

Resonance Raman Study of Cobalt(II)-Imidazole Complexes as Models for Metalloproteins¹

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Abstract: The Raman spectra of cobalt(II)-imidazole complexes of known stoichiometries and geometries are reported, using Ar⁺ laser excitation. The excitation frequency dependence of Raman peak intensities, which differs for octahedrally- and tetrahedrally-coordinated complexes, indicates a slight resonance of the vibrations with the ${}^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$ Co(II) d-d transition in the octahedral complex, and with a uv charge-transfer state in the tetrahedral complexes. In the latter complexes, the more pronounced resonance effect of the ring vibrations supports assignment of a number of peaks to ring modes rather than C-H vibrations. Resonance enhancement in the tetrahedrally-coordinated Co(II)-histidine complex is very minor. The low Raman intensity and only slight resonance effects observed in the model complexes make it unlikely that Co(II)-substituted zinc proteins would exhibit a resonance-enhanced Raman spectrum of the cobalt-protein chromophore.

In resonance Raman (rR) spectra² of metalloproteins only those molecular vibrations associated with the metal-ligand chromophore are observed to undergo intensity enhancement. In the case of heme proteins³⁻⁵ laser excitation wavelengths near the α and β absorption bands of the protein produce a different set of resonance-enhanced Raman peaks than excitations near the γ band. The resonance-enhanced peaks are assigned to vibrations of the porphyrin macrocycle. The internal vibrations of ligands attached at the 5 and 6 positions of the central iron atom of the heme are not observed since the orbitals of these ligands are not involved in electronic transitions giving rise to α , β , or γ absorption bands. In the case of iron-sulfur proteins, the rR spectra reveal principally the Fe-S vibrations of cysteine and inorganic sulfur ligands.^{6,7}

Cobalt(II)-substituted zinc enzymes are potentially suitable for rR spectroscopic studies because these enzymes possess visible absorption spectra due to the Co(II)-protein complex, whereas the native Zn enzymes are colorless. Cobalt has been substituted for zinc in the enzymes carbonic anhydrase,⁸ alkaline phosphatase,⁹ and carboxypeptidase¹⁰ with little or no loss in catalytic activity. The amino acids which have been implicated as metal ligands in the Zn-containing enzymes are two histidines and one glutamic acid in carboxypeptidase¹¹ and thermolysin¹² and three histidines in carbonic anhydrase.¹³ Since histidine is the predominant ligand, any rR spectrum of a Co(II)-substituted zinc protein should be principally due to the Co(II)-histidine chromophore.

We have investigated the Raman spectral properties of Co(II)-imidazole and -histidine complexes of known stoichiometries and geometries to determine those ligand vibrations that undergo rR enhancement.

Experimental Section

Histidine (Sigma), imidazole (J. T. Baker), and the metal salts (Mallinckrodt) were of the highest purity available from the respective suppliers and were used without further purification.

Cobalt(II) complexes with imidazole, C₃H₄N₂ (Im), and imidazolate anion, C₃H₃N₂⁻ (Im'), were prepared as follows:¹⁴ Co^{II}Im₂Cl₂ and Co^{II}Im₆(NO₃)₂ from CoCl₂·6H₂O and Co(NO₃)₂·6H₂O, respectively, with imidazole in neutral ethanol solution and precipitated by the addition of ether; Co^{II}Im'₂ from CoCl₂·6H₂O and imidazole in ethanol with sufficient ethanolic KOH added to precipitate the imidazolate complex.

Bis complexes of cobalt(II) with histidine, C₆H₉O₂N₃ (His), and histidinate anion, C₆H₈O₂N₃⁻ (His'), were prepared,¹⁵ respectively, from CoSO₄·7H₂O and histidine in neutral aqueous solution or with sufficient added NaOH to produce the purple histidinate complex. Solids were isolated by evaporating to dryness in a

rotary evaporator. All steps were carried out in a glove box purged with chromous perchlorate scrubbed N₂.

