Mater. Res. Soc. Symp. Proc. Vol. 845 © 2005 Materials Research Society AA6.5

Synthesis and Application of Gold Nanoparticles Functionalized with Collagen Mimetic Peptides

Xiao Mo, Seungju M. Yu

Department of Materials Science & Engineering
The Johns Hopkins University
Baltimore, MD 21218

ABSTRACT

Collagen is the principal tensile element of the extra-cellular matrix in animals and is the basic scaffold for cells and tissues. Abnormalities in its structure are known to result in a number of debilitating human diseases. Collagen mimetic peptides (CMPs) with repeat unit of (Pro-Hyp-Gly) are capable of forming right-handed triple-helical structures similar to that of the collagen triple helices. Recently, our group has shown that CMPs exhibit specific binding affinity to natural collagen under controlled thermal conditions. Using solid phase peptide synthesis, we have prepared a CMP cysteine derivative that was used to modify gold nanoparticles. Transmission electron microscopy (TEM) shows that the Cys-CMP functionalized gold nanoparticles have affinity to collagen fibers. We are investigating the interactions between Cys-CMP functionalized gold nanoparticles and collagen fibers. The Cys-CMP conjugated nanoparticles can potentially be used as a tool to visualize and understand unstable domains of collagen fibers which are related to a number of pathological conditions of extra cellular matrices.

INTRODUCTION

Collagen is the most abundant protein in animal and is the major structural protein in the extra-cellular matrix. Collagen molecules are composed of repeat units of (X-Y-Gly) sequence, where X and Y are often Proline (Pro) and 4-hydroxyproline (Hyp), respectively. In nature, three collagen chains intertwine around one another forming a tight triple helix. It has been reported that collagen mimetic peptides (CMP) with repeat units of (Pro-Hyp-Gly) have collagen-like triple helices [1].

Modification of collagen has received much attention since collagen has been widely used in drug delivery and tissue engineering [2]. Chemical coupling reaction [3] through amino acid side chain has been developed; however, chemical reactions between exogenous components and collagen molecules are hard to control since collagen molecules are very large and complex biopolymers.

Our group has recently shown that CMP has strong propensity to associate with natural collagen in forming triple helices. We developed a "physical method" to attach exogenous component to collagen [4]. Fluorescence labeling was used for visualization of CMP-collagen complexes. But due to the intrinsic resolution of fluorescence microscope, the sub-micron structure of CMP-collagen complexes cannot be revealed.

In this work, we present the synthesis of CMP gold nanoparticles conjugates and its interaction with collagen fibers. Gold nanoparticles serve as a visualization tool for CMP-collagen complexes in transmission electron microscopy (TEM).

EXPERIMENTAL PROCEDURE

Synthesis and characterization of gold nanoparticles: Gold nanoparticles were synthesized by citrate reduction method according to the literature [5]. Tri-sodium citrate solution (38.8 mM, 50 mL) was quickly added to a refluxing aqueous solution of $HAuCl_4$ (1 mM, 250 mL) with vigorous stirring. The color of the solution changed from pale yellow to deep red after adding the citrate solution. The solution was refluxed for an additional 15 min, cooled, and filtered through a glass filter.

Synthesis and purification of CMP cysteine derivative: Cys-CMP, Cys-$(Pro\text{-}Hyp\text{-}Gly)_7$, was synthesized by solid phase peptide synthesis method using Fmoc chemistry. Peptides were purified to >90% purity by reverse phase high performance liquid chromatography (HPLC). Mass spectrometry was used to confirm its molecular weight. Circular dichroism was used to characterize the formation of collagen-like triple helices. CD melting behavior experiment was performed by monitoring the ellipticity at 225-nm as a function of temperature (0.1°C/min).

Surface functionalization of gold nanoparticles with CMP: Purified Cys-CMP was added to gold nanoparticle solution and the reaction mixture was incubated at room temperature for 24 hours. Excess CMPs were removed by repeated centrifugation and washing by deionized water.

TEM analysis of interaction between collagen fiber and Au-CMP: A drop (8 μL) of collagen solution (type I fibers in PBS containing 1% BSA) was added to a holey carbon TEM grid. Au-CMP solution (in PBS containing 1% BSA) was added to the grid and incubated for 5 minutes at room temperature followed by washing with deionized water. Uranyl acetate was used to stain the sample.

