is a protein whose activity requires the mandatory dimerization of two identical subunits. Most of the driving force for the dimerization derives from an interdigitating N- and C-terminal, four-stranded, antiparallel β-sheet. HIV-1 protease plays a critical role in viral replication and, consequently, is a key target for the design of inhibitors as potential anti-HIV drugs. Inhibition may result from binding at the active site or, because of the dimeric nature of the protein, from binding at the dimerization interface, which leads to the disruption of the active dimeric species.[38] Work by the group of Chmielewski[39] has shown that peptides containing similar sequences as those present at the N- and C-termini of each monomer of HIV-1 protease, and cross-linked by a C_{14}-hydrocarbon spacer, display significant antiviral activity. We reasoned that the reversible control of the distance between the two tethered peptides in such an inhibitor would lead to the modulation of its activity. For this purpose we connected two peptides (analogous to those reported by Chmielewski et al.) by a rigid aromatic spacer to the Tren template. Molecular modeling predicted that the distance between the two N-termini would be close to 10 Å (presumably required for optimum activity) only in the presence of this spacer and when a Zn^{II} ion was complexed by the Tren template (Figure 10). In the design of the system the third arm, which is necessary to impart rigidity to the Zn^{II} complex but is not used for enzyme recognition, was functionalized with a naphthalene unit in order to have a fluorescent readout for the protonation and/or Zn^{II} complexation of the Tren template. We were able to verify our hypothesis by using a fluorogenic substrate assay, which revealed an enhancement of the inhibition of HIV-1 protease activity upon the addition of Zn^{II}. Control experiments revealed that the addition of EDTA (ethylenediaminetetraacetate) reversed this effect, because of depletion of the Zn^{II} ion from the Tren template.

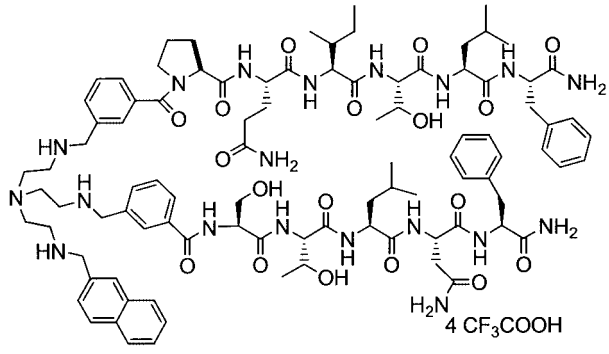

Figure 10. Peptide template used by us in HIV-1 protease inhibition; the most active species is the Zn^{II} complex

Kawai et al.[40] have used the cyclic decapeptide Gramicidin S [cyclo(-Val-Orn-Leu-(D)Phe-Pro)$_2$], which adopts an antiparallel β-sheet conformation, as a rigid template. The ornithine residues were functionalized with bis(2-pyridylmethyl) groups. The Zn^{II} dinuclear complex proved to be a good catalyst for the cleavage of 2-hydroxypropyl-*p*-nitrophenyl phosphate (HPNP). The suggested mechanism requires the cooperativity between the two metal centers, whose relative positions are partly controlled by the rigid cyclopeptide scaffold.

A very appealing method for the construction of very short, but highly organized peptides is the use of C^{α}-tetrasubstituted amino acids. Research by the group of Toniolo[41] has demonstrated that most of the C^{α}-tetrasubstituted amino acids, and in particular α-aminoisobutyric acid (Aib), are very strong promotors of the 3_{10}/α-helical conformations. The main factors that determine the type of helix formed are the main-chain length, the fraction of Aib, and the amino acid sequence. Although the formation of a stable 3_{10}-helix is quantitative in low polarity solvents, in polar solvents like water folding generally results in mixed 3_{10}/α-helices. Recently, we were able to synthesize a heptapeptide containing five Aib residues that prevailingly adopts a 3_{10}-helical conformation also in aqueous solution.[42] For the remaining positions ATANP (vide supra) was used, which imparts a very high water-solubility on the structure. The 3_{10}-helical conformation in water was confirmed by CD and NMR spectroscopy.

Interestingly, we were able to show for a different heptapeptide that the α/3_{10}-helix correlates quantitatively with the solvent polarity. In polar solvents, including water, this heptapeptide prevails as the α-helix, while in less-polar solvents, such as 2-propanol, the conformation is that of a 3_{10}-helix. A possible driving force for the control of the conformation could be the solvation requirements of the apolar substituents in the lateral arms of the amino acids: in the α-helix the peptide is shorter and the distance between the substituents is smaller, making them less exposed to the surrounding medium. The opposite is true when the peptide adopts a 3_{10}-helical conformation. Because of this change in elongation (the helical pitch is about 5.6 and 6.2 Å for the α- and 3_{10}-helix, respectively) the molecule can be regarded as a solvent-driven molecular spring (Figure 11).[43]

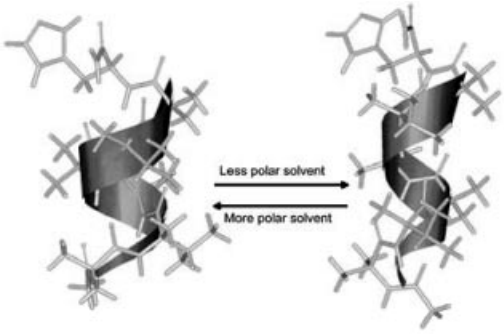

Figure 11. Heptapeptide shifting from an α- to a 3_{10}-helix according to the polarity of the solvent