Ultraviolet spectra were recorded on a Cary 16 spectrophotometer. All Raman spectra were recorded on polycrystalline samples mixed with KCl as a bulk carrier and with either K₂SO₄ or NO₃⁻ as internal intensity standards. Mixtures were lightly pressed into a 5 cm diameter disk and spun at 2000 rpm. A Jarrell-Ash 25-300 Raman spectrometer with a Coherent Radiation Laboratories Model 52 Ar⁺ laser was used. No sample decomposition was observed even after several hours of continuous irradiation with up to 750 mW of power at the sample. Spike filters were used to eliminate laser plasma lines. Intensity data are based on peak height measurements, and ratioed to the $\nu_1(A_1)$ vibration of SO₄²⁻. For Co^{II}Im₆(NO₃)₂ the $\nu_1(A_1)$ vibration of NO₃⁻ served as the internal standard. Since we observed a slight (~10%) increase of $I_{\nu_1(\text{NO}_3^-)}/I_{\nu_1(\text{SO}_4^{2-})}$ with decreasing wavelength of excitation in KNO₃-K₂SO₄ mixtures, the Co^{II}Im₆(NO₃)₂ data are corrected to sulfate.

Results

The Raman spectrum of Co^{II}Im₆(NO₃)₂ (Figure 1a) is nearly identical with that of Co^{II}Im₂Cl₂ and both are very similar to the spectrum of free imidazole¹⁶ (Table I). Some dissimilarities between free and complexed imidazole are observed in the 1075-1175-cm⁻¹ region. Co^{II}Im'₂ has a Raman spectrum resembling that of the free imidazolate anion (Table I, Figure 1b).

At neutral or slightly acidic pH, Co^{II}His₂ forms a pink-colored complex characteristic of octahedral coordination. The reactivity of this material toward atmospheric oxygen, forming a brown Co(III)-peroxo complex,¹⁷ prevented us from obtaining its Raman spectrum. In strongly basic solutions, however, the deep violet Co^{II}His'₂ complex having tetrahedral coordination is formed.¹⁵ This complex is sufficiently stable in air to permit recording of its Raman spectrum. While the overall spectrum is weak, the predominant peaks between 1000 and 1600 cm⁻¹ with 476.5-nm excitation appear at: 1002 (w), 1063 (w), 1082 (m), 1172 (m), 1210 (m), 1260 (s), 1282 (w), 1308 (m), 1335 (m), 1391 (w), 1425 (w), 1470 (w), 1489 (w), and 1559 cm⁻¹ (w).

The intensities of strong peaks in the spectra of the complexes were measured relative to the intensity of the internal standard and are plotted against excitation wavelength in Figures 2, 3, and 4. These relative intensities, normalized to the relative spectral intensities using one of the excitation wavelengths, are listed in Tables II, III, and IV.

Discussion

Vibrational Assignments. The elemental analyses reported for the complexes, Co^{II}Im₂Cl₂ and Co^{II}Im₆(NO₃)₂,

Table I. Raman Spectral Frequencies (cm^{-1}) of Imidazoles, Co(II) Complexes of Imidazoles, and Spectral Assignments between 900 and 1550 cm^{-1}

Im ^a	Co ^{II} Im ₂ Cl ₂ ^b	Co ^{II} Im ₆ (NO ₃) ₂ ^b	Co ^{II} Im' ₂ ^b	Im' ^c	HIm ⁺ ^d	Assignments ^e
1532 w	1534 w	1540 w 1517 w				Ring
1486 m	1495 w	1495 w	1489 m	1457 m	1460 s	Ring
1430 m	1421 m 1350 w	1439 m				Ring
1328 s	1322 m	1331 m	1332 vw 1313 w	1330 w 1307 w		Ring
1258 s	1253 s	1258 s	1273 s	1249 s	1215 s	Ring
1160 m	1129 w	1147 m				$\delta(\text{N-H})$
1130 m	1165 s	1173 s 1134 w	1163 s	1143 s	1135 w	Ring
1095 w	1100 w	1105 m	1103 w	1100 w	1106 m	$\delta(\text{C-H})$
1066 w		1068 w	1085 w	1075 w	1068 vw	$\delta(\text{C-H})$
	950 w		950 vw	945 w		
915 vw	913 w	920 w			913 w	$\delta(\text{C-H})$

^a Aqueous solution of imidazole, $\text{C}_3\text{H}_4\text{N}_2$, 514.5-nm excitation. ^b Co(II) complex, polycrystalline, 457.9-nm excitation. ^c Alkaline solution ($\sim 3 \text{ M KOH}$) of imidazole, 476.5 nm. ^d Acidic solution ($\sim 6 \text{ M HCl}$) of imidazole, 476.5 nm. ^e $\delta(\text{C-H})$ and $\delta(\text{N-H})$ assignments from ref 16 and 18; ring assignments from ref 16, 18, and this work.

