PICOSECOND TIME SCALE OF HETEROGENEOUS EXCITATION ENERGY TRANSFER FROM ACCESSORY LIGHT-HARVESTING BACTERIOVIRIDIN ANTENNA TO MAIN BACTERIOCHLOROPHYLL *a* ANTENNA IN PHOTOACTIVE PIGMENT–PROTEIN COMPLEXES OBTAINED FROM *CHLOROBIUM LIMICOLA*, A GREEN BACTERIUM

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Received 11 April 1980

1. Introduction

To understand the structure and function of the photosynthetic unit, pigment–protein complexes have been extracted from photosynthetic membranes. In such studies with green bacteria, the progress of preparative biochemistry proved to be decisive [1–8]. The available data provide information about the homogeneous excitation energy transfer from antenna bacteriochlorophyll *a* (BChl *a*) to P840 reaction centers of green bacteria [5,6,9], whereas hardly anything is known about the heterogeneous energy transfer from the major antenna component, bacterioviridin (BVr), to the minor one, BChl *a*. In intact cells, BChl *a* constitutes only 5% of all light-harvesting pigments; 95% of the antenna pigments are represented by BVr. A knowledge of heterogeneous energy transfer parameters is necessary for describing the model of energy transfer dynamics within the photosynthetic unit of a bacterial cell.

Here, data on the rate and quantum efficiency of heterogeneous energy transfer from BVr to BChl *a*, using a new method developed specifically for phase fluorometry, is reported.

2. Materials and methods

Chlorobium limicola cells (3–5-days-old) were grown anaerobically under illumination [10]. Photochemically active complexes enriched in P840 were isolated by sonication and subsequent centrifugation as in [3]. Light-induced changes in the relative fluorescence yield and the absorption changes corresponding to the photobleaching of P840 were recorded as in [11]. Absorption spectra were measured with a Hitachi EPS-3 spectrophotometer. Fluorescence spectra were measured with the instrument in [12].

The phase fluorometer operating at $\nu = 12.3 \times 10^6$ Hz was used for fluorescence lifetime measurements [6]. Time resolution was $\sim 5 \times 10^{-11}$ s.

3. Results and discussion

To study the heterogeneous energy transfer, we used photochemically active pigment–protein complexes (PPC) in which the BChl *a* and BVr fluorescence components are almost equal. In all samples the ratio between the concentrations of BChl *a* and P840 was constant and equal to 90:1, as in vivo. The BVr concentration was variable (3–20-times lower than that in intact cells), with variations depending on the sonication regime. The absorption and fluorescence spectra of PPC are shown in fig.1. The photochemical activity of PPC did not depend on the BVr concentration. This is shown in fig.2: the quantum yields of P840 photooxidation were determined for two PPC samples, with different BChl *a*/BVr ratios, from the dependence of the rate of P840 photo-oxidation on the concentration of photo-oxidized P840. This method, as well as the justification for its application, are detailed in [12]. An analysis by the time-lever method of light dependence of the relative fluorescence yield and lifetime of BChl *a* and of the photooxidized portion of P840, as in [6], showed that in

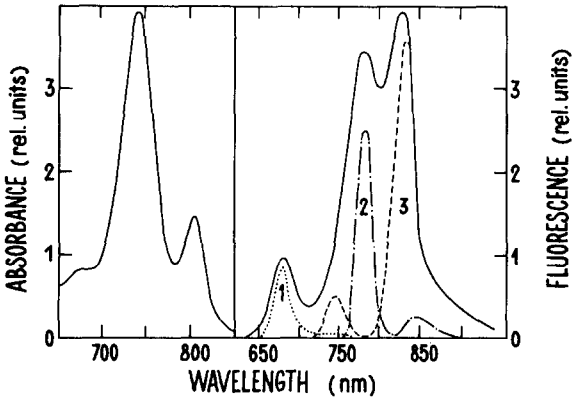

Fig.1. Absorbance and uncorrected fluorescence spectra of the photochemically active pigment–protein complex obtained from *Chlorobium limicola*. Excitation with mercury line 436 nm was used. Absorbance peak near 750 nm and fluorescence peak near 770 nm belong to bound antenna BVr, absorbance peak near 810 nm and fluorescence peak near 820 nm belong to antenna BChl *a*. Absorbance peak near 670 nm and fluorescence peak near 680 nm belong to BVr both in monomeric and pheophytinized forms. (1–3) Transmittance spectra of three filter combinations used for the examination of BVr and BChl *a* fluorescence.

