A NOVEL SHORT-LIVED EMISSION FROM THE PHOTOSYNTHETIC BACTERIUM *RHODOSPIRILUM RUBRUM*

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1. Introduction

When growing a mutant strain of *Rhodospirillum rubrum* (carotenoid-less mutant blue-green strain G-9) it was observed that the culture emits red light strong enough to be seen even directly in day light. The emission was also observed from dilute suspensions of chromatophores. Examination with a fluorimeter revealed that both the wild type and the mutant exhibited emission; however, the mutant's emission was about six times greater. This emission appeared in addition to the ordinary infrared emission of bacteriochlorophyl, and could not be attributed to bacteriochlorophyl due to the considerable mismatch between its wavelength and the absorption of bacteriochlorophyll, which occurs at much longer wavelengths.

A search into the literature did not reveal any observations of such fluorescence, except for a single report [1] on weak delayed light (in the msec time range) of similar spectrum, from chromatophores of *Rhodospirillum rubrum* and *Rhodopseudomonas spheroides*. This delayed light was only detectable under anaerobic conditions. Surprisingly, no mention was made in this report [1] of in vivo prompt fluorescence, although the authors reported that similar fluorescence is emitted by an isolated compound (according to them Mg-Protoporphyrin IX) in vitro. In this communication we wish to establish and characterize the red fluorescence from *R. rubrum*.

2. Materials and methods

The growth and isolation of chromatophores was as previously described [2–4]. The mutant was grown in the same way as the wild type except that after innoculation the cells were stored for 24 h in the dark so as to exhaust all oxygen by respiration, and only then illuminated. Bacteriochlorophyll was determined using absorbance coefficients in vivo as given by Clayton [5]. The standard sample contained Tricine NaOH buffer (10 mM, pH 7.5) $MgCl_2$ (3.3 mM) and chromatophores (9 μM bacteriochlorophyll). Anaerobiosis was maintained by the addition of the enzymatic system glucose/glucose oxidase which traps O_2 (30 mM glucose, 500 μM glucose oxidase). Fluorescence measurements were carried out in the Hitchi Perkin Elmer MPF 3 spectrofluorimeter (excitation and emission spectra).

A search for possible changes of the fluorescence yield with time and with light intensity was carried out in a locally built fluorimeter, as described in [6]. The instrument for the measurement of fluorescence decay kinetics was of the type described by Hundley et al. [7] and modified as described elsewhere [8]. The fluorescence was excited by a Deuterium flash lamp TRW, with light of 420 nm isolated by a Jarrel Ash double monochromator (Model 82-410) band width 14 nm. The fluorescence of the chromatophore suspension was passed through a combination of Schott KV 470 and Balzers K-5 filters.

The fluorescence decay data were analyzed by the method of non linear least squares as described by Grinvald and Steinberg [9], on an IBM computer Model 370/165 using the NLIN computer program [10].

3. Results and discussion

Fig.1 depicts the emission spectrum between 500 and 700 nm resulting from excitation at 422 nm of

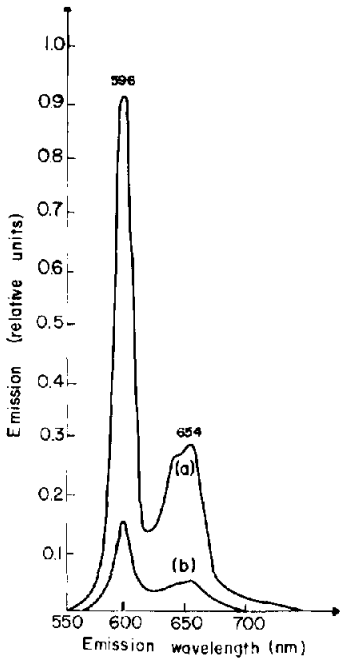

Fig.1. Fluorescence emission spectrum excited at 422 nm: (a) carotenoid-less mutant, (b) wild type. The spectra were obtained at equal absorbance at 422 nm (0.35 O.D.) Conditions as in Materials and methods.

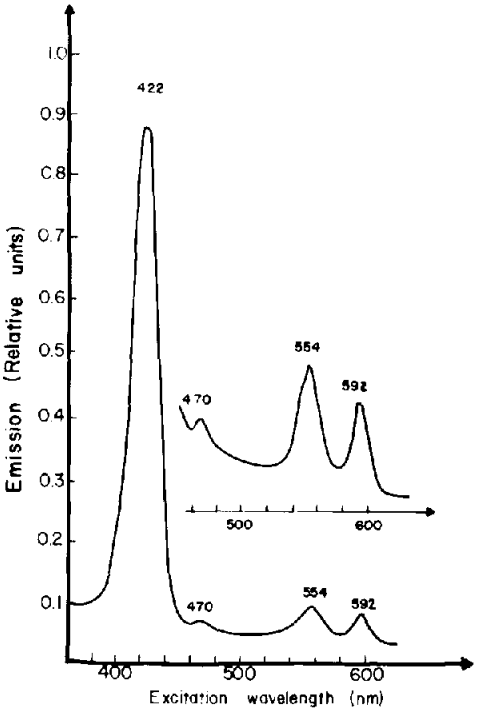

Fig.2. Excitation spectrum of the 654 nm emission band in the carotenoid-less mutant. Conditions as for fig.1.

