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Electronic Spectral and Magnetic Susceptibility Studies of Nickel(II) and Cobalt(II) Carboxypeptidase A Complexes

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Abstract: The electronic absorption spectrum of nickel(II) carboxypeptidase A exhibits weak bands at 9430, 14,600, and 24,250 cm^{-1}, which are assigned to the three spin-allowed d-d transitions ($^3A_{2g} \rightarrow {}^3T_{2g}$, $^3A_{2g} \rightarrow {}^3T_{1g}(F)$, $^3A_{2g} \rightarrow {}^3T_{1g}(P)$) of octahedrally coordinated nickel(II). Comparison of the ligand field parameters $Dq = 923$, $B = 755$ cm^{-1} for Ni^{II}CPA with those of a variety of octahedral Ni(II) complexes suggests either an N_2O_4 or NO_5 donor-atom set in the resting enzyme. Addition of fluoride ion or the model substrate glycyl-L-tyrosine does not change the octahedral nature of the Ni(II) center. The electronic spectrum of the complex between Ni^{II}CPA and the inhibitor β-phenylpropionate (βPP), however, is substantially different, as two visible bands of moderate intensity and a low-energy feature (<6500 cm^{-1}) are observed. The position and molar extinction coefficient of the principal peak (23,350 cm^{-1}; $\epsilon \sim 50$) are suggestive of five-coordinate Ni(II). A formation constant of $K_1 = 0.37 \times 10^4$ M^{-1} was measured by spectroscopic methods for the Ni^{II}CPA·βPP complex. Magnetic susceptibility and electronic absorption spectral data for Co^{II}CPA are analyzed in detail. The $\mu_{eff} = 4.77$ BM and the intensity of the principal visible absorption band (18,000 cm^{-1}; $\epsilon \sim 150$) are more compatible with five coordination than with a distorted tetrahedral Co(II) center. The fact that substantially different coordination geometries are adopted by various fully active CPA derivatives demonstrates the considerable flexibility of the binding site for metal ions.

Carboxypeptidase A (CPA) is a zinc metalloenzyme of molecular weight 34,600.[1,2] The enzyme catalyzes the hydrolysis of the C-terminal amino acid of a polypeptide chain. Enhanced activity is observed for C-terminal residues that have aromatic or branched aliphatic side chains or are in the L configuration. Esterase activity subject to these criteria has also been observed.[3]

A combination of high resolution X-ray[4-6] and complete amino acid sequence[7] studies has provided structural explanations for the peptide substrate specificity of CPA. These studies have also established His 69, Glu 72, and His 196 as the protein ligands of the active-site zinc ion and that the probable coordination geometry is distorted tetrahedral, with the fourth ligand a water molecule in the resting enzyme. The zinc ion in CPA is thought to bind to the carbonyl oxygen of the peptide bond to be hydrolyzed, thereby withdrawing some electron density from the adjacent carbon and rendering it more susceptible to nucleophilic attack by other groups in the enzyme.[5,6,8] Dipositive first-row transition metal ions[9] such as Mn^{2+}, Co^{2+}, Ni^{2+}, and Cu^{2+}, as well as the vanadyl ion,[10] VO^{2+}, can be substituted for the zinc ion in CPA with varying degrees of retention of activity. Only Co^{II}CPA and Ni^{II}CPA, however, retain full peptidase activity.[9]

The spectral properties of Co^{II}CPA have been extensively studied. The electronic absorption,[11] circular dichroism,[11] magneto circular dichroism,[12] and low temperature electron paramagnetic resonance[13] spectra of Co^{II}CPA have been interpreted as being consistent with distorted tetrahedral coordination about the metal center. However, except for the absorption spectra, extensive model system data are simply not available for Co(II) systems, making attempts to assign structure based on results from the various physical measurements subject to considerable uncertainty.

The size of the magnetic moment, or rather the magnitude of the unquenched orbital angular momentum, is a well-established indicator of the coordination number and geometry in high-spin Co(II) complexes.[14] We have previously shown that both Co^{II}CPA and Ni^{II}CPA have high-spin ground states.[15] Here we report a detailed analysis of the magnetic properties of Co^{II}CPA and demonstrate how distorted four-, five-, and six-coordinate geometries can be distinguished by low temperature magnetic susceptibility and electronic absorption spectral results. We also report our results on the solution properties of resting Ni^{II}CPA and its complexes with a model substrate, an inhibitor, and fluoride ion.

