Phosphorescence of tryptophan from parvalbumin and actin in liquid solution

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Ca^{2+}-dependent phosphorescence *Phosphorescence, anaerobic conditions* *Phosphorescence, long-lived*

1. INTRODUCTION

Phosphorescence from tryptophan at room temperature offers several features which make it a potentially interesting spectroscopic tool for the study of proteins:

(i) In contrast to fluorescence, phosphorescence arises from a forbidden transition, which has a consequence that the emission is very long lived. This opens a new time frame for the study of protein dynamics, namely, the millisecond time scale.

(ii) It is apparent from [1–3] that very few tryptophan moieties within proteins exhibit long-lived phosphorescence in liquid solution. This can be advantageous when studying a complex containing several tryptophans; the use of time resolution allows one to effectively monitor a single tryptophan.

We report here that phosphorescence can be obtained under anaerobic conditions from two muscle proteins, parvalbumin and actin.

2. MATERIALS AND METHODS

Parvalbumin was prepared from frozen cod fish as a mixture of I, II and III types as in [4]. G-actin and myosin from rabbit muscle, were obtained from Sigma Chemical Co. (St Louis MO) and used without further purification. Sarcoplasmic reticulum of white skeletal rabbit muscle was prepared as in [5].

Phosphorescence spectra were obtained with an SLM spectrofluorometer (Urbana IL) which was modified by the addition of a mechanical shutter on the excitation side. The shutter could be opened and closed under control of a Hewlett-Packard computer. The computer was also used to synchronize the triggering of the SLM electronic components and the monochrometer stepping motor, thereby allowing for the automatic acquisition of time-resolved phosphorescence spectra. For measurement of phosphorescence lifetimes, a Perkin-Elmer MPF-2A fluorometer was used with the modifications in [6]. For all measurements the sample was kept in a glass-stoppered quartz cuvette and deoxygenated as described using the conditions given in fig.1 [6].

Fluorescence spectra were obtained with Perkin-Elmer 650-105 Fluorescence Spectrophotometer.

3. RESULTS

Several muscle proteins were examined for tryptophan phosphorescence at room temperature and normal assay conditions.

The phosphorescence emission of parvalbumin to which calcium is bound is shown in fig.1. At −196°C the emission spectrum shows maxima at 412 nm and 435 nm, characteristic of tryptophan emission. At 4°C and 20°C the emission yield is less than at −196°C, but shows the general features of tryptophan emission. It is of note, however, that the O–O emission at 412 nm is attenuated and broadened at the higher temperatures. This was also observed for the emission of alkaline phosphatase at room temperature [6]. The yield and lifetime of tryptophan phosphorescence emission of parvalbumin depends upon temperature: the lifetime was 5.1 s in the frozen state at