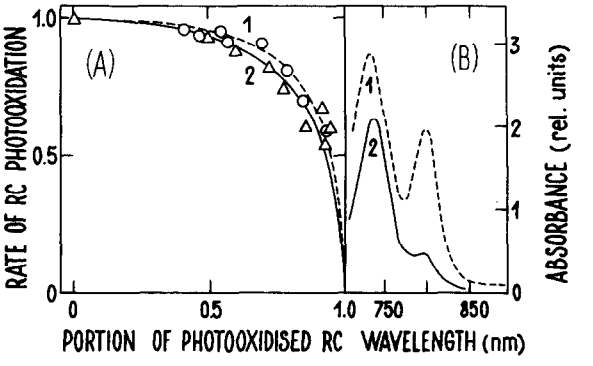

Fig.2. The rate of P840 photooxidation as a function of the portion of oxidized P840 (A); circles and triangles: experimental points for two different samples of pigment–protein complexes, corresponding to two theoretical curves (1,2) for the quantum yield of P840 photooxidation, equal to 93% and 92%, respectively. The portion of oxidized P840 was determined as a normalized absorbance change at 828 nm (one of the characteristic maxima of long wave P840 photobleaching). (B) Absorbance spectra of the two samples of pigment–protein complexes used.

the PPC investigated ~10% of the BChl *a* molecules are inactive in photosynthesis and emit fluorescence with a quantum yield of ~0.1 and a lifetime of ~2 ns; ~90% of BChl *a* molecules transfer the excitation energy to the photosynthetic chain and emit fluorescence, the quantum yield and lifetime of which are governed by the redox state of P840 and are equal to 0.001–0.002 and 20–60 ps, under the conditions of light-unsaturated photosynthesis.

The character of light dependence of the relative quantum yield (ψ_m) and lifetime (τ_m) of BVr and BChl *a* fluorescence in the PPC proved to be analogous, i.e., with increasing excitation light intensity, causing P840 photobleaching, increase in the measured relative fluorescence yields of BChl *a* and BVr was accompanied by a decrease in their fluorescence lifetime (table 1). The antibatic character of the light dependence of ψ_m and τ_m of BVr indicates that the measured BVr emission is heterogeneous [6,13]. The heterogeneity of BVr emission can be proved independently by comparison of two experimentally determined ratios: ψ_m/τ_m for the fluorescence of BVr in the PPC and ψ_s/τ_s for the fluorescence of BVr solution. These two values for a homogeneous fluorescence system must be equal, as the intrinsic lifetime value $\tau_o = \tau/\psi$ remains unaltered on transition from an in vivo to an in vitro system [12]. Acetone and methanol were used as solvents for BVr. Our experiments showed that the ψ_m/τ_m value for BVr in the PPC differed significantly (in β time) from the ψ_s/τ_s value for BVr in solution (see table 1 and [18]).

The antibatic character of the behaviour of ψ_m and τ_m for BVr in the PPC shows [13] that the measured fluorescence of BVr results from a superposition of the two components: the constant long-living component with constant quantum yield (ψ_c) and lifetime (τ_c) values and the variable, short-living component with variable quantum yield (ψ_v) and lifetime (τ_v) values governed by the redox state of P840. Then, the ψ_m and τ_m values measured with a phase fluorometer must satisfy the following equations [13]:

Table 1
The measured characteristics of BVr emission in the pigment–protein complex

