Bioinspired polymeric materials: in-between proteins and plastics

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Chemical and biological researchers are making rapid progress in the design and synthesis of non-natural oligomers and polymers that emulate the properties of natural proteins. Whereas molecular biologists are exploring biosynthetic routes to non-natural proteins with controlled material properties, synthetic polymer chemists are developing bioinspired materials with well-defined chemical and physical properties that function or self-organize according to defined molecular architectures. Bioorganic chemists, on the other hand, are developing several new classes of non-natural oligomers that are bridging the gap between molecular biology and polymer chemistry. These synthetic oligomers have both sidechain and length specificity, and, in some cases, demonstrate capability for folding, self-assembly, and specific biorecognition. Continued active exploration of diverse backbone and sidechain chemistries and connectivities in bioinspired oligomers will offer the potential for self-organized materials with greater chemical diversity and biostability than natural peptides. Taken together, advances in molecular bioengineering, polymer chemistry, and bioorganic chemistry are converging towards the creation of useful bioinspired materials with defined molecular properties.

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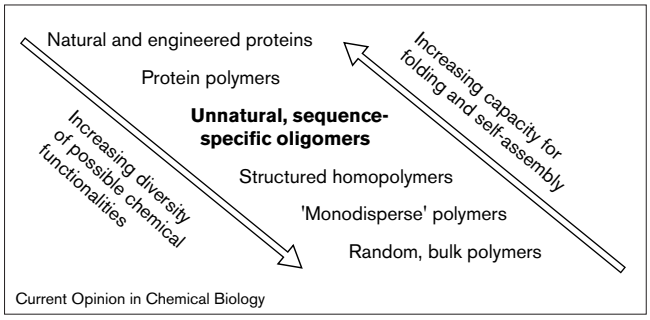
Abbreviation
CD circular dichroism

Introduction

Information encoded in the sequences of natural proteins is sufficient to drive the adoption of complex three-dimensional architectures. Although they are based upon unique linear arrangements of just 20 different monomers, folded proteins nonetheless achieve a tremendous breadth of physical and chemical activities, ranging from exquisitely specific room-temperature catalysis to the formation of unusually strong and tough biomaterials such as collagen and spider silk. Active, folded proteins are typically challenging to produce in commodity amounts. By contrast, man-made polymers are typified by random monomer arrangements and broad molecular-weight distributions, and can be manufactured in bulk at low cost with a wide diversity of backbone and sidechain chemistries and high molecular weights. Without precise control over sequence and chain length, however, complex folded architectures cannot be designed.

Figure 1



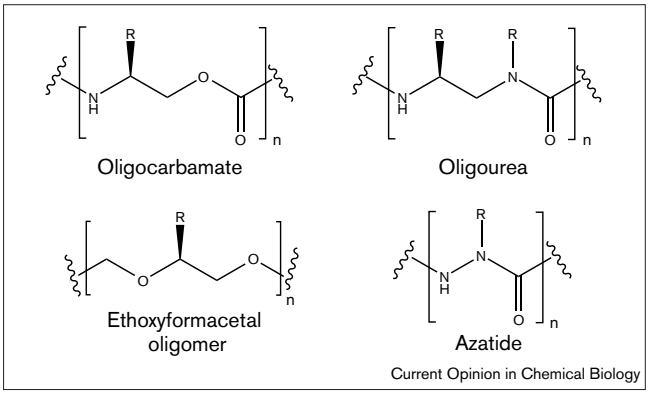
The spectrum of polymeric materials, ranging from proteins that are sequence-specific and monodisperse, to polymers that have random sequences and are polydisperse. Non-natural, sequence-specific oligomers represent a new 'middle ground' between these extremes.

As shown schematically in Figure 1, polymeric materials can be considered to fall along a 'spectrum' of increasing chemical diversity on one hand (greatest for man-made polymers), and increasing capacity for adoption of secondary and tertiary structures on the other (greatest for biological polymers). Molecular biologists, polymer chemists, and bioorganic chemists are taking different approaches to the development of new materials with controlled properties intermediate between those of biological heteropolymers (proteins) and man-made homopolymers (plastics). In this review, we discuss some converging advances in these fields that promise an improved class of non-natural, bioinspired polymers that can effectively mimic protein structures, activities, and/or material properties, and yet can be obtained at lower cost and with greater chemical diversity and biological stability. We give special attention to the progress of synthetic bioorganic chemists toward the creation of folded, sequence-specific oligomers with novel backbone and sidechain chemistries.

