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Luminescent Metallothioneins: Emission Properties of Copper, Silver, Gold and Platinum Complexes of MT*

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Abstract

Observations of luminescence from complexes of the metals Cu(I), Ag(I), Au(I), Pt(II), and cisplatin and the protein metallothionein, are reported. In each case, emission intensity is observed in the 500–600 nm region. The luminescence properties of these metal complexes can be interpreted in terms of the presence of similar, lowest energy, $d \rightarrow s$ and $d \rightarrow p$ excited states, that are metal-centered states in origin. These data show that the emission spectrum provides a new probe to study metal binding to metallothionein.

Introduction

The protein metallothionein (MT) has been isolated from many different organs in man and animals, as well as from a variety of other species [1]. Major sources of the protein in animals are livers and kidneys [1]. The protein peptide chain comprises 20 cysteines out of 61 amino acids, and no aromatic amino acids and disulfide bonds are present [1]. Studies on the binding of cadmium and zinc have provided a focus for the role of MT in the physiological chemistry of group 12 metals [2]. An extensive chelation chemistry for MT can be illustrated by the remarkably wide range of metals that are already known to bind to the protein [1, 3]. Particularly exciting has been the recognition of the formation of clusters through metal–thiolate bonding to give stoichiometries of Cd_4S_{11} (α-domain) and Cd_3S_9 (β-domain) [4, 5].

Although it is well known that MT can bind a range of d^{10} metals both *in vivo* and *in vitro*, detailed structural information on how the binding sites are organized is available for only Cd, Zn and Co [1, 4–9]. With the inherent 'chromophoric silence' of metals like Cd(II), Zn(II), Cu(I), Ag(I) and Hg(II), there exists a problem of finding a suitable spectroscopic method. We have previously been able to apply the spectroscopic techniques of circular dichroism (CD) and magnetic circular dichroism (MCD) to the measurements of the ligand-to-metal charge transfer transitions observed in the 250–320 nm range for the Cd- and Hg-containing protein in dilute solutions [7, 8, 10].

We have also found that while the CD spectrum of Cu-containing MT is poorly defined [9], the emission intensity recorded during titrations of the protein with Cu^+ was directly related to the formation of copper(I)–thiolate clusters, with a maximum emission intensity being observed for Cu_{12}–MT. Similarily, we have recently observed [11] that a maximum emission intensity occurs for an Ag_{12}–MT species formed on reaction of MT with Ag^+. It appears then that the emission spectrum of some metallothioneins can offer an important new view of metal–thiolate cluster formation.

We report here the characteristics of the luminescence observed from Cu(I)-, Ag(I)-, Au(I)- and Pt(II)-substituted metallothionein. These emission spectra provide new and detailed probes of the binding of these important metals to MT. For metals like Cu(I), Ag(I) and others, this technique may be the only readily available technique from which to obtain such structural information. No detailed spectral data have been reported previously for silver, gold and platinum containing metallothioneins.

Experimental

A mixed-metal, Zn,Cd–MT isoform 1 was isolated from rat liver following exposure of the animal to Cd^{2+} salts. Zn_7–MT isoform 2 was isolated from rabbit liver following induction procedures described previously with Zn^{2+} salts [12]. Apo-MT 1 and apo-MT 2 were prepared as described previously [7]. Solutions of apo-MT 2 in water were titrated with Cu^+, using $Cu(CH_3CN)_4$ dissolved in 70% water: acetonitrile solution to form Cu(I)–MT 2. Aliquots of Ag^+ ($AgNO_3$) were added to the solutions of

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apo-MT 2 under nitrogen to form Ag(I)–MT 2. Au(I)–MT 1 was prepared by incubation of the apo-MT 1 with an excess of sodium aurothiomalate (Na_2AuTM) in 0.02 M phosphate buffer at pH 8.0 under nitrogen. Pt(II)–MT 1, *cis*-Pt(II)–MT 1 and *trans*-Pt(II)–MT 1 were prepared by incubation of the apo-MT 1 in 0.02 M phosphate buffer at pH 8.0 under nitrogen, with an excess of potassium tetrachloroplatinate, *cis*-dichlorodiammine platinum and *trans*-dichlorodiammine platinum, respectively. Portions of these solutions were frozen in liquid nitrogen and added to a quartz tube used for emission measurements. Emission spectra were recorded on a Perkin-Elmer MPF-4 equipped with a red-sensitive Hamamatsu R-928 phototube. The excitation and emission spectra were corrected for monochromator and photomultiplier efficiency. Due to large Stokes shifts there was no distorting overlap between the light absorption and emission, and therefore no inner-filter effect was present. The spectral data were digitized directly from the spectrometer and replotted with Spectra Manager [13].