Experimental Section

Materials. Crystalline carboxypeptidase A, isolated by the Cox procedure,[16] was obtained from Sigma Chemical Co. and used without further purification. The Cox procedure was chosen because it yields very little of the undesirable CPA_γ form of the enzyme.[17] Metallocarboxypeptidase A derivatives were prepared by the method of Coleman and Vallee.[18] Extreme care was taken to prevent contamination of CPA by adventitious metal ions.[19] Plastic labware was used, and all the Tris-HCl buffers were repeatedly extracted with dithizone in CCl_4 prior to use. Solutions of Co^{2+} or Ni^{2+} were made up by dissolving the pure metal in metal-free HCl. Minimum 99.8% pure metal powders were obtained from Alfa Inorganics, Inc. Samples of CPA were checked for peptidase activity[20] and metal content both before and after metal replacement. Hippuryl-L-phenylalanine (0.994×10^{-3} *M*) (Schwarz/Mann) was used as the substrate in all assays (assay conditions 0.5 *M* NaCl, 0.05 *M* Tris·HCl, pH 7.5, 25°). Substrate hydrolysis was followed spectrophotometrically at 256 nm. A residual zinc level of from 0.01 to 0.03 g-atom/enzyme was found in all derivatives. Peptidase activity ratios were found to fall in the following ranges: $Co^{II}CPA/Zn^{II}CPA$ = 1.7–1.9, $Ni^{II}CPA/Zn^{II}CPA$ = 0.7–0.9 (these ratios refer to activity of the Co(II) or Ni(II) derivative relative to the native enzyme *before* Zn(II) removal). The D_2O buffer used in the spectral experiments was freed of metal ion impurities by passing it through a column of Chelex-100. D_2O (99.8%) was obtained from Columbia Organic Chemical Co. Deuterated apoCPA was obtained by dialyzing apoCPA against at least five changes of deuterated buffer. Sodium β-phenylpropionate (NaβPP) was prepared by neutralizing an alcoholic solution of hydrocinnamic acid (Eastman Chemical Co.) with sodium hydroxide. The NaβPP was recrystallized from ethanol–hexane solution. Metal analyses were performed on a Varian Techtron Model AA-5 atomic absorption spectrometer equipped with a Jarrell-Ash Model 82-000 monochromator and Varian Techtron element-specific hollow cathode lamps.

Spectral Measurements. Near-infrared and visible absorption spectra of Ni^{II}CPA were measured on a Cary 17I recording spectrophotometer which had been modified to run at constant slit width; this modification considerably reduces baseline variation in the near-infrared region on the 0.0–0.1 OD slide-wire. Because of the low optical densities of Ni^{II}CPA solutions, spectra were measured by a difference technique utilizing 50-mm matched cells (vol. 1.95 ml) obtained from Helma Cell, Inc. A baseline spectrum was determined with solutions of apoCPA of the same concentration in both sample and reference cells. This baseline spectrum was subtracted, point by point, from the spectrum obtained when a small volume (~20 μl) of first the metal ion and then substrate or inhibitor was added to the sample cell. An equivalent volume of metal-free buffer was added to the reference cell in order to keep the protein concentrations equal. Spectra were measured in 1 *M* NaCl and 0.05 *M* (pH 7.8) deuterated Tris buffer at temperatures between 7 and 12°. At the pH of this medium, Ni^{II}CPA has the maximal peptidase activity.[18] Corrections for enzyme dilution were applied to all the spectral data. Enzyme concentrations were measured spectrophotometrically at 278 nm.[20]

Magnetic Susceptibility Measurements. The magnetic susceptibility of Co^{II}CPA as a function of temperature was measured on a magnetometer of ultrahigh sensitivity, utilizing a superconducting, quantum mechanical sensor, by methods described previously.[15] In order to obtain a suitable sample of Co^{II}CPA, a suspension of crystalline Co^{II}CPA was spread on a glass slide and allowed to dry in a scrupulously dust-free enclosure and then transferred to a quartz tube. Only samples containing less than one Co^{2+} per enzyme molecule were used. Air-dried crystalline Co^{II}CPA retains its red-purple color. The visible absorption spectrum of a pellet of this air-dried Co^{II}CPA exhibited a band maximum at 558 nm, in agreement with the position of the principal peak in the solution spectrum. Following the susceptibility measurements, a total cobalt analysis showed that the 24.4-mg sample of Co^{II}CPA contained 29.7 μg of cobalt (~0.7 Co/CPA).

Results

Ni^{II}CPA. Magnetic susceptibility measurements have established a $^3A_{2g}$ ground state for Ni^{II}CPA.[15] The absorption spectrum of Ni^{II}CPA in D_2O buffer is shown in Figure 1. Three bands with low molar extinction coefficients attributable to electronic transitions in Ni^{II}CPA are observed. The sharp features in the spectrum below 8000 cm^{-1} are undoubtedly due to the vibrational overtones of residual water. The observed electronic absorption bands of Ni^{II}CPA may be assigned unambiguously to the three spin-allowed d–d transitions predicted by ligand field theory for an octahedral, $^3A_{2g}$ Ni(II) center: $^3A_{2g} \rightarrow {}^3T_{2g}$, 9430; $^3A_{2g} \rightarrow {}^3T_{1g}(F)$, 14,600; $^3A_{2g} \rightarrow {}^3T_{1g}(P)$, 24,250 cm^{-1}. Neither fluoride ion nor the slowly hydrolyzed substrate glycyl-L-tyrosine changes the octahedral-like character of the absorbing species, as the same pattern of low-intensity bands is observed when these substances are added to a solution containing Ni^{II}CPA. The spectrum of Ni^{II}CPA in a 0.5 *M* NaCl, 0.5 *M* KF Tris buffer medium is slightly red shifted from that observed in the sodium chloride buffer solution, indicating that fluoride ion is coordinated to the metal (Figure 2).

