Biol Metals (1990) 3:137-140

BIOLOGY OF METALS



# Binding of azide and thiocyanate ligands to copper(II) model complexes

**Luigi Casella, Michele Gullotti, Gianfranco Pallanza, and Morena Buga**

Dipartimento di Chimica Inorganica e Metallorganica, Università di Milano, Via Venezian 21, I-20133 Milano, Italy

**Summary.** Binding of azide to a series of copper(II) complexes has been investigated by absorption, CD and EPR spectroscopy. Axial binding of azide to Cu(II) can be differentiated from equatorial binding through the lower intensity and lack of optical activity of the LMCT band. The affinity of azide for Cu(II) increases with the overall positive charge of the complex. The preliminary data on thiocyanate binding to Cu(II) seem to agree with the trends observed for the corresponding azide adducts.

**Key words:** Copper(II) complexes - Azide binding studies - Thiocyanate binding studies - Spectroscopic studies

## 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 (Dooley 1987). Binding of anions such as azide, cyanide, thiocyanate and bromide to copper(II) is generally accompanied by the appearance of LMCT transitions in the visible or near-ultraviolet region and produces changes in the EPR spectra that can be used for empirical correlations between spectra and structure.

The azide ion has been used, for instance, in binding studies to amine oxidases (Dooley and Coté 1985), superoxide dismutase (Dooley and McGuirl 1986), galactose oxidase (Bereman et al. 1977), hemocyanins (Himmelwright et al. 1980a), tyrosinase (Himmelwright et al. 1980b) laccase (Spira-Solomon and Solomon 1987) and ascorbate oxidase (Casella et al. 1988). However, the spectral features exhibited by the azide complexes of these proteins are so diverse that it is often difficult to infer the mode of coordination of the ligand to the metal centres. This difficulty is due to some extent to the structural arrangement that may be assumed by the copper sites in the proteins, but depends also on the lack of firm correlations based on the coordination behaviour of the ligand to suitable model complexes. We have therefore initiated a systematic investigation of the binding of azide and thiocyanate ions to a series

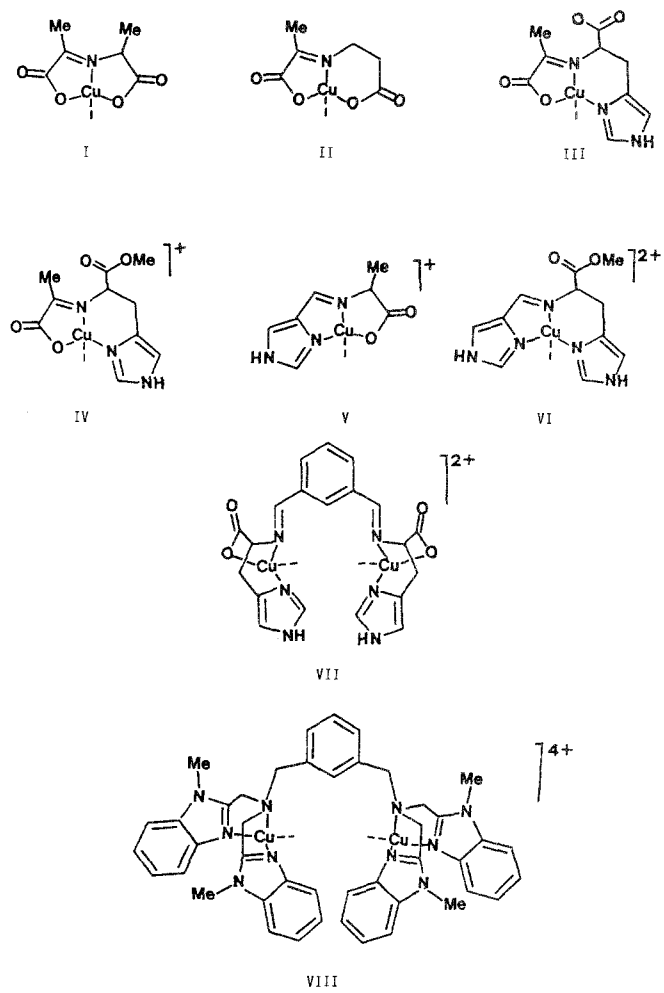


**Fig. 1.** Structures of the complexes I–VIII

*Offprint requests to:* L. Casella

of copper(II) complexes with ligands of possible biological relevance. The structures of the complexes are reported in Fig. 1.

