Isocyanides as Ligand-Directed Indicators of Cu(I) Coordination in Copper Proteins. Probing the Inequivalence of the Cu(I) Centers in Reduced Dopamine-β-monooxygenase

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Abstract: The use of isocyanides as ligand-directed probes of Cu(I) coordination in proteins has been investigated. Reaction of 2,6-dimethylphenyl isocyanide (DIMPI) with reduced dopamine-β-monooxygenase (DβM) indicates the initial formation of monoisocyanide complexes at each of the two coppers (Cu_A and Cu_B) with different frequencies (2148 and 2129 cm^{-1}) indicative of inequivalent Cu(I) coordination at each copper. However, further addition of DIMPI leads to formation of a species containing multiple isocyanide ligands, believed to be a trisisocyanide adduct with a single IR band at 2160 cm^{-1}. This titration behavior can be interpreted by the active site model $Cu_A^I(His)_2X$-$Cu_B^I(His)_2Y$ (X = His; Y = Met) where the first stage of the reaction with isocyanide is the formation of a mono-DIMPI four-coordinate complex at each Cu, giving rise to the two observed IR bands (2148 and 2129 cm^{-1}) provided the protein ligands X and Y are different. The second stage is the displacement of protein-bound ligands by the isocyanide to form a protein-bound bis or tris complex (2160 cm^{-1}). Closely analogous chemistry involving the reaction of DIMPI with deoxyHc is described, which illustrates the generality of isocyanides as probes of Cu(I) coordination in copper proteins. A model system $[Cu^I(MePY2)(DIMPI)]ClO_4$, **II**, is also described in which identical isocyanide-binding chemistry can be demonstrated, thus validating the conclusions on the protein systems. The crystal structure of **II** is described, and the clean conversion of **II** to a trisisocyanide complex is demonstrated by FTIR and FT Raman spectroscopy.

Copper monooxygenases have been intensively studied, particularly with respect to how they bind and activate dioxygen. Impressive progress has been made in understanding the coordination chemistry of dioxygen binding to the Cu(I) centers in hemocyanin (Hc)[1] and inorganic complexes,[2] but details of dioxygen interaction with dopamine-β-monooxygenase (DβM)[3] and peptidylglycine α-hydroxylating monooxygenase (PHM)[4] are limited since these enzymes do not form stable dioxygen adducts. In previous work, we have used CO as a vibrational spectroscopic probe of dioxygen binding and have demonstrated the catalytic and structural inequivalence of the copper centers in dopamine-β-monooxygenase (Cu_A and Cu_B).[5-7] Like CO, isocyanides are potential ligand-directed probes of metalloprotein coordination due to their strong IR absorption and preference for low-valent coordination chemistry,[8] and heme-isocyanide complexes have been well studied.[9] However, although a number of inorganic Cu(I)-isocyanide complexes have been structurally characterized,[10-12] isocyanides have not previously been used as probes of Cu(I) coordination in proteins.

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Here we report the first such use of isocyanide ligands in reactions with DβM as well as the dioxygen carrier protein hemocyanin and provide further evidence for the chemical inequivalence of the copper atoms in reduced DβM.

Materials and Methods

Enzyme Preparation. Dopamine-β-monooxygenase was purified as previously described.[6] To prepare isocyanide complexes, the enzyme (0.7 mM in Cu) was degassed under argon and dialyzed anaerobically against a saturated solution of 2,6-dimethylphenyl isocyanide (DIMPI, approximate concentration 10^{-5} M) in 50 mM potassium phosphate buffer (pH 7.5) for several hours. It was then reduced anaerobically with a 2−3-fold excess of sodium ascorbate and the dialysis continued. The time course of the reaction with DIMPI was followed by removal of aliquots of the enzyme from the dialysis bag at intervals of 1, 3.5, 5, and 24 h for FT infrared analysis. Equilibrium binding experiments were carried out by dialysis of a sample of ascorbate-reduced DβM against a stoichiometric quantity (per mole Cu in DβM) of DIMPI for approximately 24 h. All dialysis was performed at 4 °C.

Hemocyanin was prepared from the hemolymph of the whelk *Busycon canaliculatum*. The hemolymph was centrifuged at 1000 rpm to remove insoluble material and coagulated protein, followed by dialysis of the supernatant against 50 mM sodium carbonate buffer (pH 9.8) and concentration to approximately 5 mM in total Cu in an Amicon stirred cell.