The ligand field parameter values which give the best least-squares fit to the observed d–d bands of resting Ni^{II}CPA are Dq = 923 and B = 755 cm^{-1}.[21] For comparison, we have calculated Dq = 779 and B = 940 cm^{-1} for Ni^{II}PGM (phosphoglucomutase) from the spectrum reported by Multani and Ray.[22] The probable donor-atom sets which determine the ligand field strengths of these two Ni(II) enzymes may be assigned by reference to the observed spectra and calculated ligand field parameters for a wide range of model octahedral nickel(II) complexes involving nitrogen and oxygen donors (Table I). The ligand field parameters of Ni^{II}PGM clearly indicate an all oxygen-donor ligand environment, as Multani and Ray previously concluded.[22] It is reasonable to assume that the coordination environment of octahedral Ni^{II}CPA includes three H_2O molecules, in addition to the protein ligands His 69, His 196, and Glu 72. We note, however, that Dq for the Ni(II) derivative of CPA falls slightly below the range observed for complexes with N_2O_4 donor-atom sets, although it is significantly greater than the values found for the model complexes that have exclusively oxygen donor atoms. Chloride ion is apparently not coordinated to the Ni(II), as the addition of fluoride causes Dq to decrease. The relatively low value of the interelectronic repulsion parameter B in Ni^{II}CPA is in line with results obtained from model complexes containing polyacetate type ligands such as HEDTA and NTA. It is possible that the geometric constraints of the metal–protein interactions are such that ligand to metal π bonding is enhanced in Ni^{II}CPA. Such an effect would tend to lower both Dq and B. Thus it is probable that the donor-atom set in Ni^{II}CPA is N_2O_4, but we cannot entirely rule out NO_5, which would occur if either His 69 or His 196 were not coordinated under the conditions of our experiment. Further, the electronic spectral data do not shed any new light on the question of Tyr 248 coordination,[23] as substitution of a $TyrO^-$ group for a ligated H_2O would not be expected to shift the d–d transitions of Ni(II) appreciably.

Table I. Electronic Spectra and Calculated Ligand Field Parameters for Octahedral Ni(II) Complexes and Enzymes (all energies are in cm^{-1})