Excitation intensity (erg/cm^2 . s)	ψ_m (rel. units)	τ_m (ns)	β
6×10^2	1.00 ± 0.03	2.60 ± 0.05	7.6–8.6
6×10^4	1.50 ± 0.04	2.20 ± 0.05	4.3–4.9

$$\tau_m = \tau_c \frac{1+\alpha x^2}{1+\alpha x}; \quad \psi_m = \psi_c \frac{1+\alpha x}{1+\alpha}$$

where $x = \tau_v/\tau_c$, $\alpha = C_v/C_c$ and C_v and C_c are relative concentrations of the corresponding fractions of BVr antenna molecules emitting the variable and constant components of BVr fluorescence, respectively. Then, the above-mentioned β value is equal to:

$$\beta = \frac{\psi_s/\tau_s}{\psi_m/\tau_m} = \frac{(1+\alpha)(1+\alpha x^2)}{(1+\alpha x)^2}$$

For the determination of the unknown parameters, τ_c, τ_v and α, only four values have to be measured: in two different conditions, under which τ_v has different values, two pairs of τ_m and β values have to be determined. Let us denote them as τ_1, τ_2, β_1 and β_2. For these four measured values a set of four equations, with four unknown values $(\tau_c, \alpha, x_1, x_2)$, can be evolved:

$$\tau_1 = \tau_c \frac{1+\alpha x_1^2}{1+\alpha x_1} \quad (1)$$

$$\tau_2 = \tau_c \frac{1+\alpha x_2^2}{1+\alpha x_2} \quad (2)$$

$$\beta_1 = \frac{(1+\alpha)\cdot(1+\alpha x_1^2)}{(1+\alpha x_1)^2} \quad (3)$$

$$\beta_2 = \frac{(1+\alpha)\cdot(1+\alpha x_2^2)}{(1+\alpha x_2)^2} \quad (4)$$

The above method of measurement of short-living fluorescence proves to be universal. The time-lever method used in [6,13] is only applicable in a particular case, since for the set of equations to be complete, use was made of a certain analytical dependence of the fluorescence quantum yield of the main light-harvesting pigment (in which the reaction centers are just embedded) on the redox state of the reaction centers; in our case the validity of the Vredenberg-Duysens model was postulated [14]. In the method used here, the above-mentioned analytical dependence is not needed. Moreover, the Vredenberg-Duysens model is to be revised in accordance with the experimental data on the quenching properties of the closed reaction centers [15–17].

By solving the set of four equations (1–4) we can determine the parameters of both BVr fluorescence components: the variable (photosynthetic) one with ψ_v and τ_v values correlating with the redox state of P840 and the constant one (background) with constant ψ_c and τ_c values. The absolute quantum yields of BVr emissions can be calculated from the relation $\psi = \tau/\tau_o$, where the intrinsic lifetime of BVr (τ_o) is equal to 18 ns [18].

The main characteristics of both BVr emission components are presented in table 2. About 90% of the BVr molecules participate in excitation energy transfer to BChl *a* and this takes place in light-unsaturated photosynthesis within 20–50 ps.

The low quantum yield of the photosynthetic fluorescence (~0.001) of light-harvesting BVr, compared to the quantum yield of BVr fluorescence in solution (~0.1), means that the efficiency of energy transfer from BVr to BChl *a* in the photosynthetic fractions of the PPC molecular antenna is >95%.

The quantum yield and lifetime of the BVr emission with absorption peak of about 670 nm were constant at all the excitation intensities investigated ($\tau \simeq 5$ ns).

Thus, the heterogeneous excitation energy transfer from BVr to BChl *a* in the PPC takes place in ps time scale with an efficiency close to 100%.

Table 2
The characteristics of both BVr fractions, photosynthetic and background, under conditions when photosynthesis is light unsaturated

Photosynthetic BVr fraction			Background BVr fraction		
τ_v (ps)	ψ_v	C_v (%)	τ_c (ns)	ψ_c	C_c (%)
20–50	$(1-3)\ 10^{-3}$	88–91	2.7–3.0	$(1.5-1.7)\ 10^{-1}$	9–12

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