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Spectroscopic and Binding Studies of Azide–Copper(II) Model Complexes†

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The characteristics of azide binding to a series of mononuclear copper(II) complexes with tridentate ligands has been investigated. The ligand donor atoms span NO_2 to N_3 donor sets, and the resulting copper(II) complexes, overall charges from 0 to +2. The spectroscopic properties of the adducts indicate that azide prefers to bind to Cu(II) in an axial coordination position when the equatorial ligand carries a dinegative charge, while it prefers to bind equatorially when the ligand is neutral or contains a mononegative donor atom in the Cu(II) plane. The equilibrium constants for the formation of the azide adducts have been determined by spectrophotometric titrations. The data show that the affinity of azide increases with the overall charge of the copper complex but depends markedly on the polarity of the medium. Very high affinities ($K > 5000\ M^{-1}$) have been found for dipositive complexes in methanol solution, while all K values are markedly lower in water solution.

Introduction

Binding of small ligand molecules to copper proteins has been widely employed as a method to obtain structural or mechanistic information on the metal sites.[1] Binding of anions such as azide, thiocyanate, and bromide to copper(II) is generally accompanied by the appearance of LMCT transitions in the visible or near-UV region and produces changes in the EPR spectra that may be potentially very useful for empirical correlations between spectra and structure.

Azide is probably the most widely used ligand probe for the copper protein sites, as shown for instance by studies on amine oxidases,[2] superoxide dismutase,[3] galactose oxidase,[4] hemocyanin,[5,6] tyrosinase,[7] laccase,[8] ceruloplasmin,[9] and ascorbate oxidase.[10] Even so, the spectral features exhibited by the azide adducts and the affinities of the ligand for these proteins are so diverse that it is usually difficult to infer the coordination characteristics of the metal center and the mode of coordination of the anion. These difficulties reflect to some extent the variety of structural arrangements that are assumed by the copper centers in the proteins but depend also on the lack of unambiguous correlations based on the behavior of azide coordination to suitable model complexes. We expect, for instance, that the spectroscopic characteristics of the azide adducts and more generally the intrinsic affinity of azide for a given copper(II) center will be markedly influenced by the nature of the other ligands in the coordination sphere and by factors such as the overall charge of the complex, the stereochemistry of the metal site, and the coordination position to be occupied by the ligand (e.g. equatorial vs axial). Additional problems arise when there is the possibility for azide to form a bridge between two metal centers, since this binding mode is not easily recognized from the spectra.

In this paper we initiate a systematic investigation of the binding of azide to copper(II) complexes with ligands of potential biological relevance. Although there has been recently a growing interest in the chemistry of copper(II)–azide complexes, the main focus has been on azido-bridged binuclear complexes in the attempt to explain the properties of the derivatives of methemocyanin active site.[11-15] The systems studied here are a series of copper(II) complexes with ligands containing nitrogen and oxygen donor atoms, which can be considered as mimics of the potential ligands of type 2 and type 3 copper centers in the proteins (Figure 1). The spectroscopic and binding properties of the azide adducts of these complexes, with 1:1 stoichiometry and terminal mode of coordination of the anion, are the subject of the present investigation.

Experimental Section

Reagents and Preparations.[16] All reagents were of the highest grade commercially available and were used as received. The peptides were products from Fluka. 4-Imidazolecarboxaldehyde was prepared according to a literature procedure.[17] The complexes [Cu(pyv-L-ala)] (**1**), [Cu(pyv-L-his)] (**3**), and [Cu(pyv-L-hisOMe)]Cl (**4**) were prepared as reported before.[18] The complex [Cu(pyv-β-ala)] (**2**) was prepared sim-

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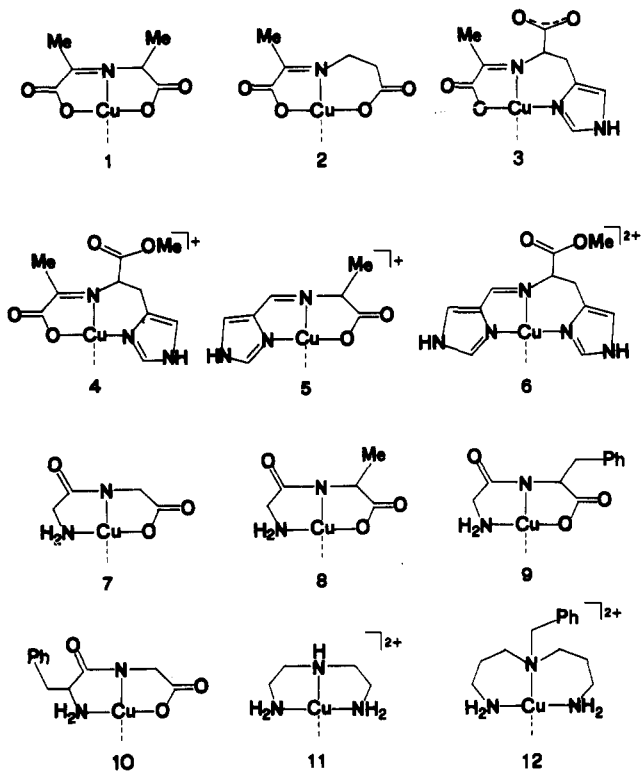

Figure 1. Structure of the copper(II) complexes investigated.

ilarly. Anal. Calcd for $C_6H_7NCuO_4 \cdot H_2O$: C, 30.19; H, 3.77; N, 5.87. Found: C, 29.98; H, 3.84; N, 5.89.

