TRIPLET ABSORPTION AND PHOSPHORESCENCE EMISSION IN ZINC CYTOCHROME *c*

Sudha N. DIXIT, Alan J. WARING+ and Jane M. VANDERKOOI

Department of Biochemistry and Biophysics, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104 and *+Department of Pathology and Biochemistry, Hahnemann Medical College, 230 North Broad Street, Philadelphia, PA 19102, USA*

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1. Introduction

The excited state properties of porphyrins have attracted considerable attention due to their relevance to the understanding of electron transfer processes in biological systems. The excited state porphyrins can transfer electrons in model systems such as the porphyrin derivatives of Ni(II), Cu(II), Mg(II), Ag(II), Zn(II), Pd(II) and Pt(II) [1–5]. In the report [6] on the steady-state and time-resolved luminescence emission in copper cytochrome *c*, intramolecular rate parameters below 80 K were significantly influenced by the porphyrin–protein interaction. Another application of substituted porphyrins is the study of the whole molecule rotational motion in membrane systems. [6]. The triplet state of rhodium-substituted cytochrome b_5 was examined and its properties used to measure the rotational diffusion of the molecule in phospholipid vesicle system [7].

Here we report the results of our measurements of decay time of transient triplet absorption and phosphorescence emission in zinc cytochrome *c*. It is known that zinc porphyrins can transfer electrons in the excited state in model systems [8]. We aim to investigate whether similar electron transfer occurs in proteins containing zinc-substituted porphyrins.

2. Materials and methods

Cytochrome *c* from horse heart, type III was obtained from Sigma Chemical Co. (St Louis MO) KCl was from J. T. Baker Chemical Co. (Phillipsburg NJ). Zinc cytochrome *c* (cytochrome *c* with iron substituted by zinc) was prepared as in [9]. Rhodamine 575 dye was obtained from Exciton Chemical Co. (Dayton OH). Methanol, absolute was a Baker analysed reagent.

2.1. *Deoxygenation of the sample*

The sample contained in a screw-top glass cuvette was bubbled with argon in a glove bag for 20 min at the rate of 1 bubble/2 s. The cuvette was then closed with a cap and kept in the glove bag until use.

2.2. *Apparatus*

Fig.1 depicts the block diagram of the set up used for the measurement of triplet absorption and phosphorescence emission. The sample was excited with 0.4 μs laser pulse, using a phase-R DL-1000 pulse dye laser. The rhodamine 575 dye was dissolved in methanol to 5×10^{-5} M. A continuous tungsten lamp (FDT, 12 V, 100 W) was employed for the triplet absorption measurement. Low temperatures were achieved by passing nitrogen gas of known pressure through a copper coil immersed in a liquid nitrogen dewar.

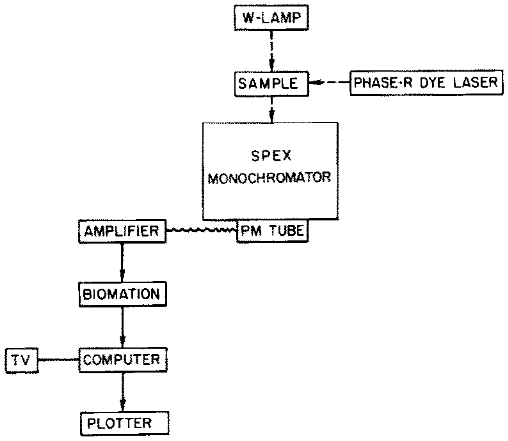

Fig.1. Block diagram of apparatus.

This cold stream of nitrogen gas was then passed from below the sample chamber to control the sample temperature and to prevent condensation on the cuvette faces. Temperature was measured with a thermister. The analog signal from the Johnson Foundation photometric amplifier was digitized by a 2805 biomation and the data transferred to an apple II computer for analyses of the decay kinetics, using a least squares program. To improve the signal/noise ration, 10 repeat signals were averaged.

