Excitation delocalization over the whole core antenna of photosynthetic purple bacteria evidenced by non-linear pump-probe spectroscopy

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Abstract Anomalously high values of photoinduced absorption changes were revealed in the antenna of photosynthetic purple bacteria. They were found to be 4–16 times greater at the bleaching peak of the antenna than at the bleaching peak of the BChl dimer of the reaction center. This is direct proof of excitation delocalization over many pigment molecules. Calculations according to the model of exciton delocalization over all core antenna BChls allow one to explain the observed phenomenon.

Key words: Exciton interaction; Bacterial photosynthesis

1. Introduction

Nowadays there are two theoretical models describing spectral and kinetic properties of the light-harvesting antenna of purple photosynthetic bacteria. The first model assumes excitation localization at the antenna BChl dimer [1] (let call it (L)ocalized (E)xciton theory or model (briefly the 'LE model'). The other model suggests exciton delocalization within a circular aggregate of N light-harvesting BChl molecules [2,3–5] (a model of exciton (D)elocalization over (C)ircular (A)ggregate or the 'DCA model'). The most recent data [6,7] shows that the antenna BChls are arranged in a form of regularly shaped ring. However, these structural data gives no information about exciton delocalization (or localization) in the antenna. Some kind of non-linear spectroscopy of the antenna presents a real possibility to discriminate between the LE and DCA models. It is known that the non-linear response of a molecular aggregate is proportional to the number of excitonically coupled pigment molecules [5]. Earlier we have noted an anomalously large value of antenna bleaching per absorbed quantum for several preparations from purple bacteria (briefly published only for the B890 complex from *C. minutissimum* [2,9]. The same phenomenon was observed for *C. vinosum* and *C. tepidum* [10].

In this paper we present results on the anomalous antenna bleaching per absorbed light quantum (or per bleaching of the BChl dimer of reaction center (RC)) for different purple bacteria and analyze them within the context of the DCA model.

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2. Materials and methods

The B890 pigment–protein complex was isolated from purple sulfur photosynthetic bacterium *C. minutissimum* according to the modified method of Moskalenko and Erokhin [11]. Cells of non-sulfur bacterium *R. rubrum* (wild-type strain No. 1 MGU) were grown and chromatophores were prepared as described previously [12]. The *R. viridis* membrane thylakoid fraction was prepared by a similar procedure as for chromatophores from *R. rubrum*. Samples were suspended in 0.05 M Tris–HCl buffer (pH 8.0). The reduced (or oxidized) state of reaction centers was controlled by sodium ascorbate with TMPD (10^{-4} M) (or ferricianide) addition. All measurements were carried out at room temperature. Sample absorbance in a 1 mm cell at the excitation wavelength was ~0.2.

The picosecond absorbance difference measurements were performed with the apparatus described in [13]. The spectrometer allowed one to measure the photoinduced absorption changes down to ~10^{-4} A units. The wavelengths of pump and probe pulses were tuned continuously and independently over the spectral range 400–1500 nm. Excitation energy could be varied over the range 10^{-11}–10^{-17} photon/cm^2 per pulse. Pump and probe pulses were well approximated by Gaussian-shape time profiles with FWHM equal to 18 ps. The repetition rate of the pulses was 1 Hz. The 0 ps tick on the time scales corresponded to the coincident pump and probe pulses.

3. Results

We are interested in the ratio $\xi = \Delta A_{ant}^{max}/\Delta A_{RC}^{max}$. In the case of *R. rubrum* chromatophores ΔA_{ant}^{max} is the antenna bleaching value at the 896 nm peak measured for the zero time delay between pump and probe ps pulses, and ΔA_{RC}^{max} is the bleaching of the BChl special pair of RC at 865 nm for a time delay of ~1000 ps. This ratio can be determined by a direct measurement of the photoinduced absorbance changes for the above wavelength/time delay pairs (direct determination) or recalculated from a single kinetic curve carried out for the 0–1000 ps time delay range at some wavelength in this spectral region.

