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Bioinspired Modular Synthesis of Elastin-Mimic Polymers To Probe the Mechanism of Elastin Elasticity

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Elastin is an important extracellular matrix protein conferring elasticity to tissues and organs.[1] The native elastin isolated from animal tissues has two domains, the repeating hydrophobic domain and the alanine-rich cross-linking region.[1] It is widely accepted that the hydrophobic domain contributes to the elasticity of elastin.[2] Because of its remarkable elasticity[1] and stimuli-responsive properties,[3] recently elastin and elastin-like polypeptides (ELPs) have been actively pursued as biomaterials for various biomedical applications[4] including protein purification,[4] drug delivery,[5] and tissue engineering.[6] For many applications, it is critical to develop efficient and scalable synthesis for ELPs.[4,7,8] Herein we report a bioinspired modular synthesis of elastin-mimic polymers (EMPs) via "click" polymerization (Figure 1).

Taking advantage of the versatility of our modular synthesis, we further proposed to permute the peptide sequence of the module to probe the mechanism of elasticity of elastin (Figure 1). Despite many elegant studies, the mechanism of elastin elasticity remains a subject of scientific debate with respect to the origin of elastomeric storing force.[2,9] Previous mechanistic studies of elastin elasticity primarily focused on direct investigation of native elastin structures or protein-based polypeptides by employing various physiochemical methods.[9] With the flexibility of our modular synthesis, we envisioned a different approach to gain mechanistic understanding through structural permutation and structure−property correlation.

A number of mechanisms have been proposed to account for elastin elasticity. First, the "random" model[9a] treats elastin as a random coil polymer with simple entropic elasticity like classical rubbers. However, this model could not explain why elastin only shows elasticity in its hydrated form. Second, the two-phase models, including "liquid-drop"[9b] and "oiled-coil" models,[9c] propose that elastin is composed of hydrophobic domains of protein with aqueous diluents confined between them. However, this model is not consistent with the highly dynamic nature of elastin chains.[10] Finally, Urry et al. proposed a molecular model in which the elasticity of elastin is attributed to a special "β-spiral" secondary structure formed as a continuous coil of type II β-turns.[11] Nevertheless, a number of studies have indicated that this model has significantly overestimated the content of β-turns and long-range order of elastin.[12]

Given the highly dynamic nature[10] and lack of long-range order[12] in elastin, we hypothesize that it may not be critical to have a continuous peptide sequence in the bioinspired design of EMPs. Therefore, we propose a bioinspired modular synthesis of EMPs in which short elastin repeating peptides (elastic motifs) are efficiently polymerized by "click" chemistry (Figure 1). Specifically, we design three EMPs, **P-1**, **P-2** and **P-3**, inspired by one of the most abundant repetitive sequences of elastin hydrophobic domain, $(VPGVG)_n$ (Figure 1).[2] The three polymers share an identical chemical composition but have different peptide sequences or D/L-proline stereochemistry. In **P-1**, the pentapeptide in the repeat unit is identical to the canonical elastic module, VPGVG, and is expected

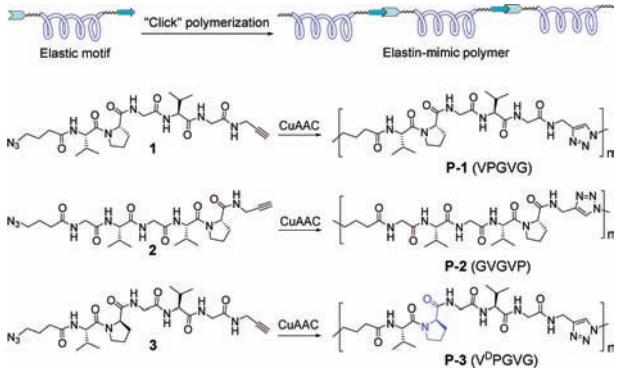

Figure 1. Bioinspired modular synthesis of elastin-mimic polymers via "click" chemistry (top). Structures of pentapeptide monomers **1** (VPGVG), **2** (GVGVP), and **3** (V^{D}PGVG) with the corresponding elastin-mimic polymers **P-1**, **P-2**, and **P-3** (bottom).

