Vibrational Raman spectroscopy of nanoscale needle shaped histidine

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ABSTRACT

Amino acids in aqueous solutions have low Raman signals and are difficult to detect at low concentrations. To increase Raman signals one often uses UV excitation. We find that nano-needels can be grown from droplets of aqueous histidine on silica substrates. We observe intense and narrow vibrational Raman bands of nanoscale needle shaped histidine using visible excitation sources. Scanning electron microscopy reveals a combined structure of folded flat leaves and regions with a dense array of needles with diameters in the 50-100nm range. The needles are formed mainly due to substrate heating, convection is found to be less important in their formation. The pH dependent measurements show their influence on the detected spectral bands. The observed spectral bands are compared with density functional calculations which taking into account of the peptide bond formation with neighbouring molecules. The C-H stretching at high frequency are identified and attributed to the imidazole ring and the back bone. C-H stretching mode of the back bone is found to be strongly conformational dependent. We show that the growth method can be generalized to other amino acids like glycine, valine or histamine.

Keywords: nanoscale needles, histidine, vibrational bands, Raman spectroscopy, scanning electron microscopy

1 INTRODUCTION

Raman spectroscopy is a powerful non-invasive tool to obtain information on structure, function and reactivity of biological targets. The vibrational spectra of amino acids in peptides and proteins depend sensitively on organisation and interaction with its environment. Histidine takes part in many biological processes such as the coordination of metal ions [1-2] or acid-base reactions and is a common residue in organisms (up to 3%). The imidazole side chain is often found as a coordinating ligand of metal ions like copper or zinc in metalloproteins. For example histidine is a very important amino acid in Alzheimer's disease because it binds copper in the amyloids plaques [3] thus contributes to the production of reactive oxygen species and finally to the death of neurons. Raman detection of histidine at relatively low aqueous concentration (mM range) is challenging using visible laser excitation. UV Raman spectroscopy has been increasingly used to enhance the Raman response of proteins by resonance excitation [4]. Recently structural and vibrational properties of L-histidine oxalate crystals have been investigated [5-6].

We show here, using Raman spectroscopy with visible laser excitation, that intense and narrow Raman signals can be observed from histidine nano-needels grown on SiO_2. Scanning electron microscopy reveals a structure of folded flat leaves and nano-needles in different orientations. We compare the experimental Raman spectra with *ab-initio* calculations taking into account two histidine molecules to include effects of neighboring molecules.

2 EXPERIMENTAL

Histidine (Sigma Aldrich) was first dissolved in 1ml of de-ionised water at a concentration of 30mM and then single droplets (15µl) were deposited on SiO_2 plates. The droplet was dried under a 40W power lamp at a distance of 30cm or on a heating plate at variable temperatures (50°C - 80°C). Raman spectra were recorded (Dilor XY 2400 spectrometer) using 488nm excitation (Spectra Physics 2017 argon ion laser, 20mW).

The solubilized histidine droplet forms when dried a deposit in the form of a ring. The ring consists of a thin film contracted into leaflets and regions with a high concentration of needles with diameters in the 50nm range and several micrometers long. Fig. 1.a shows the leaflet with a homogeneous thickness and a region with a dense array of nano-needles (Fig. 1.b). Fig. 1.c shows the nano-needels at high concentration.

The Raman spectra of the histidine nano structures differ from histidine in aqueous solution and are also different from macroscopic histidine crystals or histidine in

powder form. Figure 2 compares Raman spectra of histidine in the 800-1800 cm^{-1} spectral range, excited at 488 nm of aqueous histidine (a), of histidine in powder form (b) and histidine in the form of nano-needels (c, d). Spectrum 2.a of aqueous histidine contains little spectral information and the few observed spectral bands correspond to the most intense spectral bands for the spectra 2.b, 2.c and 2.d. Spectra 2.c and 2.d have been obtained by varying the preparation technique of the nano-needels.

