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Charge-Transfer Absorptions of Cu(II)–Imidazole and Cu(II)–Imidazolate Chromophores

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Abstract: Electronic spectra over the 50 000–20 000-cm^{-1} region are reported for well-characterized chromophores having Cu(II)-imidazole (ImH) and Cu(II)-imidazolate (Im^-) units. For tetragonal Cu(II)-ImH chromophores, *three* ligand to metal charge-transfer (LMCT) absorptions originate from the σ-symmetry nitrogen donor lone pair and from two π-symmetry ring orbitals, one having primarily carbon character (π_1) and the other having primarily nitrogen character (π_2). These σ(ImH) →, π_2(ImH) →, and π_1(ImH) → Cu(II) LMCT absorptions occur at ~220, ~260, and ~330 nm, respectively. Ligand rotation causes the π-symmetry absorptions to be broadened for solutions containing geometrically unconstrained Cu(II)-ImH complexes. The π-symmetry absorptions generally are well-resolved spectral features of crystalline complexes, and may be split when the ImH groups have nonequivalent orientations. The σ(ImH) → Cu(II) absorption at 220 nm is insensitive to ligand rotation about the Cu–N axis, and is well resolved from the ligand-localized absorption at ~205 nm. The Cu(II)-Im^- complexes exhibit an additional and characteristic broad absorption at ~375 nm for which a tentative assignment has been suggested. Tetragonal type 2 and type 3 copper protein chromophores are expected to exhibit corresponding π(ImH) → Cu(II) LMCT transitions in the near-UV region. Such absorptions are expected to be red shifted for the approximately tetrahedral type 1 copper chromophores. The reported spectra of the above types of proteins briefly are reconsidered from this point of view.

Introduction

Imidazole groups have important ligand roles when copper is complexed by histidine-containing peptides. The structural features of such bonding have been well characterized by X-ray crystallographic studies of low molecular weight Cu(II) complexes[1] and of proteins such as plastocyanin,[2] azurin,[3] and superoxide dismutase.[4] Other evidence has been used to infer Cu(II)-imidazole interactions in stellacyanin,[5] serum albumin,[6] galactose oxidase,[7] cytochrome *c* oxidase,[8] ceruloplasmin,[9] hemocyanins,[10] and other proteins. Our interest in the electronic spectra of the Cu(II) proteins has led to the synthesis and/or characterization of model complexes which have served to elucidate features of Cu(II)-thioether,[11] Cu(II)-disulfide,[12] Cu(II)-mercaptide,[13] Cu(II)-deprotonated amide,[14] and Cu(II)-superoxide[15] bonding. We report here an extension of these studies to the ligand to metal charge-transfer (LMCT) absorptions of Cu(II)-imidazole (ImH) and Cu(II)-imidazolate (Im^-) chromophores. Charge-transfer spectra of fully characterized low molecular weight Cu(II) complexes are presented and assigned. The presence of corresponding absorptions in the spectra of Cu(II) proteins is discussed briefly.

Experimental Section

Preparation of Complexes. Imidazole (ImH) and L-histidine were obtained from the Aldrich Chemical Co. and Matheson Coleman and Bell, respectively. These ligands were purified by recrystallization (thrice) from water that was distilled and deionized: The water used for recrystallization and spectral studies must be scrupulously pure. Otherwise, the spectra of the free ligands and Zn(II) complexes contain extraneous UV absorptions attributable to Cu(II) (and possibly other) impurities. $Cu(ImH)_4SO_4$,[16] bis[*cyclo*-(L-histidyl-L-histidyl)]copper(II) diperchlorate tetrahydrate ($C_{12}H_{14}N_6O_2Cu{\cdot}2ClO_4{\cdot}4H_2O$),[17] aquo-$\mu$-bis[*cyclo*-(L-histidyl-L-histidyl)dicopper(II) diperchloratemonohydrate ($H_2O(C_{12}H_{13}N_6O_2)_2Cu_2{\cdot}2ClO_4$),[18] $[Cu(\beta\text{-ala-L-his})H_2O]_2$,[19] $Cu_3(ImH)_8(Im)_2{\cdot}4ClO_4$,[20] and $Zn(\text{L-His})_2{\cdot}2H_2O$[21] were prepared according to published procedures. The densities of these complexes were measured in CCl_4–$BrCH_2CH_2Br$ gradients and compared with the values calculated from the published unit cell parameters. Agreement of the observed and calculated densities within 0.02 g/cm^3 indicated that the synthetic procedures yielded the above complexes. $Zn(\text{L-His})_2{\cdot}2H_2O$ was recrystallized five times from water. Even though the ligands already were in pure form, additional purification of the complex was required to attenuate the extraneous UV absorptions resulting from Cu(II) present in $ZnSO_4{\cdot}7H_2O$.

The imidazolate-bridged species $Im(CuR_2NCH_2CH_2NHCH_2CH_2NR_2)_2{\cdot}3ClO_4$ (R = H) was prepared by a published procedure.[22] The Cu–Im^-–Cu unit recently has been verified by a crystallographic study of a complex where R = CH_3.[23] The corresponding complex where R = C_2H_5 was prepared by adding 1 equiv of NaOH to a methanol solution containing equimolar amounts of $Cu(ClO_4)_2{\cdot}6H_2O$, $Et_2N(CH_2)_2NH(CH_2)_2NEt_2$, and ImH.

Anal. Calcd for $Cu_2C_{27}H_{61}N_8Cl_3O_{12}$: Cu, 13.76; C, 35.12; H, 6.66; N, 12.14. Found: Cu, 13.79; C, 35.05; H, 6.68; N, 12.12.