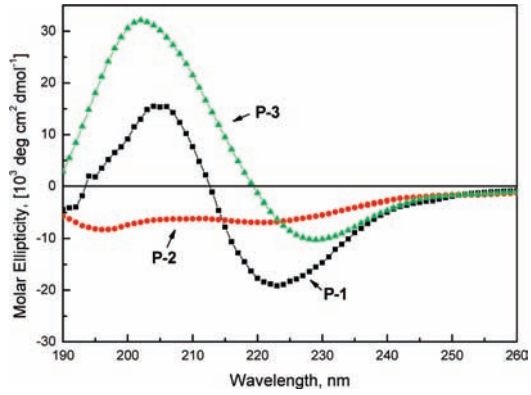

Figure 2. CD spectra of elastin-mimic polymers in trifluoroethanol (TFE) at 25 °C: **P-1**, black; **P-2**, red; **P-3**, green.

to adopt similar local conformation (type II β-turns) as the natural sequence. In **P-2**, the pentapeptide in the repeat unit is scrambled to GVGVP. Based on previous studies, the VPG triad is the core of the β-turn conformation.[11a,b] By disrupting the VPG triad sequence, the local β-turn conformation should be perturbed. In **P-3**, the D-proline residues are chosen to replace the L-proline residues in **P-1**. It is well-known that D-proline can be used as a turn-inducing moiety in peptides.[13] The peptide sequence with D-proline has the tendency to form a type II′ β-turn which results from the restricted φ ($+60° \pm 20°$) of the D-proline residue.[14]

We choose Cu-catalyzed alkyne azide cyclization (CuAAC) chemistry for polymerization because of its high efficiency and excellent tolerance of functional groups.[15] The pentapeptides were capped with azide and alkyne terminals, which enables us to efficiently make peptide polymers via CuAAC chemistry (Figure 1 and see Supporting Information for the procedure to synthesize the monomers and polymers). The click polymerization was very

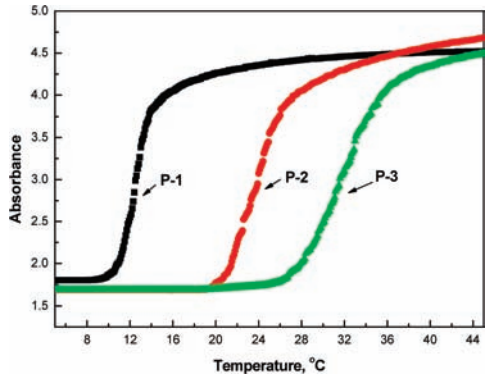

Figure 3. LCSTs of elastin-mimic polymers at a concentration of 3.0 mg/mL in water: **P-1**, black; **P-2**, red; **P-3**, green.

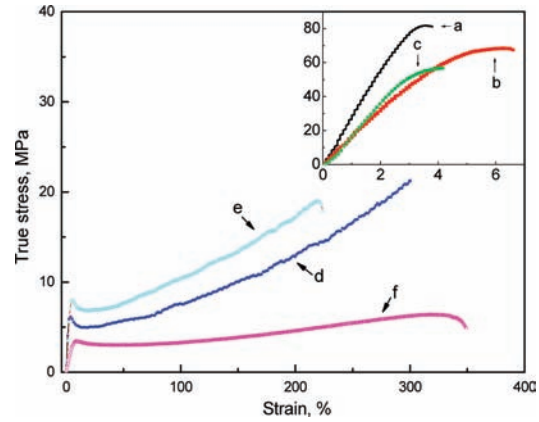

Figure 4. True stress−strain curves for the elastin-mimic polymers in dry and hydrated forms. The inset at the upper-right corner shows the curves for dry films: (a) black, dry **P-1**; (b) red, dry **P-2**; (c) green, dry **P-3**. For hydrated films: (d) blue, **P-1** with 13% water; (e) cyan, **P-2** with 13% water; (f) magenta, **P-3** with 13% water. (Percentage of water is defined by the weight of absorbed water to the weight of the dry film).

efficient, affording polymers in high yields (93%-96%). As measured by GPC using PEG standards, the polymers are of high molecular weight with $M_n = 43$, 26, and 21 kDa, for **P-1**, **P-2**, and **P-3**, respectively. The chemical structures of the polymers were confirmed by ^{1}H and ^{13}C NMR.