3. Results and discussion

The triplet lifetime measurements were made by monitoring the transient triplet absorption and phosphorescence emission signals. The measurement at various wavelengths indicated that the transient absorption maximum occurs at 462 nm and the emission at 720 nm. These are the regions typical for the zinc porphyrin triplet absorption [3] and its emission [9].

Decay curves for the triplet absorption and phosphorescence emission at 15.5°C are shown in fig.2. The solid lines correspond to the computer best fit. The decay curves could be fit to a single exponential, which is an indication of a single excited species. The results of the decay time measurements at 3 different temperatures are listed in table 1. It is evident that the lifetime for the transient triplet absorption and emission are identical under a variety of conditions. Hence, under the conditions of our measurements, only a single transient triplet energy state is involved in both the processes. The possibility of any intermediate state involved is, therefore, ruled out.

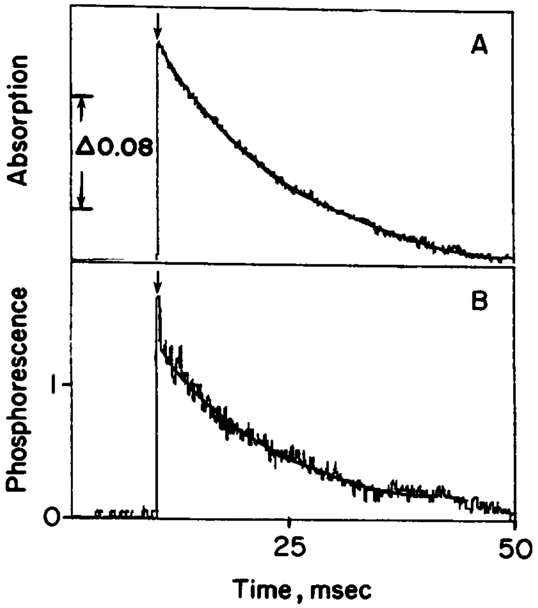

Fig.2. Decay of the transient triplet state of zinc cytochrome *c*. The sample contained zinc cytochrome *c* (64 μM), 33% glycerol and 0.15 M KCl at 15.5°C. The deoxygenated sample was excited by a 0.4 μs laser pulse at 575 nm as in section 2. (A) Absorption at 462 nm; (B) emission at 720 nm. A Schott RG 630 filter was attached to the exit slit of the monochromator. The solid lines correspond to the computer best fit.

Table 1
Temperature dependence of the triplet absorption and phosphorescence emission in zinc cytochrome *c* in 0.15 M KCl

Temp. (°C)	Solvent	Triplet absorption (τ, ms)	Phosphorescence emission (τ, ms)
22.5	33% Glycerol	11.6	11.0
15.5	33% Glycerol	14.1	14.1
−10.0	33% Glycerol	28.0	27.6
15.5	Water	7.0	7.1
−196.0	33% Glycerol	–	35.0

The triplet lifetimes observed here are in the range for zinc porphyrins [1]. It is clear from the table that the decay time is temperature dependent. In addition, the lifetime is longer in 33% glycerol than in water, which suggests that the decay kinetics depends on the viscosity of the medium. This may be due to the fact that quenchers diffuse less rapidly in a viscous medium like glycerol than in water.

We investigated the effect of laser flash intensity on the triplet lifetime. The results show that there is no effect of light intensity, indicating that there is no interaction between excited states. Changing the concentration of zinc cytochrome *c* did not cause any variation in the lifetimes. Further, the addition of ferricytochrome *c* up to 75 μM did not effect the decay time, which suggests that there is no interaction between ground states [1]. Studies on the influence of oxygen reveal that the lifetime is considerably shortened by the introduction of oxygen. For example, addition of oxygenated buffer to the degassed sample to give 0.05 mM oxygen reduced the triplet lifetime from 14.1–7.2 ms.

It is most notable that zinc cytochrome *c* exhibits a remarkably long transient triplet lifetime (14.1 ms) even at room temperature. This is due, undoubtedly, to the rigid plane of the porphyrin ring and prevention of quenching due to the protection offered by the polypeptide chain.

Acknowledgements

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