Acquiring and processing Raman spectral data for the C2-D stretching vibration of 2-deuterated histidine

Submitted by Casey Londergan, Matthew Romei, and Kevin Hoffman, Haverford College, Haverford, PA USA

We have recently reported[1] the use of a deuterium label on the 2-carbon of histidine as a reporter of the protonation state of histidine, and possibly also as a reporter of histidine's local environment. Implementation of this probe into proteins would provide an easy way to determine the pK_a of, and environment around, histidine, an important amino acid in many classes of proteins including enzymes, metalloproteins, and viral surface proteins. C-D stretching vibrations occur between 2200-2400 cm^{-1}, in a region mostly clear of other biomolecular vibrations, so C-D vibrations from selectively deuterated histidine residues provide a unique spectral reporter.

Because of histidine's importance and unique functionality, we wanted to map out the probe group's sensitivity to allow for its general use in protein related research. Solution-exposted histidine residues are well known to undergo slow but selective deuterium exchange at the C2 carbon, so sample preparation and site-specific labeling of histidine residues is quite tractable. C-H and C-D vibrations are typically very weak IR absorbers, so the signal strength provided by the C-D[2] stretching mode of 2-deuterated histidine in Raman spectroscopy was examined. Two suitable model compounds, 4-methylimidazole and l-carnosine (see Figure 1), were selected for studying the probe's solvent dependence and ability to act as a reporter of histidine's protonation state, respectively. Raman spectra of 4-methylimidazole were collected in a range of different hydrogen bond donor solvents, and Raman spectra of l-carnosine across a broad pH range were captured to establish a titration curve from which the Histidine pK_a was determined. For 4-methylimidazole, we worked at 50-100 mM concentrations in most samples. For l-carnosine in aqueous buffers, we worked at sample concentrations relevant to globular proteins, about 1-10 mM.

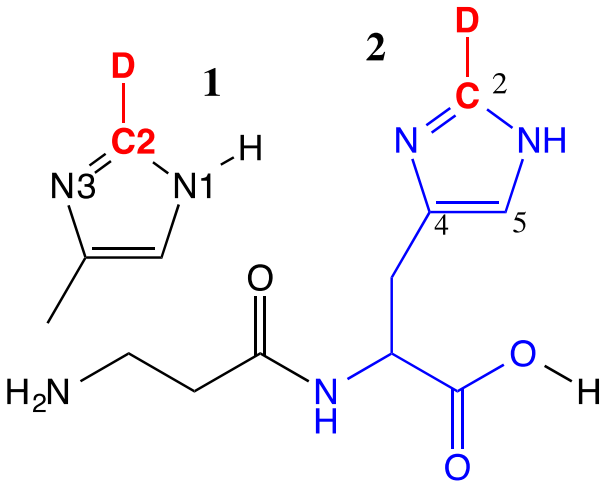

Figure 1.

*Figure 1. Structures of model compounds for 2-deuterium labeled histidine. **1** is 4-methylimidazole, and **2** is l-carnosine.*

Collecting data and isolating the C2-D stretching bands proved to be moderately challenging, both from a light collection point of view and in terms of post-collection data processing.

Experimental setup

Since the band of interest is relatively broad in liquid media, moderate (about 3 cm^{-1}) resolution was sufficient for these experiments. Since we worked with dilute, transparent samples and our main interest was in a band well-removed from the laser line, high optical throughput was the major consideration in designing our Raman system. Following excitation of a glass capillary sample at 514.5 nm and about 50-80 mW incident power, the scattered light was collected at 90 degrees via a f/1.8 Nikon camera lens, passed through a 514.5 nm long-pass filter, focused into a PI-Acton 0.5m single monochromator which separated the scattered light on a 600 grooves/mm grating, and collected in 1340 vertical spectroscopic bins on a Spec-10/100 liquid N_2-cooled CCD camera running in low-noise mode. Vertical excitation of the 1 mm-thick sample, 90° collection of the scattered light, and appropriate lenses led to the projected image of the vertical excitation line's nearly matching the vertical dimension of the monochromator slit, thus allowing us to use all 100 vertical pixels of each column on the CCD camera for data collection. The dispersion offered by the 600 grooves grating provided the right balance between signal/noise and spectral resolution for these spectra, which exhibit pH-dependent changes of 35 cm^{-1} in the C-D Raman shift. Each spectrum in our recent study was the result of one hour of continuous accumulation; the data shown in the figure are from 10 minute accumulations.

