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A NEW GLOW PEAK IN *RHODOPSEUDOMONAS SPHAEROIDES*

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Abstract—A new glow peak at 120 K has been observed in *Rhodopseudomonas sphaeroides* and in its carotenoidless green mutant. This peak (labelled Z_n), which is composed of two peaks at 120 and 150 K, appears when the bacteria are illuminated with white light while being cooled to 77 K and then warmed in darkness at a heating rate of 10 K per min. Delayed light emission and prompt fluorescence spectra show peaks around 530, 610 and 660 nm. The action spectra of light emission show a major peak at 410 nm and a smaller peak around 545 nm. The pigment responsible for the light emission is also leached out in the suspension medium. The chromophore responsible for the light emission appears to be magnesium protoporphyrin IX, not bacteriochlorophyll.

INTRODUCTION

The study of delayed light emission (DLE)† has proved to be very useful in providing information regarding the mechanism of quantum conversion, charge storage during photochemical reactions, membrane potential, etc. Fleischman and Mayne (1973) have recently reviewed how the study of light emission by photosynthetic organisms could be used in different ways to probe the mechanisms of photosynthesis. Thermoluminescence (TL)† is one of the techniques that has been used for studying DLE in photosynthetic organisms. In higher plants as well as in algal cells it has yielded valuable information about the electron transport and energy storage mechanisms (Desai *et al.*, 1975; Sane, 1975; Lurie and Bertsch, 1974; and Ichikawa *et al.*, 1975).

Arnold and Thompson (1956) were the first to discover DLE in photosynthetic bacteria *Rhodospirillum rubrum*. Fleischman (1971) observed glow peaks in *Rhodopseudomonas viridis* and delayed light in *R. rubrum*. Recently Fleischman (1974) and Carithers and Parson (1975) have made a detailed analysis of DLE from *R. viridis*. They showed that DLE is from bacteriochlorophyll and suggested it to be due to back reaction of primary reactors of photosynthesis. We present here the data relating to the appearance of a new major glow peak in the range of 120–130 K in *Rhodopseudomonas sphaeroides*. Experiments with sharp cut-off color filters indicate that this peak is not from bacteriochlorophyll, but from a chromophore emitting light in the 500–700 nm range. Delayed light emission and fluorescence spectra at room temperature and fluorescence spectra at 77 K establish that the major emission bands are at 530, 610 and 660 nm; an additional band around 635 nm is also observed in some samples. The action spectra show a major peak at 410 nm and smaller bands in the 545 nm region; an additional band at 250 nm is observed in some samples. It appears that the new glow peak discovered here is from the same chromophore (Mg protoporphyrin IX) that gives DLE from *Rhodopsirillum rubrum* and *Rhodopseudomonas sphaeroides* recently observed by Arata *et al.* (1974).

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†Abbreviations used: DLE, delayed light emission; TL, thermoluminescence.

MATERIALS AND METHODS

Rhodopseudomonas sphaeroides and its green mutant were grown in a culture medium anaerobically. Cells were cultured for 2 days in fluorescent light (two tubes of 40 W at a distance of 5–7 cm). They were centrifuged for 5 min at a speed of 5000 g. Pellets suspended in phosphate buffer (0.05 *M*, pH 7.8) were used for glow peak measurements. Thick suspensions allowed anaerobic condition to predominate. For DLE and fluorescence measurements, samples were diluted to an optical density of about 0.3 at 400 nm. Thermoluminescence peaks were measured by an instrument described earlier (Tatake *et al.*, 1971) and 10 ms delayed light emission and fluorescence spectra were measured on an Aminco Bowman Spectrophotofluorometer with phosphoroscope accessory. Front surface illumination was used for fluorescence measurements and for 77 K measurements samples were frozen in liquid nitrogen and used without the glass sample holder to avoid artifact emission from glass.

RESULTS AND DISCUSSION

Glow peaks

Figure 1 shows the glow curves for *Rhodopseudomonas sphaeroides* and its green mutant. Three peaks are observed at about 120, 260 and 290 K (Curves A and B). The 120 K peak is composed of at least two peaks as shown in the curve C. We have concentrated our studies on the 120 K peak. A peak at 120 K also appears in bacteriochlorophyll solution when irradiated with γ-rays and seems to resemble the

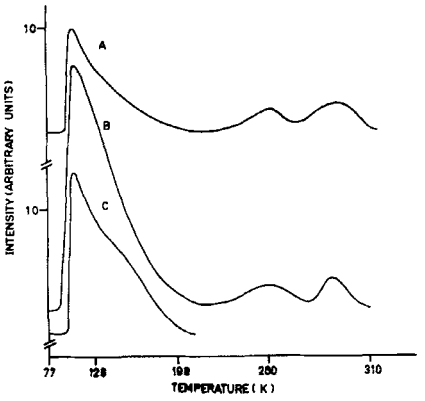

Figure 1. Glow curves of *R. sphaeroides*. (A) Wild type. (B) Green mutant. (C) Resolved glow peak showing two components of the Z_n peak.