Results and Discussion

Luminescence Properties of Metallothioneins

Neither demetallated MT, apo-MT, nor the 'natural', Zn,Cd–MT, exhibit luminescence in the visible region. The emission intensity is observed upon substitution of MT with the d^{10} metal ions. Luminescence properties have been investigated for Cu–MT [9] and only recently for Ag–MT species [11]. The common feature of the emission spectra of these species is a very large Stokes shift. Typically, the absorption spectrum of metal–MT complexes, such as Cu(I)–MT and Ag(I)–MT, is matched to some extent by the excitation spectrum, which is in the UV region (below 350 nm), while emission is observed in the 500 to 600 nm region.

Luminescence of Copper(I)–MT

Emission spectra have been reported for many metallorganic complexes of Cu(I) [14, 15], although these complexes do not resemble any known model of the MT binding site. Our previous studies [9, 16] of the emission spectra of rat liver Cu–MT 2 in solution have established that the emission maxima lie near 600 nm, and that the lifetime of the emitting state is highly dependent on temperature. Emission spectra recorded at room temperature during titrations of Zn_7–MT 2 with Cu(I) demonstrate a strong dependence of the emission intensity on the stoichiometric ratio, with a maximum being observed for Cu_{12}–MT [9]. In frozen solution Cu–MT glows a bluish colour when formed from apo-MT. This contrasts with the orange colour observed when Zn_7–MT is used as the starting species for the Cu^+ binding

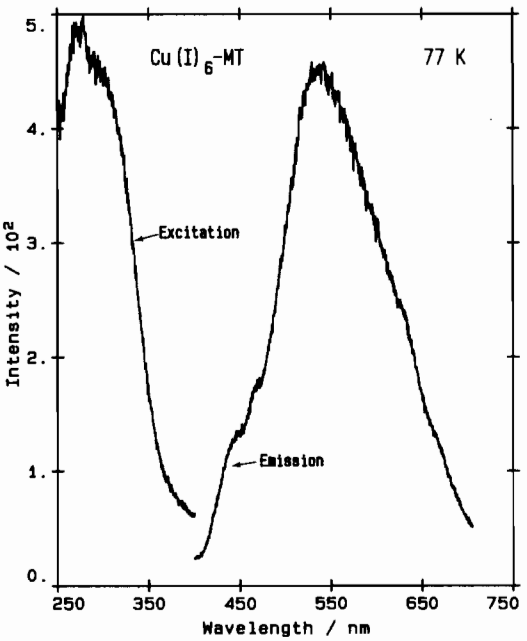

Fig. 1. Uncorrected excitation (λ_{em} = 530 nm) and emission (λ_{ex} = 300 nm) spectra recorded at 77 K, for a solution of rabbit liver apo-MT 2 (20 nmol/ml) containing 6 mol. eq. Cu^+.

[17]. Figure 1 shows the uncorrected emission and excitation spectra recorded at 77 K for a sample of rabbit liver apo-MT 2 containing 6 mole equivalents (mol. eq.) Cu^+. The peak emission intensity is found at 535 nm. In frozen solutions at low temperatures (77 K), the emission spectrum is very much dependent on both the stoichiometric ratio of Cu to MT, and on the nature of the MT species used at the beginning of the titration; that is whether it was Zn_7–MT or apo-MT.

Luminescence of Silver(I)–MT

When Ag^+ is added to apo-MT, a greenish luminescence is observed at 77 K for a broad range (1 to 20) of molar ratios of Ag(I) to MT. Figure 2 shows the uncorrected emission and excitation spectra recorded at 77 K for a sample of rabbit liver apo-MT 2 containing 14 mol. eq. Ag^+. The peak emission intensity is found at 570 nm. The emission intensity measured at 570 nm is dependent on the Ag^+:MT stoichiometry. From the dependence of the maximum intensity of the emission on the Ag^+:MT ratio it appears [11] that the distinct species forms when between 10 and 12 mol. eq. Ag^+ have been added to MT solution, and that this species involves Ag(I)–thiolate cluster formation. No emission is observed at room temperature for Ag(I)–MT in this spectral region even following extensive bubbling with N_2.