To gain information of local secondary structures of the polymers, circular dichroism (CD) spectra are collected for the three polymers (Figure 2) and compared with the CD spectrum of polypentapeptide $(VPGVG)_n$ reported in literature, which shows a positive $\pi-\pi^*$ band at 206 nm and negative $n-\pi^*$ band at 224 nm in trifluoroethanol (TFE) solution.[16] Polymer **P-1** shows a very similar CD spectrum to that of the native polypentapeptide $(VPGVG)_n$, implying that polymer **P-1** has similar local secondary structures to natural $(VPGVG)_n$. Thus the introduction of the triazole ring apparently does not interrupt the inherent local conformation of the VPGVG pentapeptide. This is not surprising because both molecular dynamic simulation and the proposed β-spiral model indicate that each repeat unit forms a β-turn independently with no specific interaction between adjacent repeat units.[11] Therefore, individual VPGVG pentapeptide units in **P-1** can still adopt a similar conformation as in the natural system. In contrast, scrambling the pentapeptide sequence to GVGVP results in a dramatic change in the CD spectrum for polymer **P-2**. The CD spectrum for **P-2** is noncharacteristic with the negative peak at ~195 nm suggesting a random coil conformation in solution.[17] This agrees with our hypothesis that breaking the VPG triad core should disrupt the β-turn conformation for the pentapeptide. A noticeable change is also seen in the spectrum for polymer **P-3** compared to **P-1**, but the curve for **P-3** still keeps the feature of a β-type secondary structure.

One important characteristic of elastin and ELPs is their temperature responsive behavior in aqueous solution. For example, polypentapeptide $(VPGVG)_n$ ($n = \sim 200$) has an LCST of ca. 25 °C at a concentration of ~5 mg/mL in water.[18] Since both the LCST behavior and elasticity of elastin share the same entropic driving force from interactions of water molecules with the hydrophobic side chains,[12] it would be important to find out if the bioinspired polymers preserve the LCST behavior in water. We measured the temperature responsive behavior for the EMPs in water (Figure 3); excitingly, all polymers show elastin-like LCST behavior with transition temperatures of 12, 24, and 32 °C for **P-1**, **P-2**, and **P-3**, respectively. The LCST behavior for EMPs is due to temperature-dependent water hydration of hydrophobic side chains. Below LCST, water molecules form ordered clathrate-like structures around the hydrophobic side chains. Above LCST, expulsion of water molecules associated with the hydrophobic side chains is thermodynamically favorable due to entropic gain, leading to hydrophobic collapse and precipitation in a process analogous to that for other ELPs.[3,12b] Apparently, the introduction of triazole linkages onto the peptide polymer backbone does not destroy the LCST behavior. Compared to polypentapeptide $(VPGVG)_n$, the slight decrease in LCST transition temperature for **P-1** and **P-2** can be attributed to the increased hydrophobicity due to the nonpeptido linkages. The difference in LCST transition temperature for **P-1**, **P-2**, and **P-3** suggests that the local peptide conformation can perturb their interactions with water molecules.

One of the most important properties of elastin is its excellent elasticity for fulfilling its biological functions.[1] Despite great efforts to elucidate the link between structure and property for elastin,[9,11] the molecular mechanism for its elasticity remains controversial. One major disagreement is about the contribution of the specific peptide secondary structure to the macroscopic elasticity. Another important factor is the role that water molecules play in the elasticity. Recently, a number of new studies, including NMR spectroscopy,[12a,19] molecular dynamics simulations,[12c] and single molecule force spectroscopy,[20] have revealed the significance of "hydrophobic hydration" for elastin elasticity.[9d] Indeed, dehydrated elastin has the properties of a brittle polymer, while the hydrated form is highly elastic, pointing to the profound importance of water to elastin's mechanical response.[1b]