Molecular bioengineering

Molecular bioengineers are working to design and produce proteins with polymer-like properties, exploiting the biosynthetic machinery of microorganisms to produce non-natural 'protein polymers' with defined structural and folding propensities. This can be achieved through bacterial expression of synthetic genes produced by end-to-end enzymatic linking (concatemerization) of synthetic oligonucleotides, yielding repetitive protein polymers [1]. This approach was first taken by Ferrari and Cappello (see [2] and references therein) to produce novel silk-like materials. An advantage of the biosynthetic approach is that gram quantities of monodisperse, sequence-controlled polypeptides comprising more than 250 monomers (with a typical oligomeric repeat being

Figure 2



Examples of non-natural oligomers composed of a specific sequence of diverse monomers. These are primarily of interest for combinatorial drug discovery efforts.

20–25 amino acids) are produced at low cost. These methods have been used recently to produce protein polymer mimics of spider silk [3,4] and elastin [5,6], as well as protein-based hydrogels with tunable properties [7•]. Repetitive heteropolymers are primarily of interest for their properties as biomimetic and biocompatible materials [8], rather than as folded protein catalysts.

There are limitations to using the unmodified biosynthetic apparatus for the production of non-natural protein polymers. For reasons that are often not well understood, some non-natural genes are incompatible with high-level expression in *Escherichia coli* (e.g. pure homopolypeptides are difficult to express) [2]. Furthermore, unless special provisions are made, protein polymers are restricted to the 20 natural amino acids. To expand the range of properties achievable in protein-based materials, Tirrell and co-workers [9•,10] have developed methods to incorporate non-natural amino acid analogs with structural similarity to natural amino acids. This strategy relies upon mutant bacterial strains unable to synthesize a particular amino acid. The bacteria can then be fed the synthetic amino acid analog of interest. Recently, Van Hest and Tirrell [9•] have selectively introduced analogs with allyl functionalities, complementing previous introductions of non-natural sidechains that are selenated, fluorinated, electroactive, conformationally constrained, and olefinic [10]. In a different approach, Schultz and co-workers (see [11••]) have worked to modify the biosynthetic machinery of *E. coli* to include an additional, engineered codon that will enable *in vivo* synthesis of proteins containing non-natural amino acids. Recently, Liu and Schultz [11••] have succeeded in synthesizing a non-natural tRNA and aminoacyl–tRNA synthetase pair orthogonal to any existing natural pairs. The development of novel technologies for the introduction of non-natural amino acids into proteins and protein polymers will expand the range of useful and interesting molecules that can be obtained by harnessing biosynthesis.

Polymer chemistry

Whereas molecular biologists work to increase diversity in biosynthesis, polymer chemists are developing polymers with more protein-like properties. In particular, they strive to narrow polymer molecular-weight distributions, control monomer sequence, and develop functionalized polymers with well-defined molecular architectures and conformations.

In order to control molecular weight and to some degree the monomer sequence, polymer chemists have developed the method of 'living' polymerization [12]. With this method, chain initiation events are well-controlled, while chain-transfer and chain-termination reactions are suppressed. Under ideal living polymerization conditions, polymer chains grow at a uniform rate until the supply of monomer is exhausted, yielding relatively narrow molecular weight ranges (though distributions are still quite broad compared to natural proteins) [12]. Different 'blocks' of a given monomer can be added sequentially, with the average block length controlled by the amount of monomer that is added. Although much of this work has been done with hydrophobic polymers, the technique is now being used for protein mimicry. Recently, Deming [13,14•] has found the first clean route to the synthesis of high molecular weight block copolypeptides with relatively tight molecular weight distributions, employing living, ring-opening polymerization reactions with improved catalysts. His group is presently investigating block copolypeptides for their ability to mimic mussel adhesive proteins, which form an underwater 'glue' with properties that promise to make them useful as surgical adhesives [15].