The helical scaffold of 3_{10} helical sequences may be used to place appropriate functional groups in the side arms of the constituent amino acids at a precise reciprocal distance. For instance, when placed in positions *i* and *i*+3, they will face each other with a separation of about 6.2 Å, which is the pitch of the 3_{10}-helix (vide supra). A good example is the heptapeptide Ac-Aib-L-ATANP-$(Aib)_2$-L-ATANP-$(Aib)_2$-OMe, which folds into a 3_{10}-helix in water. The two ATANP residues not only provide water solubility, but can also form strong complexes with transition metals such as Zn^{II} and Cu^{II}.[42,44] The dinuclear Zn^{II} complex turned out to be a good catalyst for the cleavage of both an RNA model substrate (HPNP)[45a] and plasmid DNA.[45b] In the latter case, cooperative action between the metal centers was demonstrated by comparison of the activity of the mononuclear and the dinuclear complex. A tentative mechanism was proposed which requires the formation of a supramolecular DNA–peptide complex, as shown in Figure 12. The k_{ψ} for the cleavage process (approx. 1×10^{-5} s^{-1}) allows the estimation of a rate acceleration of about ten million times with respect to the uncatalyzed cleavage process.

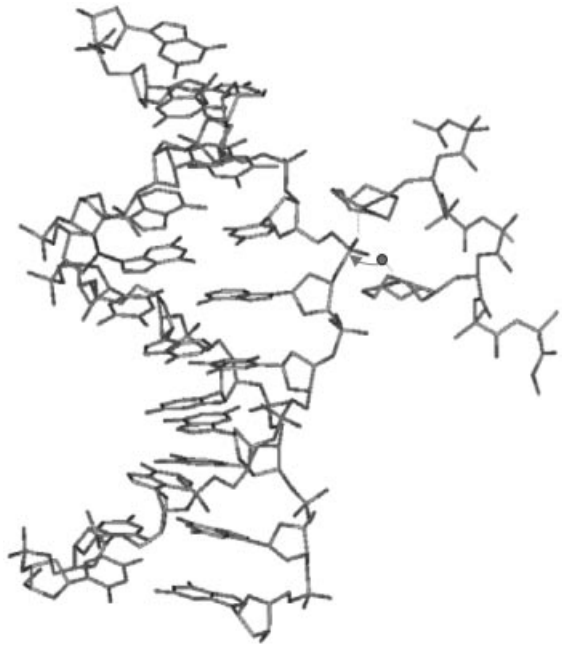

Figure 12. Proposed mechanism for the cleavage of DNA by the dinuclear Zn^{II} complex of the heptapeptide reported by us and incorporating two ATANP units

Finally, we will give an example of an artificial multinuclear metallonuclease that combines all the structural elements present in the previous examples. A tripodal apopeptide was synthesized by connecting three copies of the heptapeptide H-Iva-Api-Iva-ATANP-Iva-Api-Iva-$NHCH_3$ [where Iva is (*S*)-isovaline and Api is 4-amino-4-carboxypiperidine] to the Tren template. The oligopeptides contain C^{α}-tetrasubstituted amino acids to induce helicity and ATANP to introduce a ligand for metal binding. The apopeptide is able to bind up to four metal ions (Cu^{II} or Zn^{II}): one in the Tren subsite and three in the azacyclononane subunits.[46] In analogy with what has been observed for the previous examples, the binding of metals to the Tren platform induces a change from an open to a closed conformation in which the three helical peptides are aligned in a parallel manner, thus creating a pseudo-cavity with the azacyclononane units pointing inward (Figure 13). However, at variance with the previous systems this tripodal template shows a very peculiar behavior in the transphosphorylation of phosphate esters. The tetrazinc complex catalyzes the cleavage of HPNP, while the free ligand is a catalyst for the cleavage of an oligomeric RNA sequence with a selectivity

for pyrimidine bases. In the case of HPNP, Zn^{II} acts as a positive allosteric effector by enhancing the catalytic efficiency of the system. In the case of the polyanionic RNA substrate, Zn^{II} switches off the activity, thus behaving as a negative allosteric regulator. Our explanation for this different behavior is that the small cavity formed upon metal–ligand coordination is able to accommodate HPNP, but not the larger RNA oligomer. Consequently, the tetranuclear complex is a catalyst for the cleavage of HPNP, but not for the cleavage of the oligonucleotide.

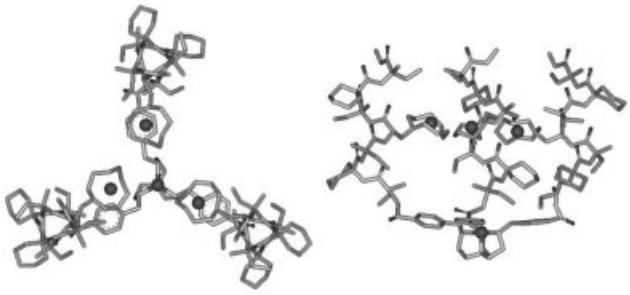

Figure 13. Tripodal trispeptide template subject to allosteric control by Zn^{II} ions and active in the cleavage of phosphate esters

Concluding Remarks

In conclusion, the selected examples discussed here illustrate the significant progress made in synthesizing short oligopeptides with a well-defined secondary structure, and, more importantly, with (artificial) functional groups in key positions. Although nature remains the champion to be beaten, artificial model systems have shown impressive efficiencies in mimicking their biological counterparts. In addition, they have revealed valuable mechanistic insights in these processes. In this respect, the fact that chemists are not restricted to the natural set of amino acids is of great help and appears to be of fundamental importance for designing peptide structures that have a function not present in natural systems.

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