The complexes [Cu(imd-L-ala)]ClO_4 (**5**) and [Cu(imd-L-hisOMe)]$(ClO_4)_2$ (**6**) were prepared according to the following procedure. 4-Imidazolecarboxaldehyde (1 mmol) was dissolved in methanol (20 mL), and the free L-amino acid (1 mmol) was added under stirring. The stoichiometric amount of copper(II) perchlorate hexahydrate dissolved in methanol (5 mL) was added to the solution of the Schiff base, and the mixture was left under stirring for about 2 h. In the case of [Cu(imd-L-ala)]ClO_4, 1 equiv of methanolic sodium hydroxide was also added immediately after the copper salt. The resulting solution was cooled to 5 °C to allow crystallization of the product. This was collected by filtration and dried under vacuum. Anal. Calcd for [Cu(imd-L-ala)]ClO_4 ($C_7H_8N_3CuClO_6$) (**5**): C, 25.54; H, 2.45; N, 12.77. Found: C, 25.25; H, 2.90; N, 12.59. Anal. Calcd for [Cu(imd-L-hisOMe)]$(ClO_4)_2$ ($C_{11}H_{13}N_5CuCl_2O_{10}$) (**6**): C, 25.92; H, 2.57; N, 13.74. Found: C, 25.45; H, 2.80; N, 13.66.

The peptide complex [Cu(glygly)] (**7**) was obtained according to a literature method.[19] The complex [Cu(L-phegly)] (**10**) was prepared similarly by adding a solution of copper(II) perchlorate hexahydrate (1 mmol) in water (3 mL) to a warm solution of L-phenylalanylglycine (1 mmol) in water (10 mL). The pH was raised to 6.78 by addition of a few drops of concentrated sodium hydroxide; then dimethylformamide (3 mL) was added, and the mixture was concentrated to half-volume under vacuum. The product precipitated upon cooling of the solution for several hours in a refrigerator. The complex [Cu(gly-L-phe)] (**9**) was prepared in a similar way but by adjusting the pH of the aqueous solution to 6.92 and omitting the addition of DMF. The complex [Cu(gly-L-ala)] (**8**) was also obtained by following the same procedure but by omitting the DMF addition, concentrating to a small volume the aqueous solution, and adding ethanol to the residue. Anal. Calcd for [Cu(gly-L-ala)]·$2H_2O$ ($C_5H_{12}N_2O_5Cu$) (**8**): C, 24.64, H, 4.96; N, 11.49. Found: C, 24.39; H, 4.88; N, 11.32. Anal. Calcd for [Cu(gly-L-phe)]·H_2O $C_{11}H_{14}N_2O_4Cu$) (**9**): C, 43.77; H, 4.68; N, 9.28. Found: C, 43.58; H, 4.60; N, 9.20. Anal. Calcd for [Cu(L-phegly)]·H_2O ($C_{11}H_{14}N_2O_4Cu$) (**10**): C, 43.77; H, 4.68; N, 9.28. Found: C, 43.45; H, 4.58; N, 9.25.

The complexes [Cu(dien)]$(ClO_4)_2$ (**11**) and [Cu(bdipn)]$(ClO_4)_2$ (**12**) were prepared by mixing methanolic solutions of diethylenetriamine or *N*-benzyldipropylenetriamine[20] (1 mmol) and copper(II) perchlorate hexahydrate (1 mmol) under stirring. The blue precipitates formed were collected by filtration, washed with methanol, and dried under vacuum. Anal. Calcd for [Cu(dien)]$(ClO_4)_2$ ($C_4H_{13}N_3CuCl_2O_8$) (**11**): C, 13.14; H, 3.58; N, 11.50. Found: C, 13.10; H, 3.44; N, 11.40. Anal. Calcd for [Cu(bdipn)]$(ClO_4)_2$ ($C_{13}H_{23}N_3CuCl_2O_8$) (**12**): C, 32.27; H, 4.79; N, 8.69. Found: C, 32.12; H, 4.83; N, 8.61.

Azide Titrations and Data Analysis. Azide binding studies were performed by adding concentrated aqueous or methanolic solutions of sodium azide to solutions of the complexes in the same solvent. In all cases it was found that binding of the anion is fast, so that no incubation of the mixtures was necessary before the spectroscopic measurements. In some instances precipitation of material took place at relatively high azide concentration. In most cases, however, we did not try to achieve saturation of the ligand-binding sites because of the possible formation of adducts with stoichiometry above 1:1 at high azide complex ratios.

The equilibrium constant for the copper(II) complex–azide adduct can be obtained according to the following equation assuming 1:1 stoichiometry:

$$K = [ML]/[M][L] = \Delta A/(\Delta A_\infty - \Delta A)[L] \quad (1)$$

where [M], [ML], and [L] represent the concentrations of unbound copper complex, azide adduct, and free azide, respectively. ΔA is the absorbance change at the λ_{max} of the LMCT band caused by the addition of azide, and ΔA_∞ is the absorbance change for complete formation of the azide adduct (at infinite ligand concentration). Equation 1 can be modified as follows:

$$\frac{1}{\Delta A} = \frac{1}{K\Delta A_\infty}\frac{1}{[L]} + \frac{1}{\Delta A_\infty} \quad (2)$$

A plot of $1/\Delta A$ against $1/[L]$ should thus yield a straight line with a slope of $1/K\Delta A_\infty$ and x and y intercepts of $-K$ and $1/\Delta A_\infty$, respectively. Formation of adducts with 1:1 stoichiometry was established by use of the Hill equation in logarithmic form:

$$\log [\Delta A/(\Delta A_\infty - \Delta A)] = n \log [L] + \log K \quad (3)$$

The value of ΔA_∞ to be used in this equation was obtained from the double reciprocal plot. Thus, a plot of $\log [\Delta A/(\Delta A_\infty - \Delta A)]$ against $\log [L]$ should yield a straight line with slope $n = 1$ in case of single binding of the anion to the copper(II) complex.