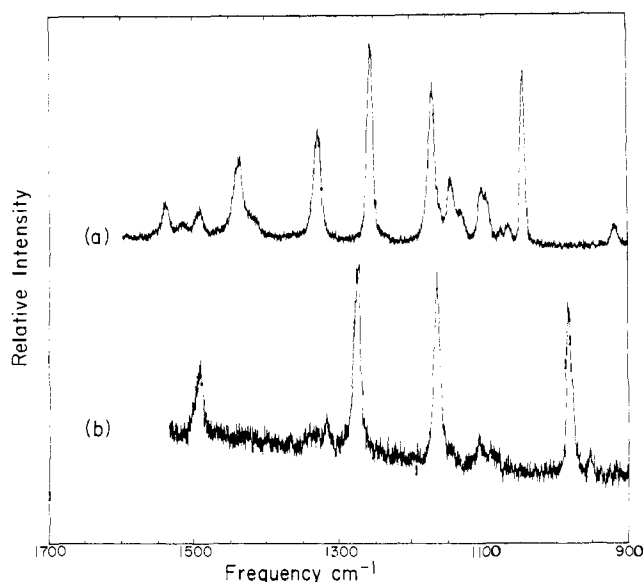


Figure 1. Raman spectra of cobalt(II)-imidazole complexes with 457.9-nm excitation: (a) $\text{Co}^{\text{II}}\text{Im}_6(\text{NO}_3)_2$ (s), peak at 1045 cm^{-1} is ν_1 (NO_3^-); (b) $\text{Co}^{\text{II}}\text{Im}'_2$ (s), peak at 980 cm^{-1} is ν_1 (SO_4^{2-}).

show that one nitrogen in the imidazole is protonated as in the free ligand.¹⁴ Upon complexation of the imidazole to Co^{2+} through the other nitrogen, the symmetry (C_s) of the ligand is not altered. For very strong ligand-cobalt coordination, one would expect that the Raman spectra of the complexes would resemble that of the imidazolium cation (HIm^+). However, the similarity of the observed spectra to that of free imidazole implies that the ligand is only weakly coordinated to the metal ion.

A comparison of the 1075–1175- cm^{-1} regions of the spectra of the free and complexed imidazoles suggests that the $\delta(\text{C-H})$ vibration of free imidazole assigned at 1095 cm^{-1} ^{16,18} is shifted to $\sim 1105 \text{ cm}^{-1}$ in the complexes. The moderately intense 1160 cm^{-1} $\delta(\text{N-H})$ and 1130 cm^{-1} (ring) vibrations of free imidazole are apparently shifted to 1147 and 1173 cm^{-1} , respectively, based on the resonance intensity behavior (see below).

There is disagreement in the literature concerning the assignment of the two most intense peaks in the Raman spectrum of imidazole. The 1258-cm^{-1} peak has been assigned to a $\delta(\text{C-H})$ by Perchard, *et al.*, from infrared and Raman studies¹⁶ and to a ring mode by Cordes and Walter from a

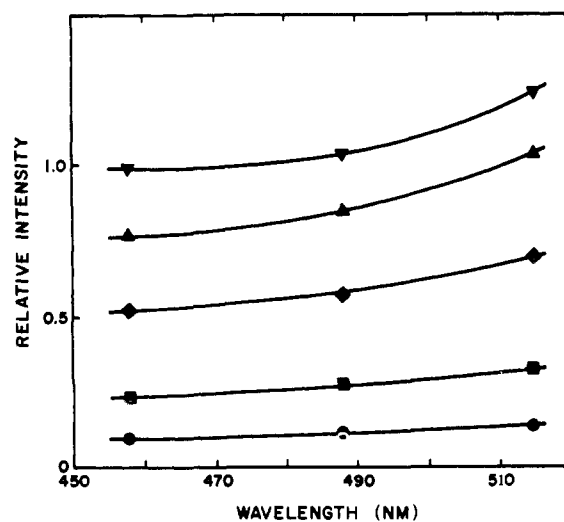


Figure 2. Dependence of Raman peak intensities of $\text{Co}^{\text{II}}\text{Im}_6(\text{NO}_3)_2$ on excitation wavelength: (●) 920; (■) 1105; (▲) 1173; (▼) 1258; (◆) 1331 cm^{-1} .

