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Luminescence Decay from Copper(I) Complexes of Metallothionein

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Abstract

Luminescence in the 600 nm region from aqueous solutions of Zn_7–metallothionein (Zn–MT) has been studied as a function of the extent of substitution of the Zn–MT with Cu^+. The steady state emission intensity shows a strong correlation with cluster formation. Analysis of the emission decay curves obtained by single photon-counting techniques using a multiexponential decay function, indicates that three components are responsible for the observed emission intensity. A short lived component exhibits a lifetime of 20 ns, while the two longer lived components exhibit lifetimes in the 1 μs range at room temperature. The longer-lived emission is assigned to the excited triplet states of the Cu–S chromophore, most probably the metal-centered $3d^9 4s^1$.

Introduction

Cu-containing metallothioneins have been found to exhibit significant luminescence which is related to the Cu(I)–thiolate chromophore [1–5]. Luminescence from a Cu(I)-containing species at room temperature in solution is highly unusual and is a further reminder of the remarkable chelation properties of metallothionein's 20 cysteine thiolate groups. While emission is known for other d^{10} systems (eg. Ag(I)), most reports concern solids, with far fewer reports describing emission data for solutions [6–8]. The emission from Cu^+ ions in solids and Cu(I) in complexes with organic ligands, has been attributed to transitions of both charge transfer and metal-centered origin. Our interest in the spectral properties of metallothioneins relates to the cluster-like metal–thiolate complex that forms between the metal (up to 12) and the 20 thiolate groups in the protein. Recently, although we have reported the first identification of a cluster-sensitive band in the circular dichroism (CD) spectrum of Cd_7–MT [9], we have been unable to find a similar transition in the optical spectra of Cu-containing metallothioneins. The sequence of energies for the lowest energy transition in Zn–, Cd–, and Hg–MTs [10] follows the red shift predicted by Jørgensen's optical electronegativity theory for ligand-to-metal charge transfer [11], but the absorption, circular dichroism and magnetic circular dichroism (MCD) of Cu-containing MT and Cu(I)–thiolate model compounds do not follow this trend nearly as clearly [12]. The luminescence observed for different Cu–MTs, with a band maximum in the 600 nm region following excitation at 300 nm [1–5], offers a possible cluster-sensitive optical property. Characterization of speciation for protein containing metals as inherently chromophorically-silent as Cu^+, is difficult, and, especially so, when the protein can bind from 1 to 20 mole equivalents of the metal.

In this paper we examine the photophysical properties of Cu–MT and we report the first analysis of luminescence decay curves for emission from Zn–MT with from 1 to 20 Cu^+ ions added.

Experimental

Zn_7–MT was isolated from rabbit liver following *in vivo* induction procedures using Zn^{2+} salts [9]. Aliquots of 10 μmol/l solutions of Zn_7–MT (isoform 2) in water were titrated with 1, 2, 6, 12 and 20 mole equivalents (mol eq) of Cu^+ in 30% water solution of CH_3CN, to form the mixed metal Cu, Zn–MT with decreasing Zn^{2+}, up to Cu_{20}–MT, where no Zn^{2+} is present.

The fluorescence and phosphorescence measurements were made on a Perkin-Elmer MPF-4 spectrofluorometer. Luminescence decay data were obtained, as described previously [13], using a PRA Model 3000 Lifetime Fluorometer which is based on the technique of time-correlated single-photon counting. A pulsed hydrogen arc lamp operating at ~30 kHz was used as the excitation source. Fluorescence lifetimes and theoretical decay curves were obtained by application of the program DECAYFIT developed in our laboratory, which uses the fast Fourier deconvolution technique [14].

Results

Figure 1 shows the emission spectra recorded at 298 K from solutions of Zn–MT-2 as up to 20 mol

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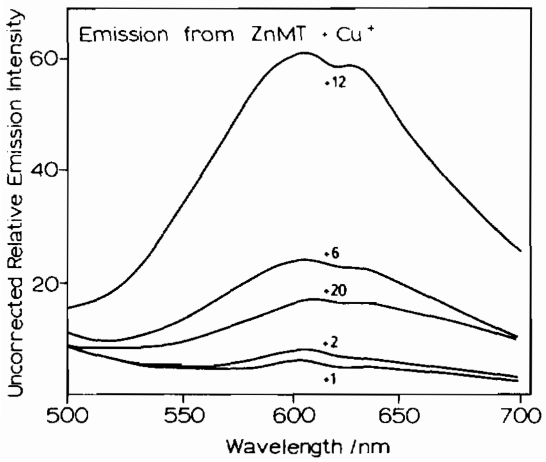

Fig. 1. Uncorrected emission spectra recorded for solutions of Zn_7–MT containing 1, 2, 6, 12 and 20 mol eq of Cu^+. The excitation wavelength was 300 nm and the luminescence was filtered out below 310 nm. Note the decrease in total luminescence intensity when 20 mol eq of Cu^+ has been added.

eq of Cu^+ were added. The excitation spectrum closely resembles those published previously for Cu–MT [4]. The emission spectrum in the 500 nm to 700 nm region does not exhibit a homogeneous emission band, but rather it is composed of at least two overlapping bands. Luminescence intensity rises as Cu^+ is added up to 11–14 mol eq, then falls rapidly as up to 20 mol eq Cu^+ are added. On the other hand, the emission spectrum itself does not change much with temperature between 273 and 323 K.