## Materials and methods

The copper(II)-pyruvate complexes derived from L-alanine (I), β-alanine (II), L-histidine (III) and L-histidine methyl ester (IV) were prepared as reported before (Casella et al. 1982). The corresponding imidazoleimine complexes derived from L-alanine (V) and L-histidine methyl ester (VI) were prepared similarly. The synthesis of the dinuclear complexes VII and VIII will be reported elsewhere (Casella et al., unpublished).

The optical spectra were recorded on an HP 8452 A diode array spectrophotometer. Circular dichroic spectra were obtained on a Jasco J-500 C dichrograph. EPR spectra were measured in frozen solutions at −150° C using a Varian E-109 spectrometer at X-band frquencies.

## Results and discussion

The complexes I–VIII were selected because they possess several useful characteristics for the development of this study. (a) The ligands contain donor groups which mimic the potential ligands of type 2 and type 3 copper centres in the proteins. (b) The ligands provide only three donor atoms to each copper(II), leaving a fourth coordination position readily accessible to the exogenous anion. (c) The electronic spectra of the complexes do not contain intense absorptions in the range of interest for investigating the LMCT transitions from $N_3^-$ to Cu(II) (300–500 nm). (d) Many of the complexes are chiral, thus allowing the dichroic behaviour of the LMCT transitions to be examined. (e) The stereochemical properties of the complexes have been studied in detail (Casella et al. 1982).

**Table 1.** Spectral data on the LMCT bands and binding constants for the azide adducts of the copper(II) complexes

| Complex | Absorption $\lambda_{max}$ [nm] ($\varepsilon$, $M^{-1}cm^{-1}$) | CD $\lambda_{max}$ [nm] ($\Delta\varepsilon$, $M^{-1}cm^{-1}$) | $K$ [$M^{-1}cm^{-1}$] |
|---|---|---|---|
| I | 356 (615) | 360 (+0.01) | 125 |
| II | 358 (830) | — | 43 |
| III | 356 (1240) | 375 (+0.7) | 41 |
| IV | 370 (1750) | 390 (+0.2) | 343 |
| V | 365 (1500) | — | 330 |
| VI | 378 (1740) | 400 (+0.4) | >5000 |
| VII | 358 (1100) | 390 (−0.2) | 190 |
| VIII | 384 (3000) | — | >5000 |

When azide is added to aqueous or methanolic solutions of the copper(II) complexes new absorption bands occur in the range between 300–400 nm. From spectral titrations it is possible to obtain the equilibrium constants ($K$) for the binding of the anion; double-reciprocal plots of absorbance against azide concentration or Hill plots can be used to determine $K$ (Sono et al. 1982). A summary of the data of the $(N_3)^- \rightarrow$Cu(II) LMCT bands and the binding constants are given in Table 1. For the mononuclear complexes the number of azide ions bound to Cu(II), obtained from the slopes of the Hill plots, were generally close to unity. A representative example of the spectral titration is reported in Fig. 2. The changes produced by coordination of azide to copper(II) were also followed by EPR, recording spectra in frozen solutions of the complexes. The spectral parameters are collected in Table 2.

From the data reported in Table 1 it is apparent that while the position of the LMCT band is relatively little affected by the nature of the complex, its intensity and chiroptical behaviour is more variable. A comparison between the azide adducts of the complexes derived from pyruvate and L-alanine (I) and that derived from