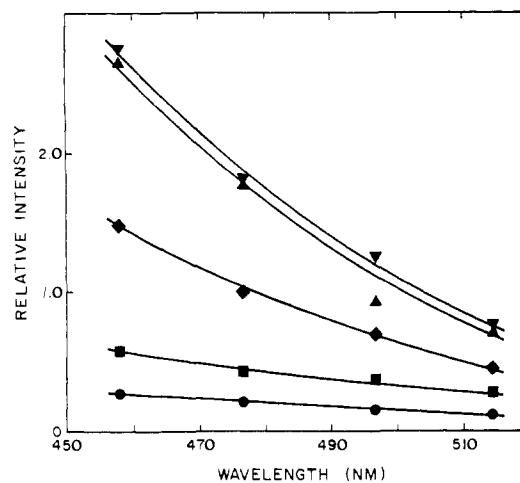


Figure 3. Dependence of Raman peak intensities of $\text{Co}^{\text{II}}\text{Im}_2\text{Cl}_2$ on excitation wavelength: (●) 913; (■) 1100; (▲) 1165; (▼) 1253; (◆) 1322 cm^{-1} .

normal coordinate treatment of ir data.¹⁸ On the other hand, the 1331-cm^{-1} peak has been assigned to a $\delta(\text{C-H})$ mode by Cordes and Walter and to a ring mode by Per-

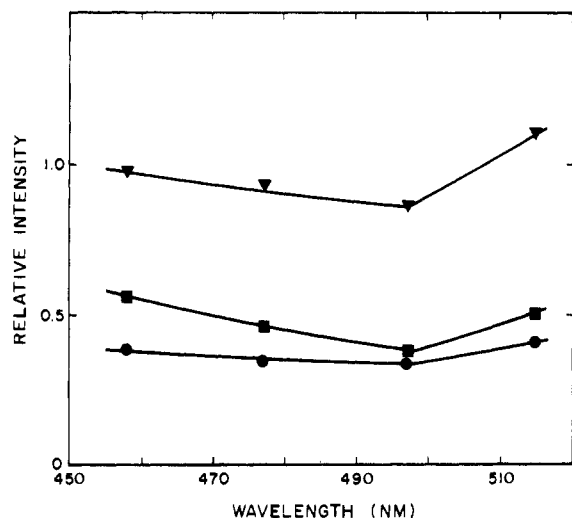


Figure 4. Dependence of Raman peak intensities of $\text{Co}^{\text{II}}\text{His}'_2$ on excitation wavelength: (●) 1180; (■) 1218; (▼) 1267 cm^{-1} .

chard, *et al.* Since the 1331- cm^{-1} peak decreases considerably in intensity upon conversion of imidazole to imidazolate anion but is unaffected by deuteration of the nitrogen in free imidazole,¹⁶ it is more likely that the most significant contribution to this peak is from ring vibration rather than C-H or N-H deformation. The resonance behavior (see below) of both the 1258- and 1331- cm^{-1} peaks is consistent with assignment to ring modes.

The three most intense Raman peaks of $\text{Co}^{\text{II}}\text{Im}'_2$ are observed at 1163, 1273, and 1489 cm^{-1} . In the ir spectrum of this complex, Cordes and Walter¹⁸ have assigned peaks at 1160 and 1485 cm^{-1} to ring vibrational modes and a peak at 1273 cm^{-1} to a combination band (948 + 340 cm^{-1}). In the Raman spectrum, however, the peak at 1273 cm^{-1} is the most intense and, consequently, should be assigned to a fundamental vibration and not to a combination band.