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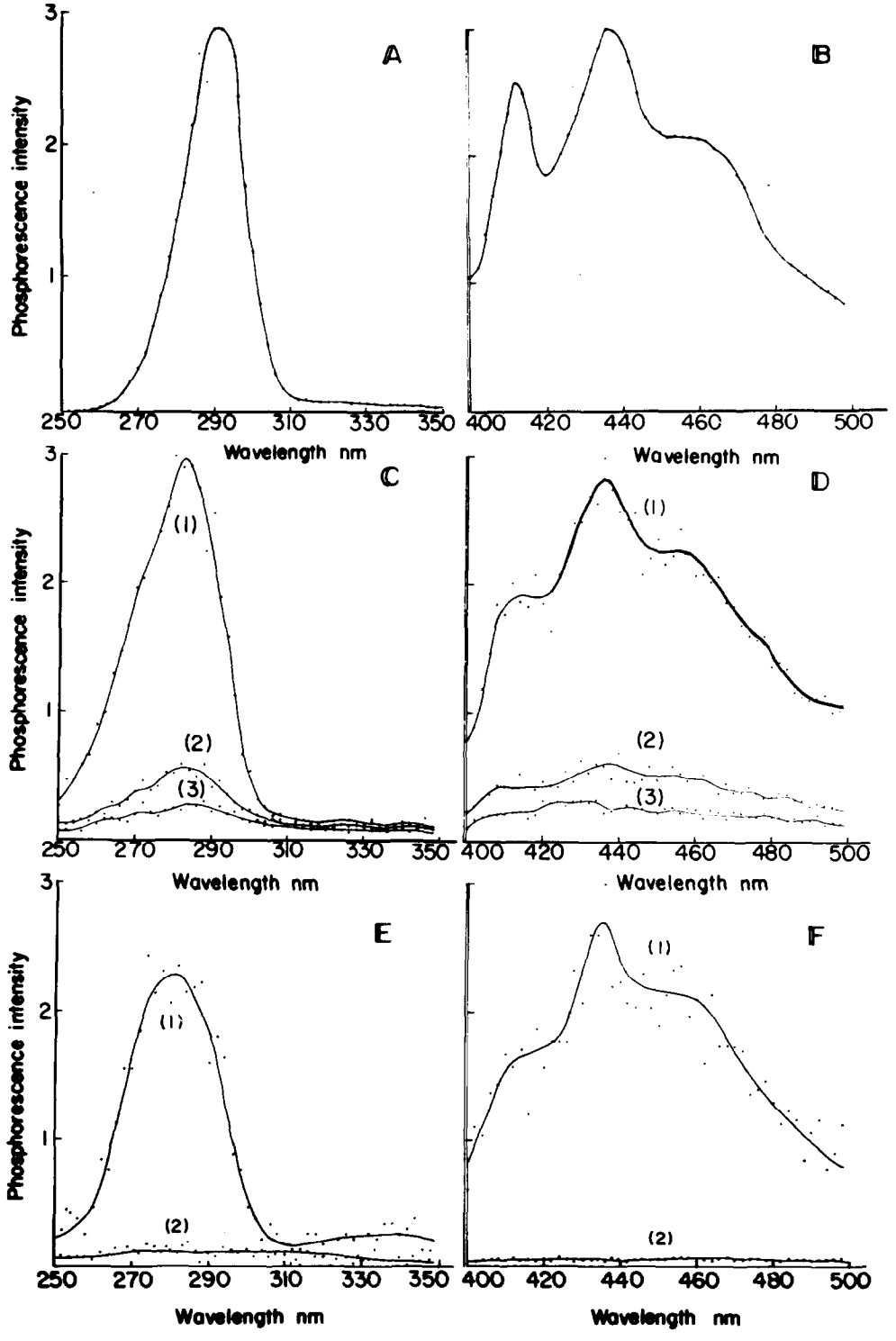
A
B
C
D
E
F
Phosphorescence intensity
Wavelength nm
(1)
(2)
(3)
250 270 290 310 330 350
400 420 440 460 480 500