When the affinity for the ligand is high, [L] differs significantly from the added amount of the anion, $[L]_0$, so that the following correction is required:[21]

$$[L] = [L]_0 - [M]_0\Delta A/\Delta A_\infty \quad (4)$$

where $[M]_0$ is the total concentration of the complex, bound and unbound, $\Delta A/\Delta A_\infty$ represents the fractional conversion of the adduct, and $[M]_0\Delta A/\Delta A_\infty$ is the concentration of ligand consumed in the formation of the adduct. In these cases a good estimate of ΔA_∞ by direct determination is generally possible because saturation of the ligand-binding sites is achieved at relatively low ligand concentrations. The Hill equation (3) is then used for determination of the equilibrium constant and stoichiometry of the adducts. All measurements were done at 23 °C. Rectilinear regression lines were obtained by least-squares fittings using a computer program that takes into account the volume changes of the

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(16) Abbreviations employed for the ligands: *N*-pyruvylidene-L-alaninato dianion = pyv-L-ala; *N*-pyruvylidene-β-alaninato dianion = pyv-β-ala; *N*-pyruvylidene-L-histidinato dianion = pyv-L-his; *N*-pyruvylidene-L-histidine methyl ester anion = pyv-L-hisOMe; *N*-(4-imidazolylmethylidene)-L-alaninato anion = imd-L-ala; *N*-(4-imidazolylmethylidene)-L-histidine methyl ester = imd-L-hisOMe; L-phenylalanylglycine dianion = L-phegly; glycylglycinato dianion = glygly; glycyl-L-alaninato dianion = gly-L-ala; glycyl-L-phenylalaninato dianion = gly-L-phe; diethylenetriamine = dien; *N*-benzyldipropylenetriamine = bdipn. PIPES = piperazine-1,4-bis(2-ethanesulfonic acid).

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Table I. Spectral Data for the LMCT Bands and Binding Constants for the Azide Adducts of Copper(II) Complexes

complex	solvent	abs λ_{max}, nm (ϵ, M^{-1} cm^{-1})	Cd λ_{max}, nm ($\Delta\epsilon$, M^{-1} cm^{-1})	K, M^{-1}	n
[Cu(pyv-L-ala)]	MeOH	355 (850)	356 (+0.01)	125	0.96
	H_2O	348 (1200)	350 (+0.01)	49	1.02
[Cu(pyv-β-ala)]	H_2O	358 (1300)		63	1.08
[Cu(pyv-L-his)]	H_2O	357 (2200)	370 (+0.68)	33	0.98
$[Cu(pyv-L-hisOMe)]^+$	MeOH	370 (2000)	390 (+0.29)	345	1.13
	H_2O	358 (1510)	380 (+0.44)	100	1.04
$[Cu(imd-L-ala)]^+$	MeOH	375 (1700)		405	1.08
	H_2O	358 (1650)		104	1.02
$[Cu(imd-L-hisOMe)]^{2+}$	MeOH	380 (2200)	405 (+0.65)	13700	0.95
	H_2O	364 (1800)	385 (+0.22), 460 (+0.05)	289	0.99
[Cu(glygly)]	H_2O	334 (1800)		35	0.98
[Cu(gly-L-ala)]	H_2O	334 (1850)	335 (+0.04)	46	1.04
[Cu(gly-L-phe)]	H_2O	338 (1800)	346 (+0.06)	38	0.90
[Cu(L-phegly)]	H_2O	336 (1680)	340 sh	59	1.00
$[Cu(dien)]^{2+}$	MeOH	358 (2200)		13400	1.20
	H_2O	344 (1900)		140	1.00
	PIPES/NaCl[a]	344 (2100)		56	0.98
	Phosphate[b]	344 (1600)		10	0.99
$[Cu(dien)OH]^+$[c]	MeOH	356 (1600)		300	1.04
$[Cu(bdipn)]^{2+}$	MeOH	380 (1700)		8200	0.96

[a] 0.02 M PIPES and 0.1 M NaCl, pH 6.5. [b] 0.1 M phosphate buffer, pH 7.0. [c] Obtained in situ from $[Cu(dien)]^{2+}$ with 1 equiv of methanolic NaOH.

solutions following each addition of the titrant; the correlation coefficients were >0.995 in all cases.

Physical Measurements. Elemental analyses were by the microanalytical laboratory of the University of Milano. Optical absorption spectra were recorded on a HP 8452 A diode array spectrophotometer. Circular dichroism spectra were obtained on a Jasco J-500 C dichrograph. EPR spectra were measured in frozen solutions at −150 °C by using a Varian E-109 spectrometer operating at X-band frequencies and a V-4000 variable-temperature-control apparatus. Potentiometric titration of $[Cu(dien)H_2O]^{2+}$ was performed by titrating an aqueous solution of diethylenetriamine (3.30×10^{-2} M), perchloric acid (0.10 M), and copper(II) perchlorate (3.30×10^{-2} M) with sodium hydroxide (1.0 M) at 23 °C. The ionic strength was maintained at 0.5 M with sodium perchlorate; changes in pH were followed by using a glass electrode and an Amel Model 328 pH meter.

Caution! Although the compounds reported in this paper seem to be stable to shock and heat, extreme care should be used in handling perchlorate salts. Azide complexes are also potentially explosive, but we had no problems studying the adducts in solution.