chromatophores from the cariotenoid-less mutant 1(a) and the wild type 1(b). The spectra were obtained at equal absorption at 422 nm for both species. The emission is characterized by a main band centered at 596 nm and a second broader band which was lower in intensity, centered at 654 nm. The emission spectra from the wild type and the mutant seem completely similar. However, the emission intensity from the wild type is 6-fold less than the emission from the mutant.

The excitation spectrum of the 654 nm emission peak is shown in fig.2. It consists of a large sharp peak centered at 422 nm, and three satelite bands at 470, 554 and 592 nm. The excitation spectra for the wild type and the mutant were found to be quite similar.

The decay curve for the emission F(t), excited by a short flash of 7 nsec duration, G(t), is presented in fig.3. An extensive treatment of the methods of analysis of the fluorescence decay curves appears in [9]. According to this analysis the mean life time for the wild type and the mutant emission was found to be equal (5.7 ± 0.9 nsec for the wild type and 6.9 ± 1 nsec for the mutant) enabling to classify the emission as short lived fluorescence.

A more exact analysis, searching for several exponential contributions of the fluorescence decay [9], reveals that very probably the decay in the mutant is composed of two main exponential factors (about 2/3 of the decay has a decay time 1 nsec and the remaining 1/3 has a decay time of 9.3 nsec). Unfortunately the signal/noise ratio in the wild type fluorescence was too low to yield a reliable estimation of two exponentials. Still, however, the mean life time in the wild type is quite accurate.

We have searched for possible changes in the fluorescence that would indicate whether it may be linked in a dynamic way to the photosynthetic process. Such changes were observed with bacteriochlorophyll fluorescence (e.g. fluorescence induction: change in quantum yield with the time of irradiation [11]) and are generally typical of chlorophyll fluor-