With our EMPs sharing identical chemical composition but having subtle differences in secondary structures, we reason that investigation of their bulk mechanical properties should provide useful insight to the origin of elasticity of elastin. Particularly, we want to examine the importance of two factors, i.e., peptide local conformation and hydration, on the polymer elasticity. For this purpose, films were cast from the solution in methanol for **P-1**, **P-2**, and **P-3** and their tensile mechanical properties were tested in both dry and hydrated forms. The dry films were immediately subjected to mechanical testing after vacuum drying, while the hydrated films were prepared by equilibrating the films in a closed chamber saturated with water vapor for a controlled period before testing immediately. Figure 4 shows the true stress−strain curves of the elastin-mimic polymers in dry and hydrated forms. In the dry form the films are brittle with relatively high moduli but very small extensibility (inset in Figure 4). For example, the Young's moduli for dry samples are 2.80, 1.53, and 1.95 GPa for **P-1**, **P-2**, and **P-3**, respectively, while the maximal strains are only ~4%, ~6%, and ~4% for **P-1**, **P-2**, and **P-3**, respectively. Hydration of the samples results in dramatic changes in mechanical properties.

The 13% hydrated films (w/w, weight of absorbed water to weight of the dry film) clearly show that the hydrated films are much more extensible. Young's modulus decreases significantly with hydration: ~200 MPa for polymers **P-1** and **P-2** with 13% of hydration. Polymer **P-3** is much softer after hydration. Young's modulus is ~50 MPa for the hydrated films of **P-3**. After yielding at 4−5% strain, the hydrated samples undergo a large deformation. The films can be pulled up to 3 times its length before rupturing for the hydrated films of **P-1** and **P-3** (curves d and f in Figure 4). While these samples are not recoverable because of the lack of cross-links like those found in the noncross-linking ELPs, the transition from brittle to highly extensible behavior is what one would expect for a noncross-linked elastomeric polymer, as reported for native and synthetic ELPs.[21]

While the EMPs in dry form are very stiff and nonextensible, partial hydration (13 wt %) significantly lowers Young's modulus and dramatically increases their extensibility. Due to the lack of any cross-linking, further hydration makes the samples too soft for stress−strain analysis. Instead, the fully hydrated **P-1** was characterized by rheological analysis. The results show that it has typical viscoelastic properties at 40 °C. Its storage modulus ($G' \approx 5$ MPa) is close to the value of native elastin in fully hydrated form.[22] The relatively small tan δ value (~0.1) for **P-1** also strongly supports that the gel has good elastomeric properties (see Figure S4 in Supporting Information).

Two important observations are worth noting in the physical property studies of the EMPs. First, despite their dramatic difference in local secondary structure in solution (Figure 2), **P-1**, **P-2**, and **P-3** display similar behavior in LCST and mechanical performance, suggesting that local secondary structure is not essential for elasticity in EMPs. This agrees with the highly dynamic nature and lack of long-range order for elastin. A recent investigation of $(VPGVG)_3$ peptides by solid state NMR confirmed that an ensemble of dynamic conformations coexists in solid state with only a minor fraction existing in a compact β-turn conformation.[12a] Second, our results confirm that hydration plays a critical role in the elasticity of elastin. Similar to natural elastin, simple hydration converts **P-1**, **P-2**, and **P-3** from very brittle into very ductile. Presumably, solvation of backbone amide bonds by water reduces main chain/main chain hydrogen bonds, hence, making the polymer chains more dynamic. In the meantime, the interaction of water molecules with hydrophobic side chains, i.e., hydrophobic hydration, provides the entropic driving force for both the elasticity and LCST behavior observed.[12,19]

In summary, we have demonstrated a novel bioinspired synthesis of EMPs. The unique molecular design enables us to probe important mechanistic questions and assess the structure−property relationship of EMPs. Our results indicate that polymer conformation is not essential for the elasticity of EMPs. Instead, our data confirm that hydrophobic hydration, as opposed to an organized secondary structure, plays a critical role for the elasticity. The bioinspired polymers can be conveniently prepared through a modular approach using "click chemistry". Despite the introduction of nonpeptido linkages, the bioinspired EMPs fully preserve critical features of native elastin: the LCST behavior in aqueous solution and high elasticity in bulk. The simple modular synthesis provides an efficient approach to access a broad range of elastin-mimic polymers for many potential biomaterials applications.

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Supporting Information Available: Experimental details of synthesis and characterization of monomers and polymers. This material is available free of charge via the Internet at http://pubs.acs.org.

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