For Cd^{2+}, Zn^{2+} and Hg^{2+}, CD and MCD spectra show a characteristic spectral pattern for a molar ratio of 7 [7, 8, 10, 18]. This coincides with the

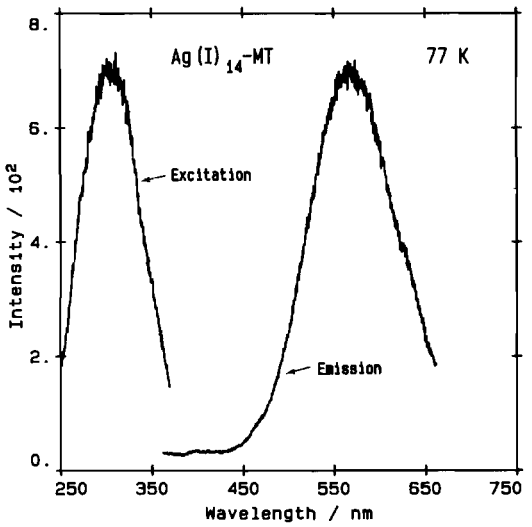

Fig. 2. Uncorrected excitation (λ_{em} = 570 nm) and emission (λ_{ex} = 300 nm) spectra recorded at 77 K, for a solution of rabbit liver apo-MT 2 (10 nmol/ml) containing 14 mol. eq. Ag^+.

structural predictions of tetrahedral symmetry around each atom of these metal ions [1, 4, 5]. For Cu^+ [9] and Ag^+ [19] there exist characteristic CD spectra with maximum intensities at both 6 and 12 mol. eq. of the metal ions. It appears from both CD and emission data that Cu^+ and Ag^+ bind to MT with a similar geometry. The increase in emission intensity as the molar ratio of Cu^+ or Ag^+ approaches 12, suggests that as the metal ions bind to the protein, solvent is extruded, which reduces the radiationless transitions that are otherwise expected to quench the luminescence. The binding site region becomes more hydrophobic. When more metal ions are added the protein expands and solvent once again can quench the emission. We associate the luminescence intensity maximum at molar ratios of metal ions to MT of 12, with distinct metal–sulphur cluster formation of Cu(I) or Ag(I) with the 20 thiolate groups that make up the metallothionein peptide chain.

Luminescence of Gold (I)–MT

Figures 3 and 4 show absorption, excitation and emission spectra of Au(I)–MT 1, respectively, formed by addition of an excess of Au^+ to apo-MT 1. The corrected excitation spectrum matches quite closely the absorption spectrum of Au(I)–MT. The emission spectrum of Au(I)–MT, with a maximum at 600 nm, is red-shifted when compared with the spectrum of the Na_2AuTM complex.

Luminescence of Platinum(II)–MT

The binding of platinum to metallothionein is of particular interest because of the comparisons that can be made with crosslinking in DNA in its anti-

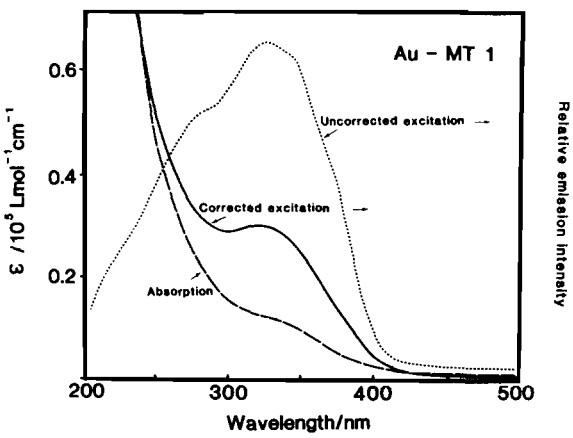

Fig. 3. Absorption spectrum (broken line), uncorrected (dotted line) and corrected (solid line) excitation spectra of Au(I)–MT 1 frozen at 77 K. Emission intensity was measured at 600 nm.

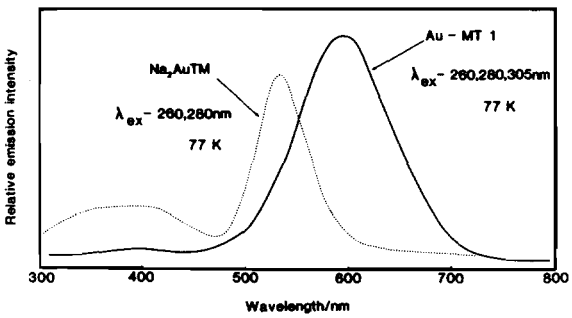

Fig. 4. Emission spectra of Au(I)–MT 1 (solid line) and sodium aurothiomalate, Na_2AuTM (dotted line) solutions at 77 K. Essentially the same emission spectra were obtained for Au(I)–MT for excitations at 260, 280 and 305 nm.

cancer role. We expect crosslinking between different cysteine residues in MT to be the mode of binding for Pt–MT. Figure 5 shows absorption and excitation spectra of *cis*-Pt(II)–MT. This complex is formed following incubation of apo-MT 1 with *cis*-platin. The general luminescence characteristics, Fig. 6, for this d^8 metal–MT complex are found to be quite similar to those of the d^{10} metal–MT complexes, with the λ_{max} dramatically red-shifted to 600 nm. The emission spectra for the series of Pt(II)–MT complexes, *cis*-Pt(II), *trans*-Pt(II)–MT and Pt(II)–MT, vary to such an extent, that we suggest that the metal binding site in the MT molecule changes as a function of the complexation of the precursor Pt complex.

