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OEGylated collagen mimetic polypeptides with enhanced supramolecular assembly

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ABSTRACT

Collagen mimetic polypeptides (CMPs) pendanted with linear or dendritic oligoethylene glycols (OEG) via ether linkage were synthesized, and their thermoresponsiveness, secondary structures and supramolecular assembly investigated. High molar masses of OEGylated CMPs were achieved by polyamidation of tripeptide precursors through activated ester strategy. Pendanted linear OEGs confer CMP with enhanced triple helix conformation, while pendanted dendritic OEGs offer CMPs with characteristic thermoresponsive properties. In order to examine the effects of OEGylation, CMP with naked hydroxyl groups was also prepared. These OEGylated CMPs show intrinsic supramolecular assembly behavior in solutions which was explored with UV/vis and CD spectroscopies, as well as dynamic light scattering. Effects of polypeptide structures, molar masses, solvent polarity and substrates on the supramolecular assembly of the OEGylated CMPs were investigated, and we propose the solvent-favorable dendrons on the periphery and solvent-unfavorable polypeptide backbone form radial amphiphilicity, which enhance the supramolecular assembly of OEGylated CMPs to form long fibers.

1. Introduction

The main text of the article should appear here with headings as appropriate. Collagens are the most abundant proteins in human body [1]. It comprises of 28 types of proteins to participate into different bio-functions [2]. The various roles in vivo are all based on different featured self-assembly morphologies nevertheless share the same subunit of collagen triple helix. The helix consists of three polyproline II helices to wind around each other to form a right handed super helix. Each strand of the helix constitutes of a repeating X-Y-glycine sequence, where X and Y are majorly occupied by proline and hydroxyproline, respectively. Due to its excellent gel forming ability and biocompatibility, collagen has been widely used in bio-scaffold and biomedical materials [3]. However, the use of animal derived collagen is limited by some intrinsic shortages like thermal instability, possible contamination and difficulty in post modification. Therefore, designed collagen mimetic peptides (CMPs) have been explored widely.

Side chain modification are first studied to explore the fundamental mechanisms for collagen triple helix forming. Important factors that influence the conformation stability of collagen triple helix can be included by (a) inter-strand hydrogen bonding [4,5], (b) the ring puckering of the proline rings [6–8], (c) *cis*-trans conformation transition of the amide bonds [6–8], and (d) suitable steric hindrance [9], and they all can be controlled by substitution on C4 of proline residue [10,11]. On the basis of these pioneered efforts, side chain modification, which is believed to efficiently change the inter-strand interactions both in strength and style, is further utilized to confer novel and versatile self-assembly. So far, fibers [12–17], discs [17–20], and hollow sphere [19] have been achieved.

Thermoresponsive collagen have drawn considerable interest last decades for its promising prospects in bio-functional materials. Among the limited works reported, one method to confer CMPs with thermoresponsiveness is to utilize the reversible triple helix folding-unfolding transition property of relative-short CMPs [21]. $(POG)_n$ ($n < 10$) is first linked to a multi-handed linker so as to construct a crosslinking system and form hydrogel. The mechanical properties of hydrogel can be tuned by changing temperature. However, the phase transition temperature can only be limitedly controlled by the length of collagen peptides. An alternative method is to introduce thermoresponsive moieties, including elastin peptide sequence VPGVG [22] and PDEGMEMA [23] to CMPs

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by end modification. The phase transition temperatures can be tuned by changing the species and ratio of thermoresponsive moieties. All works mentioned above adopt an end-modification method. However, using side chain modification to realize thermoresponsiveness should generate more versatile results yet remains barely explored. More recently, a modified type-I collagen with temperature-dependent, reversible self-assembly was achieved by side chain methacrylation [16]. It is noted this kind of collagen remains the D-periodic fiber forming ability of natural collagen which should provoke more effects in developing side chain modification CMPs to achieve bio-functional smart materials.

Oligo(ethylene glycol)s (OEGs) are well-known biocompatible moieties which have been used to solubilizing and stabilizing protein or peptide-based biomaterials. Furthermore, OEG chains, especially OEG dendrons, have been used as efficient scaffolds to confer characteristic thermoresponsiveness to polypeptides [24,25]. Based on our continuous efforts on this direction, we here report on the synthesis and characterization of novel OEGylated collagen mimetic polypeptides through polyamidation. These OEGylated CMPs are prepared via polycondensation of active ester from the corresponding OEGylated tripeptide precursors. Three types of OEGylated CMPs carrying linear OEG (PDEG) and methoxyl (PMe) or ethoxyl (PEt) dendritic OEG pendants were prepared to examine structure effects on thermoresponsive properties, secondary structures, and their aggregation behavior (Fig. 1). CMP with naked hydroxyl groups (PPOG) was also prepared for comparison. The supramolecular assembly of these OEGylated CMPs was investigated from various solvents on mica or HOPG substrates and directly visualized by AFM.

