LUMINESCENCE OF Cu-CYTOCHROME *c*

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Received 10 February 1978

1. Introduction

A recent approach to the study of electron transfer in cytochrome *c* has been to replace the central iron by other metals. The resulting metal derivatives by virtue of their electron paramagnetic resonance [1,2] or luminescence [3–5] allow specific questions concerning the conformation of the protein and interactions of the protein with neighbouring proteins to be answered

In this paper we report that copper cytochrome *c* exhibits luminescence characteristic of copper porphyrins. We suggest that this luminescence may be a useful indicator to monitor interactions of cytochrome *c* with cytochrome oxidase.

2. Procedures

Cytochrome *c* in which the iron is replaced by copper was prepared according to the general procedure for making other metal cytochrome *c* derivatives [2,4]. Iron-free cytochrome *c* (1 mM) [3] was incubated with 10 mM $CuCl_2$, pH 6.0, at 25°C. After 30 min, no metal free cytochrome *c* remained as indicated by the absence of the characteristic porphyrin fluorescence. Free $CuCl_2$ was removed by passage over a Sephadex G-50 column. The Cu cytochrome *c* than applied to a CM cellulose column and eluted with 0.15 M NaCl and 20 mM PO_4, pH 8.0.

Beef heart mitochondria were prepared as in [6] and stored frozen at −30°C until use. Cytochrome *c* depletion of thawed mitochondria was done as in [7] which removed approx. 95% endogenous cytochrome *c*. Depleted mitochondria were suspended in a medium containing 225 mM mannitol, 75 mM sucrose, 20 mM phosphate, pH 7.4 and 30% ethylene glycol to give final conc. 20–40 mg protein/ml. Copper cytochrome *c* was added to the cytochrome *c*-depleted mitochondria at room temperature. For 'reduced' mitochondria 10 mM succinate was added. After 10 min at room temperature the sample was frozen in liquid nitrogen for fluorometric assay. For 'oxidized' mitochondria the suspension was stirred in the presence of oxygen gas followed by rapid freezing in liquid nitrogen.

Luminescence spectra were obtained using a Perkin-Elmer MPF 2A spectrofluorimeter equipped with a Hammamatau 928 photomultiplier. A cold-finger liquid nitrogen dewar was used as sample holder.

3. Results and discussion

The emission properties of copper porphyrins have been described theoretically and experimentally. Theory states that the single unpaired d electron of copper couples to the normal porphyrin excited states such that the singley becomes a doublet while the triplet splits into 'trip-doublet' and quartet states [8,9].

Cu cytochrome *c* exhibits luminescence typical of copper porphyrins. On fig.1. this is illustrated. The excitation spectrum is experimentally indistinguishable from the absorption spectrum. The emission spectrum shows a maximum at 688 nm shoulders at 715 nm and 762 nm.

It is interesting to note that there are subtle changes in the spectrum of copper cytochrome *c* when this protein binds to the mitochondria (fig.2). There is both a diminution of the overall signal and a

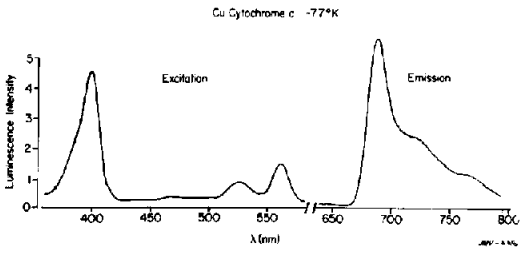

Fig.1. Excitation and emission spectra of Cu cytochrome *c*. The sample contained 30 μM Cu-cytochrome *c* in 50% ethelene glycol. Temp. 77°K; Excitation wavelength, 400 nm; emission wavelength, 690 nm. Half-maximal band pass, 8 nm.

change in the spectrum itself when cytochrome *c* is bound. This is especially evident in the spectrum of Cu cytochrome *c* bound to reduced mitochondria (fig.2C).

The result indicates that there is interaction between cytochrome *c* and cytochrome oxidase which can be physically detected. There are several possible interpretations of the results:

1. A conformational change may occur when cytochrome *c* binds. This change may effect the position of Cu relative to the plane of porphyrin such that coupling to the porphyrin excited states is changed.

2. The excited state of porphyrin may be affected by the electrons in the neighboring oxidase. This is suggested since the spectrum of cytochrome *c* differs when bound to oxidized or reduced mitochondria. Since the absorption of the visible copper of oxidase is in the spectral region where Cu cytochrome *c* is emitting [10], dipolar interactions are possible.

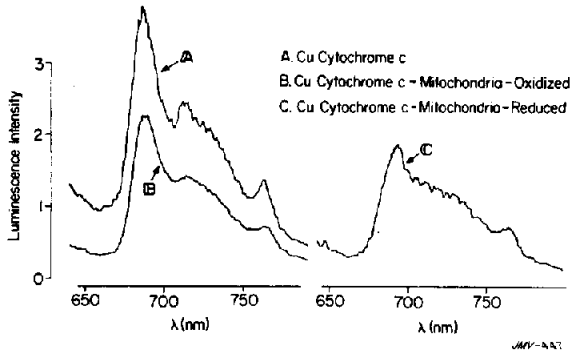

Fig.2. Emission spectrum of Cu cytochrome *c* in the presence of mitochondria. All samples contained 3 μM Cu cytochrome *c*, 225 mM mannitol, 75 mM sucrose, 20 mM phosphate, pH 7.4 and 30% ethelene glycol. Samples were prepared as in section 2. (A) no mitochondria; (B) 20 mg/ml 'oxidized' mitochondria; (C) 20 mg/ml 'reduced' mitochondria.

To distinguish between these two possibilities the luminescence is being examined in terms of quantum yield, spectrum and lifetime as a function of temperature and redox potential of the oxidase.

Acknowledgements

This work was supported by USPHS GM12202 and ESO1628. J.V. is supported by Career Development Award USPHS GM-0053.

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