BIOMEDICAL RAMAN MICROSPECTROSCOPY AND IMAGING: PATHOLOGIC APPLICATIONS

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Abstract: Two pathologic applications of near-infrared (NIR) Raman microspectroscopy and imaging are presented: the analysis of hair shaft and prostate tissue from patients exhibiting trichothiodystrophy (TTD) and prostate cancer, respectively.

Near-infrared (NIR) Raman microspectroscopy and Raman imaging are vibrational techniques capable of probing the structure, function and pathology of human tissue. Specifically, hair shaft and prostate tissue from patients exhibiting trichothiodystrophy (TTD) and adenocarcinoma of the prostate, respectively, were examined. Hair samples from TTD patients reflect both an extreme brittleness and a significantly reduced sulfur content compared to normal hair [1]. The disulfide stretching mode region of both normal and TTD affected hair was examined by NIR Raman microspectroscopy employing line focus illuminations; dominant contributions are due to three Raman bands at 510 ± 5 cm^{-1}, 525 ± 5 cm^{-1} and 540 ± 5 cm^{-1} which were assigned to the *gauche-gauche-gauche* (g-g-g), *gauche-gauche-trans* (g-g-t) and *trans-gauche-trans* (t-g-t) disulfide conformers [2] (Figure 1). This vibrational assignment was unambiguously confirmed by density functional calculations using two different hybrid-exchange correlations functionals, B3PW91 and B3LYP, in combination with the 6-311++G(d,p) Pople basis set; characteristic molecular properties such as relative energies, disulfide bond distances and vibrational frequencies of the disulfide stretching modes representative of the three conformers were calculated. The (g-g-g) conformer is the energetically most stable form and the introduction of each *trans* linkage leads to an increase in relative energy of 1.5 – 2.0 kJ mol^{-1}. The relative contributions for the (g-g-g), (g-g-t) and (t-g-t) forms, determined from band area ratios in the Raman spectra of hair samples of 3 volunteers and 3 TTD patients, are as follows:

(73 ± 5.9) %, (20 ± 5.5) % and (6.9 ± 0.4) % for the normal samples and
(33 ± 3.0) %, (40 ± 3.5) % and (27 ± 3.2) % for the TTD samples.

This quantitative spectral analysis shows significant increases in the contributions from the two energetically less favored (g-g-t) and (t-g-t) conformers for TTD hair compared with normal hair, indicating a strong association with hair brittleness observed for this rare hereditary disorder [3].

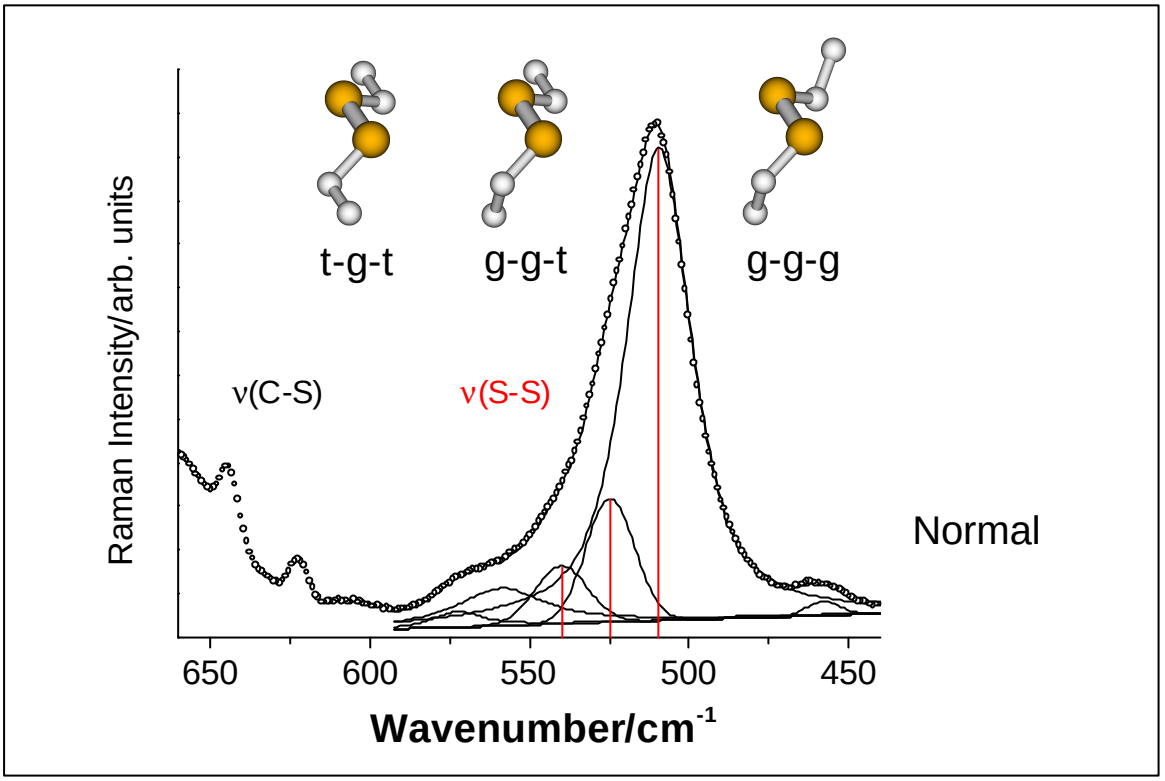

Fig. 1.: Disulfide stretching mode region of the NIR Raman spectrum obtained from normal hair. The energetically most stable (g-g-g) form exhibits the largest relative contribution of the three disulfide conformers.

Tissue microarrays with both benign and cancerous epithelial regions of human prostate tissue from the same patient were examined using NIR microspectroscopic line mapping at the 1.0 – 1.5 µm spatial resolution level. The spectroscopically resolved data allow the generation of Raman images in the wavenumber interval 1800 – 400 cm^{-1}. In particular, the chemical images associated with a DNA and RNA marker band at 782 cm^{-1} display the spatial distribution of nucleic acids. For benign epithelial tissue of the prostate, round and localized nuclei are observed. Epithelial tissue of the same patient exhibiting well differentiated adenocarcinoma of the prostate [4], classified as Gleason Grade 6, shows a significantly altered, delocalized nuclear distribution which is attributed to an increased activity in cell division.

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