Physical Measurements. Electronic spectra were recorded on Cary Model 14, 18, and 17 spectrophotometers. The latter instrument has been interfaced with a Tektronix computer. Solid samples were dispersed in mineral oil mulls and KBr pellets for electronic spectral studies. Evaporation of aqueous $Cu(ImH)_4{\cdot}SO_4$ on quartz flats yielded blue-violet crystalline films of the complex. The films were composed of elongated rectangular plates which were oriented in approximately the same direction, and exhibited well-resolved electronic spectra. Detailed single-crystal studies were not conducted because their usefulness is limited by the low symmetry of the space group (*C*2/*c*) in which the complex crystallizes.[16]

Results and Discussion

Electronic Structure of Imidazole. An analysis of Cu(II)-ImH LMCT spectra necessarily must consider the electronic

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Table I. Ionization Potentials (eV) of Imidazole

orbitals				
π_1	π_2	n	method	ref
9.87	11.51	11.62	CNDO/S	29
8.36	9.49	9.52-9.82	PPP	27
8.35			PPP	26
7.74	8.15		PPP	28
9.19	11.09	11.77	ab initio	25
8.92 (11.17)[a]	10.15 (12.71)[a]	10.25 (12.83)[a]	ab initio	24
8.78	10.3	10.3	exptl (PES)	24

[a] The actual calculated IPs are given in parentheses. The authors of ref 24 recommend that these values should be reduced by a factor of ~0.8.

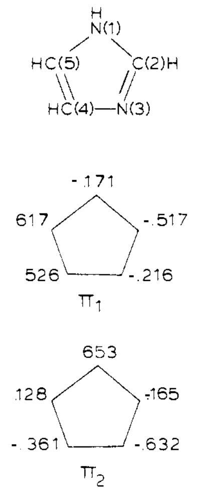


Figure 1. Numbering scheme for imidazole along with the MO coefficients of Del Bene and Jaffé.[29]

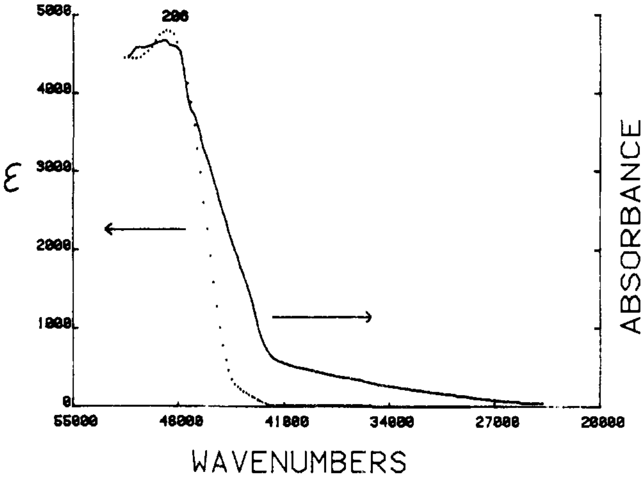


Figure 2. Aqueous solution (···) and mineral oil mull (——) spectra of imidazole at 298 K.

structure of the free ligands and the perturbation, if any, in their absorption spectra by a spectroscopically inactive dipositive ion such as Zn(II). The electronic structure of imidazole has been probed by calculations employing ab initio,[24,25] modified Pariser-Parr-Pople,[26-28] and CNDO/S[29] techniques. All of the calculations yield the pleasant result that there are three relatively high energy occupied molecular orbitals which are well removed from the core orbitals. A view of ImH along with the MO coefficients reported by Del Bene and Jaffé[29] is shown in Figure 1; relevant calculated ionization potentials are summarized in Table I. All the calculations show that the HOMO is a π-symmetry orbital (π_1), which is located primarily on the carbon atoms. A second π-symmetry orbital (π_2) and σ-symmetry orbital (n) approximately are degenerate. The π_2 orbital is composed primarily of the 2p(π) orbitals of the nitrogen atoms whereas the more tightly bound n orbital consists largely of the lone pair on the pyridine-type N(3) atom. The calculated separation between the π_2 and n orbitals varied from 0.03 to 0.68 eV, and always was substantially smaller than the corresponding separation between the π_1 and π_2 orbitals. Experimental support for the calculated contents of Table I comes from the published photoelectron spectra of ImH.[24] Only two transitions are seen at energies less than 13.7 eV, and these reasonably have been assigned to ionization from the π_1 level (8.78 eV) and ionization from the π_2,n levels (broad, unresolvable absorption at 10.3 eV). Two strong π-π^* transitions at ~200 nm along with a weak n-π^* transition at lower energy are predicted by calculations of excited ImH.[30] Ethanolic and aqueous ImH exhibits an absorption in the 205-210-nm region (ϵ ~5000) which corresponds to one of the π-π^* transitions. A higher energy π-π^* process has been observed in the 178-187-nm region; the position of this transition exhibits a modest dependency upon solvent and pH.[30] A presumed n-π^* transition at 250 nm (ϵ ~60) reported by early workers could not be reproduced in more recent studies.[30] Mull spectra of solid ImH are said to exhibit a broad, featureless absorption over the 240-400-nm spectral region.[31] This result is attributable to impurities. Pure ImH is spectrally transparent over this region (Figure 2); the shoulder in the mull spectra at ~40 500 cm^{-1} slowly diminishes with repeated further recrystallization and may result from traces of Cu(II).

Electronic Structure of Cu(II)-ImH Chromophores. Complexation of the ImH unit by a first-row dipositive metal ion does not perturb the ligand spectra appreciably. The π-π^* transition of the ImH units in Zn(L-His)$_2$·2H$_2$O occurs at 203 nm. As noted above, the corresponding transition in free ImH appears in the 205-210-nm range. Moreover, the spectral transparency of ImH at $\lambda > 225$ nm is not affected by bonding to the spectroscopically inactive d^{10} Zn(II) ion.