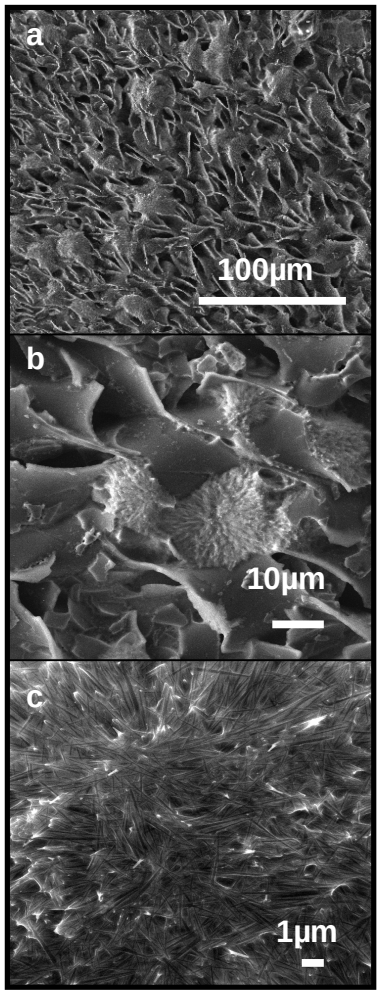

Figure 1 SEM of histidine micro crystals at low resolution (a) medium resolution (b) high resolution (c) region where needles in the diameter range of <100nm are formed.

We find that using a hotplate to evaporate the solvent, the Raman bands of the nano-needels is more intense and the bands are narrower than when the droplet has been dried using a lamp over a longer time. The evaporation using the hot plate is faster and the comparison of the two preparation techniques shows that the main contribution in the heat flow goes through the substrate and convection is not important. Using a hotplate the evaporation of the solvent is faster and the assembly and organisation of nano-needles leads to a better micro-crystalline structure. As a result the Raman signals are more intense and narrower. If we compare with histidine in powder form (fig 2.b.) the signals are less intense indicating that the molecule is organized differently in the powder. Our evaporation technique promotes a micro crystallisation of histidine in the form of needles which enhances the Raman vibrational bands using visible laser excitation.

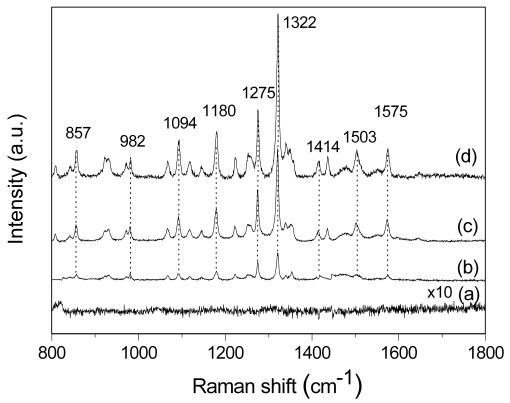

Figure 2 Raman spectra of microcrystalline histidine on SiO_2 compared with spectra in solution and in powder: (a) histidine 30mM solution between glass plates, (b) histidine powder on glass plate, (c) histidine 30mM deposited on SiO_2 dried under lamp during 25 minutes, (d) histidine 30mM deposited on SiO_2 dried on heating plate at 70°C

The intensity of the observed spectral bands and their narrow line shape shows that the nanostructures formed are crystalline. We relate the recorded Raman spectra mainly to the molecular organization in the self organized needles. The needles are comparable to the length of the excitation wavelength and as a result act as antennas which enhance the interaction with the incident beam and light couples strongly with the nano-needles. Taking into account of the concentration, drop volume and size of the trace left on the substrate, we find the recorded spectra correspond of approximately 10 nano gram quantity of histidine. Given the large recorded intensity, it becomes possible to detect histidine on surfaces using visible optical spectroscopy at the ato gram level.