These nd^{10} (n = 3, 4 and 5) metal–MT complexes, Cu(I)–MT, Ag(I)–MT and Au(I)–MT, exhibit a very large Stokes shift; with excitation at 300 nm the emission spectra have maxima in the 530 to 600 nm region. The emission lifetime of Cu(I)–MT in solution at room temperature is of the order of several μs. The lifetime increases substantialy at 77 K to become

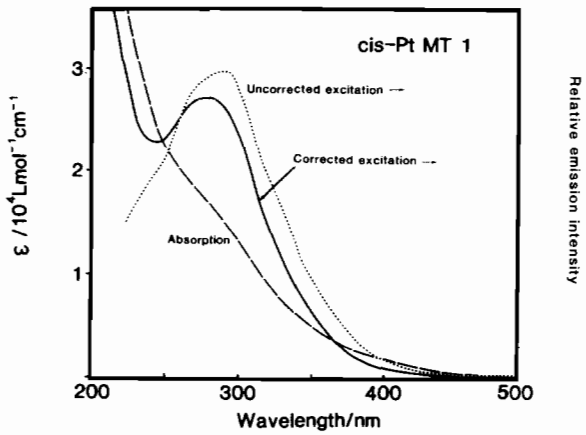

Fig. 5. Absorption spectrum (broken line), uncorrected (dotted line) and corrected (solid line) excitation spectra of *cis*-Pt(II)–MT 1 frozen at 77 K. Emission intensity was measured at 600 nm.

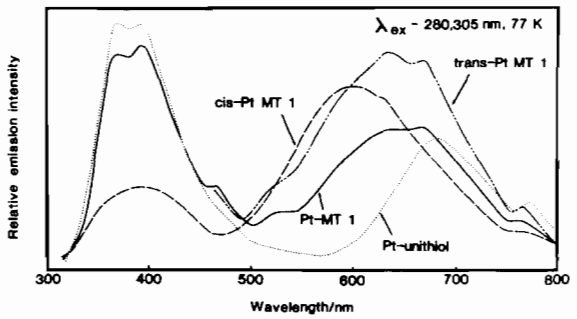

Fig. 6. Emission spectra of solutions of Pt(I)–MT 1 (solid line), *cis*-Pt(I)–MT 1 (broken line), *trans*-Pt(I)–MT 1 (combined line), and a model, thiolate complex of Pt(II) with 2,3-dimercapto-1-propanesulfonate (unithiol) (dotted line) at 77 K. Essentially the same emission spectra were obtained for Pt(II)–MTs for excitations at 280 and 305 nm.

longer than 100 μs [16]. Similarly, long lifetimes are found for the emissive states of Ag(I)–MT complexes at 77 K [11]. We have observed [16] that oxygen quenches the emissive excited state of Cu(I)–MT. This suggests that the emissive transition is spin-forbidden, and originates from a low-lying triplet state. We have attributed [16] the emission of Cu(I)–MT to a $3d^9 4s^1 \rightarrow 3d^{10}$, intra-copper(I) transition. There are few reports of luminescence from silver(I) and gold(I) complexes, and no previous reports for such luminescence from metallothionein complexes, with which to compare these results.

An analogy can be found between the d^{10} and d^8 complexes that is important in view of the similarity of the luminescence data. For certain symmetries, the $nd \rightarrow (n+1)p$ excited states become the lowest energy excited states in both the d^8 and d^{10} complexes [20–22]. The generality of these types of excited states would explain the observed luminescence properties of the range of d^{10} and d^8 metal–MT complexes reported in this work.

Our data on the luminescence properties of Cu(I), Ag(I), Au(I) and Pt(II) complexes of metallothionein, indicate that emission intensity may provide a tool for probing the nature of the binding site in the protein. Metals, such as Cu, Au and Pt, have an important physiological chemistry, in which MT may play a role in the clearing mechanisms. Although many other metals bind to MT, there is a limited number of methods available for the characterization of the complexes formed. Emission spectroscopy is well known to be sensitive to the composition of the excited states, and therefore the observation of emission from such a wide range of metals may allow a unified binding scheme to be proposed for this range of metals.

Conclusions

We report novel spectral information from metallothionein containing Cu(I), Ag(I), Au(I), or Pt(II). The luminescence properties of these nd^{10} (n = 3, 4 or 5) and $5d^8$ metal complexes of MT can be interpreted in terms of the presence of similar emissive excited states, that are $d \rightarrow s$ and $d \rightarrow p$, metal-centered states in origin. These emission data provide a new probe for metal binding with metallothionein for an important range of metals.

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