RESULT AND DISCUSSION

Gold Nanoparticles were synthesized using citrate reduction method according to the literature [5]. **Figure 1** is a typical TEM micrograph of as-synthesized gold nanoparticles. The nanoparticles are fairly monodispersed and have an average diameter of 13 nm. Cys-CMP was synthesized by manual solid phase peptide synthesis method and purified to >90% purity by reversed phase HPLC. **Figure 2A** shows the CD spectrum with a maximum near 225 nm and a minimum near 198 nm, which are characteristic peaks of collagen-like structure [6]. **Figure 2B** shows Cys-CMP has a

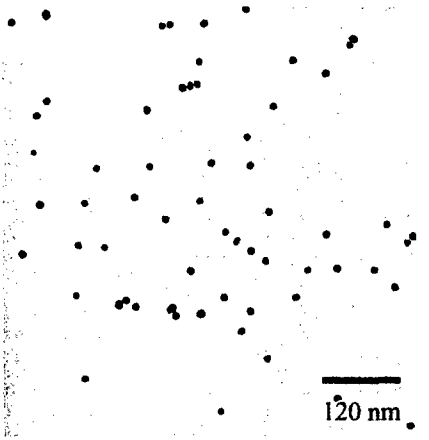

Figure 1. TEM image of as synthesized gold nanoparticles

melting transition temperature (Tm) near 39°C. CD spectrum and melting curve confirm that adding a cysteine residue at the N terminus of CMP has minor effect on the triple helical structure.

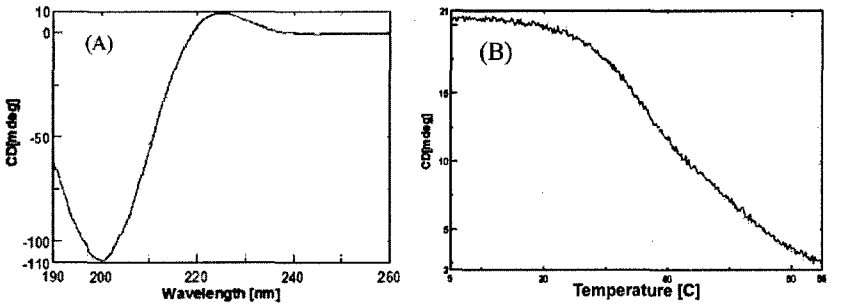

Figure 2. A: Circular dichroism spectrum of Cys-CMP; **B**: CD melting experiment of Cys-CMP.

Purified Cys-CMP was used to passivate the gold nanoparticles by thiol-gold interactions. After CMP functionalization, the UV-vis spectrum of gold nanoparticles has a slight red shift from 519nm to 525 nm (**Figure 3**). This red shift may not due to the surface modification but the centrifugation process. Similar red shift was also observed in DNA modified nanoparticles [7]. CMP functionalized gold nanoparticles were stable and no aggregation was observed even at elevated salt concentration (5M NaCl).

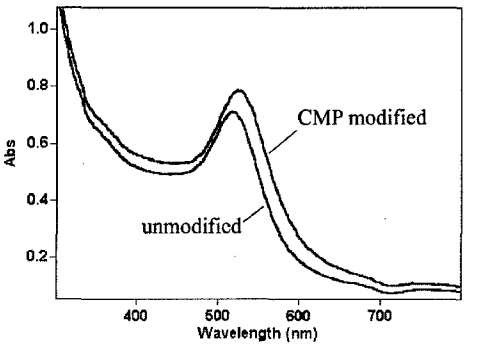

Figure 3. Comparative of UV-vis spectra of CMP modified gold nanoparticles and unmodified gold nanoparticles.

The CMP functionalized gold nanoparticles were also characterized by TEM. **Figure 4A** is a typical TEM micrograph of negatively stained (with uranyl acetate) CMP functionalized gold nanoparticles. From the TEM micrograph, most of the surfaces of gold nanoparticles were covered by CMPs, CMPs appear as white spiky bands on the

surface of gold nanoparticles due to negative staining, in oppose to unmodified gold nanoparticles, very thin and smooth white layers (**Figure 4B**).