Synthesis of [Cu(MePY2)(DIMPI)](ClO$_4$) (II). [Cu(MePY2)](ClO$_4$) (**I**) (108 mg, 0.26 mmol), synthesized in a manner analogous to the published procedure for the PF_6-salt,[13] and 2,6-dimethylphenylisocyanide (DIMPI) (35.0 mg, 0.26 mmol) were mixed together in a Schlenk flask under argon. CH_2Cl_2 (10 mL, dry, degassed) was introduced to obtain a colorless solution. After 15 min, diethyl ether was added dropwise (25 mL total), whereupon a white solid precipitated. The supernatant was removed by decantation, giving 120 mg (85%) after drying under vacuum: IR (Nujol, cm^{-1}) 2129, ν(CN), 1080, ν(ClO$_4^-$); NMR (δ 300 MHz, CD_2Cl_2) 2.37 (s, 3H, CH_3), 2.80−3.00 (s, br, 11H; NCH_3 and CH_2), 7.11−7.41 (m, 7H), 7.79−7.84 (m, 2H), 8.53 (d, 2H; py-6). Anal. Calcd for $C_{24}H_{28}ClCuN_4O_4$: C, 53.82; H, 5.23; N, 10.46. Found: C, 53.95; H, 5.15; N, 10.29.

Synthesis of [Cu(DIMPI)$_3$](ClO$_4$) (III). [Cu(CH$_3$CN)$_4$](ClO$_4$) (0.3 g) and 3 equiv of DIMPI (0.36 g) were mixed in CH_2Cl_2 (15.0 mL) under argon. After 15 min, Et_2O was introduced dropwise to obtain a colorless crystalline solid in a quantitative yield. Anal. Calcd for $C_{27}H_{27}N_3ClCuO_4$: C, 58.37; H, 4.90; N, 7.57. Found: C, 58.37; H, 4.90; N, 7.91.

Details of Data Collection and Refinement for [Cu^I(MePY2)(DIMPI)](ClO$_4$) (II). A colorless needle of **II** was obtained by slow evaporation of a MeOH solution under argon, and it was mounted on a Rigaku AFC6S diffractometer, at room temperature. The automatic centering and least-squares routines were carried out on 25 reflections in the 2θ range 22.79−26.87° and corresponded to a monoclinic cell with $a = 19.641$ (4), $b = 21.636$ (4), $c = 14.204$ (3) Å, $\beta = 121.59(1)°$, $Z = 8$, $V = 5141$ (2), space group $C2/c$. The ω-2θ data-collection technique was used, and data were collected ($3.5 \leq 2\theta \leq 50°$) at a scan speed of 8.0° min^{-1}. The structure was solved by direct methods. The nonhydrogen atoms were refined anisotropically by full-matrix least-squares technique. Hydrogen atoms were included at the calculated positions. Of 4820 reflections collected, 1918 ($I \geq 3\sigma(I)$) were used in the refinement: $R = 0.052$, $R_w = 0.64$. All measurements were made using Mo Kα ($\lambda = 0.710\,69$ Å) radiation with a graphite monochromator. Calculations were performed using the TEXSAN crystallographic software package on a VAX 3520 computer. Details of crystal and refinement data and complete lists of bond distances, angles, and structure factor tables are included in the supporting information.

Fourier Transform Infrared and Raman Measurements. FTIR spectra were recorded on Perkin Elmer 1800 and 2000 FTIR spectrophotometers. FT Raman spectra were recorded on the Perkin Elmer 2000 instrument with Raman accessory. Solution IR protein spectra were collected using a liquid nitrogen-cooled MCT detector, in 50 μm path-length calcium fluoride cells. Inorganic model complex IR data were collected using a DTGS detector at ambient temperature, in 50 μm path-length calcium fluoride cells with chloroform as solvent. Raman data were collected using a diode-pumped Nd-YAG laser (9394.698 cm^{-1}) and an InGaAs detector operating at ambient temperature, in 90° scattering geometry. Samples were contained in small glass capillaries, fitted with small septa to ensure anaerobiosis. Two hundred scans were collected for protein samples and for Raman data of models. Twenty-five scans were sufficient for IR data of model compounds. Analysis and spectral deconvolution curve-fitting were performed using the program GRAMS (Galactic Industries). In all cases, bands containing more than one component were simulated by the sum of pure Gaussian components, with the line widths constrained to well-defined limits (usually 18 ± 2 cm^{-1}).

