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Luminescent Ag_{12}-metallothionein: dependence of emission intensity on silver-thiolate cluster formation

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We report the observation of emission intensity at 77 K that is a function of Ag(I)-thiolate bonds formation within the protein metallothionein. The emission characteristics (a large, 250 nm, Stokes shift and long emission lifetime) suggests that the transition occurs from the excited triplet state. The emission intensity and circular dichroism both indicate that silver(I) clusters form with stoichiometric ratios of 12 Ag(I) to the 20 thiolate sulfur groups that are present in the protein. These data are the first to show that Ag(I)-metallothionein complexes are luminescent and that a specific Ag_{12}-MT species forms.

Emission intensity; Luminescent complex; Silver(I)-metallothionein complex

1. INTRODUCTION

The protein metallothionein has been isolated from many different organs in man and animals, as well as from a variety of other species [1]. Major concentrations of the protein in animals are found in the livers and kidneys [1]. The protein is unique in that the peptide chain comprises 20 cysteines out of 61 amino acids, no aromatic amino acids and no disulfide bonds [1]. Studies on the binding of cadmium and zinc have provided a focus for the role of metallothionein (MT) in the physiological chemistry of group 12 metals [2]. However, the potential for a much more extensive chelation chemistry for MT is illustrated by the remarkably wide range of metals that are already known to bind to the protein [1,3]. The recognition that metal binding in protein involves formation of clustered metal-thiolate bonding, with the well-known examples being Cd_4S_{11} and Cd_3S_9 [4,5], has been particularly exciting. While it is likely that many of the other metals also bind in a clustered fashion, in the absence of high resolution techniques like the ^{113}Cd NMR, it has been difficult to obtain unambiguous spectroscopic data to support clustering. We report here observation of emission intensity from the Ag(I)-substituted protein. We find that the intensity is directly related to the stoichiometric ratio, with a maximum being observed between 10 and 12 Ag. These data support cluster formation for Ag-MT, which is in line with cluster formation in Cu-MT, as proposed by Winge et al. [3]. In extensive ^{113}Cd NMR studies, Armitage and co-workers [1,4] established that for Cd_7-MT, Zn_7-MT and the native Cd_4,Zn_3-MT, the metals occupy two independent domains, named α and β, with stoichiometries of M_4S_{11} and M_3S_9. Analysis of X-ray data by Furey et al. [5] has confirmed these predictions. Although it is well known that MT can bind a range of nd^{10} metals both in vivo and in vitro, detailed structural information on how the binding sites are organised is available only for Cd, Zn and Co [1,4–6]. Part of the problem lies with the inherent 'chromophoric silence' of metals like Cd(II), Zn(II), Cu(I), Ag(I) and Hg(II). We have previously been able to exploit the circular dichroism (CD) spectrum, that is measured under the ligand to metal charge transfer

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transitions observed in 250–320 nm for the Cd- and Hg-containing protein, to identify the onset of clustering in the binding site in dilute solutions [7,8]. We also discovered that while the CD spectrum of Cu-containing MT is poorly defined, the emission intensity recording 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 [9]. It appears then that the emission spectrum can offer an important view of metal-thiolate cluster formation in some metallothioneins. For metals like Cu(I) this technique may be the only readily available technique with which to obtain such structural information.

2. EXPERIMENTAL

Zn_7-MT isoform 2 was isolated from rabbit liver following induction procedures described previously with Zn^{2+} salts [10]. Aliquots of Ag(I) were added to individual solutions of Zn_7-MT at pH 3.8 under nitrogen. Portions of these solutions were frozen in liquid nitrogen and added to a quartz tube used for emission measurements. Emission spectra were recorded from separate solutions on a Perkin-Elmer MPF-4 equipped with a red-sensitive Hamamatsu R-928 phototube. All measurements were made under a nitrogen atmosphere. The spectral data were digitized directly from the spectrometer and replotted with a Spectra Manager [11].

