Polarized Raman Anisotropic Response of Collagen in Tendon: Towards 3D Orientation Mapping of Collagen in Tissues

Abstract

In this study, polarized Raman spectroscopy (PRS) was used to characterize the anisotropic response of the amide I band of collagen as a basis for evaluating three-dimensional collagen fibril orientation in tissues. Firstly, the response was investigated theoretically by applying classical Raman theory to collagen-like peptide crystal structures. The theoretical methodology was then tested experimentally, by measuring amide I intensity anisotropy in rat tail as a function of the orientation of the incident laser polarization. For the theoretical study, several collagen-like triple-helical peptide crystal structures obtained from the Protein Data Bank were rotated ''in plane'' and ''out of plane'' to evaluate the role of molecular orientation on the intensity of the amide I band. Collagen-like peptides exhibit a sinusoidal anisotropic response when rotated ''in plane'' with respect to the polarized incident laser. Maximal intensity was obtained when the polarization of the incident light is perpendicular to the molecule and minimal when parallel. In the case of ''out of plane'' rotation of the molecular structure a decreased anisotropic response was observed, becoming completely isotropic when the structure was perpendicular to the plane of observation. The theoretical Raman response of collagen was compared to that of alpha helical protein fragments. In contrast to collagen, alpha helices have a maximal signal when incident light is parallel to the molecule and minimal when perpendicular. For out-of-plane molecular orientations alpha-helix structures display a decreased average intensity. Results obtained from experiments on rat tail tendon are in excellent agreement with the theoretical predictions, thus demonstrating the high potential of PRS for experimental evaluation of the three-dimensional orientation of collagen fibers in biological tissues.

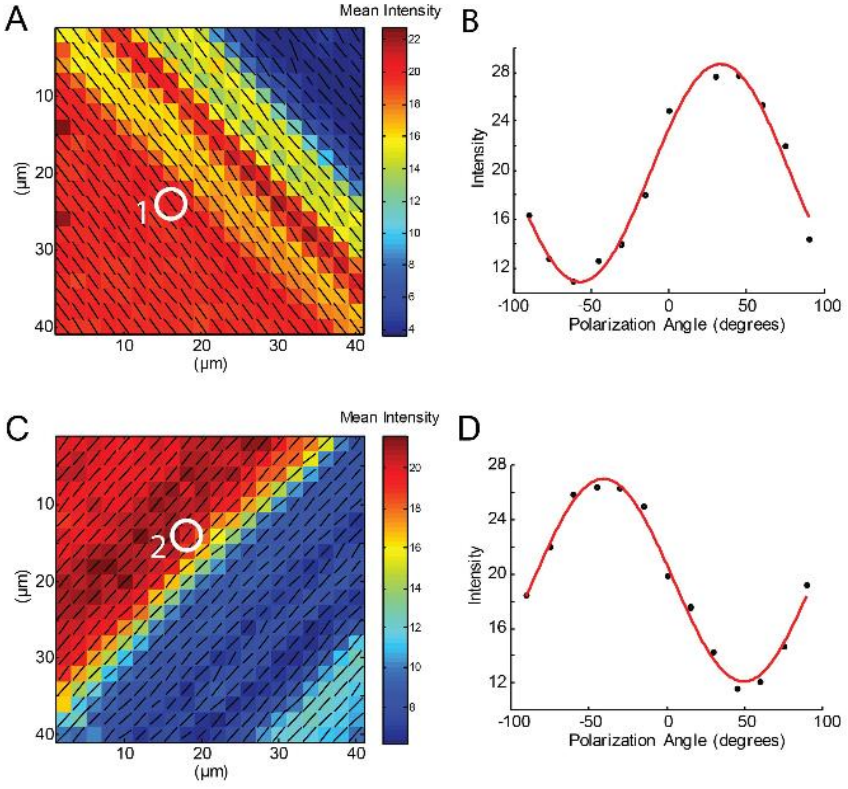

Figure 6. *In situ* PRS mapping of the collagen orientation in −45 and 45 degrees tilted dried RTT. (A) and (C) show maps obtained by fitting thirteen Raman images collected with different polarization angles of the incident laser light. The direction of arrows indicates the orientation of collagen molecules, their length represents the amplitude of the fitting curve, and the color code is the average intensity of the amide I band. (B) and (D) are example of experimental points extracted from the area marked in (1) and (2), respectively.

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