Polymer scientists are also seeking to mimic natural proteins by incorporating protein-like secondary structural elements. Helical polymers have been designed by introducing chirality into monomer sidechains [16]. In particular, Green and co-workers [17,18] have used circular dichroism (CD) spectroscopy to show that the achiral backbone of polyalkyl isocyanates can respond cooperatively to the presence of a small fraction of chiral sidechains, forming populations of conformational isomers with an easily measurable excess of one helical sense. Maeda and Okamoto [19] have additionally shown that polyphenyl isocyanates respond sensitively and cooperatively to chiral information encoded at sidechain positions quite distal to the backbone.

Polymer chemists are mimicking another fundamental property of proteins, namely their self-organization into objects of discrete shape and size. In one approach, living polymerization has been used to create low molecular weight, self-assembling oligomers with tri-block architectures [20•]. The self-assembling oligomers themselves comprise fewer than 30 monomers (in three different blocks ranging from 8–12 residues each), and associate to form discrete 'mushroom' structures that then further assemble into highly ordered, supramolecular arrays.

In a second route to discrete architecture formation, polymer backbones are hyper-branched in a highly controlled fashion to generate dendrimeric structures [21]. These spherical dendrimers are synthesized one 'shell' at a time, enabling the controlled display of multiple, identical bioactive chemical moieties on the surface [22]. Particular dendrimer designs have been demonstrated that inhibit viral adhesion [23•] and transport DNA into mammalian cells [24].

Bioorganic chemistry

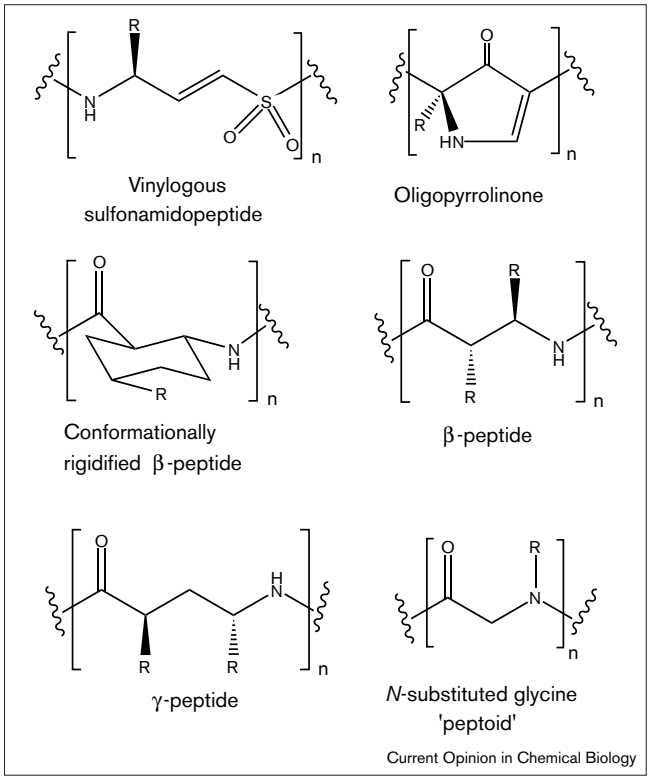
In contrast to polymer chemists, who accept some degree of randomness and polydispersity in order to access diversity and high molecular weights, bioorganic chemists keep tight control over sequence and chain length with iterative syntheses in which monomers are added one at a time. The price of this control is that high degrees of polymerization are not achieved in acceptable yield. Solid-phase methods allow iterative syntheses to be performed efficiently, and in the case of synthetic peptides and nucleic acids, solid-phase synthesis enables routine and automated preparation of sequence-specific polymers of 75–100 residues. However, most proteins and useful polymeric materials are comprised of chains much longer than 100 monomer units, so the iterative *de novo* syntheses of true protein and polymer analogs is currently not feasible. This limitation can be surpassed for relatively small proteins by chemical ligation of peptide fragments that are synthetically [25] or biosynthetically [26••] produced (see Kochendoerfer and Kent, in this issue, pp 665–671). Recently developed ligation methods allow site-specific introduction of non-natural amino acids [27,28], and coupling of unprotected peptides directly from a solid support [29,30].