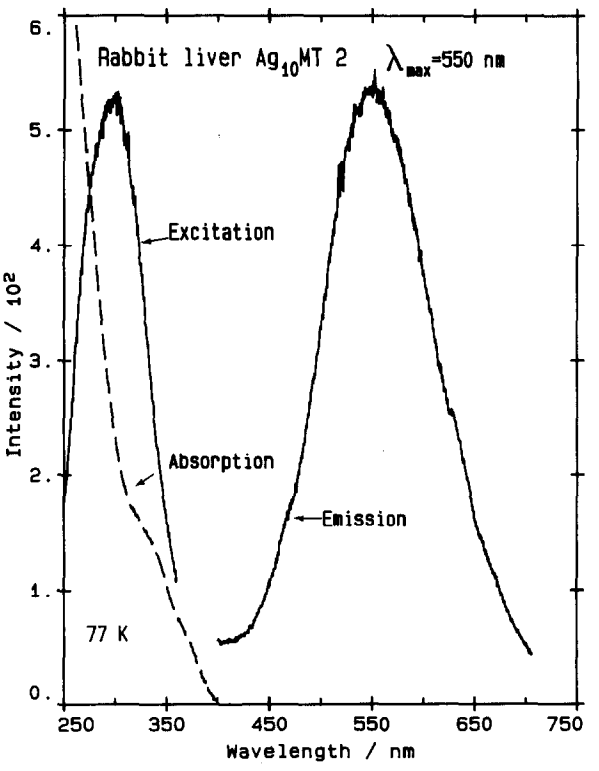

Fig.1. Uncorrected excitation (λ_{em} = 540 nm) and emission (λ_{ex} = 300 nm) spectra recorded at 77 K, for a solution of rabbit liver Zn_7-MT 2 (10 nmol/ml) containing 10 mol eq. Ag^+ added at pH 3.8. Absorption spectrum of Ag_{10}-MT is depicted using a broken line.

3. RESULTS AND DISCUSSION

Fig.1 shows the uncorrected emission and excitation spectra recorded at 77 K for a sample of rabbit liver Zn_7-MT 2 containing 10 mol equivalents (mol eq.) Ag^+. The peak emission intensity is found at 550 nm. No emission is observed at room temperature for a solution with such a low Ag(I)-MT concentration in this spectral region (the concentration was 10 nmol/ml based on MT). The silver(I)-MT exhibits a large Stokes shift: with excitation at 300 nm the emission spectrum has a maximum at 550 nm. The emission lifetime of these Ag(I)-MT complexes at 77 K is relatively long (longer than 100 μs). This suggests that the transition is spin-forbidden, for example, excited triplet $\longrightarrow$ singlet ground state. In similar luminescence that is characteristic for $Cu(I)_{12}$-MT complexes, we have attributed [12] the emission to a $3d^94s^1 \longrightarrow 3d^{10}$ intra-copper(I) transition, although we cannot rule out the $3d^94p^1$ excited state. Emission from coordinated Ag(I) complexes is rare, and has not been reported previously for Ag(I)-MT.

Fig.2a and b presents the emission data recorded at 77 K for a series of solutions of MT with increasing amounts of Ag^+ added. Curve 1 in fig.2a represents the intensity of the emission spectrum at 550 nm. The dramatic increase in the emission intensity as the molar ratio of Ag^+ to MT approaches 12, suggests that as Ag^+ binds to the protein, solvent is extruded, which reduces the radiationless transitions that would be expected to quench the luminescence. The binding site region therefore becomes more hydrophobic. We associate the maximum at 12 Ag^+, with distinct cluster formation of Ag_{12}-MT, with the 20 thiolate groups that make up the metallothionein peptide

