UVRR Spectroscopy of the Metal Receptor Site in MerR

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Although cysteine coordination to d^{10}-metal transition-metal ions is observed in an increasing number of metal detoxification[1] and metal chaperone proteins,[2] there are still relatively few methods for probing such coordination environments. These metal centers have a closed-shell electronic structure and thus exhibit few spectroscopic handles. The d^{10}-metals bound to cysteinate ligands in proteins exhibit broad and intense ligand-to-metal charge transfer (LMCT) transitions in the 200−300 nm region.[3] We demonstrate here that it is possible to use ultraviolet (UV) laser excitation into these LMCT transitions of d^{10}-thiolate centers in metalloproteins to obtain resonance enhancement of metal−ligand Raman bands. UV resonance Raman (UVRR) spectra are presented for a trigonal mercuric complex of an aliphatic thiolate $[Et_4N][Hg(SBu^t)_3]$ (**1**), and for the Hg−MerR metalloregulatory protein, a heavy metal receptor component of an Hg(II)-responsive genetic switch. Our spectroscopic data indicate that Hg(II) is trigonally coordinated in Hg−MerR and give support to results of EXAFS data,[4] site-directed mutagenesis studies,[5] and UV absorption,[3,6] most recently by ^{199}Hg NMR.[7] These results indicate that UVRR is a powerful probe of metalloprotein active sites containing d^{10}-thiolate centers.

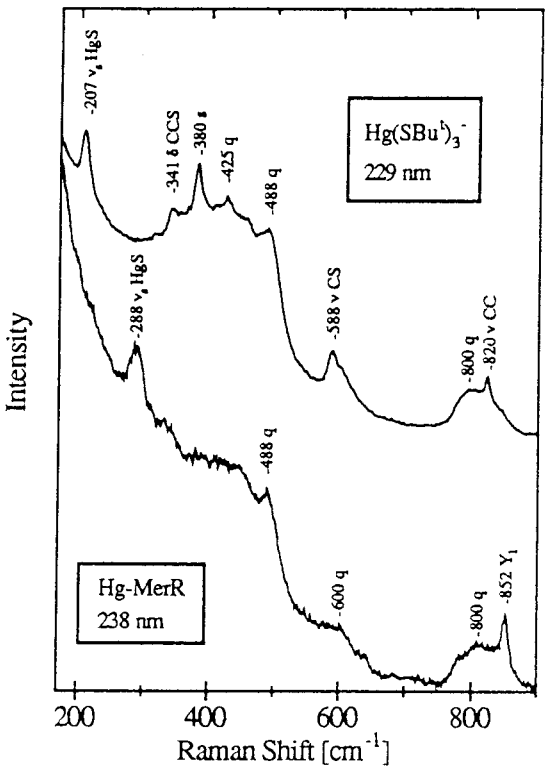

Figure 1. UVRR spectra of **1** (26 mM, acetonitrile, 229 nm excitation, top) and of Hg−MerR (238 nm excitation, bottom). Solvent and quartz bands are labeled as (s) and (q), respectively.

Table 1. Absolute Intensities[a] for Bands of $[Et_4N][Hg(SBu^t)_3]$ Solution[b] and for the 288 cm^{-1} Band of Aqueous Hg−MerR Solution[c]

exc. wavelength [nm]	σ_{207}	σ_{341}	σ_{588}	σ_{820}	σ_{288} Hg−MerR
229	76	32	42	53	83 (89)
238	42	28	5	14	81 (83)
244	45	22	0	0	117
257	37	14	0	0	115
676[d]	0.002	0	0.002	0.008	

[a] Expressed in units of cross section, [millibarns/(molecule steradian)], 1 barn = 10^{-24} cm^2. [b] Acetonitrile bands at 379 and 918 cm^{-1} as internal standard (ref 2). [c] 932 cm^{-1} band of 0.2 M $NaClO_4$ added as internal standard.[20] Values in brackets based on tyrosine modes located at 1176 (ν_{9a}), 1205 (ring-C) and 1613 cm^{-1} (ν_{8a}) with previously determined absolute intensities.[21] [d] Kr^+ (Coherent), Spex 1877 triple spectrometer equipped with an intensified diode array detector (Princeton Instruments).