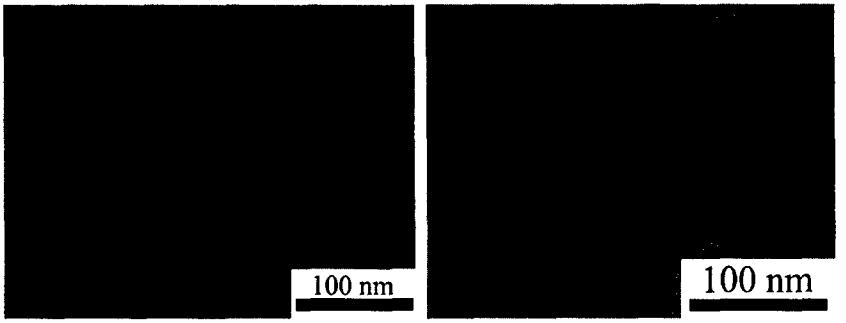

Figure 4. TEM micrographes of negatively stained CMP functionalized gold nanoparticles (A) and unmodified gold nanoparticles (B). Gold nanoparticles were negatively stained by 2% uranyl acetate.

When Cys-CMP functionalized gold nanoparticles were allowed to interact with natural collagen fibers, the gold nanoparticles exhibited strong tendency to attach to intact collagen fiber (**Figure 5A**). However, when randomized CMP (peptide with same amino acid composition as that of $(Pro\text{-}Hyp\text{-}Gly)_7$ but with scrambled sequence) were employed, we observed very little gold nanoparticles on the collagen fiber (**Figure 5B**). The results suggest that CMPs are able to attach to the surface of intact collagen fiber and that its propensity to form triple helix is essential for the binding process. The Cys-CMP conjugated nanoparticles can potentially be used as a tool to visualize and understand unstable domains of collagen fibers which are related to a number of pathological conditions of extra cellular matrices.

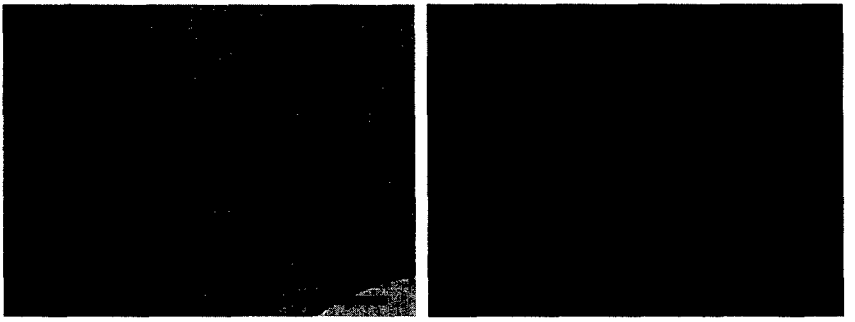

Figure 5. TEM micrographs of Collagen fiber and CMP functionalized gold nanoparticle (A) and random sequence peptide functionalized gold nanoparticles (B). The sequence of random peptide is: CGPGP*PP*PPGPPP*GP*P*PP*GP*GG. (P*= Hyp)

ACKNOWLEDGEMENT

This work was supported by National Science Foundation Grant CTS-0210220 and by Johns Hopkins Engineering School start-up grant.

REFERENCE

1 S. K. Holmgren, K. M. Taylor, L. E. Bretscher and R. T. Raines, Nature **392**, 666 (1998)
2 W. Tan and R. Krishnara, T. Desai, Tissue Eng. **7**, 203, (2001)
3 J. S. Pieper, P. B. van Wachem, M. J. A. van Luyn, L. A. Brouser, T. Hafmans, J. H. Veerkamp and T. H. van Kuppervelt, Biomaterials **21**: 1689 (2001)
4 Y. Wang, C. Chen and M. S. Yu, submitted (2004)
5 K. C. Grabar, R. G. Freeman, M. B. Hommer and M. J. Natan, Anal. Chem. **67**: 735 (1995)
6 T. Hayashi, S. Curran-Patel and D. J. Prockop, Biochemistry **18**: 4182 (1979)
7 J. J. Storhoff, R. Elghanian, R. C. Mucic, C. A. Mirkin and R. L. Letsinger, J. Am. Chem. Soc. **120**: 1959 (1998)