Data processing

See Figure 2 for a schematic of our background subtraction procedure. We took two approaches to isolate the C2-D band of interest from other signals, one involving an internal molecular standard (see Figure 2 for a schematic of this approach) and one involving a solvent band. Although the region near the C-D stretching band of interest is free of most other Raman signals from biomolecules, there is both a nonzero response from water in this region and the N_2 stretching band also appears nearby. Spectra of deuterated and non-deuterated samples (top two spectra) were collected in the same solvent or pH conditions, and then the methyl C-H stretching bands from these otherwise identical samples were used to scale the spectra for subtraction (third spectrum), yielding a subtracted sample containing the C2-D band of interest (fourth spectrum and inset) and some minor subtraction artifacts. A similar procedure could also be carried out using the O-H band of water (the large hump at high shifts in the top two spectra) to scale the spectra before subtraction.

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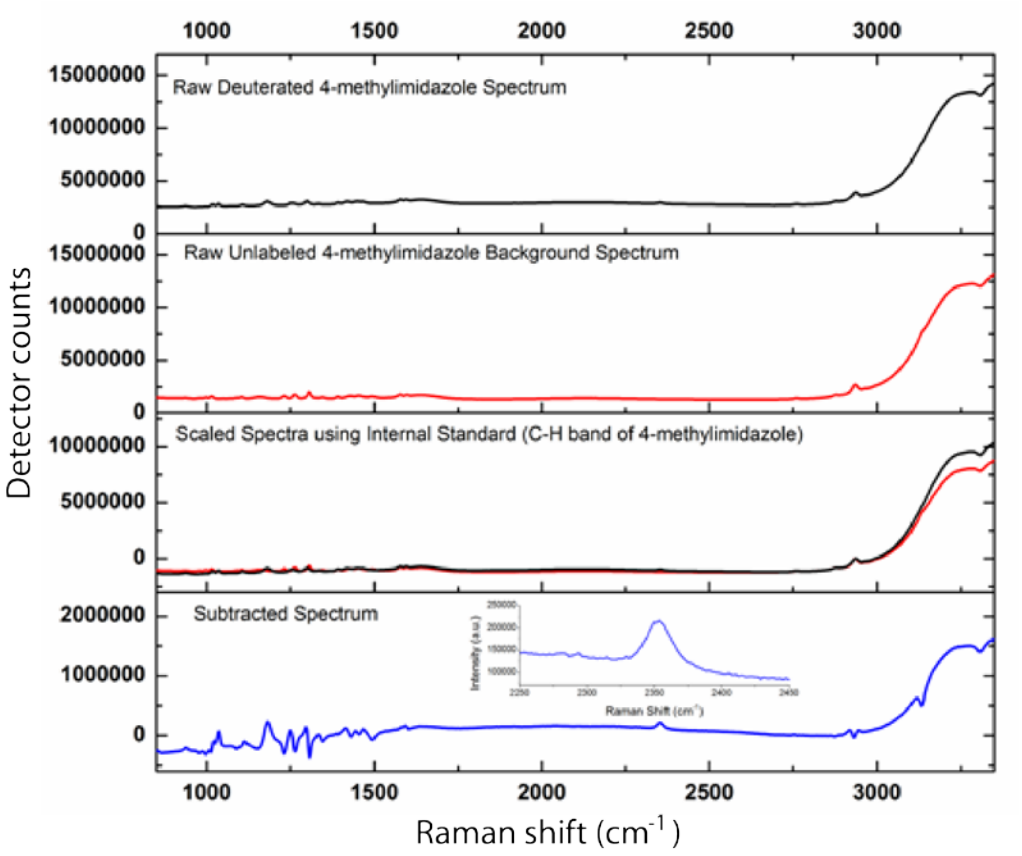
Figure 2.

Subtraction procedure for isolation of the peak of interest. The methyl C-H stretching band was used to scale the spectra of deuterated and non-deuterated samples (shown in the top two spectra and scaled together in the third) to yield a subtracted spectrum that clearly isolates the C2-D band (inset) in the fourth, resulting spectrum.



References

[1]Kevin W. Hoffman, Matthew N. Romei, Casey H. Londergan. *J. Phys. Chem. A*, **2013** *117*:5987–5996. doi: 10.1021/jp311815k

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