Results

Selection of Model Systems. Complexes **1–12** were selected because they contain ligands with three donor atoms that bind to equatorial positions and leave the fourth, strong planar coordination position of copper(II) readily accessible to the exogenous azide anion. Moreover, the electronic spectra of the complexes do not contain significant absorptions in the range of interest for investigating the LMCT bands generated by binding of azide to Cu(II) (300–500 nm). Since most of the complexes are optically active, it is also possible to investigate the dichroic behavior of the LMCT transitions. The tridentate nature of the ligands ensures the complexes have sufficiently high stability[22] to prevent ligand displacement reactions even with excess amounts of azide. The ligand donor atoms span NO_2 to N_3 donor sets, and the corresponding copper(II) complexes, overall charges from 0 to +2.

The complex $[Cu(imd-L-ala)]^+$ was obtained in an almost optically inactive form in repeated preparations due to the extremely facile racemization undergone by positively charged metal aldimine complexes of amino acids with two fused five-membered chelate rings.[23] Binding of azide to $[Cu(dien)]^{2+}$ has been studied before;[3e,5b] this system was reinvestigated in detail here because its good solubility properties gave the opportunity to ascertain the effects of various media on the azide binding characteristics. For a few other systems, when the solubility was sufficient, the binding behavior of azide was investigated in water and methanol solutions.

Equilibrium Data of Azide Binding. The addition of azide to solutions of complexes **1–12** produces the growth of a moderately intense absorption band in the range between 330 and 380 nm. From spectral titrations it is possible to estimate the strength of the binding of azide to copper(II). In general, for low- and medium-affinity binding ($K < 500$ M^{-1}) we preferred to obtain the equilibrium constants from double reciprocal plots; however, the K values derived from the corresponding Hill plots were within ±25%. Only Hill plots were used for estimating the high-affinity binding constants ($K > 5000$ M^{-1}). The optical and CD features of the $N_3^- \rightarrow Cu(II)$ LMCT bands and the equilibrium K and n values for complexes **1–12** are summarized in Table I. The Hill coefficients were all sufficiently close to unity to consider formation of azide adducts with 1:1 stoichiometry. Representative spectral and titration data are given in Figures 2 and 3.

The equilibrium constant values span a much wider range in methanol than in water solution, but in both cases it is clear that K increases primarily with the positive charge of the copper(II) complex. The experiments performed with $[Cu(dien)]^{2+}$ show that also the nature of the buffer medium has strong influence on K. Our data in PIPES/NaCl compare with those obtained earlier in similar conditions.[3e] Binding of azide to Cu(II) in aqueous medium may be affected by dissociation of coordinated water, which lowers the positive charge on Cu(II):

$$[Cu(L)H_2O]^{n+} \rightleftharpoons [Cu(L)OH]^{(n-1)+} + H^+ \qquad (5)$$

Formation of hydroxo complexes has been reported to occur with a pK_a of 9.31 for $[Cu(glygly)H_2O]$[19] and about 9.5 for $[Cu(dien)H_2O]^{2+}$ in the presence of KCl.[22c] We obtained a somewhat lower pK_a value of 9.1 for the latter complex by potentiometric titration in the presence of $NaClO_4$, but in any case it is clear that dissociation equilibria of type 5 should not affect binding of azide in neutral aqueous solution. The decrease in K due to the presence of a coordinated OH^- group is remarkable in methanol: the constant for azide binding to $[Cu(dien)OH]^+$ is about 2 orders of magnitude lower than that for $[Cu(dien)]^{2+}$ and falls within the range of the other monopositive complexes.

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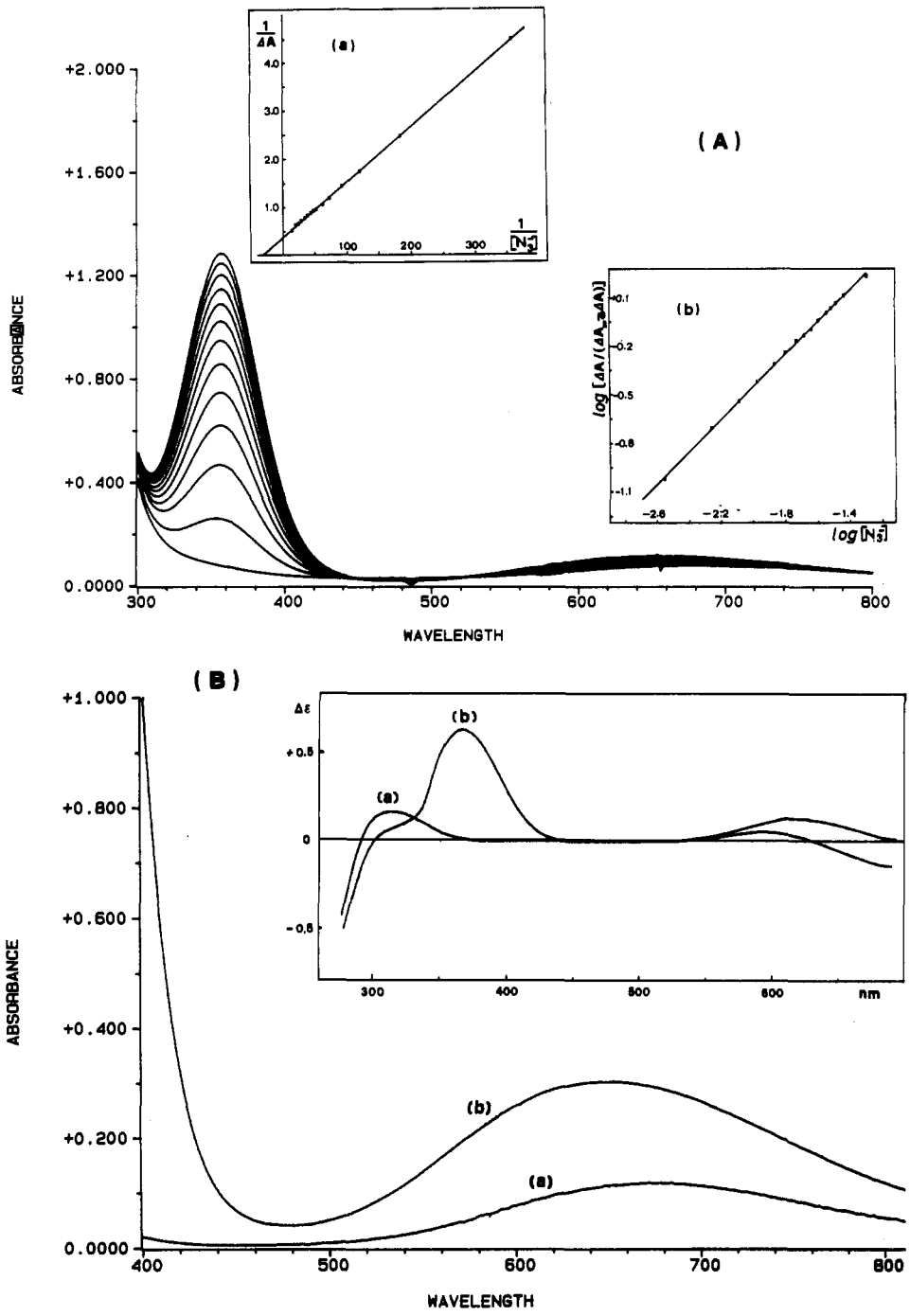