Synthetic methods for producing non-natural protein mimics are at a much earlier stage of development than peptide and oligonucleotide synthetic methods, and are under active investigation. Although much synthetic work has been done to produce conformationally — or 'solvophobically' — structured non-sequence-specific homo-oligomers (recently reviewed in [31]), we focus on non-natural oligomers that have specific sequences of diverse sidechains (Figures 2 and 3).

Unstructured sequence-specific oligomers

Several families of sequence-specific oligomers have been developed for combinatorial drug discovery (Figure 2). Schultz and co-workers [32] used solid-phase methods to synthesize a library of oligocarbamates with a variety of sidechains, and screened for binding to a monoclonal antibody that was raised with a related peptide. A number of ligands with IC_{50} values of 60–180 nM were discovered in this manner. Wang, Huq and Rana [33] found that biomimetic oligocarbamates can bind specifically to HIV trans-activation mRNA (TAR), a 59-base stem-loop structure located at the 5′ end of the nascent HIV-1 transcripts. In recent work, cyclic and acyclic oligocarbamate libraries based on 27 diverse monomers were synthesized and screened for

Figure 3



Examples of non-natural oligomers composed of a specific sequence of diverse monomers, which additionally have been shown to exhibit stable secondary structures in solution.

binding to integrin GPIIb/IIIa [34]. Two cyclic trimeric and tetrameric ligands had activities within a factor of 3 of kistrin, a 68-residue snake venom protein that effectively inhibits platelet aggregation [34], a problem in patients with arterial thrombotic diseases. Nanomolar inhibitors of α-adrenergic and opiate receptors (which modulate arterial blood pressure and pain response, respectively) have also been discovered from a diverse, 5000-member, combinatorial oligo-*N*-substituted glycine (peptoid) library (Figure 3) [35].

Oligoureas are another class of non-natural oligomers that are of interest for drug discovery because they can be made with a diversity of sidechains; two different solid-phase routes to these molecules have been described [36,37]. Tamilarasu, Huq and Rana [38] have made a biomimetic oligourea decamer and shown that it binds specifically to its intended RNA target. Another family of sequence-specific oligomers are the azatides [39]. Azatide pentamers have been made with inclusion of five different *N*-pendant sidechains. One such pentamer was synthesized with a biomimetic sequence mimicking leucine–enkephalin; its lack of binding activity was attributed to differences in allowed backbone conformations in comparison to the natural peptide [39]. Hall and Schultz [40] have investigated the ability of sequence-controlled oligoethers to specifically bind metal ions.

Three ethoxyformacetal tetramers have been prepared, incorporating four different chiral sidechains.

Structured sequence-specific oligomers

By introducing a variety of structure-inducing elements into the constituent monomers, several groups have identified oligomers that adopt defined secondary structures (Figure 3). Chiral vinylogous aminosulfonic acids are structured peptide mimics with an extended non-natural backbone that carries a strong negative charge. Gennari *et al.* [41] have synthesized vinylogous sulfonamidopeptides up to four residues in length, incorporating specific sequences of chiral sidechains. Conformational studies of oligomers in both the solution and solid phase provide evidence of an ensemble of structures predominated by hydrogen-bonded rings.

Oligopyrrolinones (Figure 3) have a stiffened backbone that incorporates 5-membered rings. Solution-phase methods have been employed to synthesize sequence-specific pentamers with a limited alphabet of proteinogenic sidechains [42,43]. Short oligopyrrolinones adopt defined conformations. X-ray crystallography has shown that the oligopyrrolinone imino group can form intramolecular hydrogen bonds with the carbonyl group of an adjacent five-membered ring to give a structure that mimics β-strands, or can form intermolecular hydrogen bonds with the carbonyl on another oligomer to mimic a β-sheet [43]. *N*-methylated 3,5-linked pyrrolin-4-ones have been shown to adopt a novel helix in solution and in the solid phase, as predicted by molecular modeling [43].