A qualitative MO diagram shown in Figure 3 indicates the essential electronic structural features of Cu(II)-ImH units. The upper occupied ligand orbitals should be stabilized somewhat by the formation of the Cu-N(3) bond. Considering the possibilities of overlap between the metal and ImH orbitals and the MO coefficients of the ImH orbitals (Figure 1), the relative stabilization of the ligand orbitals in these complexes is expected to be n > π_2 > π_1. For a typical tetragonal Cu(II) complex, LMCT transitions involve promotion of an electron from these ImH orbitals into the single d vacancy ($d_{x^2-y^2}$) localized on the metal ion. Three such LMCT transitions are expected. A relatively intense σ(ImH) → Cu($d_{x^2-y^2}$) transition should be accompanied by weaker and red-shifted π_2(ImH) → Cu($d_{x^2-y^2}$) and π_1(ImH) → Cu($d_{x^2-y^2}$) transitions. The variations in intensity of these transitions should reflect the large differences in overlap of the ImH orbitals with the d vacancy. Certain features of the LMCT spectra should vary with the orientation of the ImH rings relative to the tetragonal

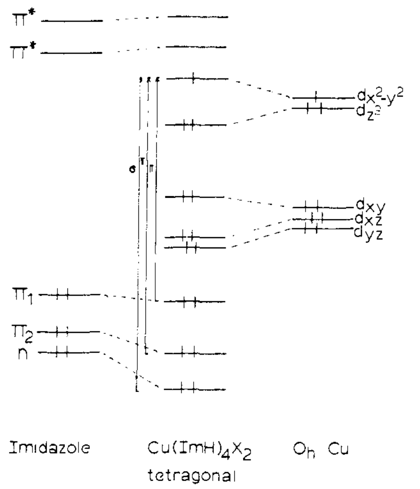
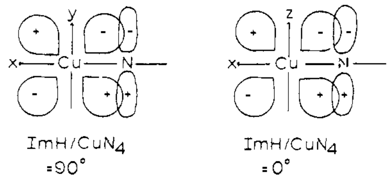


Figure 3. Qualitative MO diagram for a planar $Cu(ImH)_4^{2+}$ unit.



ImH/CuN4 =90°

ImH/CuN4 = 0°

Figure 4. Limiting π_2(ImH)-dπ(Cu) interactions for a planar $Cu(ImH)_4^{2+}$ chromophore.

CuN_4 unit. For example, the bonding between the ligand pπ orbitals and the copper dπ orbitals will depend upon the orientations of the ImH ring relative to the CuN_4 unit. Figure 4 illustrates the pπ-dπ interaction for the limiting conditions where the ImH plane is either parallel or perpendicular to the CuN_4 unit. In contrast, the σ(ImH) → Cu($d_{x^2-y^2}$) transition essentially should be independent of the rotation of an ImH ligand about its Cu-N bond. Thus, a $Cu(ImH)_4^{2+}$ complex which has two crystallographically unique ImH ligands may exhibit up to four distinct π(ImH) → Cu($d_{x^2-y^2}$) transitions as well as a single σ(ImH) → Cu($d_{x^2-y^2}$) transition.

1. $Cu(ImH)_4 \cdot SO_4$. The structure of this complex consists of centrosymmetric CuN_4O_2 units.[16] Four N(imidazole) donor atoms comprise the equatorial ligand set; the two apical Cu-O bonds result from coordination by bridging SO_4 groups. The planar CuN_4 unit (crystallographically required) contains ImH rings which have two different orientations. The dihedral angles between the CuN_4 plane and the ImH rings are 29.7 and 81.0°. Crystalline films of this complex exhibit the electronic spectra shown in Figure 5. The absorptions at ~195 and ~205 nm correspond to ligand-localized transitions of free ImH. Well-characterized Cu(II) complexes of aliphatic amines exhibit absorptions in the 240-270-nm range which result from σ(N) → Cu(II) LMCT.[11] However, the sp^2-type lone pair of the N(3) atom in ImH is stabilized relative to the sp^3-type lone pair of an aliphatic amine. For this reason, σ(ImH) → Cu(II) LMCT should be blue shifted relative to that reported for Cu(II) complexes of aliphatic amines. A blue shift of approximately 5000 cm^{-1} may be predicted from the ionization potentials (IP) observed for aliphatic amines and the N(3) lone pair of ImH. The first IP of CH_3NH_2 occurs at 78 240 cm^{-1},[11] whereas that for the lone pair on N(3) occurs at ~83 000 cm^{-1}, i.e., the broad absorption at 10.3 eV noted in Table I. Since σ(N) → Cu(II) LMCT transitions of aliphatic amine-Cu(II) chromophores occur at an average energy of ~40 000 cm^{-1}, the transition of $Cu(ImH)_4 \cdot SO_4$ at ~220 nm (45 500 cm^{-1}) reasonably may be assigned as a σ(ImH) → Cu($d_{x^2-y^2}$) transition. Polycrystalline films of this complex also exhibit distinct doublets at 240, 260 and at 305, 335 nm. These bands exhibit interesting polarization effects. Intensities of the bands at 240 and 305 nm are maximized when those of the bands at 260 and 335 nm are minimized, and vice versa. We assign all four transitions to π(ImH) → Cu(II) LMCT of crystallographically distinct ImH ligands (vide supra). In particular we assign the bands at 240 and 260 nm to two π_2(ImH) → Cu($d_{x^2-y^2}$) LMCT absorptions and those at 305 and 335 nm to two π_1(ImH) → Cu($d_{x^2-y^2}$) LMCT absorptions.

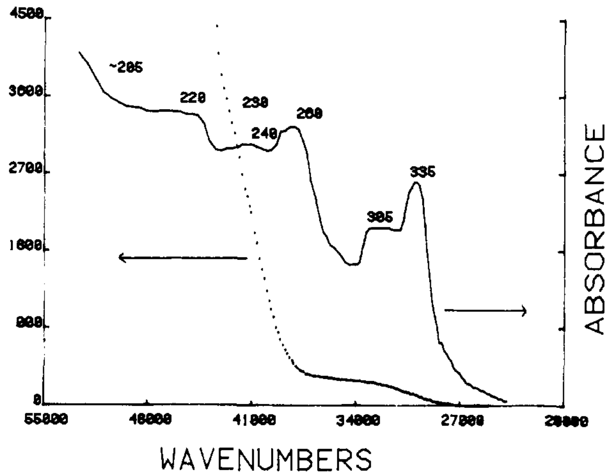


Figure 5. Electronic spectra at 298 K of $Cu(ImH)_4SO_4$ as a polycrystalline film (—) and in aqueous solution (·····).