Results and Discussion

2,6-Dimethylphenyl isocyanide (DIMPI) was chosen as the isocyanide ligand in the present study because it is a solid and easy to handle. In addition, it is only sparingly soluble in aqueous medium (~10^{-5} M); thus, dialysis against a saturated solution containing excess solid ensured a constant concentration of ligand low enough to inhibit multiisocyanide complex formation (see below), yet maintaining a sufficient mole fraction of isocyanide for stoichiometric binding to the enzyme. DIMPI complexes of reduced dopamine-β-monooxygenase were prepared by two separate routes. In one series of experiments, the ascorbate-reduced enzyme was dialyzed against a saturated solution of DIMPI in 50 mM phosphate buffer (pH 7.5) and the progress of the reaction monitored by removal of samples for IR analysis at specified intervals. Under these conditions, it appeared that at longer dialysis times there was a tendency toward formation of complexes containing more than one isocyanide per Cu. In a second series of experiments, an attempt was made to halt the reaction at an intermediate stage where the predominant species were monoisocyanide complexes. In these experiments, the reduced DβM was dialyzed exhaustively against stoichiometric or substoichiometric amounts of isocyanide ligand.

Figure 1d shows the FTIR spectrum of the final product after 24-h dialysis against a saturated solution of DIMPI (method 1). One strong band is present at 2160 cm^{-1}. After 1 h (Figure 1a), the main peak is at ~2129 cm^{-1} with a shoulder at ~2148 cm^{-1}. Figure 1 (parts b and c) (measured after 3.5 and 5 h, respectively) shows that the 2160-cm^{-1} band grows in as the reaction proceeds, accompanied by the simultaneous disappearance of the 2129-cm^{-1} band. Spectral deconvolution (data not shown) indicates the presence of a third band in all the spectra at ~2148 cm^{-1}, which forms after 2129 cm^{-1}, but is then itself also converted into the 2160-cm^{-1} species.

Figure 2 shows FTIR spectra obtained when reduced DβM was dialyzed against 0.5 molar equiv of DIMPI per Cu (method 2). These results show that at substoichiometric ratios two well-resolved IR bands are observed at 2129 and 2148 cm^{-1}. Spectral deconvolution shows approximately equal contributions from the two major bands as well as a small contribution from the 2160-cm^{-1} band. These results are consistent with those of Figure 1 and suggest that the 2129- and 2148-cm^{-1} features represent Cu(I)-monoisocyanide complexes, whereas the 2160 cm^{-1} band is probably a species containing multiple isocyanide ligands.

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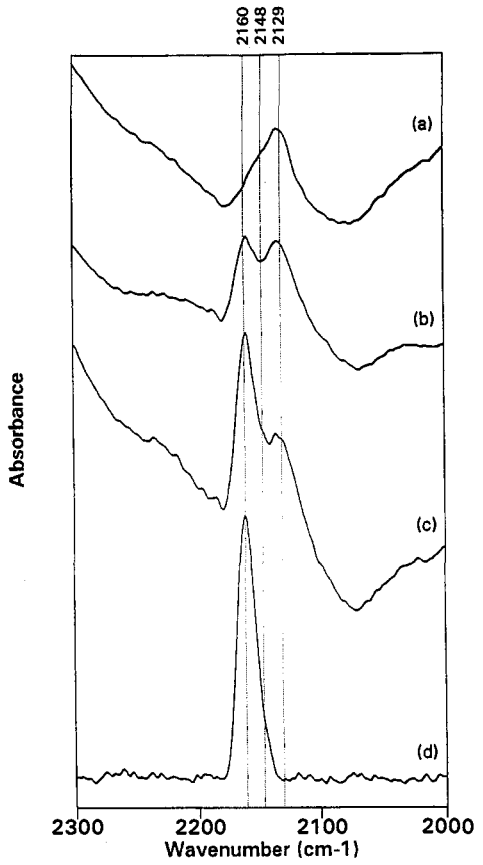

Figure 1. FTIR spectra of DIMPI binding to reduced DβM. Time course of dialysis of reduced DβM (0.7 mM in total copper) against a saturated solution of DIMPI in 50 mM potassium phosphate buffer, pH 7.5: spectra recorded at (a) 1 h, (b) 3.5 h, (c) 5 h, and (d) 24 h dialysis times.

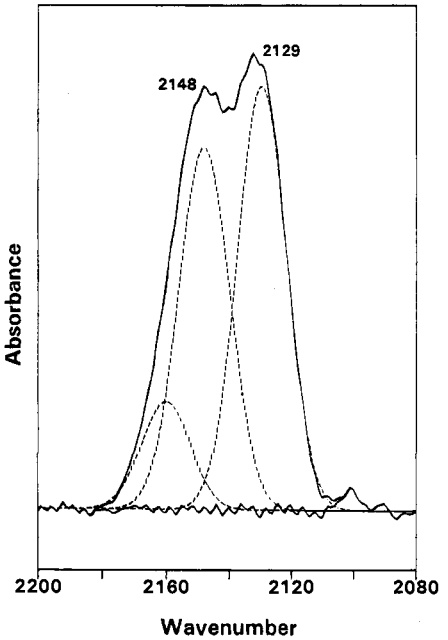

Figure 2. FTIR spectrum of reduced DβM dialyzed against 0.5 molar equiv of DIMPI in 50 mM phosphate buffer, pH 7.5: the solid line represents experimental data, the dashed lines represent individual components of the absorption envelope obtained by curve fitting using the program GRAMS. Spectral deconvolution curve fitting utilized pure Gaussian line shapes, with line widths constrained to 16–19 cm^{-1}. The baseline represents the residual of experiment minus theory.