Complex	Donor atom set	ν_1 ($^3A_{2g} \rightarrow {}^3T_{2g}$)	ν_2 ($^3A_{2g} \rightarrow {}^3T_{1g}(F)$)	ν_3 ($^3A_{2g} \rightarrow {}^3T_{1g}(P)$)	Dq	B	Ref
$Ni(en)_3^{2+}$	N_6	11,600 (7)	18,350 (7)	29,000 (9)	1150	855	*a*
$Ni(im)_6^{2+}$	N_6	11,100	18,150	28,700	1131	849	*b*
$Ni(tn)_3^{2+}$	N_6	10,900 (6)	17,800 (11)	28,200 (15)	1110	836	*c*
$Ni(CH_3CN)_6^{2+}$	N_6	10,700	17,400	27,800	1085	836	*d*
$Ni(NH_3)_6^{2+}$	N_6	10,750 (4)	17,500 (5)	28,200 (6)	1080	886	*a*
$Ni(NC_2H_5)_6^{2+}$	N_6	10,500 (7)	17,100 (7)	27,500 (12)	1063	841	*e*
$Ni(C_2H_5NH_2)_6^{2+}$	N_6	9,662	16,666	27,027	1006	881	*f*
$Ni(n\text{-}C_3H_7NH_2)_6^{2+}$	N_6	9,826 (6)	16,502 (5)	26,809 (14)	1006	862	*f*
$Ni(C_5H_5N)_6^{2+}$	N_6	10,150 (4)	16,500 (5)	27,000 (10)	1022	852	*c*
$Ni(i\text{-}C_3H_7NH_2)_6^{2+}$	N_6	9,480	15,267	26,042	939	881	*f*
Ni(his)$_2$	N_4O_2	10,700 (7)	18,000 (8)	28,000 (10)	1109	826	*g*
$Ni(tren)(H_2O)_2^{2+}$	N_4O_2	10,500 (15)	17,800 (9)	27,800 (2)	1092	834	*c*
$Ni(C_5H_5N)_4(CF_3CO_2^-)_2$	N_4O_2	9,874 (5)	15,870 (10)	28,080 (19)	988	821	*h*
Ni(NTA)(en)$^-$	N_3O_3	9,400 (11)	16,900 (8)	27,200 (13)	1004	901	*c*
$Ni(gly)_3^-$	N_3O_3	10,100 (10)	16,600 (8)	27,600 (14)	1018	906	*a*
Ni(NTA)(gly)$^{2-}$	N_2O_4	9,900	17,100	26,200	1043	772	*c*
Ni(HEDTA)$^-$	N_2O_4	10,100	16,785	26,455	1038	792	*i*
Ni(asp)$_2$	N_2O_4	9,810 (7)	16,340 (5)	27,930 (13)	990	967	*j*
Ni(glygly)$_2$	N_2O_4	9,270 (7)	16,130 (5)	27,030 (9)	983	907	*j*
$Ni(gly)_2(H_2O)_2$	N_2O_4	9,270 (7)	16,050 (6)	27,100 (9)	979	917	*j*
$Ni(Tris)_2(H_2O)_4$	N_2O_4	9,540	15,750	25,975	965	846	*i*
$Ni(NTA)(H_2O)_2^-$	NO_5	9,500 (16)	16,000 (7)	25,600 (13)	980	799	*c*
$Ni(CH_3OH)_6^{2+}$	O_6	8,431 (2)	14,226 (4)	25,000 (6)	853	905	*k*
$Ni(H_2O)_6^{2+}$	O_6	8,500 (2)	13,800 (2)	25,300 (5)	834	947	*l*
$Ni(DMF)_6^{2+}$	O_6	8,500 (6)	13,600 (5)	25,000 (15)	826	933	*m*
NiO in MgO	O_6	8,600	13,500	24,600	827	901	*l*
$Ni(C_2H_5OH)_6^{2+}$	O_6	8,180 (3)	13,404 (3)	24,795 (7)	806	941	*j*
$Ni(CH_3CO_2)_2$ in Li^+, Na^+ acetate glass	O_6	7,800	13,200	24,200	786	919	*n*
$Ni(DMSO)_6^{2+}$	O_6	7,728 (3)	12,970 (3)	24,038 (10)	773	920	*o*
$Ni(DMA)_6^{2+}$	O_6	7,690	12,900	23,920	769	916	*m*
$Ni(SO_4)$ in $K_2(SO_4)$, $Zn(SO_4)$	O_6	7,300	12,200 (6)	23,100 (21)	726	903	*p*
$KNiF_3$	F_6	7,250 (6)	12,530 (11)	23,810 (23)	737	943	*q*
Ni(quinoline)Cl_2	N_2Cl_4	6,400 (5)	11,100 (9)	21,100 (12)	652	837	*r*
			Ni(II) Enzymes				
Ni^{II}CPA	N_2O_4?	9,430 (3)	14,600 (7)	24,250 (24)	923	755	*s*
Ni^{II}PGM	O_6?	7,692 (5)	13,160 (6)	24,390 (23)	779	940	*t*
Ni^{II}CA	N_3O_3?		16,000 (10)				*u*

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Addition of the inhibitor sodium β-phenylpropionate dramatically alters the spectrum of Ni^{II}CPA, as is shown in Figure 3. Both visible absorption bands are red shifted and enhanced in intensity. In the near-infrared region, the $^3A_{2g} \rightarrow {}^3T_{2g}$ band of Ni^{II}CPA is replaced by a new absorption feature above 1400 nm. The exact position and intensity of this new peak are hard to establish, as this region of the near-infrared is obscured by absorption due to water (1430 nm) and the onset of the effective low-energy cutoff in D_2O (1550 nm). The peaks at 1420 and 1520 nm appear to be vibrational overtones of the solvent superimposed on a broader electronic absorption band, which falls below 1540 nm (6500 cm^{-1}). The spectrum of Ni^{II}CPA·βPP is the same in both chloride and fluoride ion media, which indicates that F^- does not bind effectively to the nickel(II) when inhibitor is present. In this regard it is of interest to note that nmr relaxation experiments have shown[24] that the exchange between F^- and the inner coordination sphere of Mn^{II}CPA is greatly attenuated upon addition of βPP. Our results in the Ni^{II}CPA system add some support to one possible interpretation of the nmr experiments, namely, that the fluoride binding site is lost upon formation of Mn^{II}CPA·βPP.

Following the procedure of Furman and Garner,[25] the changes in the absorption spectrum of Ni^{II}CPA on addition of NaβPP may be used to evaluate the formation constant K_I for the Ni^{II}CPA·βPP complex. As the absolute absorbance changes are so small, linear plots of $(E \times I/A_\lambda - pE \cdot \epsilon_{Ni^{II}CPA})$ *vs.* $(E + I)$ were obtained only in the region of greatest absorbance change (405–460 nm) (E and I are the total enzyme and inhibitor concentrations, respectively; A_λ is the observed absorbance at wavelength λ, and p is the pathlength of the cell in centimeters). Some representative plots are shown in Figure 4. A value of $K_I = (3.7 \pm 1.7) \times 10^3\ M^{-1}$ was obtained, and the absorbance maximum of Ni^{II}CPA·βPP was calculated to fall between 425 and 430 nm (~23,350 cm^{-1}), with a molar extinction coefficient of roughly 50.