A referee wondered whether these four bands all may be assigned to π_1 → Cu(II) transitions. We have difficulty reconciling such an assignment with the fact that the complex contains only two crystallographically unique types of ImH ligands. Moreover, reasonable separations between the complexed ImH orbitals may be drawn from our assignments. Using a simple one-electron picture (i.e., ignoring the contributions of coulombic integrals, electron correlation effects, etc., to the LMCT energies) and using average positions for the doublets in Figure 5 (i.e., 250 and 320 nm), our scheme yields *approximate* π_1-n and π_1-π_2 separations of 14 300 and 8750 cm^{-1}, respectively. The π_1-π_2/n separation in free ImH was observed experimentally to be ~12 200 cm^{-1},[24] and the calculations (Table I) suggest that the n orbital is more stable than the π_2 orbital by >1000 cm^{-1}.

The above doublets are not evident in the spectra of aqueous $Cu(ImH)_4^{2+}$. A featureless broad absorption (ε ~300) over the 260-340-nm range presumably arises because the ImH rings freely rotate about the Cu-N bonds. These spectra were measured under conditions which ensure that essentially all of the Cu(II) is present as the $Cu(ImH)_4^{2+}$ complex. This artifact of solution spectra need not apply to geometrically constrained Cu(II)-ImH chromophores such as those present in proteins. Moreover, we have observed that aqueous Cu(II) complexes of 4,5-disubstituted imidazoles exhibit well-resolved π_2(ImH) → Cu(II) and π_1(ImH) → Cu(II) LMCT bands.[32]

2. $Cu(C_{12}H_{14}N_6O_2)_2 \cdot 2ClO_4 \cdot 4H_2O$. The CuN_4 unit of this interesting complex results from coordination by four ImH units, two from each cyclic dipeptide.[17] For the convenience of the reader, a view of the $Cu(C_{12}H_{14}N_6O_2)_2^{2+}$ ion has been reproduced as Figure 6. Since the copper atom lies on a twofold axis, two crystallographically unique (although nearly equivalent) ImH rings are present. The dihedral angles between the ImH rings and the best CuN_4 plane are 45 and 46°. The CuN_4 fragment is nonplanar and distorted toward a tetrahedral configuration; the dihedral angle N(2)-Cu-N(6)/N(2′)-Cu-N(6′) of 29° provides one measure of this distortion. In contrast to the $Cu(ImH)_4 \cdot SO_4$ complex, the CuN_4 unit in $Cu(C_{12}H_{14}N_6O_2)^{2+}$ is puckered and its ImH rings essentially

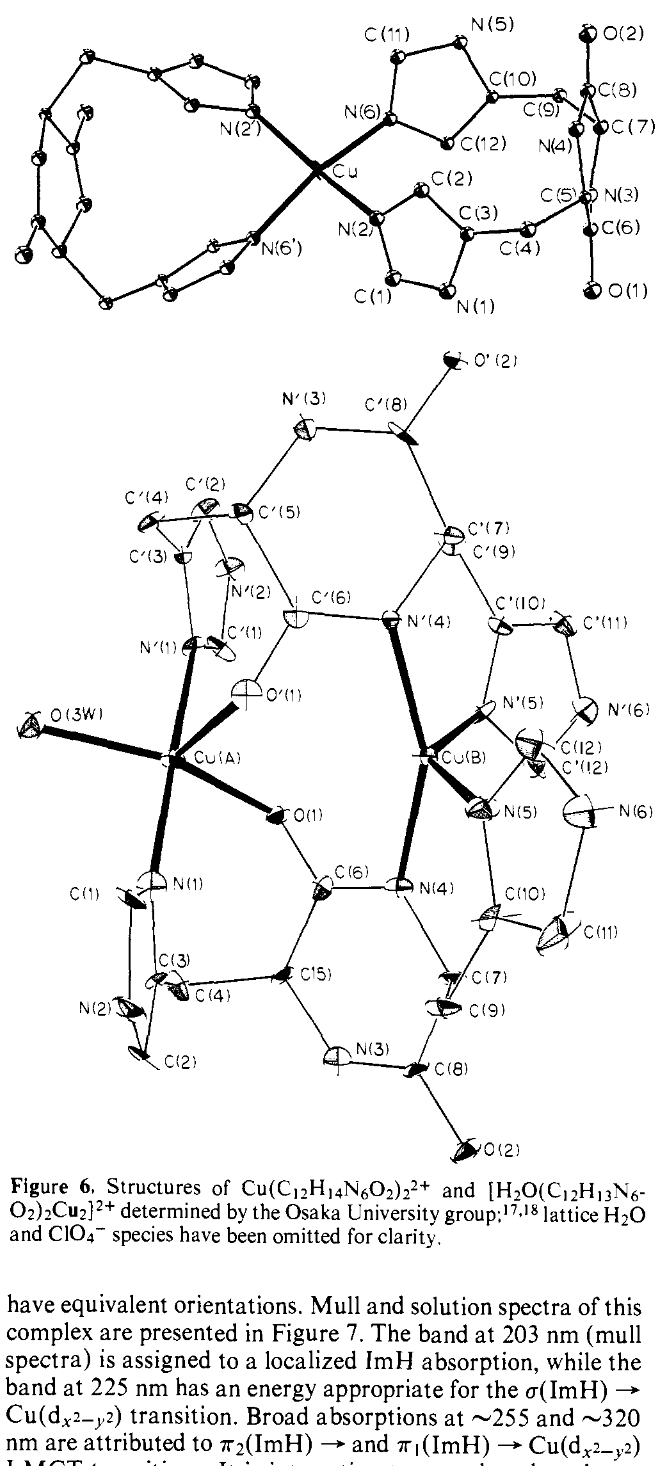


Figure 6. Structures of $Cu(C_{12}H_{14}N_6O_2)_2^{2+}$ and $[H_2O(C_{12}H_{13}N_6O_2)_2Cu_2]^{2+}$ determined by the Osaka University group;[17,18] lattice H_2O and ClO_4^- species have been omitted for clarity.