We have investigated the generality of the reaction of isocyanides with Cu(I) centers in proteins by carrying out similar

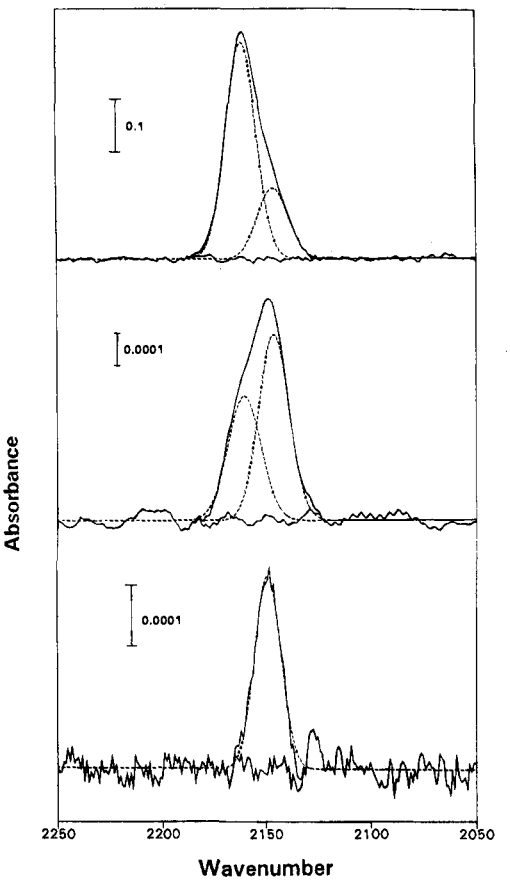

Figure 3. FTIR spectra of the reaction of DIMPI with *B. canaliculatum* deoxyHc. Bottom spectrum: deoxyHc (5.0 mM in Cu, 50 mM sodium carbonate buffer, pH 9.8) dialyzed for 3 days vs 2.0 equiv of DIMPI per copper. Middle spectrum: as for bottom spectrum but with the mole fraction of DIMPI increased to 2.5 mol per copper. Top spectrum: final product after exhaustive dialysis for 5 days against excess a saturated solution of DIMPI (solid present). Spectral deconvolution of curve fitting utilized pure Gaussian line shapes, with line widths constrained to 16–19 cm^{-1}. For each spectrum, the baseline represents the residual of experiment minus theory.

studies on hemocyanin (Hc), the oxygen-carrying protein of molluscs and crustacea. The structures of both the deoxy and oxy forms of the protein are known.[1] The deoxy form contains a dinuclear Cu–Cu center with each Cu coordinated to three histidine ligands. In the oxy form, dioxygen bridges the two copper atoms in a $\eta^2:\eta^2$ (side-on) fashion. The availability of the crystal structure and the ability of deoxyHc to bind small neutral molecules like O_2 and CO make it an attractive protein model for investigating isocyanide binding to Cu(I) centers. Exhaustive dialysis (3–5 days) of deoxyHc against limiting concentrations of DIMPI demonstrated reactivity patterns similar to those observed for DβM, as shown in Figure 3. The lowest ratio of DIMPI to Cu (2.0 mol per Cu) resulted in the FTIR spectrum shown in Figure 3 (bottom) which could be fit to a single Gaussian band centered on 2149 cm^{-1}. On increasing the ratio of 2.5 mol DIMPI per Cu and continuing the dialysis, a second band grew in as shown in the two-component spectrum of Figure 3 (middle) (curve fitting, two Gaussian components centered at 2160 and 2146 cm^{-1}). Finally, exhaustive (5 days) dialysis against a large excess of DIMPI gave the IR spectrum shown in Figure 3 (top), which is best represented by a major Gaussian component centered on 2160 cm^{-1} and a minor band centered on 2146 cm^{-1}. The reaction chemistry of hemocyanin with DIMPI thus appears to involve initial formation of a species with IR absorption at 2146–2149 cm^{-1}, followed by the conversion of this complex to a second species absorbing at