The luminescence decay curves measured at 298 K for samples containing 1, 2 and 6 mol eq of Cu^+ per mol of MT-2 are shown in Fig. 2a. The samples were excited at 310 nm and the wide-band emission was detected with $\lambda > 560$ nm by using a Corning CS 2-73 high-energy cut-off filter. The experimental data (points) could not be fitted adequately with less than a three-exponential decay function; using this function we obtained the lifetimes, and contribution to the overall emission intensity, of three components. The lifetimes calculated for each decay curve shown in Fig. 2, appear to be the same for each curve, within experimental error, and were calculated to be 20 ns, 0.7 μs and 3.5 μs. However, the contribution from the individual components to the overall emission envelope varies significantly between the addition of 1 Cu^+ and 6 Cu^+. Changes in the lifetimes are observed for Zn–MT heavily substituted with Cu^+ (with more than 12 Cu^+).

Figure 2b shows the effect of dissolved oxygen on the luminescence decay. Oxygen also quenches the steady state emission recorded at room temperature.

Figure 2c shows the luminescence decay curves at two temperatures. The emission from the red-edge of the spectrum was analysed in this case by cutting off the light with $\lambda < 600$ nm (using the Corning CS 2-60 filter). Elimination of the emission with $\lambda < 600$ nm, results in the contribution of the fast 20 ns lifetime component becoming insignificant. Lowering

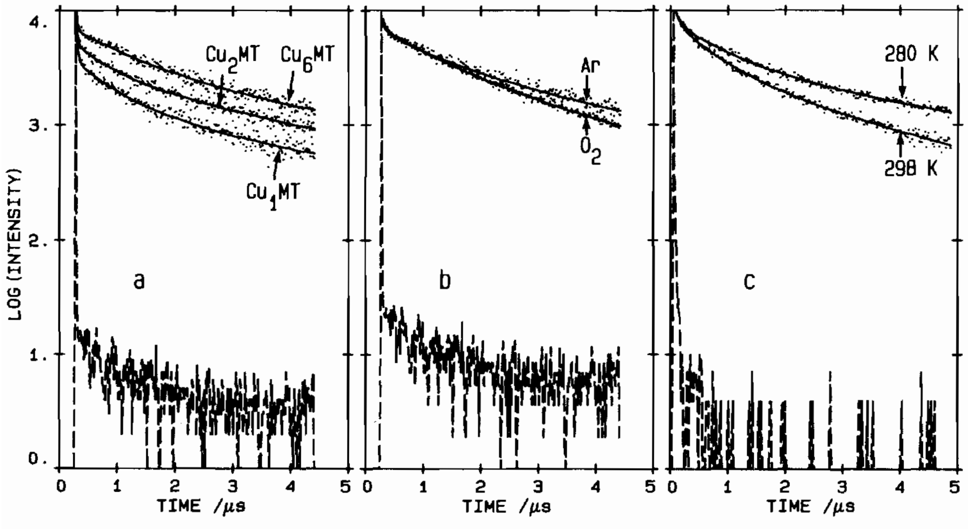

Fig. 2. (a) Luminescence decay curves recorded from solutions at 298 K for samples containing 1, 2 and 6 mol eq of Cu^+ per mol of Zn–MT-2. The samples were excited at 310 nm and only emission with $\lambda > 560$ nm was detected. (b) Luminescence decay curves for Zn_7–MT containing 6 mol eq of Cu^+, in the presence and absence of oxygen. The samples were excited at 310 nm and only emission with $\lambda > 560$ nm was detected. (c) Luminescence decay curves recorded at 280 K and at 298 K for Zn_7–MT containing 12 mol eq of Cu^+, in the absence of oxygen. The samples were excited at 310 nm and only emission with $\lambda > 600$ nm was detected. Broken lines represent the lamp profile. Experimental data (points) were fitted with a three-exponential decay function (solid lines).

the temperature from 298 K to 280 K increased the lifetimes of the slower decaying components to about 0.9 μs and 4.5 μs, respectively. The lifetime increases significantly at 77 K to well beyond the range of the detection method used in this study ($\tau >$ several μs).

Discussion

Cu–MTs from variety of sources are known to emit in solution at room temperature in the 570–620 mm region [1–5, 12]. Because most Cu^+ complexes do not emit under these conditions, this makes the metallothionein system unusual. It also means that there is little data available from potential model compounds for comparison. The appearance of emission from Cu–MT provides an extra assignment criterion that can be used in the characterization of the species that form as Cu^+ is added to Zn–MT. From biochemical studies, Winge and coworkers [15], have demonstrated that 6 Cu^+ preferentially bind in the β domain of the protein. Emission intensity that is Cu-dependent offers a means of following speciation *in vitro* as each Cu^+ is added. For the well-documented Cd, Zn–MT species, both metals are coordinated in tetrahedral sites using bridging thiolates to form clusters with stoichiometry of M_4S_{11} in the α domain, and M_3S_9 in the β domain [16]. For Cu^+ binding, the coordination is not as well known. Stoichiometric measurements that indicate Cu_6S_9 for the β domain [15], are complemented by emission and CD titration experiments that indicate that 12 mol eq of Cu^+ can be bound by the protein to form a single species [12]. The symmetry of the metal in such clusters may well be trigonal rather than tetrahedral.