have equivalent orientations. Mull and solution spectra of this complex are presented in Figure 7. The band at 203 nm (mull spectra) is assigned to a localized ImH absorption, while the band at 225 nm has an energy appropriate for the σ(ImH) → Cu($d_{x^2-y^2}$) transition. Broad absorptions at ~255 and ~320 nm are attributed to π_2(ImH) → and π_1(ImH) → Cu($d_{x^2-y^2}$) LMCT transitions. It is interesting to note that these latter band positions correspond to the midpoints of the split π_1- and π_2-Cu(II) LMCT bands observed for polycrystalline $Cu(ImH)_4\cdot SO_4$. Moreover, absorptions at ~315 (ϵ ~400) and ~270 nm (shoulder) are exhibited by aqueous $Cu(C_{12}H_{14}N_6O_2)_2^{2+}$. We interpret this result to mean that the aqueous and solid state $Cu(C_{12}H_{14}N_6O_2)_2^{2+}$ chromophores structurally are rather similar. In particular, the free rotation of the ImH units presumed for aqueous $Cu(ImH)_4^{2+}$ may be attenuated for the fairly rigid cyclic dipeptide ligands present in $Cu(C_{12}H_{14}N_6O_2)_2^{2+}$.

3. $H_2O(C_{12}H_{13}N_6O_2)_2\cdot Cu_2\cdot 2ClO_4$. The unit cell of this complex contains two nearly identical ligand-bridged Cu(II) dimers.[18] One of these has been reproduced in Figure 6. Cu(B) has a flattened tetrahedral donor set comprised of two ImH N atoms and two deprotonated amide N atoms; the dihedral angle N(4)-Cu(B)-N(5)/N'(4)-Cu(B)-N'(5) is 51.2°. Cu(A) has an approximately square-pyramidal N_2O_3 donor set comprised of two ImH N atoms, two amide O atoms, and a water molecule. Preliminary electronic spectra and magnetic features of this complex have been reported earlier.[14] Relative to the N(5)-Cu(B)-N'(4) plane, the nearly equivalent ImH ligands have dihedral angles of 71 and 75°. The other ImH ligands have dihedral angles of 61 and 71° relative to the Cu(A)-N(1)-N'(1)-O(3w) plane. Mull and solution spectra of this complex are presented in Figure 8. Spectra of the free ligand consist merely of the ImH absorption at 200 nm (ϵ ~3800). A shoulder in the mull spectra at ~220 nm may be assigned to σ(ImH) → Cu(II) LMCT absorption, whereas the doublets at 257, 277 and 327, 337 nm most likely arise from two sources of π_2- and π_1 → Cu(II) LMCT transitions. Although these spectra closely resemble those exhibited by $Cu(ImH)_4SO_4$ (Figure 5), the doublets most likely reflect differences between Cu(A) and Cu(B) rather than from ImH ligands which have substantial geometric nonequivalency. The lack of definition of the absorption at 220 nm may result from the overlapping σ(ImH) → Cu(II) LMCT absorptions of each Cu(II) site. As noted previously,[14] there are no spectral features of the dimer which may be attributed to a π(N-amide) → Cu(II) absorption of the Cu(B) chromophore. The spectra of the dimer may be assigned fully by reference to the spectral features of $Cu(C_{12}H_{14}N_6O_2)_2\cdot 2ClO_4$ and $Cu(ImH)_4SO_4$.

4. [Cu(β-Ala-L-His)·H₂O]₂. The approximately square-pyramidal Cu(II) ions of this dimeric complex are crystallographically equivalent, and have equatorial bonds to N(ter-

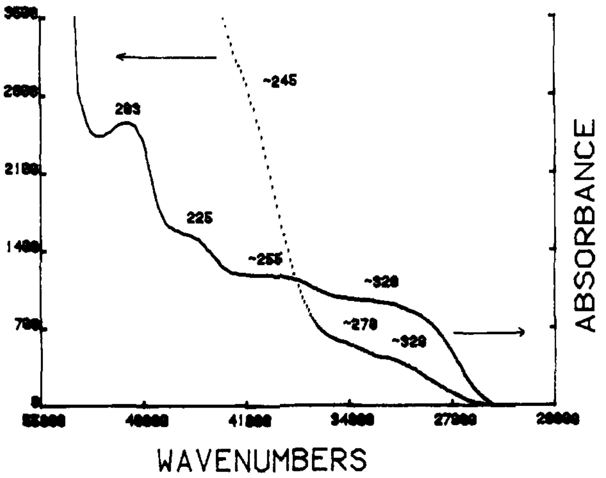


Figure 7. Electronic spectra at 298 K of $Cu(C_{12}H_{14}N_6O_2)_2\cdot 2ClO_4\cdot 2H_2O$ as a mineral oil mull (——) and in aqueous solution (·····).

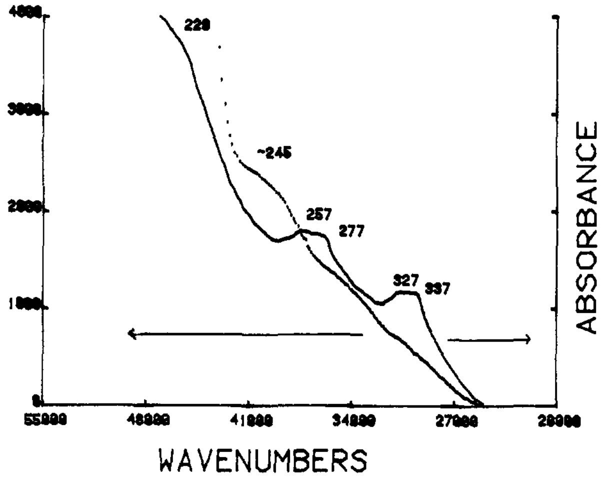


Figure 8. Electronic spectra at 298 K of $H_2O(C_{12}H_{13}N_6O_2)_2Cu_2\cdot 2ClO_4$ as a mineral oil mull (——) and in aqueous solution (·····).

