Detection of Metal-Bound Histidine in Ultraviolet Resonance Raman Spectra: Superoxide Dismutase

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We report detection of resonance-enhanced Raman bands associated with metal-bound imidazole, using ultraviolet excitation. These bands hold promise for probing the environment of ligating histidine side chains in metalloproteins.

Histidine is one of the most versatile of protein residues. Its imidazole side chain has a pK_a near neutrality and frequently serves as a general acid or base in catalysis.[1] It also binds to transition metal ions and is a common ligand in metalloproteins. Ultraviolet resonance Raman [UVRR] spectroscopy has proven to be a useful probe of local environment for the aromatic side chains of phenylalanine, tyrosine, and tryptophan.[2,3] However, the resonance enhancement of histidine is disappointingly low,[2−4] and histidine vibrations are not normally detected in UVRR spectra of proteins.

Recently we reported a prominent signal at 1408 cm^{-1} for protonated histidine in D_2O, whose intensity can be used to quantitate the average histidine protonation state in proteins.[5] The band arises from an imidazolium ring mode whose eigenvector closely matches the excited-state distortion in the resonant $\pi-\pi^*$ state, when the NH protons are replaced by deuterium.[6] We now find that D_2O exchange also leads to intensification of metal-bound imidazole ring modes. The 229 nm-excited RR spectra are compared in Figure 1 for bovine superoxide dismutase [SOD], an enzyme having Zn^{2+} and Cu^{2+} at its active site.[7] The Cu^{2+} is bound tetragonally by four histidine side chains, one of which simutaneously ligates the Zn^{2+}. Two other histidine and one aspartate side chains provide the remaining Zn^{2+} ligands.

The UVRR spectra contain well-characterized bands[2,3] arising from phenylalanine [F12, 1004 cm^{-1}; F18a, 1032 cm^{-1}], tyrosine [Y9a, 1178 cm^{-1}; Y7a, 1205 cm^{-1}], and the amide III band [1238 cm^{-1}; strongly shifted in D_2O by peptide NH/D exchange] of the peptide backbone. In addition, the prominent feature at ∼1285 cm^{-1} was earlier detected and assigned to the bridging histidine side chain by Harada and co-workers,[8] who did not, however, resolve its doublet structure. This bridging histidine band is present in both H_2O and D_2O [the bridging imidazolate ring has no exchangeable protons] but is missing in the apoprotein [Figure 2]. The remaining bands in this region of the holoenzyme UVRR spectrum are very weak in H_2O, but equilibration with D_2O reveals prominent new bands, most of which are not seen for the apoprotein in D_2O [Figure 2]. The holoenzyme shows a cluster of bands between 1410 and 1310 cm^{-1} [Figure 1] and additional bands at ∼1110, 1050, and 986 cm^{-1}. These features are also observed, albeit with somewhat altered frequencies and relative intensities, for a D_2O solution of the model complex Cu[4-MeImH]$_4^{2+}$ [4MeImH = 4-methylimidazole]. They are not present in the UVRR spectrum of a D_2O solution of histidine [Figure 2], which does, however, contain broad features at ∼1320 and 1370 cm^{-1}. These features are more prominently enhanced in a D_2O solution of the apoprotein, which has a total of eight D_2O-exchanged histidine residues. They are not present in H_2O solutions of either apoprotein or histidine.

We conclude from these observations that metal binding to imidazole augments the UVRR enhancement of ring modes when the proton on the remaining ring NH is replaced by deuterium. The situation is somewhat analagous to NH/D replacement in the imidazolium chromophore,[5,6] although the additional intensity