Resonance Effects. Albrecht and Hutley¹⁹ have shown that when the incident radiation is near or within an absorption band of a molecule, the Raman scattering intensity of specific vibrations can be enhanced either through vibronic coupling with the single active excited electronic state in resonance (the A term of the vibronic theory) or through interaction of the major active state with electronic states at higher energies coupled to it *via* the enhanced vibration (the B terms). For a diagonal element in the polarizability derivative tensor, the frequency dependence on incident radiation can be factored out of the A and B terms, giving frequency factors

$$F_A = \nu^2(\nu_e^2 + \nu_0^2)/(\nu_e^2 - \nu_0^2)^2 \quad (1)$$

$$F_B = 2\nu^2(\nu_e\nu_s + \nu_0^2)/(\nu_e^2 - \nu_0^2)(\nu_s^2 - \nu_0^2) \quad (2)$$

where ν_0 is the incident frequency, $\nu = \nu_0 - \Delta\nu$, $\Delta\nu$ is the vibrational frequency, ν_e is the major active virtual electronic state for the vibrational mode at $\Delta\nu$, and ν_s is an average frequency for all other active virtual states assumed to lie at much higher energy than ν_e . Vibrational intensities are proportional to F_A^2 or F_B^2 .

It is thus possible to obtain information on the source of the resonance enhancement of a given vibrational mode by comparing the observed intensity *vs.* wavelength dependence to that predicted from eq 1 and 2. These equations should be valid for the totally symmetric vibrations of the complexes studied. The observed and calculated relative intensity behavior is presented in Tables II-IV.

The Raman spectrum of imidazole was recorded with four different laser excitations (λ 457.9, 476.5, 496.5, and

Table II. Raman Intensities of $\text{Co}^{\text{II}}\text{Im}_6(\text{NO}_3)_2$ (Relative to the Internal Standard) Normalized to the Relative Intensities using the 457.9-nm Excitation Wavelength, and the Calculated Relative Intensity Enhancement, F_A^2 (eq 1)

Raman freq, cm^{-1}	Wavelength of excitation, nm		
	457.9	488.0	514.5
1540	1.00	0.92	1.52
1439	1.00	1.11	1.44
1331	1.00	1.09	1.34
1258	1.00	1.04	1.25
1173	1.00	1.11	1.36
1147	1.00	0.94	1.45
1105	1.00	1.19	1.41
920	1.00	1.24	1.48
Calcd for 1000 cm^{-1} , ν_e 1060 nm	1.00	1.16	1.35

Table III. Normalized Raman Intensities of $\text{Co}^{\text{II}}\text{Im}'_2$ Relative to 514.5-nm Excitation (see Table II and Text), and Calculated Values of F_A^2

Raman freq, cm^{-1}	Wavelength of excitation, nm		
	457.9	476.5	514.5
1489	2.38	1.98	1.00
1273	2.73	1.72	1.00
1163	3.27	2.29	1.00
Calcd for 1000 cm^{-1} , ν_e 395 nm	13.03	4.62	1.00
ν_e 271 nm	2.79	1.93	1.00

Table IV. Normalized Raman Intensities of $\text{Co}^{\text{II}}\text{Im}_2\text{Cl}_2$ Relative to 514.5-nm Excitation (see Table II and Text), and Calculated Values of F_A^2

Raman freq, cm^{-1}	Wavelength of excitation, nm			
	457.9	476.5	496.5	514.5
1534	4.54	3.43	2.46	1.00
1421	3.69	2.74	1.76	1.00
1322	3.26	2.19	1.53	1.00
1253	3.74	2.47	1.70	1.00
1165	3.75	2.49	1.32	1.00
1100	2.17	1.58	1.32	1.00
913	1.91	1.80	1.35	1.00
Calcd for 1000 cm^{-1} , ν_e 425 nm	55.37	9.18	2.46	1.00
ν_e 314 nm	3.70	2.29	1.45	1.00
ν_e 250 nm	2.54	1.82	1.31	1.00

514.5 nm) to determine the ν_0 dependence of peak intensities in free imidazole. All peak intensities remained unchanged relative to the ν_1 vibration of SO_4^{2-} . Since the Raman spectra of pure aqueous imidazole show no excitation frequency dependent intensity variations, any variation observed in the spectra of the $\text{Co}(\text{II})$ complexes must arise from an electronic state ν_e which involves the combined cobalt(II)-imidazole moiety.