The observed band pattern, and particularly the substan-

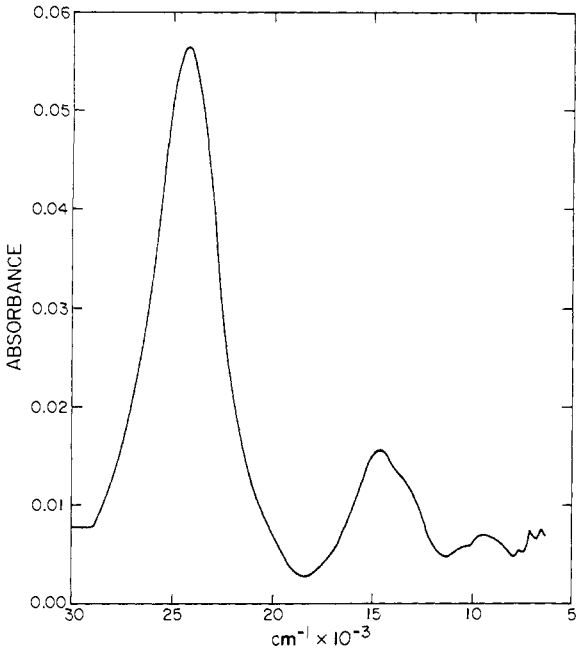

Figure 1. Near-infrared-visible absorption spectrum of 4.7×10^{-4} *M* $Ni^{II}CPA$ between 7 and 12° in 1 *M* NaCl, 0.05 *M* (pH 7.8) deuterated Tris buffer.

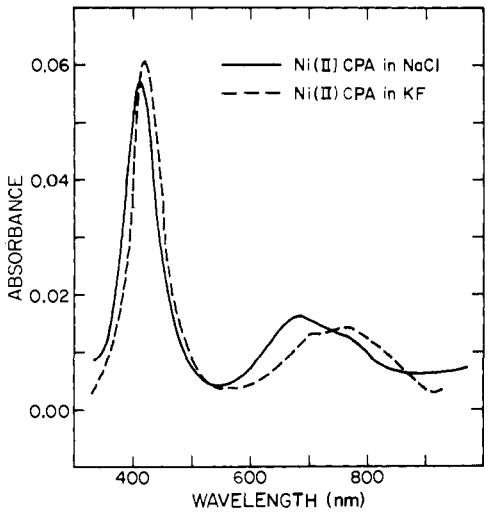

Figure 2. Visible absorption spectra of $Ni^{II}CPA$ between 7 and 12° in 0.05 *M* (pH 7.8) Tris buffer: ——, $[Ni^{2+}] = 4.71 \times 10^{-4}$ *M*, 1 *M* NaCl; - - - -, $[Ni^{2+}] = 3.35 \times 10^{-4}$ *M*, 0.5 *M* NaCl, 0.5 *M* KF.

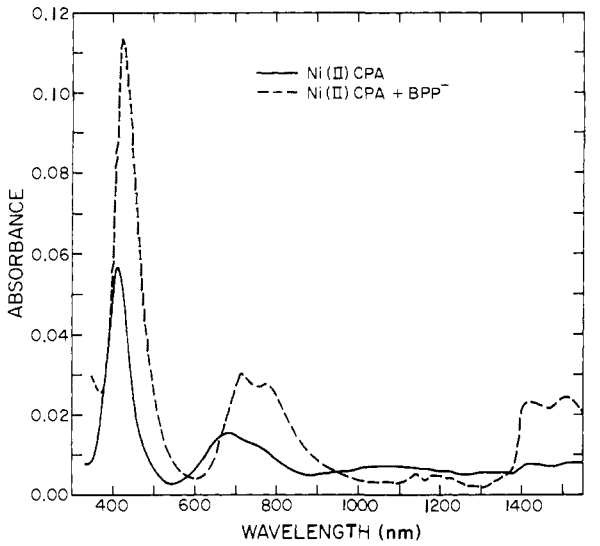

Figure 3. Effect of added NaβPP on the near-infrared-visible absorption spectrum of $Ni^{II}CPA$ between 7 and 12° in 1 *M* NaCl, 0.05 *M* (pH 7.8) deuterated Tris buffer: ——, $[Ni^{2+}] = 4.71 \times 10^{-4}$ *M*; - - -, $[Ni^{2+}] = 4.20 \times 10^{-4}$ *M*, $[NaβPP] = 8.48 \times 10^{-3}$ *M*.