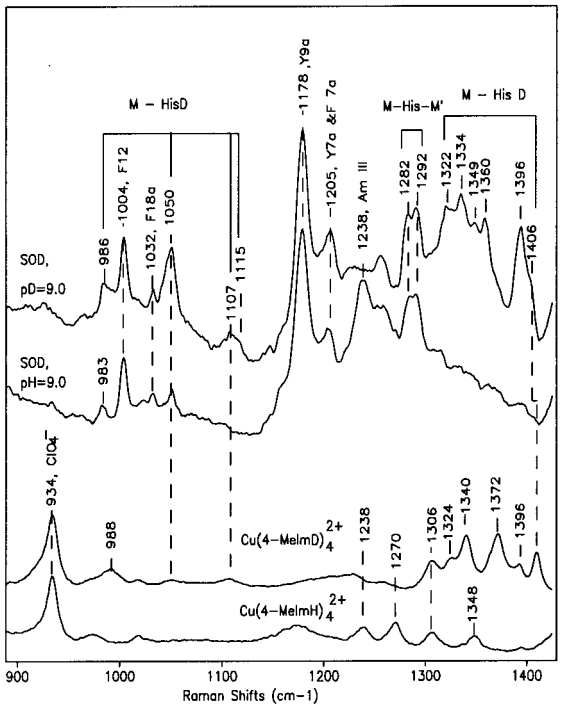

Figure 1. 229-nm excited UVRR spectra of 0.3 mM SOD and 1 mM Cu(4-MeImH)$_4^{2+}$ in H_2O and D_2O. The spectra were scaled via the 934 cm^{-1} ClO_4^- (0.25 M) band, present as an internal intensity standard for Cu(4-MeImH)$_4^{2+}$, and to the 1004 cm^{-1} Phe band of SOD; the ClO_4^- and Phe bands were normalized in a separate experiment. The 229-nm cw laser excitation (∼ 0.35 mW at sample) is the shortest wavelength (closed to resonance with the imidazole $\pi-\pi^*$ transitions) available from the intracavity frequency-doubled Ar^+ laser (Coherent, FreD). The UVRR spectra were acquired with a Spex 1269 single monochromator equipped with an intensified photodiode array detector.[12] SOD was purchased from Sigma and prepared by established procedures.[9,10] $CuSO_4 \cdot 6H_2O$ and 4-methylimidazole were purchased from Aldrich, and Cu(4-MeImH)$_4^{2+}$ was prepared according to the published method.[13]

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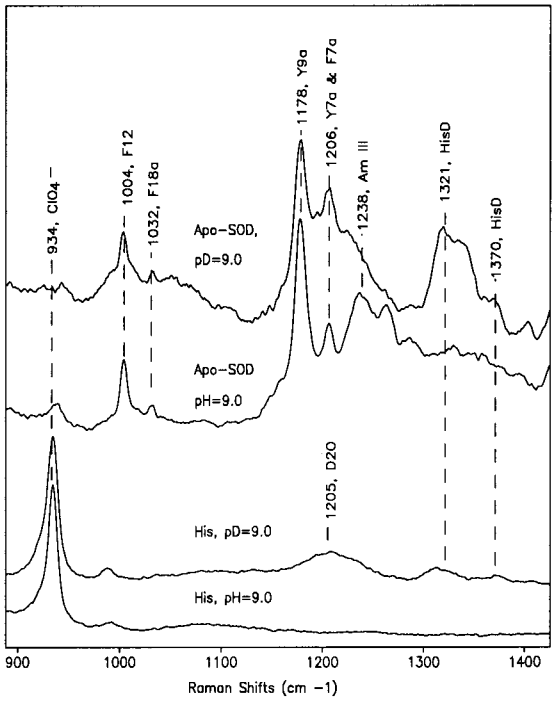

Figure 2. UVRR spectra of 0.3 mM Apo-SOD and 1 mM histidine with 0.25 M ClO_4^- excited at 229 nm.

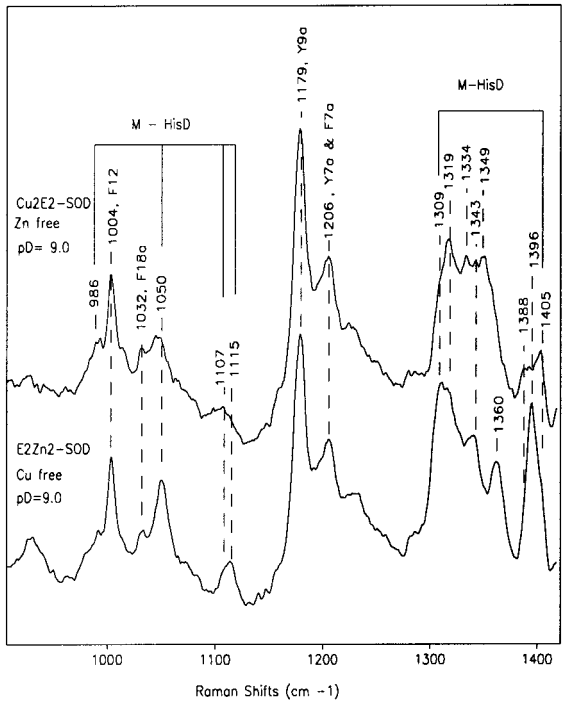

Figure 3. 229-nm excited UVRR spectra of 0.3 mM Zn- or Cu-free SOD derivatives. Samples were prepared as described in the literatures.[9,10]

associated with metal binding is distributed among a number of moderately enhanced modes, instead of being concentrated in a single dominant mode. The enhancement is nevertheless sufficient to reveal the bound histidine band structure quite clearly in the protein.

These new modes are likely to provide interesting structural details of the metal binding site in metalloproteins. When the Zn^{2+} or Cu^{2+} are selectively removed from SOD,[9,10] the UVRR signals of the remaining bound histidines differ markedly [Figure 3]. It appears that the Zn^{2+} site is responsible for most of the intensity of the bands at 1396, 1360, and 1050 cm^{-1}, while the Cu^{2+} is the main contributor to the 1406 and 986 cm^{-1} bands. These differences could arise from the differing polarization of the imidazole ring by the metal ions or from differences in the H-bond status of the coordinated imidazoles. Studies are in progress to elucidate the dependence of the UVRR spectral pattern on these factors.

We note that Takeuchi and co-workers[11] have recently assigned a 1342 cm^{-1} band to bound histidine in the off-resonance [514.5 nm excitation] Raman spectrum of a small zinc protein, but the signal is hard to detect in the background of unenhanced scattering from the protein.

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