LUMINESCENCE OF THE COPPER–CARBON MONOXIDE COMPLEX OF *NEUROSPORA* TYROSINASE

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1. Introduction

Spectroscopic studies of tyrosinase and hemocyanin have indicated that the copper-sites of both proteins are very similar. Thus the stoichiometry of oxygen binding [1,2], EPR measurements on various derivatives [3,4], magnetic susceptibility measurements [5,6] and resonance Raman spectroscopy [7] suggest the presence of a pair of antiferromagnetically coupled copper-ions separated by 3–6 Å. Moreover hemocyanin and tyrosinase both bind carbon monoxide [8,9].

We have reported that the carbon monoxide complex of hemocyanins is luminescent [10,11]. Upon excitation in the ultraviolet region, a strong emission band is observed above 500 nm. Excitation and difference absorption spectra, as well as CO titrations, clearly indicate that the emission originates from the copper–CO chromophore.

In view of the above similarities between hemocyanin and tyrosinase, we have studied the binding of CO to *Neurospora* tyrosinase by fluorescence spectroscopy, and observed that the luminescence characteristics of CO–tyrosinase are similar to those reported for CO–hemocyanins [10,11]. This result emphasizes the close structural relationships existing between the active site of these two classes of proteins, notwithstanding the overall functional differences; moreover it confirms the potentialities of fluorescence spectroscopy of the CO complexes of these proteins, to investigate the structure of their binuclear copper sites.

2. Materials and methods

Neurospora tyrosinase was prepared and stored as in [12]. Before use the protein was passed through a Sephadex G-25 column equilibrated with 0.1 M phosphate buffer (pH 7.2). The protein was reduced with NH_2OH in a molar ratio of 3·1 over copper. Difference absorption spectra were recorded on a Perkin-Elmer E-350 spectrophotometer. Fluorimetric measurements were performed as in [10].

3. Results and discussion

The fluorescence properties of deoxygenated *Neurospora* tyrosinase are similar to those observed for hemocyanins [13], in so far as the tryptophan emission, with a maximum centered at ~330 nm, is substantially quenched (~60%) when the protein solution is equilibrated with air. This quenching in hemocyanin was ascribed to energy transfer from tryptophans to the copper–oxygen complex [13]. A similar explanation may be valid for tyrosinase in view of the overlap between the donor emission and the Cu–O_2 band (λ_{max} at 330 nm, $\epsilon = 17.2 \times 10^3\ M^{-1}\ cm^{-1}$ [12]).

Reduced potato tyrosinase binds CO with a stoichiometry of 1 CO/2 Cu [9]. For mushroom tyrosinase the partition coefficient between oxygen and carbon monoxide was found to be 1.7 [14]. The difference absorption spectrum in fig.1 shows that CO binding to tyrosinase is associated with an absorp-

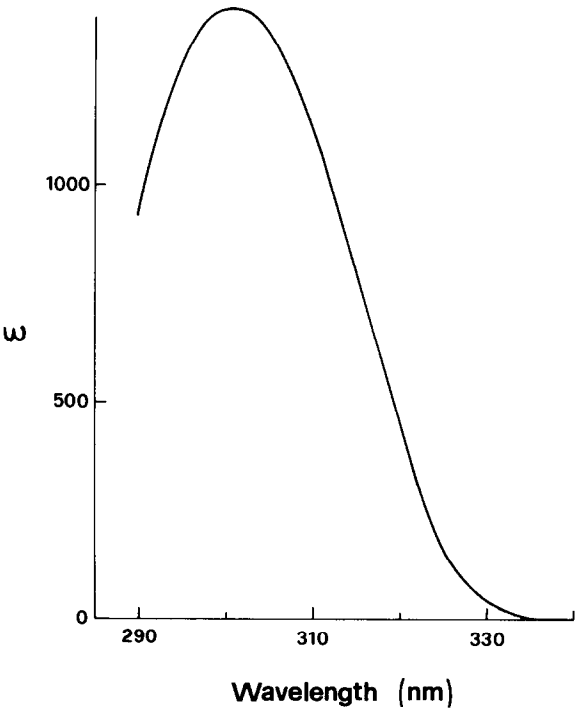

Fig.1. Difference absorption spectrum of the carbon monoxide complex of *Neurospora* tyrosinase, in 0.1 M phosphate (pH 7.2) and 20°C. ϵ = molar extinction coefficient for 1 cm lightpath.