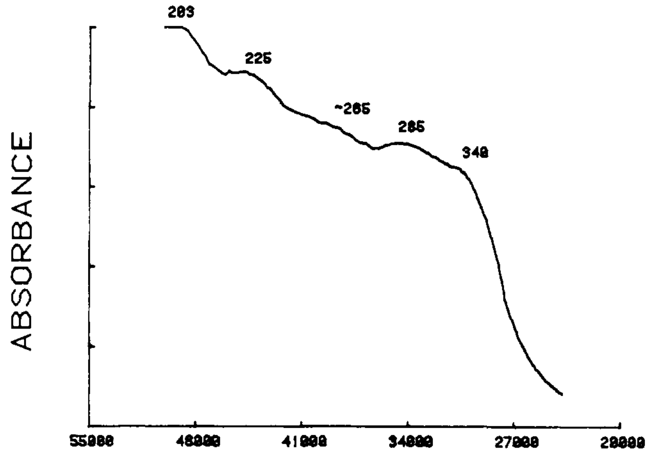


Figure 9. Electronic spectra at 298 K of $[Cu(\beta\text{-Ala-L-His})\cdot 2H_2O]_2$ as a mineral oil mull.

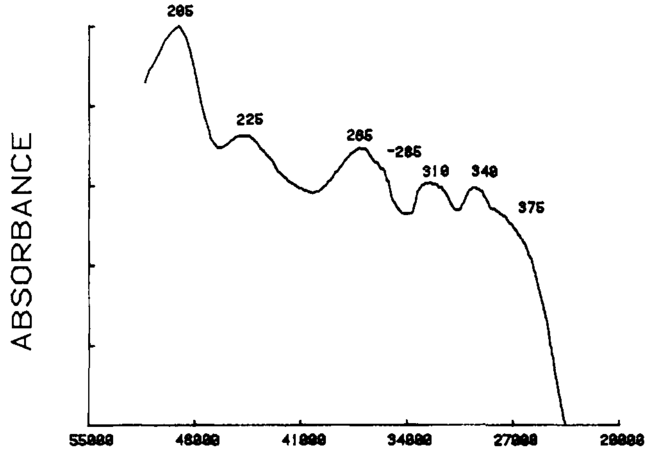


Figure 10. Electronic spectra at 298 K of $Cu_3(ImH)_8(Im^-)_2\cdot 4ClO_4$ as a mineral oil mull.

minal NH_2), O(carboxylate), N(deprotonated amide), and N(ImH from an adjacent dipeptide).[19] Coordination is completed by the apically bound H_2O molecules. The dihedral angle between the ImH ring and the approximately planar N_3O equatorial ligand set is 24.6°. Mull spectra reveal absorption maxima at 203, 225, 265, 285, and 340 nm. Similar absorption maxima at 220, 260, 290, and 340 nm were reported in a study of $[Cu^{II}(\beta\text{-Ala-L-His})\cdot H_2O]_2$ by conventional absorption spectroscopy and circular dichroism.[33] A number of other workers have noted that absorptions in this spectral region are a general feature of Cu(II) ligated by ImH, histamine, histidine, and histidine-containing peptides.[34-37] Even though the mull spectra in Figure 9 bear a similarity to those exhibited by $Cu(ImH)_4^{2+}$, a full interpretation is difficult because this complex Cu(II) chromophore contains five types of ligand donor atoms. Furthermore, the relatively intense $\sigma(ImH) \rightarrow Cu(II)$, $\sigma(NH_2) \rightarrow Cu(II)$, and $\sigma(\text{O-carboxylate}) \rightarrow Cu(II)$ LMCT transitions all fall in the spectral range 220–270 nm. The spectra in Figure 9 reflect the characteristic absorptions of a Cu(II)–ImH unit along with overlapping absorptions of the two types noted above. Despite this complication, the absorptions at 285 and 340 nm are clear evidence of $\pi_2(ImH)$- and $\pi_1(ImH) \rightarrow Cu(II)$ LMCT, respectively. The fact that these π-symmetry LMCT transitions are well removed toward lower energies enhances the value of these absorptions as an electronic spectroscopic probe.

Electronic Structure of Cu(II)–Imidazolate Chromophores. The electronic structure of metal ion–imidazolate (Im^-) chromophores is of interest because Im^--bridged metal ion systems have been identified in superoxide dismutase[4] and postulated for cytochrome *c* oxidase.[8] Ligand imidazole readily may be deprotonated at physiological pH;[38] the resulting Im^- ion can be bidentate. For the case of superoxide dismutase, a ligand-bridged $Zn(II)–Im^-–Cu(II)$ unit is formed at the active site. Since Zn(II) spectroscopically is inactive the electronic spectral features of these ligand-bridged dimers are those of the $[Cu(Im^-)(ImH)_3]^+$ subunit. These features may be predicted from studies of model Cu(II) complexes having appropriate ligand donor sets. A particularly useful model is the mixed ligand complex of composition $Cu_3(ImH)_8(Im^-)_2\cdot 4ClO_4$. Its structure[20] consists of trinuclear Cu_3 species which are joined into chains by bridging ClO_4 groups. The central Cu(II) atom (Cu(A)) of this unit lies on a center of symmetry; the other two Cu(II) atoms (Cu(B)) are related by this center. Im^- bridges link the Cu(A) atom, which has a $N_2(ImH)$-$N_2(Im^-)$ donor set, to the Cu(B) atoms, which have $N_3(ImH)N(Im^-)$ donor sets. Two apical Cu–O bonds on each Cu(II) atom complete the tetragonal coordination geometry. The dihedral angles between the best $Cu(A)N_4$ plane and the ImH and Im^- rings are 30.9 and 62.8°, respectively. Corresponding dihedral angles between the best $Cu(B)N_4$ plane are 59.7° for the Im^- ligand and 31.1, 24.7, and 72.4° for the three ImH ligands. Mull spectra of this complex (Figure 10) reveal absorptions at 205 and 225 nm along with distinct doublets at 265, 285 and 310, 340 nm. Since these spectra closely mimic those observed for $Cu(ImH)_4\cdot SO_4$ (Figure 5), an identical assignment (vide supra) is suggested. The sole spectral feature not exhibited by the simple $Cu(ImH)_4^{2+}$ chromophore is the broad shoulder at ~375 nm (Figure 10).