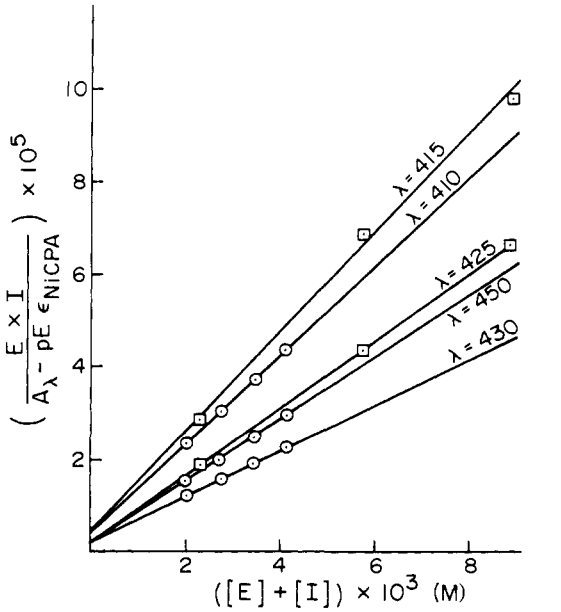

Figure 4. Plots of $(E \times I)(A_\lambda - pE\,\epsilon_{NiCPA})$ *vs.* $(E + I)$ for the reaction of $Ni^{II}CPA$ with NaβPP (λ in nm).

tial increase in the molar extinction coefficient at 23,350 cm^{-1} suggest some type of five-coordinate geometry for $Ni^{II}CPA{\cdot}\beta PP$. A distorted tetrahedral structure, which could also explain the low energy of the near-infrared band,[26-28] would not be expected to give rise to a d-d transition above 20,000 cm^{-1} for a mixed nitrogen-oxygen donor-atom set.[26] Stronger field ligands, such as phosphines, are required[29] to place the highest energy d-d transition in a distorted tetrahedral Ni(II) complex at about 23,000 cm^{-1}, which effectively rules out this coordination geometry for $Ni^{II}CPA{\cdot}\beta PP$.

Many high-spin five-coordinate Ni(II) complexes containing weak-field donor-atom sets exhibit relatively intense absorption bands at energies greater than 20,000 cm^{-1}.[28] Of particular note is the close similarity of the absorption spectrum of the N_2O_3 complex $[Ni(dacoda)(H_2O)^-]$[28a] to that of $Ni^{II}CPA{\cdot}\beta PP$. The comparison is sufficiently good to conclude that the probable donor-atom set in the enzyme-inhibitor complex is also N_2O_3. The implied contraction of the metal pocket could be caused by small conformational changes associated with the binding of the aromatic part of the inhibitor to the enzyme. It should be noted that the observed formation constant of $Ni^{II}CPA{\cdot}\beta PP$ is two orders of magnitude greater than a typical value for binding an acetate-type ligand to aquo nickel ion,[30] which signals the presence of favorable interactions between enzyme and inhibitor over and above those provided by ordinary carboxylate coordination to the metal.

$Co^{II}CPA$. The temperature dependence of the effective magnetic moment of $Co^{II}CPA$ between 30 and 120°K is shown in Figure 5. The magnetic moment is 4.77 ± 0.15 BM over the temperature range studied, which establishes a high-spin ground state.[15]

The band positions in the absorption spectrum of a pellet of air-dried $Co^{II}CPA$ are the same as those observed for $Co^{II}CPA$ in solution.[11,15] However, the possibility remains

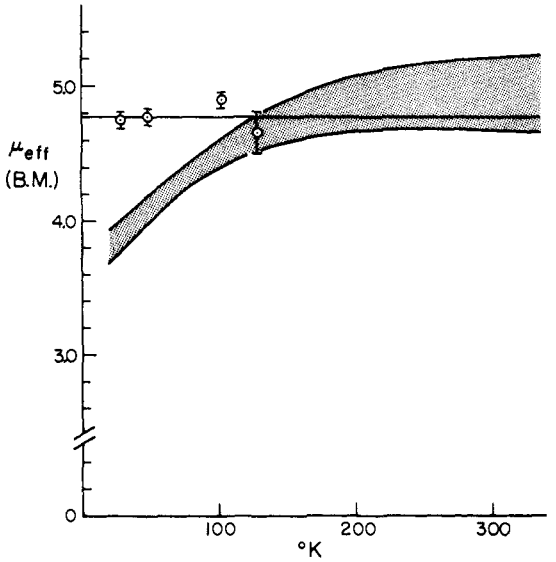

Figure 5. Comparison between the experimental μ_{eff} values for Co^{II}CPA (30–120°K, Φ) and calculated ranges (shaded area) of μ_{eff} for octahedrally coordinated Co(II). Calculations were performed using the standard theoretical expression[14] and the free-ion spin-orbit coupling constant for Co^{2+}. The upper boundary of the shaded area represents the weak-field limit (A = 1.5), and the lower boundary the strong-field limit (A = 1.0).