$\text{Co}^{\text{II}}\text{Im}_6(\text{NO}_3)_2$. A slight dependence of peak intensities on excitation frequency is observed (Figure 2, Table II) for $\text{Co}^{\text{II}}\text{Im}_6(\text{NO}_3)_2$. The measured intensities increase as the excitation wavelength increases. That is, the electronic transition responsible for the intensity enhancement (ν_e) must lie at the red end of the spectrum. The stoichiometry, visible absorption spectrum, and magnetic moment measurements indicate octahedral coordination of this complex.¹⁴ At the high wavelength end of the visible spectrum, the most intense absorption band is observed at 1060 nm and has been assigned to the ${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{T}_{2g}(\text{F})$ d-d transition of $\text{Co}(\text{II})$.¹⁴ The frequency factor F_A^2 calculated using the 1060-nm transition as the active electronic state is compared with the measured relative intensities in Table II. The agreement between the calculated and measured intensities

shows that this single term is quite successful in representing the experimentally observed wavelength dependence of the imidazole peak intensities.

We have observed a very intense absorption band with maximum below 250 nm in the uv spectrum of an ethanolic solution of the complex. This transition and the ${}^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ d-d transition at 487 nm,¹⁴ however, appear to contribute little to the observed peak intensity variation.

The slight resonance enhancement is reasonable in view of the low energy of the 1060-nm band and reflects minimal mixing of this d-d state with ligand electronic levels at higher energy (<250 nm).

Co^{II}Im₂ and Co^{II}Im₂Cl₂. A more pronounced frequency-dependent intensity enhancement is observed for Co^{II}Im₂ (Table III) and Co^{II}Im₂Cl₂ (Figure 3 and Table IV) with the peak intensities increasing as the Raman excitation wavelength decreases. Based on magnetic moment measurements and absorption spectra, cobalt in these two complexes is found to be approximately tetrahedrally coordinated.¹⁴ For Co^{II}Im₂Cl₂ the geometry is actually C_{2v} or lower with two nitrogen and two chlorine atoms surrounding each cobalt. The cobalt in Co^{II}Im₂ is surrounded by four nitrogen atoms from four different imidazole ligands. The two most intense d-d transitions for these tetrahedral-like complexes, ${}^4A_2 \rightarrow {}^4F_1(F)$ and ${}^4A_2 \rightarrow {}^4F_1(P)$, occur at 1004 and 615 nm in Co^{II}Im₂Cl₂ and at 1010 and 575 nm in Co^{II}Im₂,¹⁴ at the wrong spectral extreme to account for the observed intensity behavior. Very weak absorption bands are observed at 425 nm for Co^{II}Im₂Cl₂ and at 395 nm for Co^{II}Im₂.¹⁴ In an ethanolic solution of Co^{II}Im₂Cl₂ we have also observed a very intense band with maximum below 250 nm; spectral features of this intensity were not observed in free imidazole. This band may, thus, be due to a cobalt-imidazole charge-transfer transition.

The observed resonant behavior of peak intensities for Co^{II}Im₂Cl₂ (Table IV) may be arranged into two groups: one with $I_{457.9}/I_{514.5} \approx 3.7$ and a second with $I_{457.9}/I_{514.5} \approx 2.1$. It is interesting that those vibrations with ratios ~ 2.1 have been assigned by Cordes and Walter¹⁸ and by Perchard, *et al.*,¹⁶ to C-H vibrational modes. Where both groups of investigators have agreed, assignment of peaks within the ~ 3.7 intensity ratio group has been to ring vibrational modes. Where there is disagreement in the assignment, one or the other group of investigators has assigned these peaks to a ring vibration. The intensity behavior suggests that different resonance enhancement mechanisms (*i.e.*, different electronic levels) are involved for the two groups of peaks. It appears that those vibrations with $I_{457.9}/I_{514.5} \approx 3.7$ should all be assigned to ring vibrations.