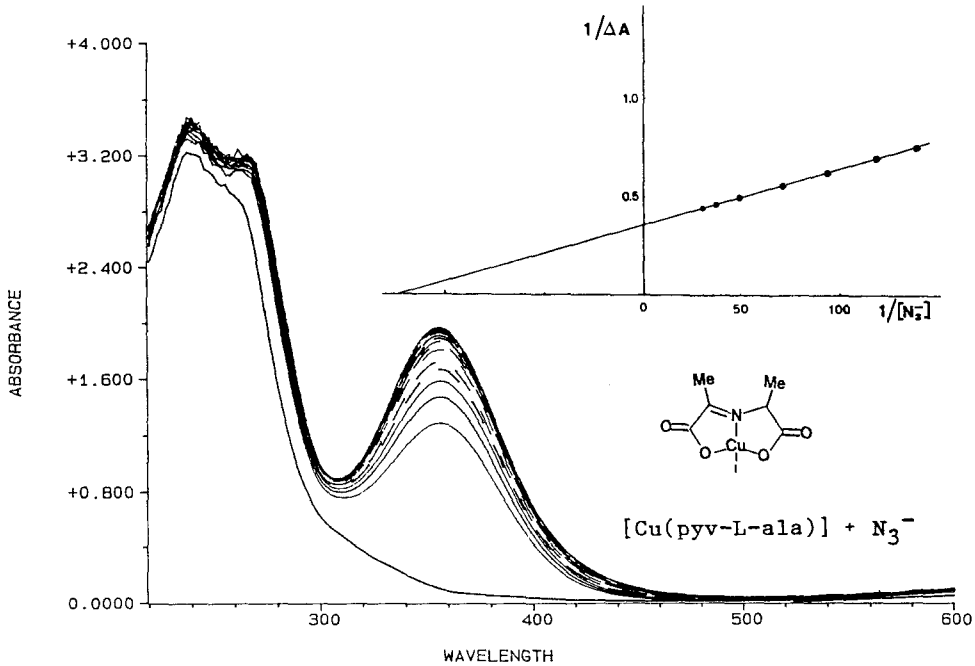


**Fig. 2.** Spectral titration of complex I with sodium azide in aqueous methanol solution

**Table 2.** EPR parameters for the copper(II) complexes and azide adducts

| Comlex | Labile ligand | $g_{\parallel}$ | $g_{\perp}$ | $A_{\parallel} \times 10^{-4}$ ($cm^{-1}$) |
|---|---|---|---|---|
| I | MeOH | 2.295 | 2.065 | 161 |
| | $N_3^-$ | 2.278 | 2.058 | 144 |
| II | $H_2O$ | 2.290 | 2.068 | 176 |
| | $N_3^-$ | 2.273 | 2.060 | 165 |
| III | $H_2O$ | 2.270 | 2.059 | 178 |
| | $N_3^-$ | 2.246 | 2.053 | 182 |
| IV | MeOH | 2.279 | 2.061 | 170 |
| | $N_3^-$ | 2.245 | 2.052 | 184 |
| V | $H_2O$ | | —[a] | |
| | $N_3^-$ | 2.255 | 2.056 | 150 |
| VI | MeOH | | —[a] | |
| | $N_3^-$ | 2.235 | 2.046 | 177 |
| VII | $H_2O$ | 2.246 | 2.06 | 183 |
| | $N_3^-$ | 2.240 | 2.06 | 188 |
| VIII | MeOH | | —[a] | |
| | $N_3^-$ | | —[a] | |

[a] Mixture of species

**Table 3.** Spectral data on the LMCT bands and binding constants for the thiocyanate adducts of the copper(II) complexes

| Complex | Absorption $\lambda_{max}$ [nm] ($\varepsilon$, $M^{-1}cm^{-1}$) | CD $\lambda_{max}$ [nm] ($\Delta\varepsilon$, $M^{-1}cm^{-1}$) | $K$ ($M^{-1}cm^{-1}$) |
|---|---|---|---|
| I | 332 (350) | — | 195 |
| II | 332 (190) | — | 77 |
| III | 328 (280) | 330 (+0.1) | 16 |
| IV | 348 (100) | 355 (+0.2) | 722 |

**Table 4.** EPR spectral parameters for the thiocyanate adducts of the complexes

| Complex | $g_{\parallel}$ | $g_{\perp}$ | $A_{\parallel} \times 10^{-4}$ ($cm^{-1}$) |
|---|---|---|---|
| I | 2.282 | 2.062 | 165 |
| II | 2.285 | 2.062 | 149 |
| III | 2.240 | 2.049 | 183 |
| IV | 2.254 | 2.052 | 187 |

pyruvate and L-histidine (III) illustrates these points. The LMCT band occurs at the same energy in the two cases, but for complex I its is about half as intense as for complex III, and only in the latter case does the band display significant optical activity. These differences can be related to the mode of binding of the anion to I and III by examining the EPR spectra of the complexes and their azide adducts. In both cases solvent replacement by azide produces a decrease in the $g_{\parallel}$ values, indicative of increased covalency in the adducts, but while $A_{\parallel}$ decreases for I it increases for III. This shows that azide binds to Cu(II) axially in I and equatorially in III (Peisach and Blumberg 1974).