Fig.1

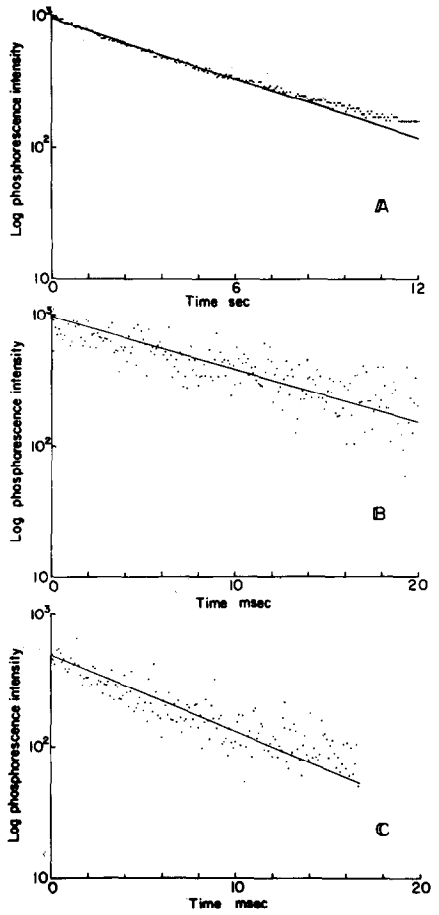

Fig.2. The phosphorescence decay curves of parvalbumin: (A) −196°C; (B) 4°C; (C) 20°C; excitation wavelength, 285 nm; emission wavelength, 435 nm. Other conditions are the same as in fig.1. The computer least squares fits: 5.1 s, 11.9 ms and 8.8 ms at −196°C, 4°C and 20°C.

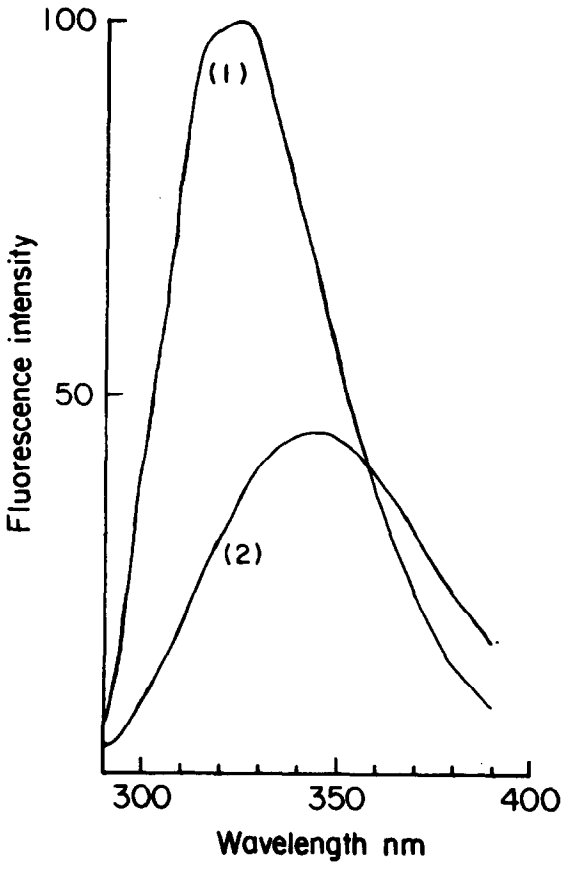

Fig.3. Tryptophan fluorescence spectra of parvalbumin with and without calcium. The emission spectra were measured at room temperature with excitation wavelength at 285 nm. Fluorescence intensity is given in arbitrary units. The medium was 0.02 M phosphate buffer (pH 7.4). The optical density of the parvalbumin at 280 nm was 0.4. (1) without EDTA, (2) with 1 mM EDTA.

77 K, 11.9 ms at 4°C, and 8.8 ms at 20°C (fig.2).

It is known from X-ray analysis that calcium-containing parvalbumin has a buried tryptophan [7]. This tryptophan apparently becomes exposed to the solvent when Ca^{2+} is removed from the protein, as can be seen in the change in fluores-

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Fig.1. The phosphorescence excitation and emission spectra of parvalbumin. Excitation (A,C,E) and emission (B,D,F) spectra were measured at emission wavelength 435 nm and excitation wavelength 285 nm, respectively. Phosphorescence intensity is given in arbitrary units. The medium contained 0.02 M phosphate buffer (pH 7.4) containing 0.3% glucose, 72 μg glucose oxidase/ml and 12 μg catalase/ml for the experiments at 4°C (C,D) and 20°C (E,F) or only phosphate buffer at −196°C (A,B). The absorbance of the parvalbumin at 280 nm was 0.4. Spectra A and B represent the light emitted during a 1 s interval after the excitation shutter was closed. Spectra C and D represent the following time intervals after closure of shutter: (1) 0–100 ms; (2) 100–200 ms; (3) 400–500 ms. Spectra E and F were accumulated from 0–100 ms. Spectra (1) in E and F were accumulated from 0–100 ms. Spectra (2) in E and F represent the background.