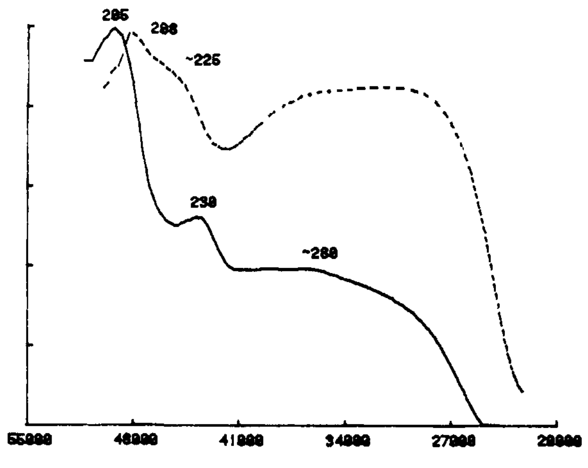
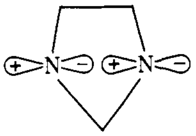


Figure 11. Electronic spectra at 298 K of $[R_2N(CH_2)_2NH(CH_2)_2NR_2Cu]_2Im^-\cdot 3ClO_4$ as mineral oil mulls; R = H (——); R = Et (- - - -).

The spectra of two Im^--bridged Cu(II) dimers (the nonbridging nitrogenous ligands on each Cu(II) are tridentate $R_2N(CH_2)_2NH(CH_2)_2NR_2$ molecules with R = H, Et) are presented in Figure 11. A dimer with the homologous R = CH_3 ligand recently has been characterized by other workers.[23] Both dimeric complexes exhibit absorptions in the 225–230-nm region which reasonably may be attributed to $\sigma(N) \rightarrow Cu(II)$ LMCT transitions involving the triamine and Im^- donor ligands. Absorptions at lower energies unfortunately lack the definition exhibited by the polycrystalline Cu(II)–ImH chromophores. However, corresponding $\pi(\text{ligand}) \rightarrow Cu(II)$ LMCT absorptions of these $Cu(II)–Im^-–Cu(II)$ dimers may result in the broad absorption maximum at ~280 nm and the tailing toward lower energies. All three $Cu(II)–Im^-$ species described above exhibit a broad absorption at ~375 nm which has not been observed for Cu(II)-ImH chromophores. A possible assignment for this band is suggested by CNDO/2 molecular orbital calculations of Im^- reported by other workers.[39] The calculations indicate that the highest occupied σ-symmetry molecular orbital primarily arises from an antibonding combination of nitrogen orbitals, i.e.



Using these results, the band at ~225 nm could correspond to LMCT associated with the $(\sigma_N + \sigma_N)$ combination, whereas the band at ~375 nm would correspond to the $(\sigma_N - \sigma_N)$ combination. LMCT associated with a corresponding antibonding combination of S(thioether) orbitals has been reported by other workers.[40]

Applications to Cu(II)-Protein Spectra.[41] Our study of model complexes has revealed that constrained tetragonal Cu(II)-ImH chromophores exhibit absorptions at ~230, ~260, and ~330 nm, assignable to $\sigma(N) \rightarrow Cu(II)$, $\pi_2(ImH) \rightarrow Cu(II)$, and $\pi_1(ImH) \rightarrow Cu(II)$ LMCT transitions, respectively. The latter two bands may be split if geometrically nonequivalent Cu(II)-ImH units are present. Moreover, these π-symmetry absorptions may be severely attenuated when the Cu(II)-ImH units undergo free rotation. The π-absorption bands are prominent spectral features of polycrystalline (i.e., rigid) Cu(II)-ImH chromophores. In contrast, free rotation within chromophores such as aqueous $Cu(ImH)_4^{2+}$ essentially destroys the usefulness of these bands as electronic spectral probes. Rotation apparently may be suppressed by appropriate substituents on the ImH ring.[32]

Other workers recently have compared the electronic spectra of aqueous $[Cu(N\text{-}Me\text{-}ImH)_4]^{2+}$ with those of the free ligand and ligand·HCl.[42] The only spectral difference noted between these materials was the absorption of the complex at ~300 nm (ϵ ~500). Inspection of Figure 5 indicates that this feature is the remnant of $\pi(ImH) \rightarrow Cu(II)$ LMCT absorption for an unconstrained $[Cu(ImH)_4]^{2+}$ chromophore. Moreover, since the absorption of the $[Cu(N\text{-methyl-}ImH)_4]^{2+}$ at ~250 nm (ϵ ~1500) was considered a UV absorption which also was exhibited by the free ligand and ligand·HCl, these latter materials presumably were contaminated by Cu(II). This study of $Cu(N\text{-}Me\text{-}ImH)_4^{2+}$ spectra has led other workers to discount the possibility of near-UV ImH → Cu(II) LMCT for type 1 blue proteins[43] and a met form of hemocyanin.[44]

