LUMINESCENCE PROPERTIES OF *NEUROSPORA* COPPER METALLOTHIONEIN

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1. Introduction

Metallothioneins are low M_r, cysteine-rich proteins, which bind high amounts of Zn, Cd and/or Cu [1–3]. They are widely distributed in eucaryotic organisms where they are synthesized de novo upon exposure to various metal ions [4,5]. From amino acid sequence studies of metallothioneins of different origin the positions of the abundant cysteinyl residues have been found to be highly conserved in agreement with their involvement in metal binding [3,6,7]. The metal ions are bound as metal–thiolate complexes responsible for the characteristic charge transfer bands located in the ultraviolet region of the absorption spectrum [3,7]. The spectral features are attributed solely to these charge-transfer transitions, since metallothioneins completely lack aromatic amino acids [3].

In contrast to the metallothioneins from higher eucaryotic organisms which bind different metal ions, the fungal metallothioneins were reported to contain exclusively copper [8,9]. From X-ray photoelectron-, EPR- and absorption spectroscopy, the copper was shown to be bound in the form of a Cu(I)–thiolate complex [10,11]. Since a number of small M_r Cu(I) complexes displaying charge transfer transitions were reported to give rise to characteristic luminescence in the visible region [12–15], we examined the fluorescence properties of copper metallothionein.

Here we show that the copper metallothionein from *Neurospora crassa* displays an orange luminescence which is attributed exclusively to the Cu(I)–thiolate metal binding mode.

2. Materials and methods

Neurospora copper metallothionein was prepared according to [9] and used either immediately after isolation or after storage at –80°C. For spectral measurements, the protein was passed through Sephadex G-25 in 10 mM Tris–HCl (pH 8.0). Absorption spectra were recorded at 10°C in a Hitachi-Perkin Elmer Mod. 340 recording spectrophotometer. Fluorescence spectra were measured with a Perkin-Elmer MPF 2A spectrofluorimeter at 10°C in ratio mode. The spectra were corrected for the inner filter effect and buffer contribution. The excitation spectrum was corrected for the photon output of the lamp, according to [16] using a concentrated solution of rhodamine 6G in ethanol. The quantum yield was calculated by comparing the area under the emission spectra obtained for the protein solution and an alcoholic solution of rhodamine 6G excited at the same wavelength and normalized for the same absorbance. Phosphorescence measurements were carried out at 77 K using the model MPF 2A phosphorescence accessory according to [17].

Metal analyses were performed on a IL 157 atomic absorption spectrophotometer using acid-washed glassware. Buffer solutions were rendered metal free by passage through Chelex 100.

3. Results

During the isolation of *Neurospora* copper metallothionein it became apparent that the fractions containing copper metallothionein display a characteristic orange fluorescence when observed with a ultraviolet lamp ($\lambda \sim 300$ nm). Upon excitation of a solution of the purified protein at 305 nm an emission spectrum with a rather broad band (half bandwidth = 62 nm) centred at 565 nm is found (fig.1). Fig.1 also shows the observed excitation spectrum obtained at the maximum of emission, i.e., $\lambda = 565$ nm. The quantum yield was calculated to be 0.013 assuming a value of

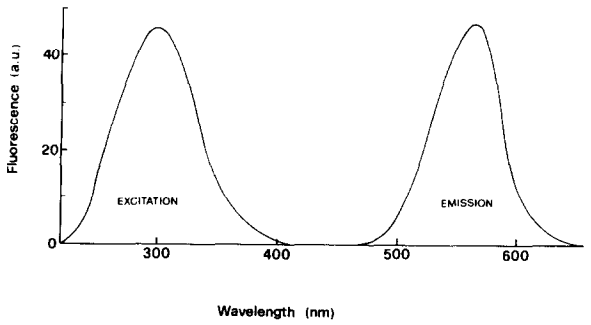

Fig.1. Observed excitation and emission spectra of *Neurospora* copper metallothionein: excitation at 305 nm; emission at 565 nm; protein 31.5 μM in copper; 10 mM Tris–HCl (pH 8.0); temp. 10°C.