In investigating the resonance Raman effect in diphenylpolyenes, Schmid and Brosa have observed that those vibrations associated with the ring vibrational modes undergo considerable resonance intensity enhancement.²⁰ The intensities of the C-H vibrational modes, on the other hand, were observed to be independent, within experimental error, of the excitation frequency. This nonuniform behavior was interpreted by assuming that the Raman scattering tensor can be separated into two parts: one dependent upon the π electrons and the second upon the σ electrons.

The relative peak intensities (F_A^2) that are calculated when the active electronic state is chosen to be 425, 395, or 250 nm are all considerably different from the observed values (Tables III and IV). The best fit of the data is obtained with $\nu_e = 31,850 \text{ cm}^{-1}$ (314 nm) for the 3.7 ratio group of Co^{II}Im₂Cl₂ and $\nu_e = 36,900 \text{ cm}^{-1}$ (271 nm) for Co^{II}Im₂. Experimentally, however, peaks are not resolved at these wavelengths in the spectra of these complexes. Calculated values of F_B^2 using $1/\nu_e = 425$ or 395 nm and $1/\nu_s$ values out to 100 nm also did not reproduce the experimen-

tal intensity behavior. The poor fit between the calculated and experimental intensity ratios indicates that a single term cannot adequately represent the observed frequency dependence of the peak intensities. The actual peak intensities appear to result from the summation over several electronic states of the tetrahedral-like complexes.

Metal-ligand charge transfer (CT) transitions are often the active electronic states for resonance enhancement of Raman peak intensities.²¹⁻²³ In these two tetrahedral-like complexes, a cobalt-imidazole CT transition or transition near 250 nm appears to be the principal contributor to the intensity variations while cobalt d-d transitions have no significant effect on the intensities as would be expected if there were little mixing of ligand orbitals with cobalt(II) d orbitals.

Histidine Complex. The relative Raman peak intensities of the tetrahedrally-coordinated histidine complex show a slight increase toward both ends of the visible spectrum with the minimum intensities occurring with 496.5-nm excitation (Figure 4). This double maximum indicates that these vibrations can vibronically couple with electronic levels which are primarily cobalt d states (at 558 and 1115 nm)¹⁵ or with charge transfer states which should lie in the near ultraviolet. A similar excitation profile has been reported for the 1157 cm^{-1} C-C single bond stretching vibration in lycopene by Rimai, *et al.*²⁴

Conclusions

Compared with resonance Raman spectra of other metal complexes or metalloproteins, the cobalt complexes in this study show only slight vibrational resonance effects. For example, at the 1:3 ratio of cobalt complex to internal standard (K₂SO₄) used here, the most intense imidazole peak has comparable intensity to the ν_1 sulfate peak, whereas in the heme protein and carotenoid resonance spectra, complex to standard ratios of 1:1000 to 1:10000 yield comparable peak intensities for complex and standard. The weak resonance effects are due to the absence of much involvement of ligand orbitals in the visible transitions, which are largely forbidden d-d bands, and the high energy of the ligand-cobalt charge transfer states. In the case of oxyhemocyanin we were able to observe resonance enhancement at a copper to standard ratio of 1:10 when the excitation frequency was within an absorption band.²⁵

Co(II)-substituted zinc proteins appear to be similar to the model cobalt imidazole complexes with comparably low absorptivities for their visible d-d transitions.⁸ Because of the low Raman intensities and only slight resonance effect observed in the model complexes, it is doubtful whether Co(II)-substituted zinc proteins would produce resonance enhanced Raman spectra of the cobalt-protein chromophore with laser excitation in the visible region. Experiments in our laboratory with Co(II)-substituted carbonic anhydrase and carboxypeptidase have confirmed this conclusion.²⁶

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Synthetic Analogs of the Active Sites of Iron-Sulfur Proteins. XI.¹ Synthesis and Properties of Complexes Containing the Fe₂S₂ Core and the Structures of Bis[*o*-xylyl- α,α' -dithiolato- μ -sulfido-ferrate(III)] and Bis[*p*-tolylthiolato- μ -sulfido-ferrate(III)] Dianions