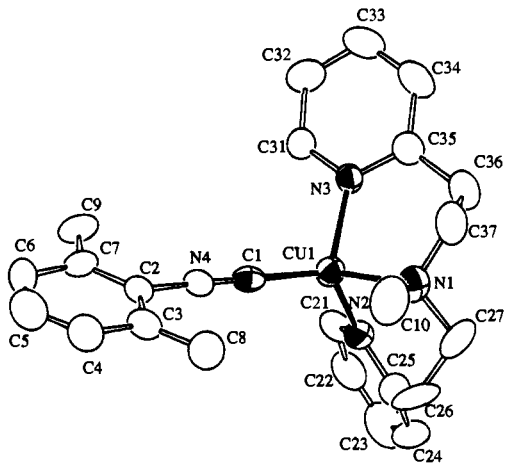

Figure 4. ORTEP (20% ellipsoid) view of the cationic portion of [Cu^{I}(MePY2)(DIMPI)](ClO$_4$) (**II**): Selected interatomic distances (Å): Cu1–N1 = 2.132 (7), Cu1–N2 = 2.032 (7), Cu1–N3 = 2.053 (6), Cu1–C1 = 1.828 (9), C1–N4 = 1.166 (9) Å. Angles (deg): Cu1–C1–N4 = 177.0 (8), C1–Cu–N1 = 114.8 (3), C1–Cu1–N2 = 121.5 (3), C1–Cu1–N3 = 113.2 (3), N1–Cu1–N2 = 98.0 (3), N1–Cu1–N3 = 199.4 (3), N2–Cu1–N3 = 106.9 (3).

2160 cm^{-1}. As for DβM, the most probable assignment is that the 2146-cm^{-1} species represents a monoisocyanide complex, while the 2160-cm^{-1} species contains multiple isocyanide coordination.

In order to assign the FTIR bands with more certainty, we have investigated a model system which shows analogous behavior. Three-coordinate [Cu^{I}(MePY2)](ClO$_4$) (**I**) (MePY2 = bis(2-pyridylethyl)methylamine)[13] reacts stoichiometrically with 1 mol of DIMPI to form the isocyanide adduct complex [Cu^{I}(MePY2)(DIMPI)](ClO$_4$) (**II**). The structure of **II** consists of a [Cu^{I}(MePY2)(DIMPI)]$^{+}$ monocation and a well-separated perchlorate anion. An ORTEP view of **II** is displayed in Figure 4. The copper(I) ion is tetracoordinate with ligation to the tridentate MePY2 ligand, a tertiary alkylamino, and two pyridyl nitrogen atoms plus a monodentate carbon of DIMPI. While rather few isocyanide-copper(I) complexes have been reported,[10] the DIMPI ligand displays the expected linear Cu^{I}–C–N–R coordination and short Cu^{I}–C bond length (Figure 4). The Cu1–N distances vary from 2.032 (7) to 2.132 (7) Å. The coordination geometry is pseudotetrahedral, but perhaps trigonal pyramidal is a better description, where N2, N3, and C1 comprise the basal plane; the copper ion is displaced 0.49 Å from this N2,N3,C1 plane, while it sits between 0.58 and 0.92 Å above planes defined by the other sets of three ligating atoms. Structures intermediate between tetrahedral and trigonal pyramidal are seen in analogous LCu^{I}–X tetracoordinate complexes (with the bis(2-pyridylethyl)amine tridentate moiety), with X = CO,[14] MeCN,[15] pyridyl,[16] phenolato,[17] and PPh$_3$[18] donors. Dihedral angles are as expected for either coordination geometry: N1Cu1C1/N2Cu1N3 = 92.0°, N1Cu1N3/N2Cu1C1 = 94.6°, and N1Cu1N2/C1Cu1N3 = 92.3°. Both Cu–N(pyridyl) and Cu–N(alkylamino) bond lengths are also in accord with those observed in the other related structures.[14–18]