1.0 for rhodamine 6G as a standard [18]. Addition of 1 N HCl to the solution (final pH 0.5), which is known to displace copper by protonation of the thiolate groups [19], leads to a complete disappearance of the absorption band at 245 nm (fig.2) and concomitantly the luminescence at 565 nm. In addition, the luminescence is also completely absent in a sample where both the cysteinyl residues and the Cu(I) ions have become oxidized spontaneously in the presence of air [20]. The corrected excitation spectrum is presented in fig.2 together with the absorption spectrum of the same sample normalized to the same scale.

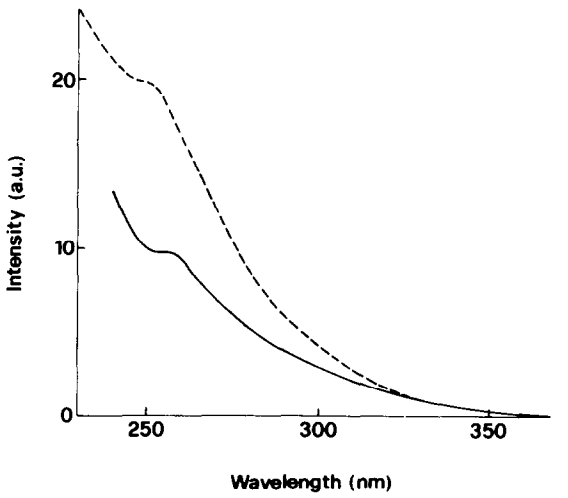

Fig.2. Absorption (– – –) and corrected excitation (——) spectra of *Neurospora* copper metallothionein; conditions as in fig.1.

Table 1
Emissive properties of *Neurospora* copper metallothionein and Cu(I)–mercaptide complexes [12]

	Emission λ_{max} (nm)	Half bandwidth (nm)
Neurospora copper metallothionein	565	62
Cu(I)–glutathione reduced	575	51
Cu(I)–cysteine	585	54

4. Discussion

The luminescence properties of *Neurospora* copper metallothionein shown here were found to be strictly related to the integrity of the Cu(I)–thiolate complex. All modifications, like protonation of thiols or spontaneous oxidation of the complex in the presence of air resulted in the complete disappearance of the luminescence. The emission spectrum of *Neurospora* copper metallothionein shows a rather broad band which is characterized by a large Stokes shift. Similar spectral properties have been reported for several low M_r Cu(I) complexes containing different ligands [12–15] and also for the CO complexes of hemocyanin and *Neurospora* tyrosinase [18,21]. In particular the emission spectra of a number of Cu(I)–mercaptan complexes [12] were found to be very similar to the emission spectrum of *Neurospora* copper metallothionein (table 1).

As pointed out [14], metal complexes involving metal ions which are diamagnetic, easily oxidized and display charge-transfer transitions are expected to be luminescent. As *Neurospora* copper metallothionein fulfills all these criteria the luminescence could be attributed to transitions of the charge-transfer type of the Cu(I)–thiolate complex. This conjecture is further supported by the corrected excitation spectrum which is very similar in shape to the absorption spectrum (fig.2). The observed discrepancy in the intensity of the two normalized spectra can be explained on the basis that transitions contributing to the absorption spectrum do not give rise to fluorescence. On the other hand, the possibility that some transitions deactivate via triplet state is rather unlikely because no phosphorescence could be observed at 77 K.

Preliminary studies on copper metallothioneins from the common mushroom *(Agaricus bispora)* and

from bovine foetal liver have indicated the same luminescence also in these proteins [20]. These findings thus strongly support the view that this spectral feature is an inherent property of all copper metallothioneins.

In conclusion, the luminescence properties of copper metallothioneins should be useful not only to follow the purification of this protein but also as a spectroscopic probe to investigate the mode of metal binding and molecular structure.

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