The 229-nm excited UVRR spectrum of **1** (Figure 1, top),[8,9] prepared by a literature method,[10] contains bands at 820, 588, 341, and 207 cm^{-1}, in addition to background features due to the quartz sample tube [marked q], and an acetonitrile solvent band at 380 cm^{-1}. The bands at 588 and 820 cm^{-1} are assigned to the C−S and the C−C stretching mode, respectively, by comparison with spectra and assignments of pure liquid *tert*-butanethiol.[11,12] The band at 207 cm^{-1} is assigned to the symmetric HgS stretching mode.[9] The band at 341 cm^{-1} is assigned to an internal ligand mode, most likely CCS bending, since bands at similar positions are seen in solid-state Raman spectra of $Hg(SBu^t)_2$ and the

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(8) UVRR spectra were excited with the lines of an intracavity doubled Ar^+ laser (Coherent Innova 300 FRED). Laser power was between 2 mW (229 nm) and 10 mW (257 nm excitation). A 135° backscattering geometry was used, and a polarization scrambler was placed in front of the monochromator. The scattered light was collected using a 1.29 m single monochromator (SPEX 1269) equipped with a diode array detector (Princeton Instruments). Raman intensities, expressed as cross sections, σ, were determined from integrated intensity ratio, I_N/I_S, of the sample (N) and internal standard (S) vibrational bands by using $\sigma_N = \sigma_S (I_N/I_S)(C_S/C_N)$ where C_S/C_N is the concentration ratio. Integrated band intensities were determined with the "curvefit" program of the Labcalc software (Galactic) using sums of Gaussian- and Lorentzian-type model bands and including linear baseline in the optimization process.
(9) ^{199}Hg NMR spectroscopic data for a 5 mM solution of $Hg(SBu^t)_3^-$ in acetonitrile indicate that the predominant species in solution is three-coordinate, and contributions to Raman band intensities arising from dissociated ligands are considered to be negligible for solutions of equal or higher concentrations. Natan, M. J.; Millikan, C. F.; Wright, J. G.; O'Halloran, T. V. *J. Am. Chem. Soc.* **1990**, *112*, 3255−3257.
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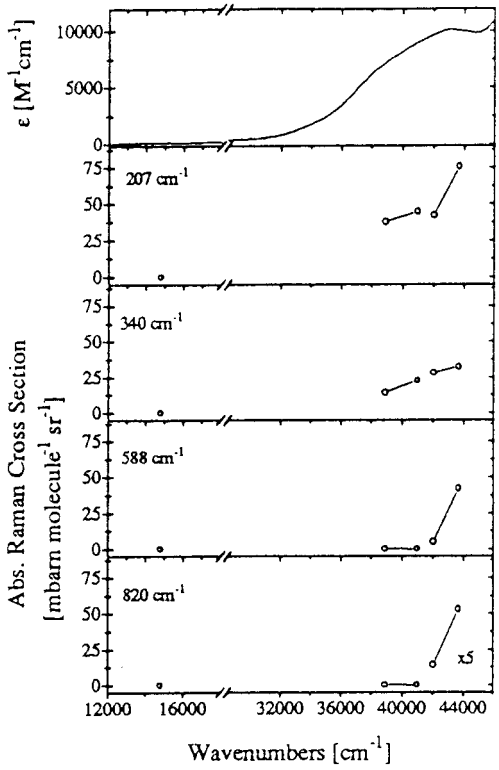

Figure 2. UV-absorption spectrum (top) and absolute Raman intensities for bands (lower panels) of **1**.

corresponding Cd(II) and Zn(II) compounds, and they were assigned to a mode of the SBu^t group.[13] An alternative assignment to the asymmetric Hg−S stretching mode is ruled out by the excessive separation, 134 cm^{-1}, from the symmetric stretching frequency. For trigonally coordinated HgX_3^- in tri-*n*-butyl phosphate solution (TBP) the separation between ν_s and ν_{as} Hg-X is less than 25 cm^{-1} for X = Cl, Br, I. Moreover, the ν_{as} Hg−S of **1** has been assigned to infrared bands[3] at 208 (298 K) and 212 (125 K) cm^{-1}, essentially coincident with ν_s Hg−S (which is IR-forbidden).