tion band centered around 300 nm. The emission properties of the tyrosinase–CO complex are reported in fig.2. Upon excitation at 285 nm a broad emission, with a maximum centered around 550 nm, is observed. The appearance of this band is associated with a ~40% quenching of the tryptophan emission. This quenching may be tentatively ascribed to a non-radiative energy transfer from the activated tryptophans to the copper–CO chromophore. Titration of the protein with CO results in a progressive increase in the visible emission intensity and in a parallel decrease in the emission at 330 nm. The binding proceeds non-cooperatively ($n_H = 1$), with a $p_{1/2}$ (i.e., the pressure at which 50% of the change in emission is accomplished) of 4 mm Hg at 20°C. Addition of oxygen to CO–tyrosinase results in a decrease in emission at 550 nm and an additional marked decrease at 330 nm. The excitation spectrum of the emission at 550 nm shows a maximum at 280 nm, which coincides approximately with the absorption difference spectrum. The contribution of the specific absorption of the copper–CO chromophore (fig.1) is masked in the excitation spectrum, as expected from the high absorbance and energy transfer of the aromatic residues.

We may conclude that the carbon monoxide complex of *Neurospora* tyrosinase exhibits luminescence

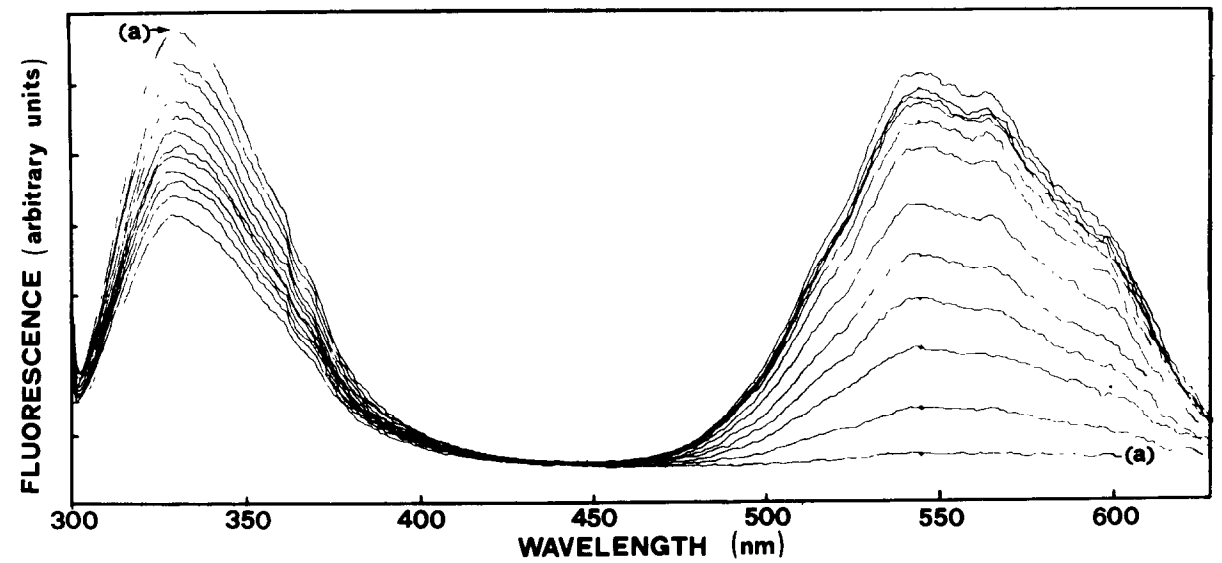

Fig.2. Emission spectra of *Neurospora* tyrosinase in the absence and presence of carbon monoxide. (a) Deoxygenated and reduced protein. The CO-emission spectra were obtained after addition of successive amounts of CO gas to the protein solution (up to a final 1 atm). A $p_{1/2}$ of 4 mm Hg and n_H (Hill-coefficient) of 1 was calculated for the CO binding. Excitation at 285 nm, protein 6 μM in copper, 0.1 M phosphate (pH 7.2) and 20°C.

properties originating from the copper–CO chromophore, similarly to that observed for hemocyanins [10,11]. Together with other information available on the functional and spectroscopic properties of hemocyanins and tyrosinases, the conclusion that their active sites are structurally very similar becomes compelling.

The copper pairs in hemocyanin and tyrosinase have commonly been designated as type 3 copper, by analogy with those described for multi-copper oxidases [15]. A number of spectroscopic observations (A_{330} and lack of EPR signal) support this idea. However functional differences between these, otherwise similar, binuclear centers are apparent in their interaction with CO. Thus, in agreement with the fact that *Rhus vernicifera* laccase is not inhibited by CO [15], no emission of the type described here was observed upon addition of CO at 1 atm to this protein, in both oxidation states. Therefore it is evident that the diamagnetic copper pairs present in various copper proteins are different. In this context the luminescent properties of the CO complexes of copper proteins appear as a powerful tool for the investigation of binuclear copper centers.

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