SPECTRAL AND ENERGETIC CHARACTERISTICS OF THE PHOTOACTIVE PARTICLES OBTAINED FROM CHROMATOPHORES OF THE GREEN BACTERIUM *CHLOROBIUM LIMICOLA*

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1. Introduction

The bulk of chlorophyllous pigments of green sulphur bacteria are represented by bacterioviridin, which is connected with a minor component-bacteriochlorophyll *a* (BChl*a*) on the energy migration level [1]. Cytochrome *c* [2] and reaction center P840 [3] were detected in green bacteria. Low content of P840 (one P840 per 1000–1500 light-harvesting chlorophyll molecule [4] makes investigations of the primary photosynthesis processes essentially difficult. The fractionation of *Chlorobium limicola* chromatophores with detergent made it possible to obtain photochemically inactive BChl*a*–protein complexes lacking in bacterioviridin [5]. On the other hand photoactive particles were prepared with French-press treatment of the chromatophores and subsequent centrifugation in the discontinuous sucrose gradient [4]. These particles contained BChl*a*, photoactive P840 and cytochrome *c*.

In the present work photochemically active particles isolated from *Chl. limicola* were optically characterized, their primary redox transitions were examined and the quantum yield of primary energy trapping was precisely measured by means of relative methods.

2. Materials and methods

Chl. limicola cells 3–5 days old were grown anaerobically under illumination [6]. The cells were washed twice in 0.01 M phosphate buffer (pH 7.6), suspended in the same buffer with 10 mM sodium ascorbate and 0.1 mM dithiothreitol present at all times. Then the following steps were carried out at 0–2°C: a) sonication during 6 min; b) centrifugation at 40 000 *g* for 10 min; c) the supernatant was then centrifuged at 40 000 *g* for 60 min, during which most of the chromatophores were pelleted; d) chromatophores were suspended in the same buffer and again sonicated during 4 min; e) the material obtained was centrifuged at 40 000 *g* for 90 min.

Both the supernatant mostly containing light particles enriched with BChl*a* and the precipitate containing heavy particles were used in experiments. Light-induced absorption changes were registered as described elsewhere [7]. Absorption spectra were measured with the Hitachi EPS-3 spectrophotometer. The phase fluorometer [8] operating at the frequency of 12.3×10^6 Hz was used for fluorescence lifetime measurements. Time resolution was about 5×10^{-11} sec. The fluorescence spectra were measured with an instrument described earlier [9].

3. Results and discussion

The absorption bands of bacterioviridin and carotenoids were observed in *Chl. limicola* chromatophores (fig. 1A, curve 1). The light subchromatophore particles also contain a large quantity of BChl*a* with absorption peaks about 600 and 810 nm (curve 2). The absorption spectrum of heavy particles (curve 3) is similar to the one for chromatophores.

The uncorrected emission spectra for the above

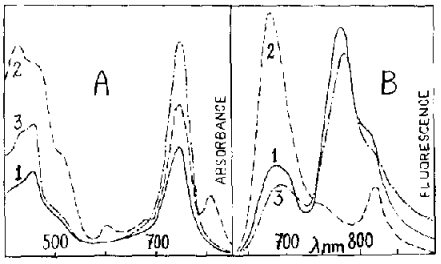

Fig. 1. Absorption and fluorescence spectra of chromatophore and subchromatophore particles from *Chl. limicola*. A) absorption spectra; B) fluorescence spectra; excitation with mercury lines 365, 404, 436 nm was used. 1 - chromatophores; 2 – light particles; 3 – heavy particles.

mentioned fractions are shown in fig. 1B. When excited with mercury lines 365, 404, 436 nm, the photoactive fraction had a major emission band about 670 nm (perhaps, the monomeric bacterioviridin), and additional band about 820 nm (BChl*a* emission). The shoulder at about 770 nm (bound bacterioviridin) was also checked.

The heavy particles exhibit very low (if any) photochemical activity. In light particles the exciting light readily caused photo-oxidation of *c*-type cytochrome and photobleaching of reaction centers (which appears to be photo-oxidation [4]) (figs. 2A and 2B). The differential spectrum corresponding to light-induced oxidation of reaction centers is characterized by two negative maxima at 828 and 845 nm. The

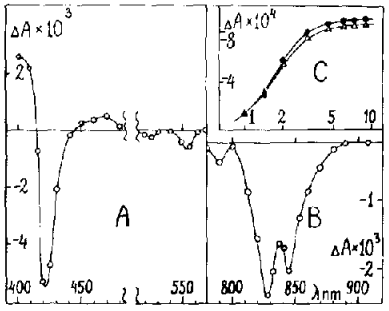

Fig. 2. The light-induced absorption changes of the light particles: A) spectrum of cytochrome *c* photo-oxidation; B) spectrum of reaction center photo-oxidation; C) light dependences of reaction center photo-oxidation measured at 815 (• – •) and 854 nm (△ – △). Light intensity in arbitrary units.

light dependences of photobleaching measured at 815 and 854 nm are shown in fig. 2C to be in close agreement. This fact may even indicate that both maxima at 828 and 845 nm belong to one species and hence the reaction center involves two associated chlorophyllous molecules (dimer or charge transfer complex) with the planes of their tetrapyrrolic rings forming a sharp angle [10]. The ratio of the bulk BChl*a* molecules to the ones photo-oxidized (we currently suggested the latter to represent reaction centers as 1:1) were obtained from the ratio of corresponding absorption band areas, and found to be equal to 100:1. Considering the BChl*a* in vivo extinction coefficient at 590 nm maximum to be equal to its in vitro value (28 $mM^{-1} \times cm^{-1}$ [11]) the 1.5:1 ratio for cytochrome *c* to reaction center was calculated using the millimolar extinction 13.3 $mM^{-1} \times cm^{-1}$ for cytochrome redox transition in α-band [12].