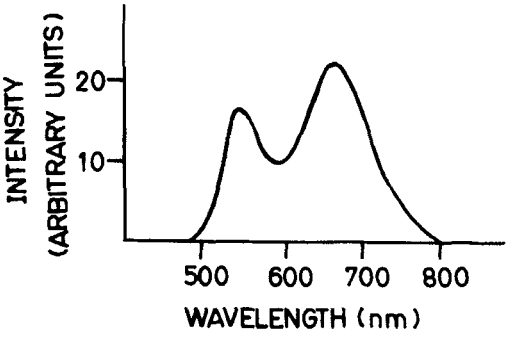

Figure 2. Delayed light emission spectrum of carotenoidless green mutant of *R. sphaeroides* at room temperature.

Z-peak observed in chlorophylls isolated from plants (Sane *et al.*, 1974). However, the glow peak observed here is not from bacteriochlorophyll as it can only be observed with S-20 (EMI 9558B) but not S-1 photomultiplier (EMI 9684B). This does not imply that a low intensity peak equivalent to *Z* peak does not appear in photosynthetic bacteria because acetone-extracted *R. sphaeroides* samples show such a peak with S-1 photomultiplier (unpublished data). In view of the differences with the usual *Z* peak, we have labelled our new peak as Z_n peak.

Table 1 shows that the emission for the Z_n peak is in the 500–700 nm range as filters transmitting light less than 500 nm and greater than 700 nm show an extreme reduction in its intensity and a full complement of this peak is observed with filters transmitting all wavelengths less than 700 nm. In order to better characterize the major emission from our samples, we present the DLE and fluorescence spectra.

Delayed light emission at room temperature

Delayed light emission spectra, as excited by various wavelengths in the 250–500 nm range, show two emission bands at 530 and 660 nm in the mutant (Fig. 2). The 660 nm band can be resolved into two bands. Similar results were obtained on using the wild type. The relative ratio of 530 and 660 nm bands was variable depending on the sample and on the wavelength of excitation, suggesting independent sources of these two emission bands. As the intensity of fluorescence is much higher than that of DLE, the rest of the work was done on fluorescence.

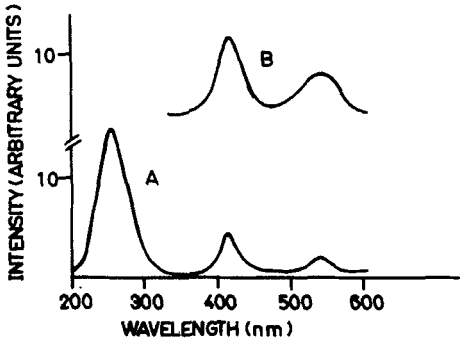

Figure 3. (A) Action spectrum of delayed light emission of carotenoidless green mutant of *R. sphaeroides* at room temperature. (B) Same as A except at 2.5× higher gain to magnify 410 and 545 nm bands.

Table 1. Spectral characteristics of Z_n glow peak appearing at 120 K

Filter		Transmission characteristics	Intensity arbitrary units
None	—		102
None	—		106
Kodak	15	Sharp cut-off; 5% at 515 nm; 50% at 528 nm; 80% at 544 nm; 88% 750 nm onwards.	80
Kodak	25	Sharp cut-off; 5% at 588 nm; 50% at 598 nm; 80% at 612 nm; 85% 630 nm onwards.	78
Kodak	29	Sharp cut-off; 5% at 608 nm; 50% at 621 nm; 80% at 637 nm; 86% 660 nm onwards.	40
Kodak	35	Peak at 410 nm (37%); 18% at 388 and 444 nm; 0% from 470 to 650 nm; 5% at 660 nm; 50% at 682 nm; 80% at 710 nm; 83% 720 nm onwards.	14
Kodak	45	Peak at 470 nm (35%); 18% at 447 and 511 nm; 0% from 540 to 695 nm; 5% to 705 nm; 50% at 732 nm; and 65% 760 nm onwards.	6
Kodak	58	Peak at 525 nm (59%); 30% at 503 nm and 570 nm; <10% from 593 nm to 726 nm; 50% at 755 nm; and 75% at 790 nm.	9