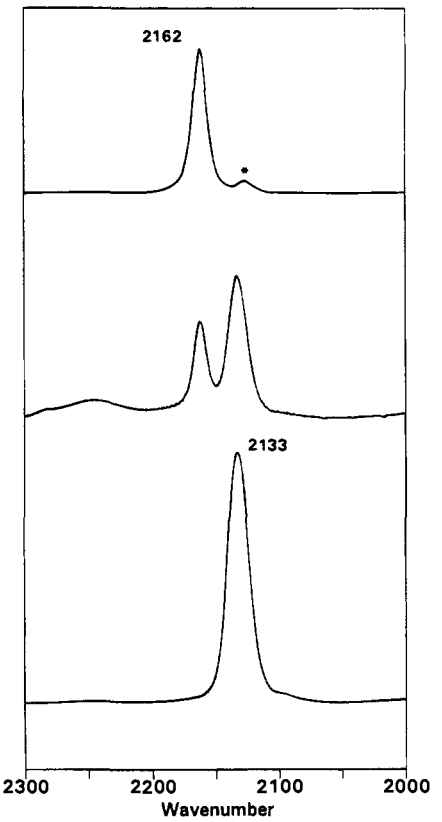

Figure 5. IR titration of complex **II** with DIMPI in chloroform. Bottom spectrum: pure **II**. Middle spectrum: **II** plus approximately 1.5 molar equiv DIMPI per copper. Top spectrum: **II** plus excess DIMPI; asterisk denotes IR band due to uncomplexed DIMPI at 2124 cm^{-1}.

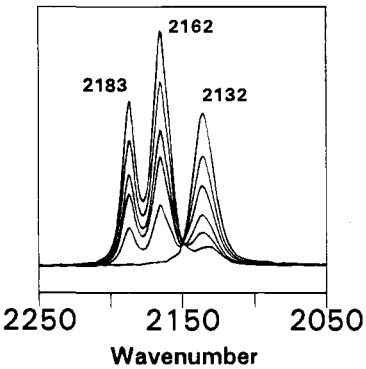

Figure 6. Raman titration of complex **II** with DIMPI in chloroform. Individual spectra correspond to addition of approximately 0.75 molar equiv of DIMPI per mole copper. Laser power, 200 mW.

The C≡N stretching frequency of **II** (measured as a ~10 mM solution in chloroform) is coincident in the IR and Raman at 2132 cm^{-1}. Titration of **II** with submolar equivalents of DIMPI in chloroform leads cleanly to formation of a new complex as shown in Figures 5 (IR) and 6 (Raman). One additional band grows in the IR at 2162 cm^{-1}, whereas two bands (2162, 2183 cm^{-1}) grow in the FT Raman, indicative of the formation of a second species similar to the behavior found in the proteins. Three lines of evidence support the assignment of the second species as a trisisocyanide complex containing only DIMPI ligands. Firstly, two additional equivalents of DIMPI (final ratio DIMPI to Cu = 3:1) are required to fully

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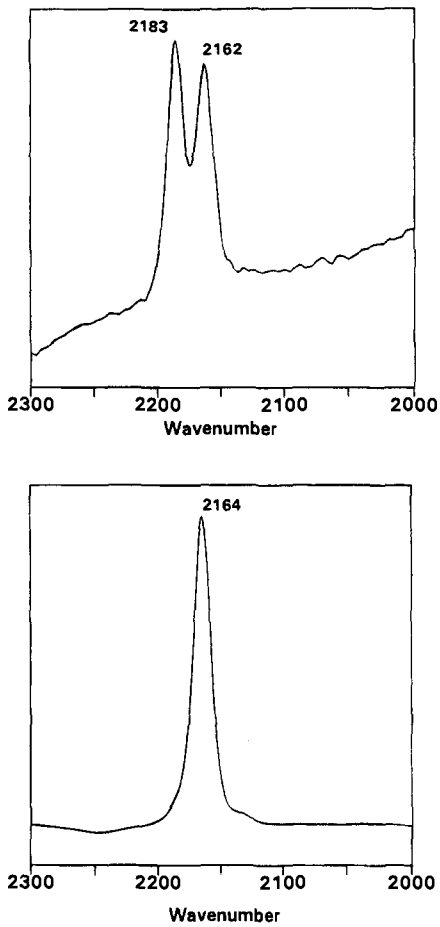

Figure 7. IR (bottom frame) and Raman spectrum (top frame) of tris-DIMPI Cu(I) perchlorate in chloroform. Raman laser power, 300 mW.

form the complex. Secondly, group theoretical considerations[19] argue strongly that the new species has 3-fold symmetry: if the new species were a bis complex, two bands would be expected in the Raman spectrum, but the pseudo C_{2v} symmetry would require each to be both Raman and IR active; despite considerable effort we have failed to find any evidence for a band at 2183 cm^{-1} in the IR. On the other hand, the observed behavior is predicted for D_{3h} or C_{3v} symmetry. Thirdly, we have prepared an authentic sample of tris-DIMPI Cu(I) perchlorate: this complex shows two Raman bands at 2162 and 2183 cm^{-1} and a single IR band at 2164 cm^{-1}, Figure 7. With the exception of a 2 cm^{-1} blue shift of the IR band, the frequencies and band multiplicities are identical to the final product of the titration of **II** with DIMPI.

