Template-Assembly of Collagen-Mimetic Peptides (CMPs) based on Collagen α_1(I) 502-507 Ligand Binding Site

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Statement of Purpose: Collagen is the principal constituent of extracellular matrices and is intimately associated with many biological processes. Mimicry of collagen structurally and biologically helps to elucidate the importance of its unique triple helical structure and provide an insight into the engineering of novel collagen-like biomaterials. Our aim is to synthesize a collagen analogue of stable collagen-like triple helical structure and biological activity as an alternative to animal-derived collagen. Towards this goal, we propose a branching peptide composed of GFGEEG (G:Glycine; F:Phenylalanine; E:Glutamate) as our template (T) to covalently link three collagen-like peptides, each of which contains residues 502-507 (GFOGER) (O:hydroxyproline and R:arginine) of collagen α_1 (I), to facilitate the folding and proper alignment of the triple helices. The GFOGER sequence is the major integrin binding locus found within type I collagen[1,2]. The Gly-Pro-Hyp triplets are used to sandwich GFOGER to form stable triple helices at relatively shorter chain length with the aid of the template.

Methods: Fmoc-GFGEEG and collagen-like peptides were synthesized by solid phase peptide synthesis method. Branching was done by coupling the C-termini of Fmoc-GFGEEG to the free amino of the peptides. Structure of the template-assembled CMPs (T-CMPs) was studied by circular dichroism (CD), nuclear magnetic resonance (NMR) spectroscopy and melting curve analyses. Biological properties of the T-CMPs were examined by cytotoxicity, cell adhesion and competition inhibition assays using Hep3B liver cells as model cell type. For competition inhibition assay, cell surface collagen receptors were pre-saturated with T-CMPs by incubating cells in serum-free medium containing T-CMPs for 30 mins prior to the seeding onto collagen-coated well plate.

Results/Discussion: T-CMP1, T-CMP2 and T-CMP3 exhibited CD spectra features characteristic of collagen-like triple helix, including a positive peak around 220-225 nm and a large negative trough near 200 nm (Figure 1). The effect of the template in promoting assembly of triple helical conformations was verified by the cooperative melting curves displayed by the T-CMPs. Melting point temperatures (T_m) of each sample are given in Table 1. 1D ^{1}H-NMR spectra of the T-CMPs showed the presence of the assembled Pro $C_\delta H_h$ signal at 3.2 ppm. In contrast, their monodispersed counterparts showed no sign of triple helical structures based on CD, NMR and melting curve analyses. Cytotoxicity assay showed noncytotoxic responses to the samples even after 3-day culture. T-CMP3 displayed about 36% cell binding activity of native collagen while almost no cells attached to CMP3 and T-CMP5 (Figure 2). The result suggests that the Hep3B cells recognize GFOGER hexapeptide and the recognition appeared to be conformation-dependent. T-CMP3 significantly inhibited cell adhesion to collagen, suggesting the cell binding to T-CMP3 involves specific collagen integrin-receptors.

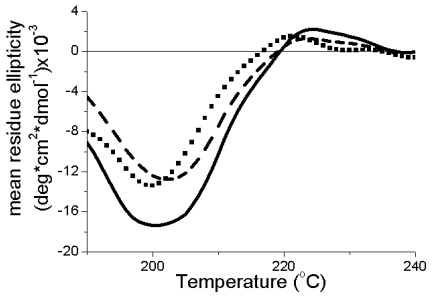

Figure 1. CD spectra of T-CMP1 (solid line), T-CMP2 (segmented line), and T-CMP3 (dotted line) at 0.5 mg/ml in H_2O at room temperature.

Table 1. Melting point temperatures (T_m)

Samples	Sequence	T_m (°C)
Collagen	Calf skin collagen	37
T-CMP1	(GFGEEG)≡[G-(POG)$_3$]$_3$	57
T-CMP2	(GFGEEG)≡[G-(POG)$_5$]$_3$	20
T-CMP3	(GFGEEG)≡[GG-GPOGFOGERGPO-GG]$_3$	17

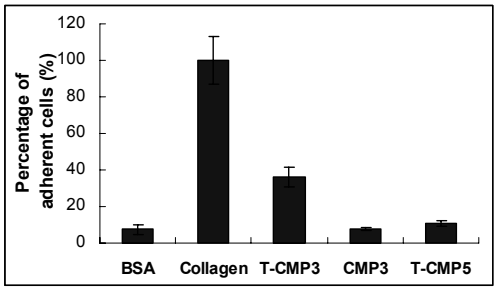

Figure 2. Cells were allowed to adhere to proteins/ peptides-coated surface for 2 hours at room temperature in serum-free medium.

Conclusions: We have successfully synthesized a peptide-based template to reinforce intramolecular folding and stabilize the triple helical conformations of our CMPs. T-CMP3 exhibiting collagen-like structure and biological activity can be used as a promising biomaterial to engineer well-defined bioadhesive matrix for medicinal and tissue engineering purposes. Our synthetic T-CMP, hence pure and reproducible, is fully constituted by only amino acids consistent with the natural protein and thus is more biocompatible and recognizable to biological cells.

References:

1. Knight et. al. J. Biol. Chem. 1998;273:33287-33294.
2. Knight et. al. J. Biol. Chem. 2000;275:35 - 40.