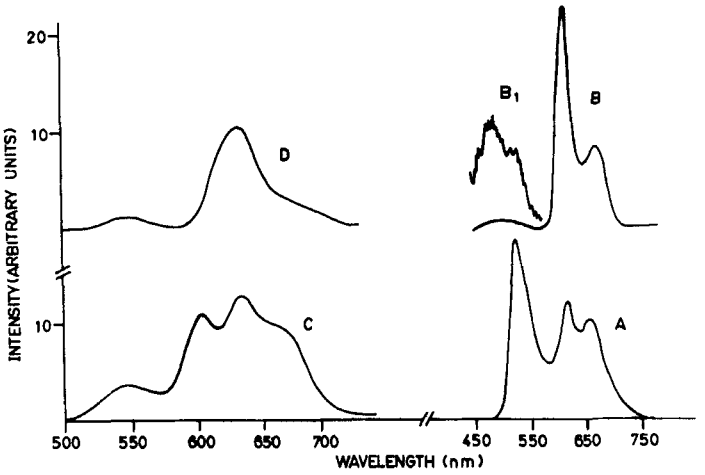

Figure 4. Fluorescence spectra of *R. sphaeroides* and its carotenoidless green mutant at room temperature using solid sample assembly. (A) Thick suspension of green mutant; excitation, 410 nm. (B) Supernatant of green mutant with 410 nm excitation. (B_1) Same as B except at 10× higher gain to bring out 480 and 530 nm bands. (C) Thick suspension of wild type, with 410 nm excitation. (D) Similar to C but with 435 nm excitation.

The action spectra of DLE show three bands at 250, 410 and 545 nm (Fig. 3). The 410 and 545 nm bands suggest that at least one of the emitting species is Mg protoporphyrin IX, as the bands coincide with the absorption spectrum of this pigment (Granick, 1961). The nature and role of 250 nm band was not investigated further.

Fluorescence

When fluorescence of *R. sphaeroides* mutant was excited with 410 nm light, three emission bands at 530, 610 and 660 nm were observed (Fig. 4, curve A). The buffer in which bacteria were suspended gave no signal even at much higher sensitivity.

Some of the fluorescent pigments must have leached out into the suspension medium, because the supernatant excited by 410 nm also gave three bands at 480, 610 and 660 nm (Fig. 4, curves B and B1). This observation is in agreement with that of Jones (1963) who has previously observed leaching of Mg protoporphyrin monomethyl ester from intact cells of *R. sphaeroides*. The emission spectra of the leached pigment and of the intact cells are somewhat different. The 530 nm band found in the cells (Fig. 4, Curve A) appears as a shoulder along with a new band at 480 nm in the supernatant (Fig. 4, Curve B_1). The ratio of 610 to 660 nm is also much higher than that in the cells; nevertheless, the general pattern is the same. The extracted form is possibly in a different environment than that in the cells. Perhaps, in the cell, the pigments are in more aggregated forms.

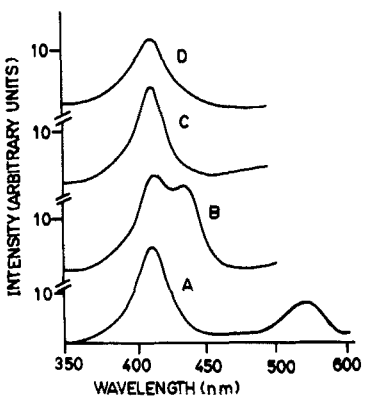

Figure 5. Action spectra of fluorescence of *R. sphaeroides* and its carotenoidless green mutant at room temperature. (A) Green mutant, fluorescence measured at both 610 and 660 nm. (B) Wild type, fluorescence measured at 635 nm. (C) Wild type, fluorescence measured at 610 nm. (D) Wild type, fluorescence measured at 660 nm; the 545 nm band is also present in the curves B, C and D, but is not shown here.

The action spectrum of the fluorescence in the green mutant gave peaks at 410 and 545 nm (Fig. 5, Curve A). These two bands are similar to the bands in the action spectra of DLE (Fig. 3), confirming the possible identity of this chromophore with Mg protoporphyrin IX.

Emission spectra of the wild type revealed an additional band at 635 nm (Fig. 4, Curve C), when 410 nm excitation is employed. This band is preferentially excited with 435 nm which does not yield other bands as prominently as with 410 nm excitation (Fig. 4, Curve D). The action spectra of fluorescence measured at 610, 635 and 660 nm reveal the main band at 410 and a minor one at 545 nm (Fig. 5). This is consistent with the assignment of the fluorescent chromophore to Mg protoporphyrin IX which has emission bands around 610 and 660 nm.

The 635 nm band, however, appears to be from another source, possibly a carrotenoid complex, as only the wild type contains carotenoids. This band is absent from carotenoidless mutant. The nature of the 530 nm band is not yet clear either. This band

reported here in fluorescence and DLE spectra is however, real and needs further study.

The spectral similarity of the TL peak and the fluorescence and DLE spectra indicate that, at least, one emitting species in photosynthetic bacteria is Mg protoporphyrin IX which is an intermediate in the biosynthesis of bacteriochlorophyll (Granick, 1961). Under the experimental conditions used the bacteriochlorophyll emission does not contribute *much* towards this glow peak. In fact, it appears that Mg protoporphyrin IX emission accounts for practically all the thermoluminescence observed around 120 K.

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