Monitoring Cysteine and Histidine Ligands in Zinc-Finger Peptides via Ultraviolet Resonance Raman Spectroscopy

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Zinc-finger peptides are small proteins, or domains of larger proteins, whose three-dimensional structure is maintained by tetrahedral coordination of cysteine and histidine side chains to a central Zn(II) ion.[1−3] They are exceedingly numerous and have been estimated to account for up to 1% of mammalian genes. Many are transcription factors and bind to specific DNA sequences, while others are involved in protein−protein recognition. There are many different structural motifs; some of the different coordination types are illustrated in Figure 1. Since all are based on coordinated cysteine and histidine side chains, spectroscopic monitors of these ligands are needed.

The advent of stable lasers operating in the deep UV region has opened up resonance Raman spectroscopy to new electronic resonances, including high-lying ligand−metal (and metal−ligand) charge-transfer transitions, and $\pi-\pi^*$ transitions of aromatic residues, including histidine. We have explored both of these regimes and have recently obtained UVRR signals associated with Hg−S bonds in the MerR mercury regulation protein,[4] and other signals associated with bound histidine ligands in Cu/Zn superoxide dismutase.[5] We now report UVRR signals for bound cysteine and histidine residues in zinc-finger peptides.

The 238 nm-excited UVRR spectra of Cd(II)-bound COM39,[6] NC3,[7] and CP1,[8] which have C_4, C_3H, and C_2H_2 coordination groups, all show a prominent band at 282 cm^{-1} (Figure 2), and CP1 also has a 247 cm^{-1} shoulder. The 282 cm^{-1} band is assignable to the metal−ligand breathing mode of the peptides. For COM39, this assignment is supported by comparison with the 261 cm^{-1} ν_1 frequency for the isoelectronic complex $CdCl_4^{2-}$.[9] Cd(II) was used in place Zn(II) because S → M charge-transfer (CT) transitions are stronger and are found at longer wavelengths for Cd(II) than for Zn(II). The Cd−S bonds are expected to weaken in the CT excited state, producing RR enhancement for Cd−S(cys) stretching.

The COM39 absorption spectrum contains a broad band at 240 nm. The 282 cm^{-1} RR band of COM39 was observable with 244 nm excitation, as well as 238 nm excitation, but not with 257 nm excitation; the last wavelength is too far removed from resonance. No other RR band was detected, and the 282 cm^{-1} band is absent in the apopeptide spectrum. For NC3 and CP1, the UV absorption decreases in proportion to the number of Cys ligands, and it blue-shifts slightly (to 235 nm for NC3 and 230 nm for CP1).

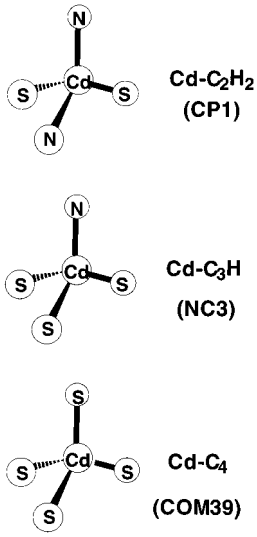

Figure 1. Coordination groups of the three zinc-finger peptides in this study.

It might have been expected that the Cd−S(cys) frequency would change from COM39 to NC3 and CP1, because of the altered kinematics in the C_3H and C_2H_2 mixed-ligand coordination groups. However, the imidazole ligand has a large effective mass, and the Cd−N(His) stretching frequency is expected in the same frequency region as Cd−S(Cys) stretching. The Cd atom does not move in the breathing mode of a tetrahedral complex, and apparently the imidazole ligands preserve the effective tetrahedral vibrational symmetry, leaving the resonance-enhanced Cd−S(cys) stretch unaltered in frequency for NC3 and CP1. However, the 247 cm^{-1} shoulder of CP1 is attributed to symmetry lowering. This is the expected frequency for the asymmetric stretch of the pseudotetrahedral complex (compare $\nu_3 = 245$ cm^{-1} for $CdCl_4^{2-}$).[9] Being non totally symmetric, this mode is not enhanced by the dominant A term RR enhancement mechanism[10] for the $Cd(Cys)_4$ chromophore of COM39, but this symmetry restriction is removed by the electronic asymmetry of the $Cd(Cys)_2(His)_2$ chromophore of CP1. However, the symmetry lowering in NC3 is apparently insufficient for significant enhancement of the asymmetric mode.