Figure 2. (A) Titration of a 2.8 mM solution of [Cu(pyv-L-his)] in water with 0.4 M azide (cell path 0.5 cm). The representative spectra correspond to the addition of successive and equal amounts of azide from 1.0 to 12.0 [azide]/[Cu] unitary ratios. Insert a shows the double reciprocal plot of absorbance at 357 nm vs azide concentration, yielding $K = 33\ M^{-1}$, and insert b the corresponding Hill plot, yielding $K = 34\ M^{-1}$ and $n = 0.98$. (B) Visible and circular dichroism spectra of a solution of [Cu(pyv-L-his)] in water (a) and after the addition of excess azide (~50:1) (b).

Optical and CD Spectra. The intensities of the LMCT absorption bands reported in Table I are the extrapolated values at infinite azide concentration obtained from double reciprocal plots, except for the systems exhibiting a high affinity for the anion (**6**, **11**, and **12** in methanol solution), where a direct determination of the extinction coefficient could be made from the spectra. Since in most cases the optical activity associated with the LMCT band was rather low, a numerical treatment of the CD data was unreliable. Therefore, the CD intensities reported in Table I for the LMCT bands were obtained from solutions of the complexes containing a large excess of azide. The position of the LMCT band shows solvent dependence, being systematically shifted to lower energy in methanol with respect to water. More importantly, while the CD λ_{max} of the LMCT band is generally red shifted with respect to the electronic absorption maximum, large red shifts are accompanied by marked increase of dichroic behavior. The sign of the optical activity within the LMCT band is always positive and probably correlates with the absolute configuration of the amino acid, since for instance the band is negative ($\Delta\epsilon_{380} = -0.30\ M^{-1}\ cm^{-1}$) for the adduct formed by [Cu(pyv-D-hisOMe)]$^+$ (data not shown). Some changes in other CD bands at higher energy of the complexes can often be noted in the presence of azide, probably for conformational changes occurring at the side chains of the amino acid residues.

The effect of azide coordination was also investigated on the optical and CD spectra of the complexes in the visible region (Table II). Due to the higher concentrations of complexes required to observe the weak bands in this range, precipitation of material in the presence of a large excess of azide occurred in most cases, thus preventing reliable titration experiments. The data

Table II. Visible Spectral Data for the Azide Adducts of Copper(II) Complexes

complex	solvent	Cu/ROH d–d abs λ_{max}, nm (ϵ, M^{-1} cm^{-1})	Cu/ROH d–d CD λ_{max}, nm ($\Delta\epsilon$, M^{-1} cm^{-1})	Cu/N_3^- d–d abs λ_{max}, nm (ϵ, M^{-1} cm^{-1})	Cu/N_3^- d–d CD λ_{max}, nm ($\Delta\epsilon$, M^{-1} cm^{-1})
[Cu(pyv-L-ala)]	MeOH	710 (45)	700 (–0.12)	725 (80)	640 (–0.09)
					750 (–0.10)
[Cu(pyv-β-ala)]	H_2O	722 (100)		738 (140)	
[Cu(pyv-L-his)]	H_2O	675 (85)	625 (+0.12)	650 (230)	590 (+0.06)
			730 (–0.04)		705 (–0.15)
$[Cu(pyv-L-hisOMe)]^+$	MeOH	670 (80)	640 (+0.11)	645 (200)	570 (+0.09)
			745 (–0.03)		695 (–0.03)
$[Cu(imd-L-ala)]^+$	MeOH	750 (80)		725 (300)	
$[Cu(imd-L-hisOMe)]^{2+}$	MeOH	655 (92)	625 (+0.18)	635 (220)	533 (+0.09)
					630 (–0.10)
[Cu(glygly)]	H_2O	636 (75)		640 (130)	
[Cu(gly-L-ala)]	H_2O	634 (70)	665 (–0.48)	640 (130)	530 (–0.20)
					680 (–0.35)
[Cu(gly-L-phe)]	H_2O	630 (70)	640 (–0.62)	635 (130)	535 (–0.25)
					665 (–0.52)
[Cu(L-phegly)]	H_2O	630 (75)	660 (+0.13)	635 (130)	660 (+0.18)
$[Cu(dien)]^{2+}$	MeOH	616 (110)		602 (200)	
	H_2O	616 (105)		604 (180)	
	PIPES/NaCl	612 (105)		602 (180)	
$[Cu(dien)(OH)]^+$	MeOH	603 (110)		614 (180)	
$[Cu(bdipn)]^{2+}$	MeOH	635 (110)		610 (200)	