that some partial denaturation may have occurred in the drying process, as accurate intensity data cannot be obtained from pellet spectra. Such partial denaturation would presumably give octahedral cobalt(II) species, which would not be spectroscopically detectable in the presence of tetrahedral or five-coordinate cobalt(II), owing to low extinction coefficients. The magnetic moment data, however, allow us to place an upper limit on the amount of octahedral cobalt(II) present. Although the room temperature magnetic moments of octahedral cobalt(II) complexes are high, 4.7–5.2 BM, they are strongly temperature dependent.[14] This temperature dependence arises because the ground state $^4T_{1g}$ term is split by spin–orbit coupling into several levels that are separated by energies roughly comparable to kT. Over the temperature range we have investigated, for example, the magnetic moments of octahedral cobalt(II) complexes are substantially reduced from their room temperature values,[31] in accord with theoretical predictions illustrated in Figure 5. Therefore, as little as 5% of an octahedral Co(II) impurity would show up in our experiment at low temperatures. The strict Curie law behavior of our sample of Co^{II}CPA strongly rules against the presence of any appreciable amounts of octahedral Co(II) species.

Both distorted tetrahedral and five-coordinate geometries are reasonable candidates for the metal ion coordination in Co^{II}CPA. Latt and Vallee have favored[11] the former structural model, whereas we have argued[15] that the available evidence is more compatible with five coordination. The issue is difficult to settle because the electronic spectra of distorted tetrahedral and five-coordinate cobalt(II) complexes are often very similar, especially when only band positions are considered.[32] We will now demonstrate, however, that a choice can be made after a detailed consideration of *both* the magnetic and spectroscopic properties associated with these modes of coordination to cobalt(II) centers.

The magnetic moments of tetrahedral cobalt(II) complexes can be calculated from the expression[14]

$$\mu_{eff} = 3.87\left(1 - \frac{4k^2\lambda}{10Dq}\right) \quad (1)$$

where λ is the free-ion, spin-orbit coupling constant, which for cobalt(II) is -180 cm^{-1}, k is the orbital reduction factor, which can take a value between 0 and 1. Ligand field parameters[21] as well as calculated and observed magnetic moments for some distorted tetrahedral and five-coordinate cobalt(II) complexes are given in Table II. The magnetic moments were evaluated from the slopes of $1/\chi_m$ *vs.* T plots or corrected for temperature independent paramagnetism when only room temperature data were available.[33] The ν_2 and ν_3 values were taken as the centers of gravity of the band multiplets that are usually observed in these systems. The magnetic moments were all calculated from eq 1, as we were interested in determining whether the experimental values for known five-coordinate Co(II) complexes could be "explained" satisfactorily on the basis of an incorrect assumption regarding stereochemistry. Inspection of Table II clearly illustrates that even the maximum "tetrahedral" magnetic moments (k = 1) fall significantly below the observed values for the five-coordinate complexes. The experimental value (4.77 BM) of the magnetic moment of Co^{II}CPA is also well above the maximum of 4.42 BM obtained from eq 1, but it does fit nicely in the range of 4.5–5.1 BM observed for the five-coordinate model complexes. Therefore, the magnetic susceptibility results strongly favor a five-coordinate assignment for the ground state of Co^{II}CPA.

The five-coordinate assignment is strengthened by an examination of the intensities of the visible absorption bands (ν_3) for the two types of model complexes. The molar extinction coefficient of ν_3 is generally greater than 250 for distorted tetrahedral complexes, whereas substantially lower values are observed both for Co^{II}CPA and the five-coordinate models ($50 \leq \epsilon_{max} \leq 225$).[15]

Discussion

We have shown how spectroscopic and magnetic data can be used to make assignments for the metal ion coordination in Ni^{II}CPA and Co^{II}CPA. Among the structural proposals, the octahedral-like character of the coordination environment in resting Ni^{II}CPA is most certain. Literally hundreds of octahedral nickel(II) complexes have been studied, and the pattern of three bands of low intensity in the near-ir and visible spectral regions is characteristic of all. The conclusions regarding the metal coordination in the resting Co^{II}CPA system must be regarded as somewhat less firm. Although our assignment of five-coordination to Co^{II}CPA is based on two independent lines of evidence, alternative structural possibilities are difficult to rule out entirely. There seems to be little doubt, however, that the metal center in Co^{II}CPA has a structure different from that of the Co(II) derivative of the enzyme carbonic anhydrase (CA). Both the low magnetic moment and the rather intense visible absorption bands of Co^{II}CA fit distorted tetrahedral coordination reasonably well, as was concluded previously by Lindskog and Ehrenberg[34] on the basis of a much more limited set of model system data. This and other comparisons we have made illustrate the considerable importance of measuring the temperature dependence of the magnetic moment in a cobalt(II) system, particularly at temperatures between 10 and 100°K, as an aid in determining the probable coordination environment.

