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Combining microRaman and microdiffraction to investigate keratin modifications under stretching

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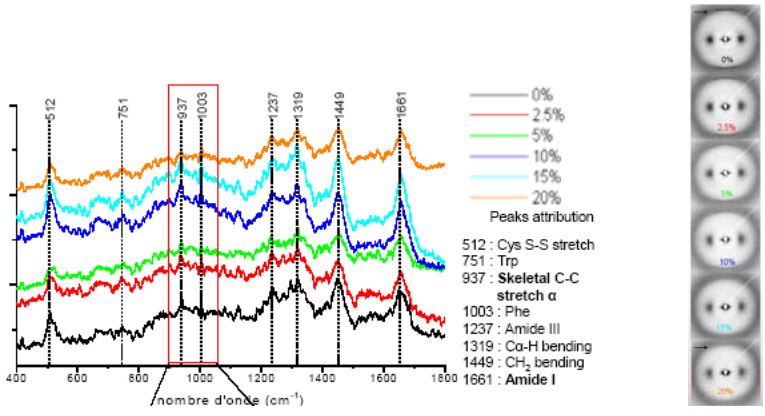
Keratin structure still raises many questions though it has been studied for more than 50 years. One reason is that this fibrous protein can not crystallize and therefore can not be determined at atomic resolution. Thus, it is directly studied in its original tissue such as hair or epidermis using fiber diffraction. This method gives rise to results as well at atomic resolution as at supramolecular scale. X-ray diffraction and Raman spectroscopy are complementary tools for investigating *in situ* proteins' structure. Raman spectroscopy provides information about atomic bonds by characterizing their vibrations, particularly by giving access to the secondary proteins' structure (random, beta-sheet, alpha-helix). It is worth noting that signals from other elements composing the tissue are integrated. In addition it is difficult to correlate the results from this two techniques. Experiments are often carried out under different conditions, sample preparation can even be different (fixation, cutting). Taking also into acount the issue of biological variability, the significance of conclusions obtained from experiments independently carried out is not straightforward.

The method we have used to overcome this difficulty consists in an innovating coupling of microdiffraction and Raman spectroscopy thanks to a facility developed at ID13 beamline. It allows to collect simultaneously Raman spectrum and diffraction pattern at the same point of the hair fiber under stretching. The laser (785 nm) and X-ray beam (13 KeV) are delivered coaxially at the same focal point with respective spot sizes of 1-2 µm and 0.2 µm. Thus, this allows to investigate microscopic samples like cells and to probe a very small volumes. We have used this method to characterize the hair keratin structure under mechanical stretching. Questioning still remains about the existence of a transition in keratin from alpha-helix to beta-sheet induced by stretching. We stretched the hair to increase its length by 20%, at room temperature and humidity. We have observed the disappearance of the diffraction peak at 5.15 A. This is correlated, in Raman spectra, with the shift of a peak assigned to C-C bond in alpha helix skeleton. These observations are interpreted in terms of unraveling of the alpha-helices during stretching. However, we have neither detected any signal from beta-sheets in Raman spectroscopy nor in X-ray microdiffraction. Thus, in our experimental conditions, we have highlighted molecular distorsions but no alpha-helix to beta-sheet transition in keratin during hair stretching.

A manuscript is presently under preparation after late confirmation of Raman results on December 2007.



micro-Raman and micro-diffraction spectra collected during hair stretching at ID13 beamline