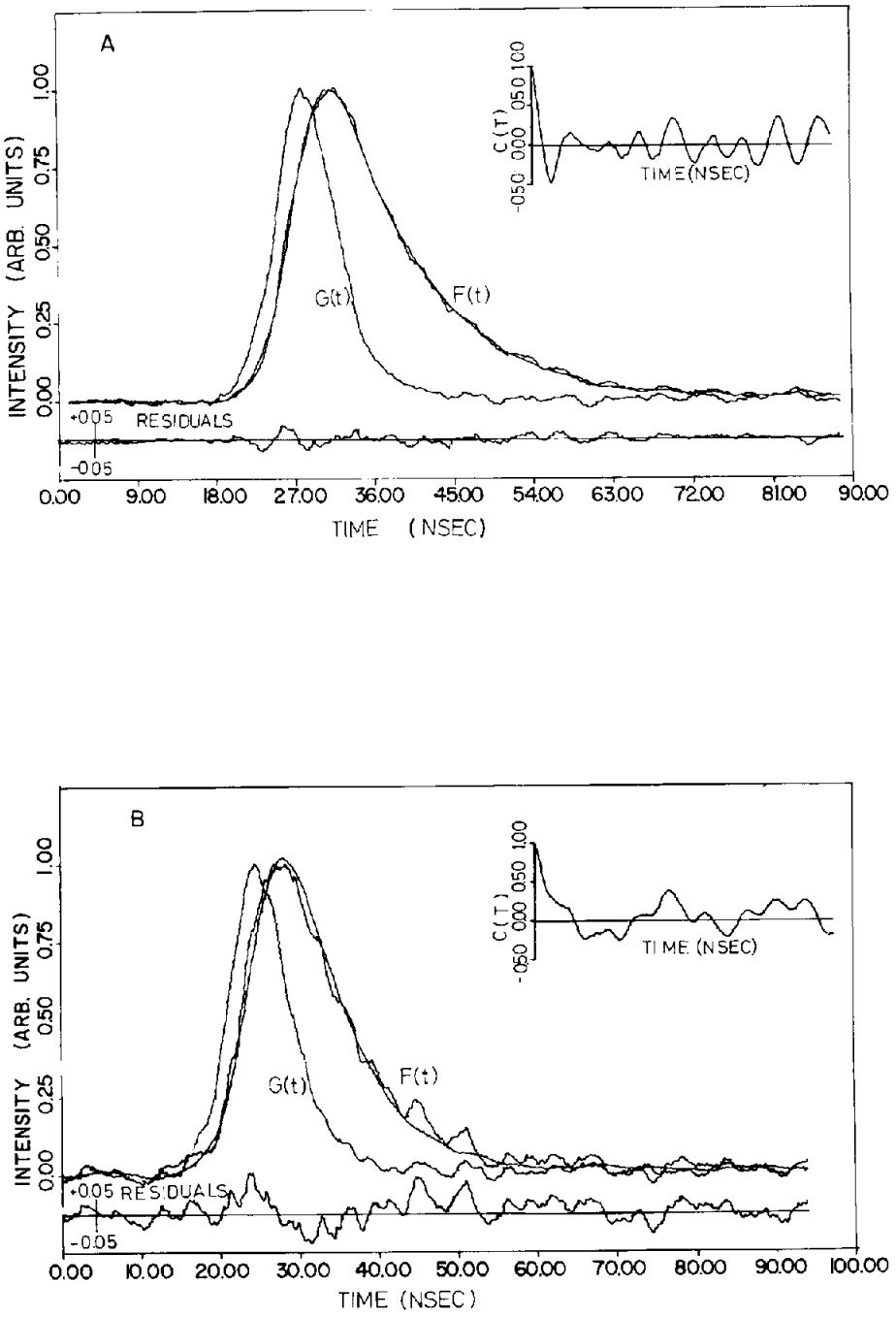

Fig.3. Fluorescence decay of the carotenoid-less mutant (A) and the wild type (B) measured at 600 nm and excited at 420 nm. F(t): the experimental fluorescence decay curve. Superimposed on F(t) is the best fitted decay curve assuming a mono-exponential decay function in (B) and bi-exponential decay function in (A). The trace of the deviations between the experimental and calculated decay curves is presented under the decay curves and is designated as 'Residuals'. (Insert) The normalized auto-correlation functions of the deviation.

escence in vivo for all types of photosynthetic organisms.

However, in the range of exciting light intensities used ($4.5 \times 10^3 - 5.0 \times 10^5$ erg/cm^2 sec) of 436 nm mercury arc line, the fluorescence intensity remained proportional to the exciting light intensity and no change with time could be observed. In this respect this emission resembles an analogous emission from the accessory pigments (phycocyanine, phycoerythrine) in the blue green and red algae.

Arata et al. [1] reported that the delayed light emitted in the same spectral region is influenced by the environment. It is seen only under anaerobic conditions and is quenched by the electron transport inhibitors orthophenanthroline, and by the uncoupler CCCP*. In our experiments the fluorescence was only slightly affected if at all by these treatments; Anaerobic conditions had no influence; 10^{-3} M orthophenanthroline [12] caused a quenching of 30% and 10^{-6} M FCCP had a negligible effect.

Since the emission was found to be six-fold greater in the carotenoid-less mutant, an attractive explanation could be that the emission in the wild type is dynamically quenched via the carotenoids. However, since no difference existed between the mean life time of emission in the two species, this possibility is not likely, due to the expected proportionality of fluorescence quantum yield and life time, which is not shown here. An alternative could be that the emitting pigment exists in relative smaller amounts in the wild type. From fig.4, which depicts the spectra obtained at equal concentrations of bacteriochlorophyll (15 μg/ml) for the mutant (a) and the wild type (b) as well as the difference between them, (c), it is clearly seen that there is a pigment absorption at 422 nm which is seen in the mutant absorption spectrum, and it is possible that this is the pigment which gives the emission described here. In the wild type the amount of this pigment is much smaller; it is not observed in the absorption spectrum and gives a relatively smaller emission.

Due to the constant value of the fluorescence yield through the range of light intensities used, and due to the absence of fluorescence induction, we are

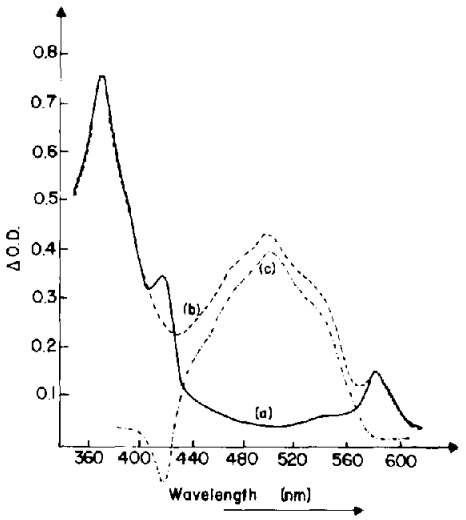

Fig.4. Absorbance spectra of the chromatophores at equal concentration of bacteriochlorophyll. (a) Carotenoid-less mutant (b) wild type (c) = b – a.

not able to determine at this stage whether the pigment responsible for the red emission plays a major role in primary photosynthetic events or whether it is of secondary importance. However, we have obtained some preliminary results indicating that 420 nm excitation alters the emission characteristics of bacteriochlorophyll (as measured at 902 nm).

Further investigations as to the nature and role of this emitting pigment is now in progress.

Abbreviations: FCCP: Carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; CCP: Carbonyl cyanide *m*-chlorophenylhydrazone.

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