Another family of structured, sequence-specific oligomers under active investigation for their ability to mimic natural proteins are the β-peptides (Figure 3), which have a backbone differing from normal peptides by the presence of an additional methylene unit. Gellman and co-workers [44,45,46••,47] taken the approach of reducing the number of allowed backbone conformations of this extended peptide by including cyclopentane and cyclohexane rings in the backbone (producing conformationally rigidified β-peptides; Figure 3) [44]. Oligomers of these molecules made by solution-phase methods have been found to form two novel types of hydrogen-bonded helical structures, in either aqueous or organic solvents. Structures of these helices in both solvent systems have been solved by two-dimensional NMR [45,46••], while organosoluble structures were recently determined by crystallography [47].

Seebach and co-workers [48–54,55•] have generated a family of enantiopure β-amino-acid monomers by homologation of the cognate α-amino acids [48,49]. This has facilitated the synthesis of β-peptides up to 12 monomers in length with incorporation of a variety of proteinogenic sidechains at either or both of the backbone methylene carbons [50,51]. Short β-peptides form a variety of stable hydrogen-bonded secondary structures in solution, including novel helices, pleated sheets, and turns [52,53]. Different secondary structures are generated by positioning sidechains on either α or β carbons, or by cyclization [54]. Recently, cyclic, structured β-peptide tetramers have been found to bind with micromolar affinities to human somatostatin receptors [55•]. Hence, they have some ability to mimic somatostatin, an endogenous peptide that plays important physiological roles as a neurotransmitter and as an inhibitor of hormone secretion.

The Seebach and Hanessian groups have recently found stable secondary structures in γ-peptide oligomers with specific sidechain sequences ([56•,57••]; Figure 3). These molecules have two additional backbone methylene units, in comparison with natural peptides, and hence allow sidechain substitution of two different positions per monomer unit. Reverse-turn and right-handed helical structures have been determined by two-dimensional NMR in two different solvent systems [58]. The helices appear to have greater conformational stabilities than either α-peptide or β-peptide helices [57••], which might not have been predicted given the highly flexible nature of the extended γ-peptide backbone. The structures are stabilized by amide proton-to-carbonyl hydrogen bonds between neighboring residues.

N-substituted glycines (peptoids; Figure 3) are presently unique among structured, sequence-specific, non-natural oligomers in that their convenient, automated synthesis can be achieved up to lengths of at least 48 monomers. Peptoids containing a diversity of alkyl, aromatic, heterocyclic, cationic, and anionic *N*-substituents have been synthesized and characterized [59••]. Although these molecules are structurally similar to α-amino-acid polymers, their backbone lacks both chiral centers and hydrogen bond donors. As for polyalkyl isocynates [17], however, the inclusion of chiral sidechains is sufficient to drive peptoids into stable, chiral helices [59••]. Oligomers as short as five residues form helical structures in organic solvents, as demonstrated by two-dimensional NMR [60]. A variety of peptoid sequences exhibit intense CD spectra, in both aqueous and organic solvents, that resemble those of peptide α-helices [59••]. Robotic peptoid synthesis efficiently generates diverse combinatorial libraries, allowing the screening of multiple sequences for a desired structure or activity. For example, 36mer cationic peptoid sequences that facilitate the delivery of DNA to cells have been discovered from a combinatorial library [61].

Conclusions

Several different areas of research are converging on the development of a new class of bioinspired materials that capture the advantages of both protein and polymer systems. While polymer chemists and molecular bioengineers push the limits of their synthetic methods, a new field in bioorganic chemistry has emerged between these disciplines. This field is still in its infancy; however, researchers have made remarkable advances. Although surprisingly short oligomers can adopt stable secondary structures and

exhibit potent biological activities, longer sequence-specific chains can also be efficiently synthesized and provide access to the realm of proteins and polymers. Continued interdisciplinary progress will enable scientists to exercise an unprecedented degree of control over the structures of polymeric materials.

Note added in proof

Two papers that describe new sequence-specific oligomer systems have recently been published [62,63]. Oligomers of chiral α-aminoxy acids up to six residues in length have been synthesized bearing a variety of aliphatic sidechains [62]. These oligomers are shown to form intramolecular hydrogen bonds that stabilize a novel helical structure. Another group has used a submonomer synthesis approach to generate a variety of trimeric hydrazinoazopeptoids [63]. These achiral oligomers were synthesized in solution using bromoacetyl bromide and a substituted hydrazine.

Acknowledgement

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