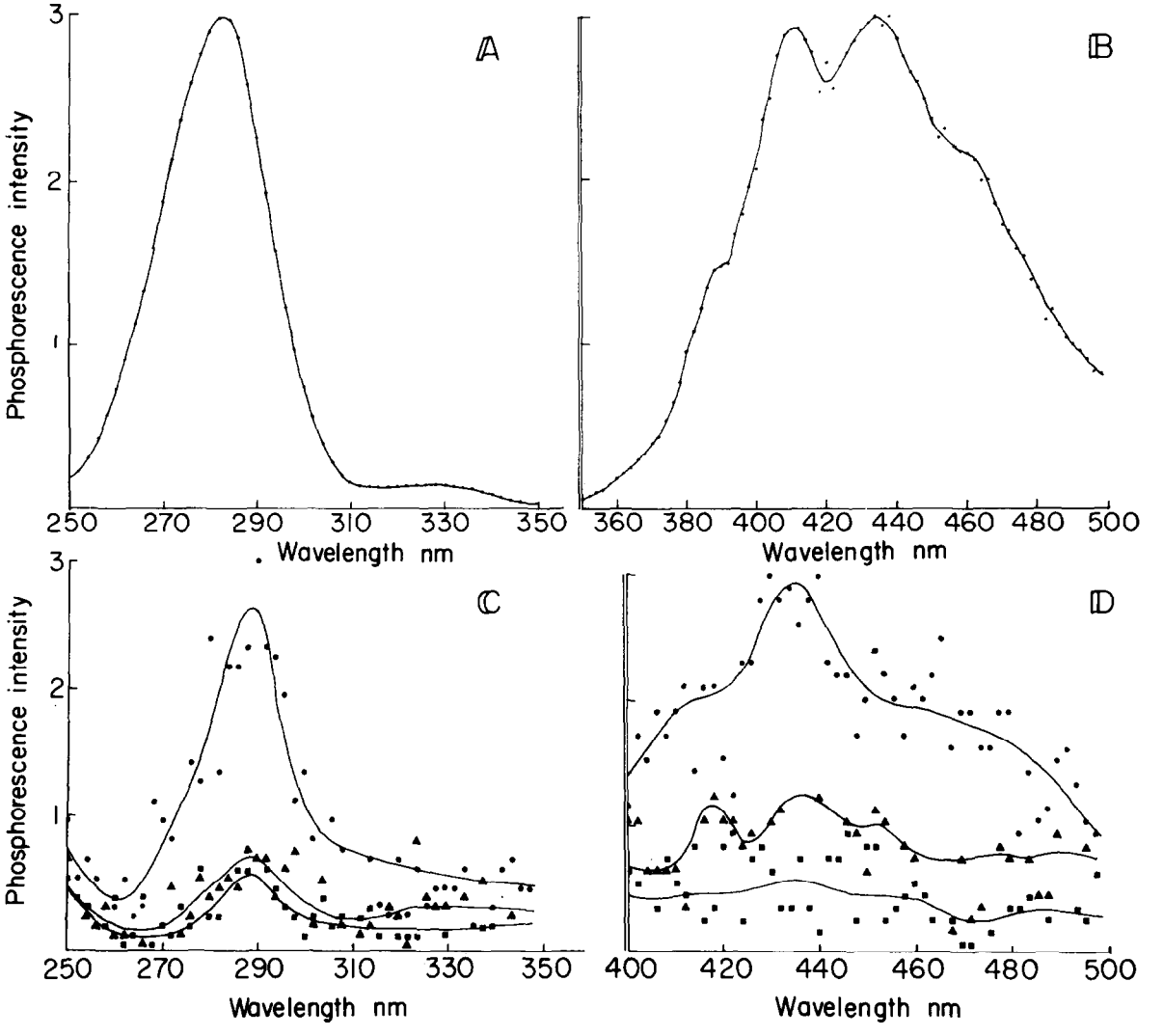

Fig.4. The phosphorescence excitation and emission spectra of G-actin. Excitation (A,C) and emission (B,D) spectra were measured at emission wavelength 435 nm and excitation wavelength 285 nm, respectively. Phosphorescence intensity is given in arbitrary units. The medium contained 2 mM Tris–HCl, 0.5 mM ATP, 0.2 mM $CaCl_2$ (pH 7.8) without the enzymes for removal of oxygen (−196°C) and with the enzymes (20°C), (see legend, fig.1). G-actin concentration was 0.17 mg/ml. Spectra A and B were measured at −196°C and recorded at 0–100 ms. Spectra C and D were at 20°C and recorded at 0–100 ms (•), 100–200 ms (▲) and 400–500 ms (■) after closing the shutter on the excitation beam.

cence maximum from 325 nm to 345 nm upon addition of 1 mM EDTA to the sample (fig.3). When calcium was removed by addition of EDTA, the phosphorescence became impossible to detect; i.e., a yield <5% of the calcium containing parvalbumin.

Phosphorescence was also observed from a sample of G-actin at room temperature. The emission spectra for frozen and liquid solutions of actin are shown in fig.4. The emission of G-actin at room temperature is again characteristic of tryptophan and, therefore, constitutes proof that tryptophan is responsible for long-lived phosphorescence. (The emission at 77 K showed a relatively broad band

for the O–O emission; however, this is a function of the broad instrumental band pass. When narrower slits were used, it was apparent that the O–O band was composed of more than one band indicating heterogeneous environments for the various tryptophans.)

Other muscle proteins were examined for phosphorescence. We did not observe phosphorescence for sarcoplasmic reticulum vesicle (in 0.1 M KCl, 5 mM $MgCl_2$ and 10 mM imidazole (pH 7.2)) or for myosin (in 0.02 M phosphate buffer (pH 7.0)). Our instrument would be incapable of measuring phosphorescence with lifetimes < 2 ms. The failure to observe phosphorescence could be due to short lifetimes, low yields, or both.