2. Results and discussion

2.1. Synthesis and characterization

Collagen mimetic polypeptides (CMPs) pendanted with either linear or dendritic OEG moieties were devised to examine possible topological effects of pendants on the secondary structures and thermoresponsive properties of CMPs. The pendanted OEG dendrons terminated with methoxyl or ethoxyl were designed to afford different hydrophilicity and thus possibly to tune the phase transition temperatures. In order to reduce the possible perturbation of the stereoelectronic effect, different OEG motifs were linked through ether linkage to the 4th position of hydroxyproline within CMPs. For comparison, CMP without pendants (with naked hydroxyl groups on hydroxyproline moieties) were also prepared with the same method to verify the structural effects. These polypeptides were prepared according to our previous report through tertiary amine-mediated polycondensation of peptidic active esters as delineated in Scheme 1 [25]. Started from amide coupling of the dipeptide **1** with glycine methyl ester and followed by saponification with LiOH, tripeptides **6a**, **6b**, and **6c** with pentachlorophenolic ester were achieved. The peptidic macromonomers **M-DEG**, **M-Me**, and **M-Et** were prepared in good yields through deprotection with hydrochloride. Pentachlorophenolic active ester is used for construction of the macromonomers to achieve high molar masses of polypeptides due to its reasonable stability during the polymerization process [25]. Polycondensation of these macromonomers in the presence of DiPEA afforded the corresponding polypeptides **PDEG**, **PMe**, **PEt**. The terminal activated ester end group of the monomers and the polypeptides was unstable and easily turned to carboxyl groups, which can be activated again with coupling reagents for further reaction with amino groups. Therefore, to achieve high molar mass polypeptides, amide coupling reagents EDC and HOBt were added after polymerization of the macromonomers for certain time. For example, **PEt** with four different molecular weights were prepared from different polymerization time and with post addition of coupling reagents (compare **PEt-A1** to **PEt-A1** and **PEt-B1** to **PEt-B2**, respectively). For comparison, **PPOG** with naked hydroxyl groups were also prepared through a similar route via amidation. All new compounds and polypeptides were characterized by ^{1}H NMR spectroscopy to ensure their chemical structures and high purities. The molar masses of polypeptides were analyzed by GPC measurements, and the results are summarized in Table 1. As shown in Fig. S1, the GPC elution curves for the polypeptides are multimodal in most cases, indicating multifarious propagations in tertiary-amine mediated polycondensation between the amines with active esters.

2.2. Thermoresponsive properties

All CMPs reported in present work are water-soluble at room temperature. Both **PPOG** and **PDEG** are too hydrophilic to show thermoresponsive behavior even up to 100 °C. However, both dendronized **PMe** and **PEt** show typical thermoresponsive behavior upon heating to elevated temperatures, and therefore, their thermoresponsiveness was investigated by using UV/vis spectroscopy and their cloud points (T_{cp}s) measured by turbidimetry at 700 nm. Their transmittance curves are shown in Fig. 2a, and the corresponding T_{cp}s are listed in Table 1. **PMe** carrying more hydrophilic methoxyl terminals shows a T_{cp} of 64.8 °C, while **PEt** pendanted with more hydrophobic ethoxyl terminals shows a slightly lower T_{cp}. Furthermore, T_{cp}s for **PEt** are dependent on its molar masses, and found to be 48.0 °C and 54.7 °C for polymers of M_n 63,000 and 31,000, respectively, indicating high molar masses facilitate efficient dehydration of the polypeptides. The thermally-induced phase transitions for all polypeptides covered large temperature ranges (Δ > 8 °C), which are much broader than the corresponding dendronized polymethacrylates [26] and polyprolines [25,27]. We attribute the broad phase transitions to the strong

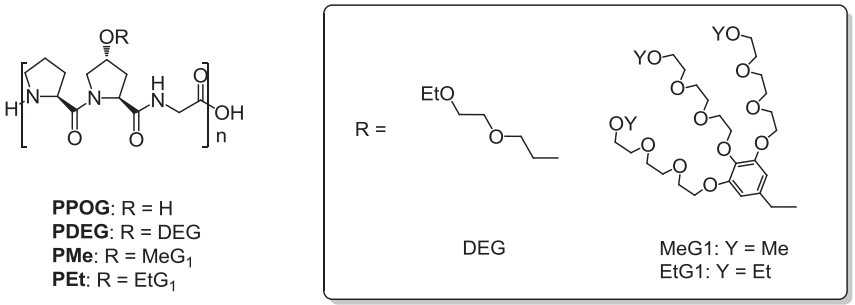

Fig. 1. Molecular Structures of CMPs discussed in present report.