Raman intensities were measured relative to solvent bands as a function of excitation wavelength (Table 1), and the resulting excitation profiles (Figure 2) are consistent with enhancement of both Hg−S and internal ligand modes in resonance with the UV absorption bands between 230 and 280 nm (43500−35700 cm^{-1}), which have previously been assigned to S → Hg LMCT transitions.[3] Thus, the LMCT state is displaced along ligand coordinates, as well as the metal−ligand stretching coordinate, as has previously been observed for Fe−S[14a] and Cu−S[14b] proteins.

The UVRR spectrum of Hg−MerR at 238 nm excitation (Figure 1, bottom) exhibits sample bands at 288 and 852 cm^{-1}, which are assigned to the symmetric Hg−S[cys] stretching mode and to the ν_1 mode of tyrosine, respectively.[15] MerR was purified[16] and its Hg(II) derivative made as previously described.[17] The absolute Raman intensity of the 288 cm^{-1} band is essentially the same as that of the 207 cm^{-1} band of **1**, when measured at 229 nm (Table 1), but is larger at longer wavelengths. LMCT transitions of Hg−MerR have previously been reported in the 200−300 nm region of absorption difference spectra.[5]

Why is the ν_s HgS frequency so much lower (81 cm^{-1}) for **1** than for Hg−MerR, even though the average Hg−S bond lengths are the same (244 pm)?[3,17] The answer lies in the large effective mass of the SBu^t ligand. For $[Hg(SMe)_3]^-$ dissolved in ethanol a Raman band at 282 cm^{-1} has been assigned to ν Hg−S, much closer to the Hg−MerR frequency. The difference is that SMe^- has only H atoms attached to the C_a, while Bu^tS^- has much heavier CH_3 groups. Indeed, it has been observed that the frequencies scale as the square root of the total ligand mass for $[Hg(SMe)_3]^-$ and $[Hg(SBu^t)_3]^-$,[11] as well as for $[Au(SMe)_2]$ and $[Au(SBu^t)_2]$.[4] However, this simple behavior is deceptive because other thiolate ligands deviate from it. For example, the ν_s frequency is much higher for $Hg(SBu^n)_2$, 325 cm^{-1}, than for $Hg(SBu^t)_2$, 223 cm^{-1}, even though the ligand masses are the same. Moreover the $Hg(SBu^n)_2$ frequency is quite similar to those of the lighter homologues $Hg(SPr^n)_2$ [329 cm^{-1}] and $Hg(SEt)_2$ [304 cm^{-1}].[17] Clearly the frequency depends not on the total ligand mass, but rather on the nature of the substituents on the C_a atom: three alkyl C atoms for SBu^t but one alkyl C atom and two H atoms for SEt, SPr^n, and Sbu^n.

This issue is important with respect to using the protein frequency as a gauge of coordination number. It is well-established that the ν_s frequency decreases as the coordination number increases. Thus, the frequency is 328, 296, and 265 cm^{-1} for $HgCl_2$, $HgCl_3^-$, and $HgCl_4^{2-}$, in TBP solvent.[17] Interestingly, the $HgCl_2$ and $HgCl_3^-$ frequencies are close to those of the $Hg(S-n\text{-alkyl})_2$ species on one hand and $Hg(SMe)_3^-$, as well as Hg−MerR, on the other. Likewise, the frequency for an oligopeptide serving as a model for 2-coordinate Hg−S[cys] proteins,[18] 327 cm^{-1}, is essentially the same as that of $HgCl_2$. Thus Cl^- serves as a good vibrational model for *n*-alkyl as well as methyl thiolates. However, the exact frequency obviously depends on the interaction of the Hg−S stretching coordinate with other coordinates of the thiolate ligands. This issue will be addressed in a separate vibrational analysis.[19]

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