As so few other Cu^+ complexes emit light in solution, the protein is clearly providing a unique environment which enhances the emission intensity of the Cu^+ chromophore. Metallothioneins do not contain aromatic amino acids that could contribute to the emission from Cu–MT. There are two main questions we wish to address. (i) Is the emission from a charge transfer state, and (ii) is the emission sensitive to cluster formation.

McMillin [6] assigned emission for triphenylphosphine complexes of Cu^+, to metal-to-ligand charge transfer transitions (MLCT). Intraligand (IL) excited states are also known [17]. However, in crystal studies, a metal-centered (MC) excited state $3d^94s^1$ that can be formed in the metal-centered (MC) transition $3d^{10} \rightarrow 3d^94s^1$, has been identified as the source of the emission intensity [18]. We can compare the nature of the emitting excited state of the Cu(I)–SR chromophores in MT with data for smaller inorganic complexes of Cu(I). There are a number of characteristic features of the Cu–MT emission.

(i) There is an extremely large difference between the absorption (excitation) and emission maxima (300 nm *versus* 600 nm).

(ii) The lifetimes of the two main components are relatively long (of the order of 1 μs at room temperature). The increase in the lifetime as the temperature falls (298 → 280 → 77 K) suggests that the transition is spin-forbidden, (triplet → singlet ground state).

(iii) The emission intensity is quenched by molecular oxygen; this also suggests that the emitting state is a triplet.

(iv) The effect of temperature in the 280–298 K range on the luminescence lifetime seems to be stronger than would be anticipated purely on the basis of a thermally-activated decay of the triplet state.

We expect charge transfer emission to be orbitally allowed and to exhibit a very short lifetime. Clearly this is not what is observed. Because of this we suggest that the luminescence can be attributed to the $3d^94s^1 \rightarrow 3d^{10}$ intra-copper(I) transition. To a first approximation, this emission is formally, orbitally forbidden, and should be observed to have a lifetime in the microsecond range. (In cubic symmetry a number of excited states, both singlets (^1E_g and $^1T_{2g}$) and corresponding triplets (^3E_g and $^3T_{2g}$), are associated with the excited state configuration of this interconfiguration transition; triplet emission from both ^3E_g and $^3T_{2g}$ has been observed [18]).

We would expect that this $3d^94s^1$ excited state of Cu(I) would be modified by any copper–copper interactions which might exist within the framework of the multicopper(I)–thiolate clusters in MT. In that case, the emission should offer a marker for cluster formation. The steady state data shown in Fig. 1, do show a very marked dependence on the amount of Cu^+ added. The increase in intensity up to Cu_{12}–MT, forming the cluster species, followed by the complete loss of intensity when the addition of more Cu^+ forms the non-clustered Cu_{20}–MT, indicates that there is considerable interaction between the excited states of the copper–thiolate clusters. The emitting state in such species can be a mixture of different excited state configurations or more than one excited state can be involved. The observation of multiexponential decay kinetics of the luminescence from Cu(I) in MT would favour the case of more than one excited state being involved. The fast component, which exhibits a lifetime of about 20 ns, probably is emission from a singlet excited state. The two slower decaying components of the decay curves can be considered to be luminescence from two different triplet excited states. The similarity in lifetimes determined for Cu_1–MT and Cu_6–MT suggest that the states themselves are Cu-related rather than Cu–SR–Cu.

An alternative explanation is to consider a nonexponential decay, for example resulting from resonance energy-exchange interaction between the copper sites in the excited state. Such a nonexponential decay can be expressed phenomenologically by the equation [19]:

$$I(t) = I(0)\exp(-kt - ct^{\alpha})$$

where k is the emission rate constant, and the two parameters α and c characterize the time-dependent, energy-transfer process. This type of kinetics should be only anticipated in the multicopper–MT complexes. However, even with only one Cu^+ present the multi-exponential decay is observed, which strongly suggests that the three-component emission arises from a single Cu–S chromophore, rather than a clustered Cu–S–Cu species.

Conclusions

Luminescence from Cu^+ substituted Zn_7–metallothionein in the 600 nm region, originates from the excited state triplets. There appears to be more than one triplet state emission component, in addition to emission from a singlet excited state at higher energies. The most probable excited state responsible for the triplet emission is the metal-centered $3d^9 4s^1$. The steady state emission intensity shows a strong correlation with cluster formation, whereas the lifetimes of the three components observed at 600 nm do not appear to change as the number of atoms of Cu^+ binding to MT increases.

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