in Table II, therefore, simply show the effect of the addition of excess amounts of azide on the ligand field absorptions of the complexes. It is clear that while in some cases (for complexes **1**, **2**, and **7–10**) the visible maximum shifts to lower energies, in others (**3–5**, **11**, and **12**) it shifts to higher energies. The intensity of the d–d band envelope invariably increases in the presence of azide, possibly because the LF transitions borrow intensity from the low-energy azide to Cu(II) LMCT transition. Some evident changes are produced by binding of azide also in the visible CD spectra, often with the appearance of new dichroic bands.

EPR Spectra. The EPR spectral changes produced by addition of azide to the complexes were followed by recording spectra in frozen solutions. The resolution of aqueous spectra was often greatly enhanced by addition of small amounts of ethylene glycol, and that of methanolic spectra by addition of chloroform. These additions did not affect the features of the electronic and CD spectra of the adducts. In the case of $[Cu(bdipn)]^{2+}$ we were unable to obtain well-resolved spectra, for the low solubility of the complex and the possible presence of several species in the frozen solutions. The latter problem arose also with [Cu(imd-L-ala)]$^+$, for which the spectrum of the azide adduct showed a single species. The spectral parameters are collected in Table III.

The EPR spectra showed the pattern typical for tetragonal symmetry, with $g_\parallel > g_\perp$ and large $|A_\parallel|$ values. In some cases the high field component of the signal exhibited a partially resolved structure (10–15 G) due to superhyperfine couplings with nitrogen nuclei and, possibly, copper hyperfine interactions, but this structure was not analyzed in detail. In general, azide coordination to copper(II) produces a decrease in the g values, as a result of the decreased positive charge in the complex and covalency in the bond with the labile ligand,[25] but two different trends in the $|A_\parallel|$ values can be noted. For complexes **1**, **2**, **7–10**, and [Cu(dien)-OH]$^+$ $|A_\parallel|$ decreases on binding azide, while it increases for **3**, **4**, and **11**.

Table III. EPR Parameters for the Copper(II) Complexes and Their Azide Adducts Obtained from Frozen Solution Spectra

complex	labile ligand	$g_\parallel$	$g_\perp$	$\lvert A_\parallel\rvert$, 10^4 cm^{-1}
[Cu(pyv-L-ala)]	MeOH	2.295	2.065	161
	N_3^-	2.278	2.058	144
[Cu(pyv-β-ala)]	H_2O	2.290	2.068	176
	N_3^-	2.273	2.059	162
[Cu(pyv-L-his)]	H_2O	2.270	2.059	178
	N_3^-	2.246	2.053	183
$[Cu(pyv-L-hisOMe)]^+$	MeOH	2.279	2.061	170
	N_3^-	2.245	2.052	184
$[Cu(imd-L-ala)]^+$	MeOH	*a*		
	N_3^-	2.255	2.056	155
$[Cu(imd-L-hisOMe)]^{2+}$	H_2O	2.254	2.059	163
	N_3^-	2.229	2.054	177
[Cu(glygly)]	H_2O	2.242	2.068	173
	N_3^-	2.224	2.045	166
[Cu(gly-L-ala)]	H_2O	2.238	2.065	182
	N_3^-	2.222	2.045	171
[Cu(gly-L-phe)]	H_2O	2.238	2.070	177
	N_3^-	2.223	2.044	171
[Cu(L-phegly)]	H_2O	2.238	2.072	172
	N_3^-	2.220	2.046	166
$[Cu(dien)]^{2+}$	MeOH	2.236	2.058	182
	N_3^-	2.213	2.053	186
	H_2O	2.246	2.068	187
	N_3^-	2.214	2.057	189
$[Cu(dien)(OH)]^+$	MeOH	2.220	2.060	192
	N_3^-	2.202	2.042	189

[a] Mixture of species.

Discussion

The highest occupied orbitals of azide are a degenerate couple of nonbonding orbitals of π symmetry.[24] These split in energy on binding to Cu(II), and in general, when the ligand is bound in a terminal mode to tetragonal complexes, one of the azide orbitals (π_b) is stabilized by forming a σ bond to the copper $d_{x^2-y^2}$ orbital, while the other (π_v) is little affected, being involved in weaker π bonding.[15] Of the possible azide to Cu(II) LMCT transitions only $\pi_b \rightarrow d_{x^2-y^2}$ is observed in practice for symmetry reasons, since the π_v orbital of azide is perpendicular to the copper x,y plane.[14b,15] This is confirmed here for the series of complexes **1–12**, where a single band is present in the spectra. The red shift of the CD maximum with respect to the absorption maximum must be due to the mechanism generating optical activity. The $\pi_b \rightarrow$ Cu(II) transition is electric dipole allowed but lacks magnetic dipole character, so coupling with other transitions is necessary to induce the optical activity.[26] No other CD band can be attributed to the magnetic dipole $\pi_v \rightarrow d_{x^2-y^2}$ LMCT transition, as for met apo-azide with the possible exception of the azide adduct formed by $[Cu(imd-L-hisOMe)]^{2+}$, where a very weak CD band at 460 nm may be assigned to such a transition.