With excitation at 230 nm, we were able to detect ring modes of metal-bound histidine (Figure 3), after H/D exchange of the remote imidazole N atom. The His mode enhancement reflects characteristics of the mode compositions and of the imidazole $\pi-\pi^*$ excited states, as modified by metal coordination.[5] A sharp band appears at 1342 cm^{-1} for either Cd(II)- or Zn(II)-CP1, which

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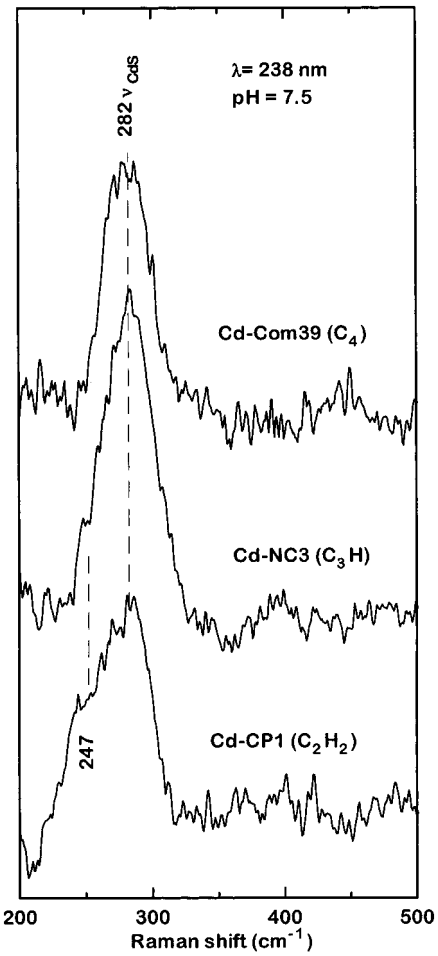

Figure 2. UVRR spectra for the indicated Cd(II)-bound peptides (1.4 mM) in TRIS buffer (pH 7.5). Background scattering was removed by digital subtraction of apopeptide spectra. Excitation (238 nm) (2 mW at the sample) was from an intracavity frequency-doubled Ar^+ laser. The Raman spectra were dispersed with a 1.25 m single spectrograph onto an intensified diode array detector, which was masked to reduce stray light. About 20 short data sets (<1 min) were co-added for different vertical positions of the sample tube.

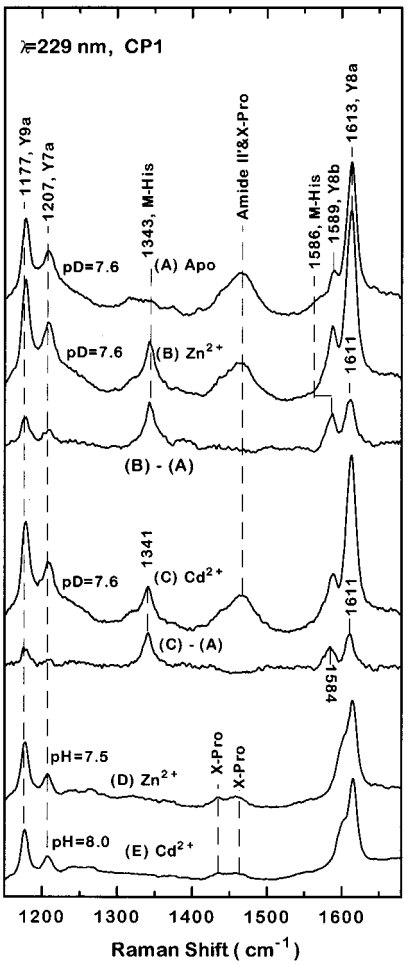

Figure 3. UVRR spectra of apo, Zn(II), and Cd(II) forms of CP1 (0.5 mM) in TRIS/D_2O buffer at the indicated pH values. $NaClO_4$ (30 mM) was added as an internal intensity standard. The difference spectra reveal the metal-induced modes of His and Tyr. The His bands are absent in H_2O; their enhancement requires H/D exchange at the remote N atom of metal-bound imidazole. Spectral acquisition was as in Figure 2, except for laser wavelength (230 nm) and power (0.35 mW at the sample).

is absent in the apoprotein, or in the holoprotein in H_2O. An additional band is superimposed on the 1589 cm^{-1} Y8b′ tyrosine band and is revealed in the holoprotein minus apoprotein difference spectra.

The difference spectra also reveal extra enhancement of tyrosine bands[11] (Y_{9a}, Y_{7a}, Y_{8a}) in the metallopeptides, probably reflecting diminished exposure of the tyrosine side chain upon peptide folding; the single CP1 tyrosine residue is close to the metal-binding site.[8] Zn(II) and Cd(II) give essentially identical His and Tyr difference signals, consistent with the same folded structure.

These results establish that cysteine and histidine coordination can be simultaneously monitored by UVRR spectroscopy and give promise for a wide range of applications to zinc-finger proteins. It will be of particular interest to examine the strength of the ligand−metal bonds as a function of solution conditions and of bonding to target DNA sequences.

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