This model chemistry demonstrates that the monoisocyanide complex is cleanly converted to the tris complex on titration with excess isocyanide ligand, with no detectable bis intermediate. This is somewhat unexpected but suggests that, whereas the four-coordinate monoisocyanide complex derives stability from the chelate effect of the tridentate MePY2 ligand, coordination of a second isocyanide and the associated displacement of one of the tridentate arms destabilizes the complex and results in a cooperative displacement of successive arms to ultimately form the $[Cu(DIMPI)_3]^+$ complex.

It is likely that similar chemistry occurs in the reaction of DIMPI with the Cu(I) centers in DβM and Hc. In previous work utilizing CO as a Cu(I) reporter ligand,[6,7] we have

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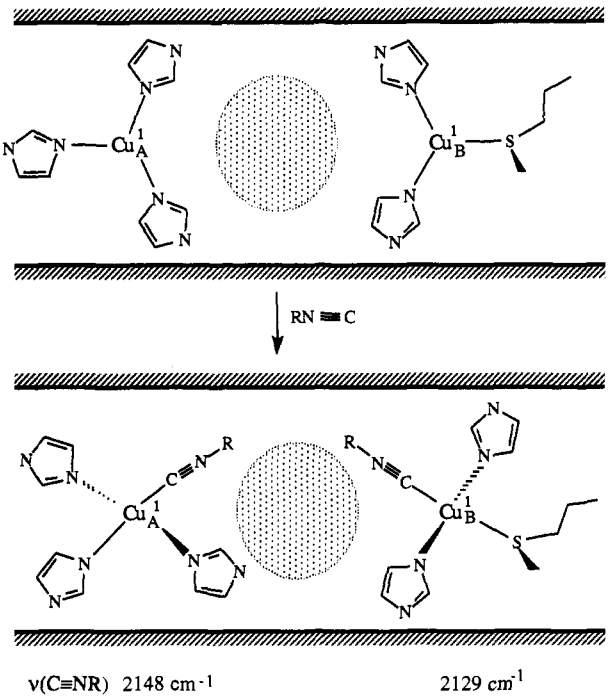
Scheme 1



suggested that the two Cu centers in reduced DβM are inequivalent and three-coordinate, with a $Cu_A(I)(His)_2X\cdots Cu_B(I)(His)_2Y$ coordination. The titration behavior of DβM with DIMPI is readily explained within this structural framework (Scheme 1). The first stage of the reaction is the formation of a mono-DIMPI four-coordinate complex at each Cu: this would give rise to the two observed IR bands (2148 and 2129 cm^{-1}) provided the protein ligands X and Y are different. In a second stage, the mono complexes are converted into a single species with a strong IR band at 2160 cm^{-1}. By analogy to the behavior of the model complex **II** with DIMPI, we believe the most plausible interpretation of the reaction chemistry of this second stage is the displacement of protein-bound ligands by the isocyanide to form the tris complex. However, the species appears to remain bound to the protein since it cannot be removed by dialysis. This raises the possibility that it may represent a species which retains some of the endogenous protein ligands. For example, displacement of ligands X and Y in our structural model for DβM by isocyanide would form a $Cu^I(His)_2(DIMPI)_2$ species at both Cu centers and thus explain the observation of a single IR band.

Analogous reaction chemistry between DIMPI and the Cu centers in Hc would again lead first to the formation of the mono-DIMPI adduct at both coppers, formulated as $[Cu^I(His)_3DIMPI]_2$ with IR absorption (ν(C≡NR)) at 2148 ± 2 cm^{-1}. Further reaction would lead to displacement of the protein-derived histidine ligands, either to form the trisisocyanide (2160 cm^{-1}) or some other species such as $[Cu^I(His)_2(DIMPI)_2]$. However, in the hemocyanin case we have been able to obtain Raman data to compliment the IR characterization of the 2160 cm^{-1} species, albeit of low signal-to-noise ratio, which show the presence of two bands at approximately 2160 and 2183 cm^{-1}, similar to the Raman frequencies of the $Cu(DIMPI)_3^+$ complex (data not shown). The absence of an IR band at 2183 cm^{-1} in the Hc data and near coincidence with the frequencies of the $[Cu(DIMPI)_3]^+$ complex seem to suggest the presence of a protein-bound tris complex. Thus, it would seem likely that in both Hc and DβM higher concentrations of DIMPI displace all of the protein-derived ligands. It remains unclear why the tris-DIMPI complex cannot be removed from either

protein by dialysis, but it is possible the tris complex remains bound to the protein surface by hydrophobic interactions.

