

MEASUREMENT OF THE SECOND ORDER HYPERPOLARISABILITY OF THE COLLAGEN TRIPLE HELIX AND DETERMINATION OF ITS PHYSICAL ORIGIN

A. Deniset-Besseau⁽¹⁾, J. Duboisset⁽²⁾, P. De Sa Peixoto⁽³⁾, E. Benichou⁽²⁾, C. Loison⁽²⁾,
G. Mosser⁽³⁾, P.-F. Brevet⁽²⁾ and M.-C. Schanne-Klein⁽¹⁾

(1) Laboratoire d'Optique et Biosciences, Ecole Polytechnique - CNRS - INSERM,
91128 Palaiseau, France.

(2) Laboratoire de Spectroscopie Ionique et Moléculaire, CNRS - Université Claude
Bernard Lyon I, 69622 Villeurbanne, France.

(3) Laboratoire de Chimie de la Matière Condensée, CNRS - Université Paris 6, 75252
Paris, France.

marie-claire.schanne-klein@polytechnique.edu

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Second Harmonic Generation (SHG) microscopy is a valuable technique to probe the three-dimensional architecture of fibrillar collagen in native and biomimetic tissues and to assess the progression of fibrotic pathologies [1,2]. However, the nonlinear optical response of fibrillar collagens is not fully characterized yet and quantitative data are required to further process SHG images. We therefore performed Hyper-Rayleigh Scattering (HRS) experiments in order to measure quantitatively the nonlinear optical response of the collagen triple helix, and to get insight into the physical origin of high SHG signals observed for fibrillar collagen in tissues.

HRS experiments determined a second order hyperpolarisability of $1.25 \cdot 10^{-27}$ esu for rat-tail type I collagen, using the internal reference method [3]. This value is surprisingly high considering that the collagen presents no strong harmonophores in its amino-acid sequence. Polarization-resolved experiments evidenced coherent contributions to the HRS signal besides the incoherent ones that are specific for molecules with dimensions much smaller than the wavelength. We therefore inferred that the high nonlinear optical response was related to the coherent summation of well-aligned basic harmonophores along the collagen molecule. This mechanism was confirmed by HRS measurements after denaturation of the collagen triple helix, and for a collagen-like short model peptide [(Pro-Pro-Gly)₁₀]₃. Moreover, measurements of the depolarization ratio indicated that the nonlinearity may originate in the peptide bond. To conclude, the high collagen nonlinear response originates in the compactedness and rigidity of the triple helix that amount to many well-aligned harmonophores and result in a coherent amplification of the nonlinear signal. This effect is similar to the coherent summation of SH radiations from all collagen triple helices within fibrils: this mechanism is responsible for high SHG signals observed in fibrillar collagens, whereas non fibrillar collagens show vanishing signals [4].

We applied these results to the monitoring of the organization processes involved in the synthesis of biomimetic collagenous matrices using SHG microscopy [5]. SHG signals were observed in concentrated solutions of collagen triple helices forming liquid crystals similar to those found in a stabilized state in living tissues. SHG images were also recorded in collagenous fibrillar gels.

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