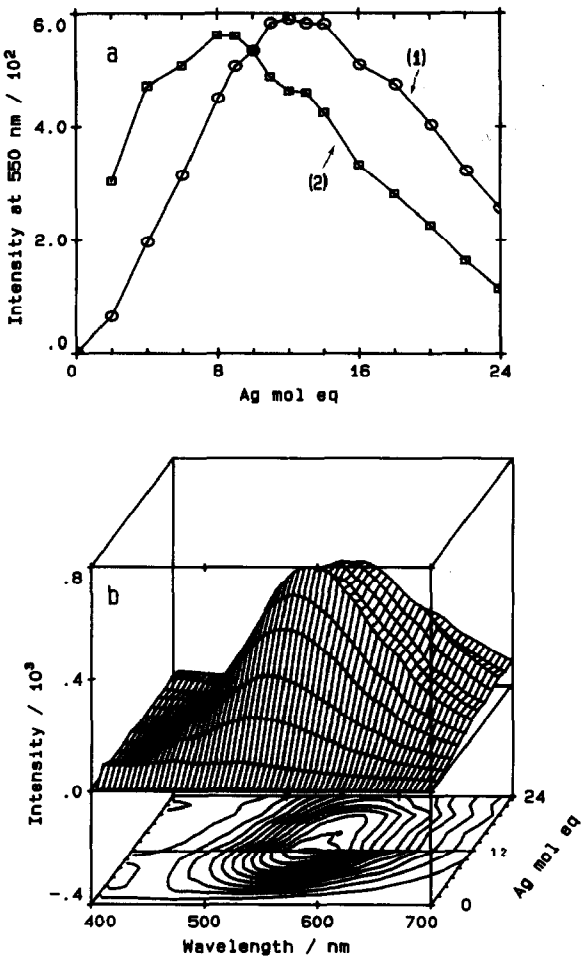

Fig.2. (a) Curve 1: dependence of the emission intensity at 550 nm on the number of mol Ag^+ added per mol rabbit liver Zn_7-MT 2 at pH 3.8. Curve 2: dependence of the normalized emission intensity at 550 nm on the stoichiometric ratio of Ag^+:protein (each intensity value was divided by the molar ratio of Ag^+). (b) A 3-dimensional representation of the emission spectral data obtained for Ag(I)-metallothionein solutions at 77 K, with λ_{ex} = 300 nm, plotted with respect to the stoichiometric ratio of Ag^+:protein.

chain. Clearly, as more Ag^+ is added, so the protein expands and solvent once again can quench the emission intensity. The relative emission yield, shown in fig.2a as curve 2, increases as the molar ratio Ag^+:protein increases towards 10. After 12 mol eq. Ag^+ have been added, the emission yield falls off rapidly. Clearly, the structure that forms between Ag_8-MT and Ag_{12}-MT significantly enhances the emission intensity.

We see from fig.2b, where the emission spectrum is shown as a function of the molar ratio Ag(I):MT, that there is a well-defined growth in intensity at 550 nm, towards a maximum in the 10–12 mol eq. region. The emission maximum red shifts towards 600 nm as Ag^+ is added in excess of 12 mol eq. Fig.2b shows that the initial growth in the 550 nm peak intensity proceeds without a shift in wavelength, which indicates that the species responsible for the emission intensity simply grows in concentration. This suggests that once past 2 Ag^+ per mol MT, the Ag(I)-S binding is of a similar nature as each Ag^+ is added. Fig.2b also shows that at low Ag(I):protein ratios, there is weak, but distinct, emission intensity in the 440–510 nm region. The contour lines in fig.2b are clearly asymmetric in the 420–500 nm region for ratios of 0 to 6 mol eq. Ag^+ per mol protein. Whether this feature is related to the binding of the first 6 Ag^+ to the protein in a single domain is not yet clear.

It appears that Cu^+ and Ag^+ bind to MT with a similar geometry [3]. For Cd, Zn and Hg, CD and MCD spectra show a particular spectral pattern with the maximal intensity at a molar ratio of 7 [7,8,13]. This coincides with the structural predictions of tetrahedral symmetry around each atom obtained for very much more concentrated solutions by NMR [1,4], and from the X-ray crystal data [5]. For Cu^+ [9] and Ag^+ (Stillman, M.J. and Zelazowski, A.J., unpublished) we have found that a characteristic CD spectrum is maximal at both 6 and 12 mol eq. of the metal ions, although the CD data for Ag^+ binding do not show such a sharp maximum as we have found in the emission data presented here.

In summary, we report novel spectral information from a silver containing metallothionein. We have demonstrated that emission intensity measured at 550 nm is dependent on the Ag^+:MT stoichiometry. The position of the maximum intensity of the emission indicates that a major species forms between Ag_{10}- and Ag_{12}-MT, and we propose that this species involves Ag(I)-thiolate cluster formation.

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