The observation of two inequivalent monoisocyanide complexes of DβM has helped to further delineate the inequivalence of the Cu_A and Cu_B centers in the $Cu_A^I(His)_2X\cdots Cu_B^I(His)_2Y$ structural model. Other data from our laboratory, using EXAFS,[6,7] FTIR,[5] and site-directed mutagenesis of the related enzyme peptidylglycine α-hydroxylating monooxygenase,[20] lend strong support to the assignment of ligand Y at the Cu_B site as methionine (Met473).[21] Assignment of ligand X at the Cu_A center is less certain, but histidine appears the most likely candidate since the 2148-cm^{-1} component corresponds closely to the band (2148 ± 2 cm^{-1}) assigned to the monoisocyanide complex of Hc (formulated as $[Cu^I(His)_3(DIMPI)]$) in the analogous experiment. We have tentatively assigned the 2129-cm^{-1} band in DβM to the mono-DIMPI complex at the Cu_B center ($Cu_B^I(His)_2Met$ (DIMPI)) since a preliminary experiment utilizing the Cu_A-depleted form of DβM gave rise to a mixture of the 2129- and 2160-cm^{-1} IR bands but no 2148-cm^{-1} band (data not shown). However, further experiments are underway to confirm this assignment.

Although the isocyanide ligand is isoelectronic with CO, its mode of bonding is rather different. Metal carbonyls exhibit strong π backbonding between the electron-rich low-valent metal and the empty antibonding orbitals of π symmetry on CO, which results in a weakening of the C≡O bond and a decrease in carbonyl stretching frequency, often well below the free-ligand value. In contrast, isocyanides are poorer π acceptors and stronger σ donors. With metals in low oxidation states, the π-acceptor effect usually predominates resulting in values of ν(C≡NR) that lie below the free ligand frequency.[8] For example, seven-coordinate monoisocyanide complexes of Ta(I) and Nb(I) have ν(C≡N) in the range 1737−1879 cm^{-1} (300−400 cm^{-1} below ν(C≡N) of the free ligands), with strongly bent M−C−N structures.[22] Similar effects are found in Fe(0), Fe(I), and Fe(II) isocyanide complexes.[23] On the other hand, metals in high-oxidation states can be stabilized by strong σ donation from the lone pair on the coordinating C atom of the isocyanide ligand(s). A recent example of this has been reported by Collins and co-workers,[24] in which trans coordination of two (CN-^{t}Bu) ligands has been found to stabilize an Fe(IV) macrocyclic complex. The Fe(IV) complex shows linear coordination of isocyanide, indicative of a markedly reduced π-acceptor interaction.

In Cu(I)-isocyanides, despite the low-valent nature of the complexes, the frequency of the C≡NR stretch generally lies above the free-ligand value, implying that the dominant mode of bonding is σ-donation with only minor contributions from π-acceptor interactions. This is probably due to the inability of the closed d^{10} configuration to effectively engage in backbonding. Given the small range of IR frequencies exhibited by Cu(I)-isocyanides and the lack of systematic structure/frequency relationships, it is hard to rationalize the frequencies that we observe in our protein and model systems in terms of electronic effects. Nevertheless, a weak trend toward an increase in frequency with basicity of coordinated ligands appears to be present in the series CuN_2X(DIMPI), as X changes from the weak donor S(methionine), DβM-Cu_B: 2129 cm^{-1}, to N(amino), complex **II**: 2132 cm^{-1}, and N(his), DβM-Cu_A and Hc: 2148 cm^{-1}. Similarly, replacement of the strong σ-donating *p*-tolylisocyanide (RNC) in the complex $[Cu(RNC)_4]^+$ by the weaker donor I^- to form $[CuI(RNC)_3]$ results in a 12 cm^{-1} decrease in ν(C≡N).[10c] Further work is necessary to extend and clarify the structure/frequency relationships in Cu(I)-isocyanide complexes in order to provide a firmer basis for structure prediction in copper protein isocyanide complexes.

In conclusion, our data demonstrate for the first time the utility of isocyanides as ligand-directed probes of Cu(I) coordination chemistry in copper proteins. In particular, the titration behavior of DIMPI with reduced dopamine-β-monooxygenase provides strong support for the active-site model with inequivalent $Cu_A(His)_3\cdots Cu_B(His)_2(Met)$ coordination as previously proposed. Our data also have begun to delineate the complex chemistry of isocyanides with Cu(I) in inorganic and biological systems.

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Supporting Information Available: Details of crystal and refinement data, positional parameters, and lists of interatomic distances and angles (Tables 1−3) for **2** (6 pages); lists of observed and calculated structure factors (Table 4) (14 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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