One issue that must be considered before applying the results of our model complexes to Cu(II) proteins is the degree to which the protein chromophores are constrained. Studies of the complexes in Figure 6 and the Cu(II)-4,5-diisopropylimidazole system[32] indicate that well-resolved $\pi(ImH) \rightarrow Cu(II)$ LMCT bands may be exhibited by solvated Cu(II)-ImH chromophores. By comparison, a Cu(II)-protein system would appear to be a relatively rigid chromophore. Another group at Rutgers has obtained high-quality spectra of superoxide dismutase, which exhibits the resolved ImH → Cu(II) LMCT features appropriate for a constrained chromophore.[45] A second issue that must be considered is the coordination geometry of our model complexes. Although we are attempting to prepare approximately tetrahedral model chromophores, the models described in this report are tetragonal. Qualitatively, the relatively large redox potential and relatively weak ligand field of the type 1 blue copper chromophores should serve to red shift the positions of $\sigma(ImH) \rightarrow Cu(II)$ and $\pi(ImH) \rightarrow Cu(II)$ LMCT bands noted above. However, the magnitude of this effect has yet to be documented by studies of well-characterized model complexes. Our current results should be directly applicable to the type 2 and type 3 Cu protein chromophores. Such species are thought to contain tetragonal Cu(II) ions whose ligation includes ImH groups.[7,44] Accordingly, a $\pi(ImH) \rightarrow Cu(II)$ LMCT band at ~330 nm ($\epsilon \approx 1000$) is expected for these chromophores. A number of Cu-N(ImH) modes are prominent features in the resonance Raman spectra[10,46] associated with the characteristic transition of oxygenated hemocyanins at ~345 nm (ϵ ~6000-9000 per Cu). Other workers have argued that ImH → Cu(II) LMCT transitions either dominate[10] or do not contribute[44] to the absorption band at 345 nm. Our studies reconcile the resonance Raman results with the view that *most* of the absorption at 345 nm does not originate from ImH → Cu LMCT transitions. An assignment of this absorption exclusively to a $O_2^{2-} \rightarrow Cu(II)$ LMCT transition has been presented by others.[44] A recent study of ascorbate oxidase indicates that type 2 copper contributes to the protein absorption at 330 nm.[47] This absorption band previously has been associated with the type 3 copper systems in multicopper proteins.[9] Assuming that the nonblue type 2 chromophores are ordinary tetragonal Cu(II) ions ligated in part by ImH residues, absorption at ~330 nm is fully expected. Moreover, similar near-UV absorption is expected for the copper chromophore in the resting form of galactose oxidase. Although ImH ligation has been identified by ESR studies,[7] it is not clear that unambiguous electronic spectra of the Cu(II) chromophore are available yet. A recent study indicates that the catalytically active form of galactose oxidase involves a one-electron oxidation of the resting Cu(II) chromophore.[48] Apparently further work may be required to produce unambiguous electronic spectra of the resting enzyme; the published spectra[49] may contain extraneous bands due to contamination by small amounts of the intensely colored active form.[48]

Finally, ImH → Cu(II) LMCT bands should be red shifted in type 1 chromophores by (a) the lower energy of the d vacancy compared to tetragonal Cu(II) species and (b) the high redox potential associated with the "soft" ligand donor set. We estimate that the red shift may be as large as ~11 000 cm^{-1}. The corresponding $\sigma(S\text{-mercaptide}) \rightarrow Cu(II)$ absorption is shifted from ~360 nm in model $Cu^{II}N_4S$ complexes[13,42] to ~600 nm in the type 1 chromophores.[50] Consequently, $\pi(ImH) \rightarrow Cu(II)$ LMCT is a strong candidate for the absorptions of the type 1 chromophores at ~450 nm, and a possible candidate for the additional absorptions of the chromophores at ~550 nm.[50]

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Visual Pigments. 11. Spectroscopy and Photophysics of Retinoic Acids and *all-trans*-Methyl Retinoate

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Abstract: The photophysics of hydrogen-bonded complexes of retinoic acid and its 9-cis and 13-cis isomers and the photophysics of the dimers of these isomers of retinoic acid were studied. The investigation indicated that complexes of retinoic acid and molecules that form hydrogen bonds with the carbonyl oxygen of retinoic acid (type I complexes) have both higher radiative and nonradiative rate constants than do hydrogen-bonded complexes of retinoic acid and molecules that form hydrogen bonds only with the hydroxyl oxygen of retinoic acid (type II complexes). For *all-trans*-retinoic acid in 3-methylpentane at 77 K, the type I complexes have radiative rate constants approximately equal to or greater than 2×10^8 s^{-1} and nonradiative rate constants greater than 3×10^8 s^{-1}. Both the radiative and nonradiative rate constants of the type II complexes of *all-trans*-retinoic acid at 77 K in 3-methylpentane are less than 1×10^8 s^{-1}. The dimer of retinoic acid (K(association) = 1×10^4 M^{-1} at room temperature for the all-trans isomer) behaves like a type I complex, and its excited-state properties are better understood in terms of hydrogen bonding than in terms of an exciton model. The photophysical properties and triplet-triplet absorption spectrum of methyl retinoate were measured. The study concluded with an examination of some of the implications of this work for the role of hydrogen bonding in the dimers and monomers of retinal and retinol.

Introduction

In recent years, there have been many theoretical and experimental studies[2-10] of the excited state of the retinyl polyenes including retinols, retinals, and retinyl Schiff bases. These studies have been motivated primarily because of the relationship these systems bear to the process of vision. Retinoic acids have so far attracted the least attention of the investigators. The absorption and fluorescence spectra of *all-trans*-retinoic acid in EPA and hydrocarbon solvents have been reported by Thomson.[9] The spectra in that work[9] were apparently considered as being due to the monomeric form of the acid.

In continuation of our interest in the model visual pigments, we undertook the spectroscopic investigation of retinoic acids and *all-trans*-methyl retinoate, henceforth called retinoyl systems when used collectively. This study also became pertinent for comparative purposes when it was observed[4,10] that aggregation (dimer formation) plays an important role in determining the photodynamical behavior of retinals and retinols under certain conditions. Moreover, recent findings regarding the potential of retinoic acids as anticancer agents[11] and the presence of retinoyl complexes as the autofluorescence component of the storage material in neurons from Batten disease[12] establish these compounds as important biomolecules.

It is generally recognized that in retinyl polyenes there are

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