It is apparent that there is considerable flexibility in the binding site for metal ions in CPA. A variety of geometrical arrangements can be accommodated, ranging from distorted tetrahedral in Zn^{II}CPA to octahedral in Ni^{II}CPA. The observed variation of coordination number from four to six while full peptidase activity is retained is not easily reconciled with the entatic state hypothesis, as it has been applied

Table II. Ligand Field Parameters (cm^{-1}) and Magnetic Moments (BM) for Co(II) Complexes

Complex	ν_2	ν_3	Dq	B	μ_{eff} (calcd; eq 1, k = 1)	μ_{eff} (obsd)	Data ref
			Distorted Tetrahedral				
$Co(EDM)Cl_2$	7688	15587	451	649	4.49	4.36	*a*
$Co(Me_4en)Cl_2$	8336	16837	490	699	4.44	4.54	*b*
$Co(MOBen \cdot NEt_2)Cl_2$	7531	16108	439	697	4.50	4.37	*c*
$Co(PA)_2$	9117	19775	531	864	4.39	4.56	*d*
$Co(Barb)_2(Im)_2$	8897	17938	523	743	4.40	4.33	*e*
$Co(BenzIm)_4(ClO_4)_2$	9009	17700	531	718	4.39	4.28	*f*
			Five-Coordinate				
β-$Co(Paphy)_2Cl_2$	7680	15850	450	669	4.49	4.79	*g*
$Co(Me_6tren)Cl_2$	9090	18333	534	760	4.39	4.51	*h*
$Co(Me_5dien)Cl_2$	9762	17487	586	644	4.35	4.61	*i*
$Co(Me_4daeo)Cl_2$	8519	16923	501	693	4.43	4.70	*j*
$Co[Py(Cy)_2]Cl_2$	7741	17168	450	761	4.49	4.85	*k*
$Co(MABenNEt_2)Cl_2$	8965	16600	534	635	4.39	4.82	*l*
			Co(II) Enzymes				
$Co^{II}CPA$	8616	17750	504	748	4.42	4.77	*m*
$Co^{II}CA$	8962	17490	529	705	4.40	4.23	*n*

[a] A. L. Lott, III, and P. G. Rasmussen, *J. Inorg. Nucl. Chem.*, **32**, 101 (1970); EDM = ethylenedimorpholine. [b] L. Sacconi, I. Bertini, and F. Mani, *Inorg. Chem.*, **6**, 262 (1967); Me_4en = *N,N,N',N'*-tetramethylethylenediamine. [c] L. Sacconi and I. Bertini, *Inorg. Chem.*, **7**, 1178 (1968); $MOBenNEt_2$ = *N,N*-diethyl-*N'*-(*o*-methoxybenzylidene)ethylenediamine. [d] R. H. Holm, A. Chakravorty, and L. Theriot, *Inorg. Chem.*, **5**, 625 (1966); PA = *N*-*tert*-butylpyrrole-2-aldimine. [e] R. C. Rosenberg, Ph.D. Thesis, California Institute of Technology, 1974; Barb = 5,5'-diethylbarbituric acid, Im = imidazole. [f] M. Goodgame and F. A. Cotton, *J. Amer. Chem. Soc.*, **84**, 1543 (1962); BenzIm = benzimidazole. [g] S. F. Lions, I. B. Dance, and J. Lewis, *J. Chem. Soc. A*, 565 (1967); Paphy = pyridine-2-aldehyde 2-pyridylhydrazone. [h] M. Ciampolini and N. Nardi, *Inorg. Chem.*, **5**, 41 (1966); Me_6tren = tris(2-dimethylaminoethylamine). [i] M. Ciampolini and G. P. Speroni, *Inorg. Chem.*, **5**, 45 (1966); Me_5dien = bis(2-dimethylaminoethyl)methylamine. [j] M. Ciampolini and N. Nardi, *Inorg. Chem.*, **6**, 445 (1967); Me_4daeo = bis(2-dimethylaminoethyl) oxide. [k] L. Sacconi, I. Bertini, and R. Morassi, *J. Chem. Soc. A*, 1570 (1968); $Py(Cy)_2$ = *N,N'*-dicyclohexyl-2,6-diacetylpyridine bisimine. [l] L. Sacconi, I. Bertini, and R. Morassi, *Inorg. Chem.*, **6**, 1548 (1967); $MABenNEt_2$ = *N,N*-diethyl-*N'*-(*o*-methylaminobenzylidene)ethylenediamine. [m] Spectral data only from ref 11. [n] S. Lindskog and A. Ehrenberg, *J. Mol. Biol.*, **24**, 133 (1967).

to acid–base catalysts.[35] According to this theory, the resting enzyme is activated in part by virtue of an unusual metal ion coordination geometry. Our findings for $Ni^{II}CPA$ are especially at odds with such ideas, as in this derivative the metal ion is coordinatively saturated and has a very ordinary ligand environment. Our results, on the other hand, accord well with mechanistic proposals in which the metal ion simply serves as one of a number of catalytically important groups.[5-8,36] It would appear that as long as a properly oriented, substitution-labile position is available at the metal center, full peptidase activity is possible. We note that in derivatives where the metal ion is expected to be inert to substitution, such as $Co^{III}CPA$, no peptidase activity is observed.[37]

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