The detection of phosphorescence in liquid samples of parvalbumin and actin depended upon the anaerobiosis of the sample. When the enzyme system was not included, or the sample was left uncovered and, therefore, exposed to atmospheric oxygen, no phosphorescence was observed.

4. DISCUSSION

Protein phosphorescence with lifetimes longer than milliseconds in liquid water, is quite unusual and apparently, can only be seen in tryptophans which are protected from the solvent by the polypeptide chain. Because the lifetime of tryptophan phosphorescence is so long, it should be very sensitive to rather small changes in the conformation of the polypeptide. A dramatic example of this is our observation that Ca^{2+} is a requirement for phosphorescence from parvalbumin. Phosphorescence may be of use to monitor the function of specific proteins in cells. For instance, by phosphorescence intensity or polarization it may be possible to monitor the aggregation state of actin in cells or the binding of calcium to parvalbumin. A limitation to the use of phosphorescence in cellular systems, is that oxygen quenches the phosphorescence and, therefore, it can only be seen in anaerobic sample. It may be possible, however, to use the lifetime of phosphorescence to measure oxygen concentrations in cells and tissues.

An interesting sidelight to these results is the possibility to explain an old observation. In 1746, Beccario reported that a long-lived luminescence was observed when a cold hand was exposed to sun-light [8]. The luminescence depended upon the ultraviolet portion of the spectrum since it could not be observed if the sun-light was first passed through glass. An interpretation is that an anaerobic condition was produced by cold and that phosphorescence from the tissue could indeed be seen by the dark-adapted eye.

ACKNOWLEDGEMENT

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