

Thermal denaturation of collagen studied by second harmonic generation microscopy

Chien-Sheng Liao^a, Zong-Yan Zhuo^a, Jiun-Yann Yu^a, Pen-Hsiu Grace Chao^b,
and Shi-Wei Chu^a

^a Department of Physics, National Taiwan University, Taipei 10617, Taiwan

^b Institute of Biomedical Engineering, National Taiwan University, Taipei 10617, Taiwan
No. 1, Sec. 4, Roosevelt Road, Taipei, 10617 Taiwan(R.O.C)
swchu@phys.ntu.edu.tw

Keywords: ligament, nonlinear microscopy, modelocked laser, thermodynamics

Recently, thermal therapies have been extensively applied to treatment of diseases. For example, by heating cornea, the induced shrinkage of collagen provides a precise way to correct the hyperopia and astigmatism. In order to reduce the damage to the bio-tissue, it is of vital importance to understand the complete molecular dynamics of denaturation. Most of the previous researches on denaturation are based on differential scanning calorimetry (DSC) and shrinkage measurement. DSC is useful to determine the energy transformation during denaturation and shrinkage measurement provides macroscopic mechanical properties. However the complete molecular model of collagen denaturation, including the molecular mechanism and the corresponding macroscopic deformation, has not yet been established with the above methods.

Type 1 collagen, the most abundant protein and the key factor in thermal therapy, has been shown to exhibit strong second harmonic generation (SHG) response. Since SHG is highly sensitive to the conformation of molecules, SHG imaging provides information on molecular deformation and enables simultaneous observation of shrinkage during denaturation. With high-speed in situ observation, we have obtained complete dynamics of collagen fibrils in ligament during thermal denaturation. A new stage, de-crimp, was identified as the initial step of thermal denaturation in collagen.

During the de-crimp step, the characteristic crimp pattern of collagen fascicles disappeared due to the breakage of interconnecting bonds between collagen fibrils, while SHG intensity remained unchanged, suggesting the intactness of the triple helical molecules. At higher temperature, shrinkage is observed with strongly reduced SHG intensity, indicating denaturation at the molecular level. By comparing the SHG intensity variation and the amount of shrinkage, a better insight of molecular dynamics of collagen during heating is revealed.