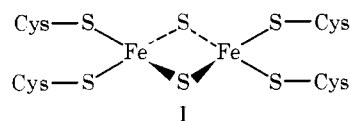
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Abstract: Although an extensive body of physicochemical data indicates that the active sites of plant ferredoxins (Fd) and similar 2-Fe proteins have the minimal formulation [Fe₂S₂(S-Cys)₄], the sites have not been defined by X-ray diffraction. Reaction of FeCl₃, *o*-C₆H₄(CH₂SH)₂, NaHS, and NaOMe in methanol affords the dimeric Fe(III) dianion [FeS(SCH₂)₂C₆H₄]₂²⁻ (**1**), isolated as its Et₄N⁺ and Ph₄As⁺ salts. The electronic spectra and other spectroscopic and magnetic properties of **1** indicate a clear electronic relationship to Fd_{ox} proteins. Ligand substitution reactions of **1** with arylthiols yield the dimers [Fe₂S₂(SAr)₄]²⁻ (Ar = Ph, *p*-tolyl (**4d**), *p*-C₆H₄Cl), isolated as Et₄N⁺ salts. The structures of **1** and **4d** (as their Et₄N⁺ salts) have been determined. Both salts crystallize with two formula units in space group C_{2h}²-P2₁/n of the monoclinic system in unit cells of dimensions *a* = 9.549 (6) Å, *b* = 13.549 (6) Å, *c* = 14.748 (6) Å, and β = 95.42 (3)° (**1**) and *a* = 8.913 (3) Å, *b* = 16.132 (3) Å, *c* = 17.583 (3) Å, β = 97.14 (1)° (**4d**). The structures consist of well-separated cations and anions, the latter being sulfide-bridged dimers with crystallographically imposed centers of symmetry. Both anions contain planar and nearly isostructural Fe₂S₂* cores with Fe··Fe and mean Fe-S* distances of 2.698 (1) and 2.691 (4d) and 2.209 (1) and 2.201 Å (4d), respectively. The coordination units FeS₂*S₂ are distorted tetrahedra with bond angles of 104.7–112.3° (**1**) and 104.6–115.8° (**4d**). The structural and other properties of **1** indicate that it is a suitable minimal representation of the oxidized protein active sites and tend to confirm the previously proposed formulation of these sites. The reaction product of **1** with *p*-Me₃N⁺C₆H₄SH is identical with the previously reported complex prepared directly from FeCl₃, the thiol, and NaHS and is shown to be the cation dimer [Fe₂S₂(SC₆H₄NMe₃)₄]²⁺. Corresponding properties of [Fe₂S₂(SR)₄]²⁻ and [Fe₄S₄(SR)₄]²⁻ complexes at parity of R substituents are compared. Dimer Fe₂S₂* cores and tetramer Fe₂S₂* faces possess a high degree of dimensional similarity. Absorption spectra in the 400–600-nm region differ significantly. Dimeric complexes, as the tetramers, are reducible to 3- and 4- anions in steps which in most cases approach reversibility in DMF under dc and pulsed polarographic measurement conditions. Potentials for the [Fe₂S₂(SAr)₄]^{2-/3-} process show an approximate correlation with σ_p . Differences in potentials for 2-/3- and 3-/4- dimer reactions (ca. 300 mV) are much smaller than those for the corresponding tetramer reactions (ca. 700 mV). The comparative spectral and electrochemical properties should allow analysis of dimer-tetramer mixtures such as might result from the extrusion of active sites from complex Fe-S proteins with aryl thiols. The preparation of several arylthiolate tetramers from Fe₂S₂(CO)₆ is described.

Within the continually expanding group of purified iron-sulfur proteins,⁴ those containing two iron and two labile sulfur (S*) atoms and obtainable from plant, mammalian, and bacterial sources are at present the most numerous. Representative 2-Fe proteins have been subjected to extensive and elegant magnetic and spectroscopic characterization, and their physicochemical properties have been summarized in detail elsewhere.⁴⁻⁸ However, unlike the active centers of 1-Fe,⁹ 4-Fe,¹⁰ and 8-Fe^{10,11} proteins, the structure of the iron-sulfur unit apparently common to most, if not all, Fe₂S₂* proteins has not been conclusively estab-

lished by X-ray diffraction methods. Critical assessment of the physicochemical data has led to the generally accepted conclusion that the active site has the minimal composition [Fe₂S₂*(S-Cys)₄] and can be represented by the structure I.



In this formulation the Fe₂S₂* core of an oxidized protein