From the data reported in Table I it is apparent that several factors affect the position, intensity, and dichroic behavior of the

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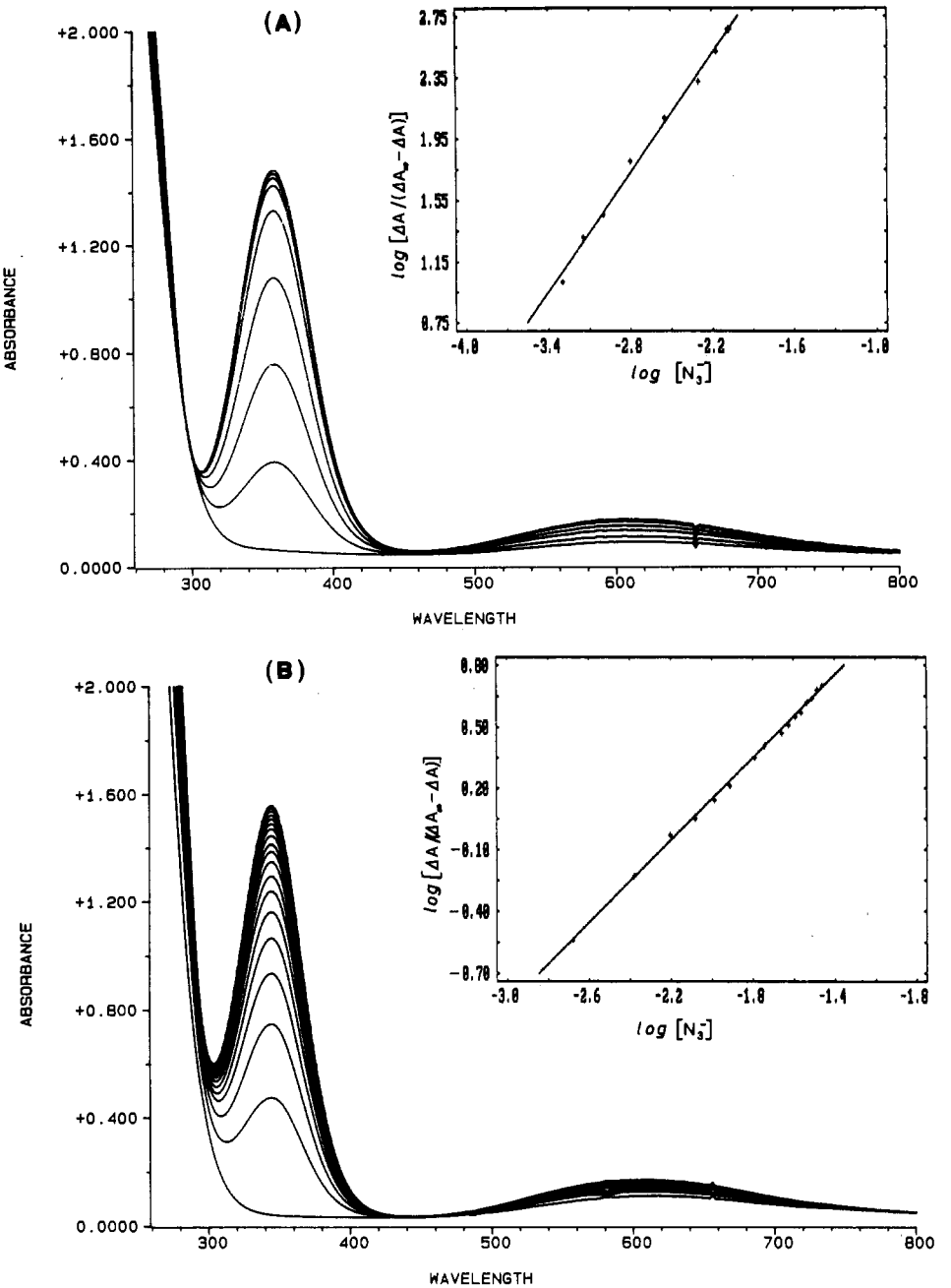

Figure 3. Titration of $[Cu(dien)](ClO_4)_2$ with azide. (A) Spectra obtained upon addition to the complex (1.4 mM) in methanol solution of successive and equal amounts of azide from 0.2 to 1.6 [azide]/[Cu] ratios. (B) Spectra obtained upon addition to the complex (2.1 mM) in aqueous solution of successive and equal amounts of azide from 1.0 to 18.0 [azide]/[Cu] unitary ratios. The insets show the corresponding Hill plots, yielding K = 13400 M^{-1} in methanol and K = 140 M^{-1} in water.

N_3^- → Cu(II) LMCT band. The adducts of the complexes [Cu(pyv-L-ala)] (**1**) and $[Cu(pyv\text{-}L\text{-}hisOMe)]^+$ (**4**) can be taken as examples to illustrate these points. The LMCT band occurs at higher energy and is less intense for **1** than for **4**, and only in the latter case it displays significant optical activity. While the energy of the band seems to be related to the charge of the complex, its intensity and optical activity depend on the coordination position occupied by the added ligand. We expect a poorer overlap between the donor (π) orbital of azide and the copper acceptor ($d_{x^2-y^2}$) orbital, and thus smaller dipole and rotational strengths in the LMCT band, when azide binds in an axial position instead of an equatorial position. Although electronic spectroscopy is difficult to use as a definitive tool to identify copper(II) structures,[27] the red shift of the d–d bands occurring upon azide binding to **1** (Table II) can be interpreted by assuming a change in copper(II) stereochemistry from square-planar to square-pyrimidal, and the blue shift observed for **4** can be interpreted as the result of a displacement of a bound solvent molecule by azide. The changes in the EPR $|A_\parallel|$ parameters[25,28] (Table III) unambiguously show that azide prefers to bind axially to [Cu(pyv-L-ala)] and equatorially to $[Cu(pyv\text{-}L\text{-}hisOMe)]^+$.