The tendency of azide to bind in an axial position seems to be related to the oxygen-rich ligand donor set, rather than to the size of the chelate rings. The azide binding behaviour to complex II, with the same chelate ring type as III, is in fact similar to the binding to complex I, whereas complex V, with the same chelate ring type as I, behaves like III or IV. Axial binding of azide is thus characterized by an LMCT band of low intensity, as expected from the poor overlap between the ligand ($\pi$) donor orbital and the metal (d) acceptor orbital, and negligible optical activity. On the other hand, the affinity of azide for the various complexes depends primarily on their overall charge. Based on the data in Table 1, we can conclude that azide binds with low affinity to neutral Cu(II) complexes ($K < 125\ M^{-1}$), with moderate affinity to complexes carrying a positive charge ($K \approx 350\ M^{-1}$), and with high affinity to complexes carrying a dipositive charge ($K > 5000\ M^{-1}$).

The azide binding behaviour to the binuclear copper(II) complexes is qualitatively in line with the expectation when independent metal centres are considered. However, the Hill plots did not give integer numbers of bound azide molecules so that a more detailed analysis of the data and, perhaps, the structural characterization of the adducts is required in order to draw firm conclusions.

Since studies on thiocyanate binding to copper proteins often flank the studies on azide binding (Dooley 1987), we thought it of interest to extend our initial investigation to some representative mononuclear copper(II) complexes. The ultraviolet and CD spectral features of the LMCT bands associated with thiocyanate binding to Cu(II) are reported in Table 3, together with the binding constants obtained from spectral titrations. The EPR spectral parameters for the thiocyanate adducts are collected in Table 4. In general, the trends observed are the same as for the binding of azide, though the intensity of the thiocyanate to Cu(II) LMCT bands is invariably much lower. A detailed comparison of the spectroscopic effects caused by azide and thiocyanate binding to Cu(II) must however await the investigation on the complete series of complexes.

## References


Bereman RD, Ettinger MJ, Kosman DJ, Kurland RJ (1977) Characterization of the copper(II) site in galactose oxidase. Adv Chem Ser 162:263–280

Casella L, Gullotti M, Pacchioni G (1982) Coordination modes of histidine. 3. Stereochemistry of copper(II) complexes related to pyridoxal catalysis. J Am Chem Soc 104:2386–2396

Casella L, Gullotti M, Pallanza G, Pintar A, Marchesini A (1988) Azide-binding studies reveal type 3 copper heterogeneity in ascorbate oxidase form the green zucchini squash (*Cucurbita pepo*). Biochem J 251:441–446

Dooley DM, Coté CE (1985) Copper(II) coordination chemistry in bovine plasma amine oxidase: azide and thiocyanate binding. Inorg Chem 24:3996–4000

Dooley DM, McGuirl MA (1986) Thermodynamics of azide and thiocyanate binding to bovine copper-zinc superoxide dismutase. Inorg Chem 25:1261–1264

Dooley DM (1987) The coordination chemistry of copper-containing metalloproteins. Life Chem Rep 5:91–154

Himmelwright RS, Eickman NC, LuBien CD, Solomon EI (1980a) Chemical and spectroscopic comparison of the binuclear copper active site of mollusc and arthropod hemocyanins. J Am Chem Soc 102:5378–5388

Himmelwright RS, Eickman NC, LuBien CD, Lerch K, Solomon EI (1980b) Chemical and spectroscopic studies of the binuclear copper active site of *Neurospora* tyrosinase: comparison to hemocyanins. J Am Chem Soc 102:7339–7344

Peisach J, Blumberg WE (1974) Structural implications derived from the analysis of electron paramagnetic resonance spectra of natural and artificial copper proteins. Arch Biochem Biophys 165:691–708

Sono M, Andersson LA, Dawson JH (1982) Sulfur donor binding to ferric cytochrome *P*-450CAM- and myoglobin. J Biol Chem 257:8308–8320

Spira-Solomon DJ, Solomon EI (1987) Chemical and spectroscopic studies of the coupled binuclear copper site in type 2 depleted *Rhus* laccase: comparison to the hemocyanins and tyrosinase. J Am Chem Soc 109:6421–6432