To assign the observed spectral bands we have carried out density functional calculations using the Gaussian-03 Package [7]. All geometries and subsequent frequency determinations have been calculated within the combined Becke's three parameters exchange hybrid functional B3LYP associated with the Generalized Gradient Approximation (GGA) of Perdew and Wang [8-9]. The electronic wave functions are described by the 6-31+G** basis set. Several histidine models were considered taking into account one or two histidine molecules. Their influence on the vibrational frequencies has been studied after

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optimizing their structure. The vibrational frequencies involving the NH_2 and COOH groups of a single molecule show significant differences due to charge transfer interactions when considering a second molecule. We find that the hierarchy of the vibrational modes is strongly dependent on conformation. This dependence of the mode frequency as a function of their environment can produce discrepancies in the assignment. The interaction and folding of the two histidine molecules has clear implications on the hydrogen bonds and associated charge transfer. The CH stretching modes are shifted and influence some of the associated delocalized modes. We note that relative small changes in the position of the spectral bands are observed in the experiment. This shows that a particular conformation is favored by the symmetry of the formed structure.

Best agreement between calculated and observed frequencies is observed in the high frequency region. The C-H stretch modes in the imidazole ring are less conformation dependent. The high frequency C-H stretch mode at 3154 cm^{-1} can be assigned to the C-H stretch mode where C is bonded to two nitrogen atoms in the imidazole ring. The mode at 3138 cm^{-1} can be assigned to the CH stretching mode of C bonded to C and N.

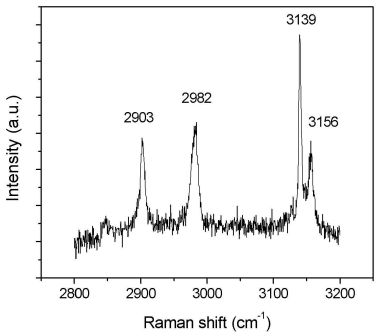

Figure 3 Raman spectrum of micro needles and leaflets in CH stretch region at high frequencies.

Side chain related C-H stretching modes are strongly dependent on conformation and cannot be assigned at this stage. Vibrational modes due to N-H vibrations and O-H vibrations at high frequency are not observed in the Raman experiment. The absence of O-H vibrations is likely due to the fact that His in aqueous solution at around neutral pH has a charged carboxylate and thus no O-H bonds are observed. The absence of this O-H is also expected after drying. However, under the same conditions N-H should be present in the ammonium group and the imidazole. Most of the vibrational modes can be assigned by using calculations with differences between theory and experiment ranging from a few wave numbers to 10 wave numbers. We note however that several modes are shifted by a much larger amount (<40cm^{-1}) and some of the intense bands observed in the experiment cannot be assigned.

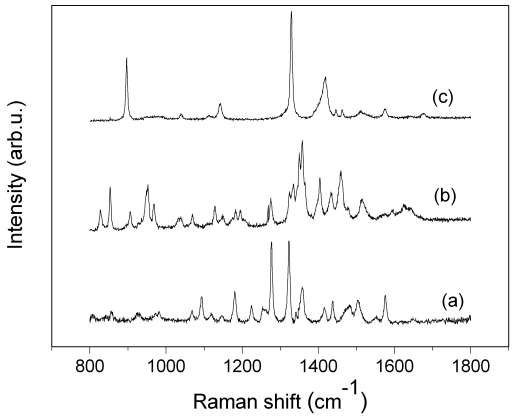

Figure 4 Raman spectra of different nano-needles (a) histidine (b) valine (c) glycine.

We carried out experiments at different pH. Histidine is an amino acid that can give off three hydrogen atoms depending on the pH of the solution. We have observed small changes in the spectral position of the vibrational bands. We also made H/D exchange reactions with histidine to observe changes in the spectra and to improve the attribution of the observed vibrational bands. Furthermore we have successfully carried out experiments to grow microcrystals from other amino acids such as glycine or valine (fig. 4).

3 CONCLUSION

We find that evaporation of histidine droplets on SiO_2 form nano-needles several micrometer in length. Their visible Raman spectra is particularly intense which makes Raman spectroscopy a viable tool to study molecular one dimensional assembly on surfaces. The needles are comparable to the length of the excitation wavelength and act as antennas enhancing the Raman signal. We have shown that the formation of the histidine nano-needles is due to substrate heating and convection plays a minor role. We find that pH influences some of the vibrational bands. Using *ab-initio* calculations we show that the calculated modes (C-H side chain vibrations) are strongly dependent on the conformation of the histidine chain. Relative small changes are seen in the vibrational spectra by changing the pH indicating that a well defined structure is formed in the small diameter needles.

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