The tendency of azide to bind in an axial rather than an equatorial position is not related to steric constraints imposed by the ligand, for instance the smaller chelate ring size of **1** with respect to **4**. The β-alanine complex **2**, with the same chelate ring

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system as **4**, behaves in fact like **1**, while [Cu(imd-L-ala)]$^+$ (**5**), with chelate rings of the same type as **1**, behaves like **4**. The main structural feature determining the coordination position preferred by the exogenous anion is the charge density accumulated by the ligand in the equatorial coordination plane: neutral or mononegative ligands allow azide to bind in the fourth equatorial position, while with dinegative ligands binding will be axial. This trend is confirmed by the series of dipeptide complexes **7–10**, which behave like **1** and **2**, perhaps with more competition between the axial and equatorial positions, and by complexes **3**, **11**, and **12**, which behave like **4** and **5**. The behavior of the complex [Cu(pyv-L-his)] (**3**) is only an apparent exception, since the ligand is dinegative too but only one of the negative charges is provided by the donors in the copper square plane, while the other is localized within the essentially nonbonding carboxylate group of the histidine residue.[18]

Another important feature of azide binding to copper(II) complexes is the affinity of this ligand for the metal center. The overall charge of the complex is the dominant factor in this respect, and the behavior of complexes **1–12** can be classified in three groups according to the order of relative affinity for azide. The *range* of K values, however, is strongly dependent on the medium. On the basis of the data in Table I, we can conclude that the affinity of azide is low for neutral complexes ($K \lesssim 125$ M^{-1} in methanol, $K \lesssim 65$ M^{-1} in water), moderate for complexes carrying a positive charge ($K \sim 350$ M^{-1} in methanol, $K \sim 100$ M^{-1} in water), and high for complexes with dipositive charge ($K > 5000$ M^{-1} in methanol, $K \gtrsim 140$ M^{-1} in water). The presence of any anionic species in solution that may act as a coordinating ligand will decrease the positive charge on Cu(II) and thereby decrease the affinity for azide. The reduction in K by the various buffers in aqueous medium can be accounted for in this way.

The decrease in binding constants from methanol to water deserves some comment, since the effect is remarkable and may have biological implications. The complexation reaction

$$\{[Cu(L)]^{n+}\}_M + \{N_3^-\}_M \rightleftharpoons \{[Cu(L)N_3]^{(n-1)+}\}_M + M\text{–}M \quad (6)$$

M = medium

involves charged or polar species and solvation effects as well as solvent–solvent interactions that may be important. The free energy change can be represented schematically by the following contributions:[29]

$$\Delta G = \Delta G_{SM} + \Delta G_{SS} + \Delta G_{MM} \quad (7)$$

where ΔG_{SM} includes all solute–medium interactions, ΔG_{SS} all solute–solute interactions and complex–azide bond formation, and ΔG_{MM} the medium–medium interactions. The ΔG_{SM} term will generally tend to be destabilizing, because the [Cu(L)]$^{n+}$–medium and N_3^-–medium interactions compete with the [Cu(L)]$^{n+}$–N_3^- interactions, while ΔG_{SS} and ΔG_{MM} will be stabilizing. We expect that the ΔG_{MM} term will be larger in water than in methanol but that the opposite will hold for the ΔG_{SS} term, because methanol has a much lower dielectric constant than water. For the same reason ΔG_{SM} should be less unfavorable in methanol. Since formation of the azide adducts becomes largely more favorable in methanol than in water as the charge of the Cu(II) complex increases, the process is apparently driven by the electrostatic interactions between charged species.

In conclusion, the present series of model complexes enables the establishment of several useful criteria to characterize the behavior of azide binding to copper(II). The stereochemistry of the binding depends on the charge carried by the ligand(s) in the equatorial coordination plane and can be deduced from the spectroscopic properties of the azide adducts: axial binding is characterized by a LMCT band of somewhat reduced intensity and very low dichroic behavior, a red shift of the d–d bands, and a decrease in the EPR $|A_\parallel|$ parameter; equatorial binding is characterized by a LMCT band of moderate intensity and dichroic behavior, a blue shift of the d–d bands, and an increase in $|A_\parallel|$. The affinity of the binding increases with the charge of the copper(II) complex but depends markedly on the polarity of the medium. The data obtained in methanol show that binding of high affinity can be achieved even with mononuclear Cu(II) complexes and the anion bound in a terminal mode.

A detailed analysis of the binding and spectroscopic data of azide to copper proteins is beyond the scope of the present investigation and will be undertaken separately. Examination of the data reported by Dooley[1] on the azide complexes of proteins containing mononuclear or isolated copper sites, e.g. the amine oxidases, galactose oxidase, and dopamine β-hydroxylase, shows a qualitative agreement with the trends expected on the basis of the model studies reported here. Some of the data are, however, outside the range of expectation, and this depends on the fact that the protein sites are usually of symmetry lower than that of the models and that the protein backbone can impose steric constraints to the azide complexes that are difficult to reproduce in the synthetic systems. Deviations from the behavior of the model complexes may actually be taken as evidence for the existence of these effects. For instance, the optical activity displayed by the LMCT band of azide–beef plasma amine oxidase[1d] is about 1 order of magnitude higher than that observed here for equatorially bound azide. This clearly reflects a high degree of immobilization of the azide complex in the active site.

Acknowledgment. This work was supported by a grant from the Italian MPI. We thank M. Bartosek for writing the computer program for determining the equilibrium constants.

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