The rate of P840 photo-oxidation against the portion of photo-oxidized P840 is shown in fig. 3.

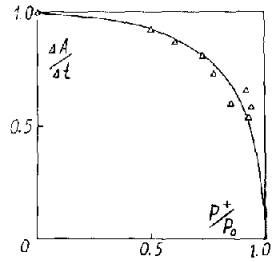

Fig. 3. The rate of reaction center photo-oxidation as a function of the portion of oxidized reaction centers; triangles – experimental points; solid line – theoretical curve for the quantum yield of reaction center photo-oxidation equal to 0.92. △ A was recorded at 828 nm.

Experimental points follow the theoretical hyperbolic curve quite satisfactorily. This highly nonlinear curve is only consistent with the multicentral type of PSU and corresponds to the quantum yield (φ_{ph}) of P840 photo-oxidation equal to 0.92. The method for φ_{ph} determination as well as the grounds for above mentioned considerations on the PSU type are described in detail elsewhere [7,13]. As the photochemical activity of light particles could be somewhat decreased in the course of their isolation procedures we consider the φ_{ph} to be:

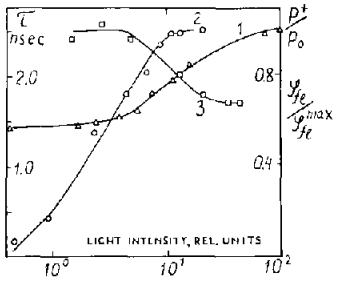

Fig. 4. Relative BChl*a* fluorescence yield in arbitrary units (1), the portion of oxidized reaction centers (2) and BChl*a* fluorescence lifetime (3) as a function of light intensity. The portion of oxidized reaction centers was determined as a normalized absorption change at 828 nm (one of the characteristic maxima of longwave photobleaching).

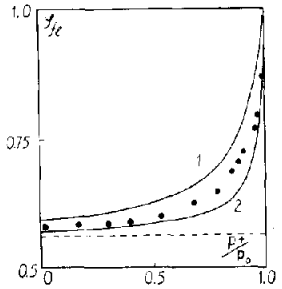

Fig. 5. Normalized BChl*a* fluorescence yield as a function of oxidized reaction centers. The experimental points restricted by theoretical hyperbolic curves (1;2) which are referred to the fluorescence background level as to the base line and correspond to φ_{ph} values equal to 0.92 and 0.97 respectively.

$$\varphi_{ph} \geqslant 0.92\ ^{+0.02}_{-0.03}$$

The quantum yield for P840 photo-oxidation was also determined by means of a fluorescence version of this relative method. The relative quantum yield of BChl*a* fluorescence (φ_{fl}^{BChl}) and P840 photo-oxidation ($\triangle A_{828}$) as functions of light intensity are shown in fig. 4. The observed changes of φ_{fl}^{BChl} and $\triangle A_{828}$ are in agreement with an idea [14] that the fluorescence and photosynthesis compete for singlet excitations. The BChl*a* fluorescence increases 1.7 times when photosynthesis becomes fully saturated. According to the theory for any PSU type it formally corresponds to a 41% quantum yield of photosynthesis sensitized by singlet excitations. However, it is in disagreement with the above value. Besides the increase in φ_{fl}^{BChl} has been accompanied by a decrease in BChl*a* fluorescence lifetime (τ_{fl}^{BChl}) under increased light intensity (fig. 4). This τ_{fl}^{BChl} behavior is an irrevocable indication that there are at least two fluorescing BChl*a* species. In fact, τ_{fl} and φ_{fl} must change proportionally to each other for homogeneous fluorescing system. The performed theoretical analysis [15] showed that the fluorescence lifetime measured on the phase fluorometer and fluorescence quantum yield may change antibatically when are at least two species of fluorescing molecules and the following conditions are satisfied: 1) background emission(s) has approximately constant $\varphi_{fl\,1}$ and $\tau_{fl\,1}$ while the values of variable 'photosynthetic' emission (coming from the major part of bulk pigment) $\varphi_{fl\,2}$ and $\tau_{fl\,2}$ increases greatly; 2) $\tau_1 > \tau_2$ 3) the intensity of the variable emission after increasing is of the same order of magnitude as the first one.

Having made an assumption that fluorescence consist of two spectrally indistinguishable emissions (the 'background' one with a constant yield and the 'photosynthetic' one with φ_{fl} depending on the redox state of P840) and performed the appropriate calculations with the experimental data (presented in fig. 4) by using method devised previously in our laboratory [7], we determined the quantum yield of photosynthesis (fig. 5):

$$\varphi_{ph} \geqslant 0.95\ ^{+0.02}_{-0.03}$$

The 20-fold increase in φ_{fl}^{BChl} when passing from active (at low intensity) to fully saturated (at high light intensity) photosynthesis strongly suggests that excitation energy migration occurs essentially *via* the singlet levels, i.e. at least on the stage of energy migration the contribution of triplet excited states is to be disregarded.

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