Fig. 1 illustrates the ξ determination from the kinetic curve for *R. rubrum* chromatophores as an example. Besides a kinetic curve, one needs to obtain photoinduced absorbance difference spectra of the antenna and RC of the preparation at hand. These spectra are used for conversion from $\Delta A_{ant}^{\lambda}/\Delta A_{RC}^{\lambda}$ to $\Delta A_{ant}^{max}/\Delta A_{RC}^{max}$, where λ is the probing wavelength. In our example λ is equal to 845 nm; the corresponding spectra for the *R. rubrum* chromatophores are shown at Fig. 1 (insert).

The accuracy of the direct measurement of ξ may be increased. To do this one has to record dependencies of ΔA_{ant}^{max} and ΔA_{RC}^{max} on the pump pulse intensity under in the low intensity limit, then to approximate the experimental points by straight lines passing through the origin of the coordinates, and finally to calculate $\xi = tg\alpha/tg\beta$. This procedure is illustrated in Fig. 2 for a membrane thylakoid fraction from *R. viridis*.

The experimentally obtained values of ξ along with the calcu-

lated data are given in Table 1. The calculations were carried out on the basis of the antenna description proposed before [2,5].

We consider an antenna which has the form of a circular aggregate of N BChl molecules with the C_N-symmetry ($N = 24$). The BChl dimer (special pair) of RC is located at the center of the ring. The transition dipole moment of a BChl molecule is $\overline{d}$ and the transition dipole moment of the lowest exciton level of the special pair is $\overline{d}_R$. Dipoles $\overline{d}$ and $\overline{d}_R$ make angles ψ and ψ_R with the plane of the ring.

Another model is the C_N-symmetry ring consisting of N BChl dimers ($N = 12$). In this case $\overline{d}$ corresponds to the lowest exciton level of a dimer (we suppose a parallel dimer with forbidden higher level). Notice that all our main results are the same for these two models.

If polarization of pump and probe pulses is determined by vectors $\overline{e}_p$ and $\overline{e}$ respectively, then the difference absorption (per quantum) in the antenna and RC is equal to:

$$\Delta A_{ant} = \langle A_{01}(\overline{e}_p)[A_{12}(\overline{e}) - A_{10}(\overline{e}) - A_{01}(\overline{e})]\rangle \langle A_{01}(\overline{e}_p)\rangle^{-1} \quad (1)$$

$$\Delta A_{RC} = \langle A_{01}(\overline{e}_p)[-A_{01}^R(\overline{e})]\rangle \langle A_{01}(\overline{e}_p)\rangle^{-1} \quad (2)$$

where A_{01} is the absorption from the ground state of the antenna aggregate; A_{12} and A_{10} correspond to the excited state absorption and stimulated emission of the circular aggregate (see analytical expressions in [2,5]); A_{01}^R is the absorption from the ground state of the special pair (A_{12}^R and A_{10}^R can be neglected due to the fast oxidation of the excited state of the special pair); $\langle ... \rangle$ indicates the averaging over all possible orientations of $\overline{e}_p$ and $\overline{e}$.

The numerical calculations were made for the C_{12}-symmetry model with $d/d_R = 1$ (oscillator strength of the antenna dimer is equal to that of the lowest level of the special pair); $\psi = \pi/10$ and $\psi_R = \pi/10$. The gap between the two lowest exciton levels of the antenna is $\omega_1 - \omega_0 = 200$ cm^{-1}; the corresponding steady-state populations at room temperature are $p_1 = 0.212$,

Fig. 2. Dependencies of the absorbance difference changes of the antenna at 1035 nm (opened circles) and RC at 960 nm (closed squares) in the membrane thylakoid fraction from *R. viridis* on the intensity of the 1064 nm pump pulse. The straight lines passing through the origin of the coordinates fit experimental points according to the lowest square method.

$p_0 = 0.576$; the line shape of the antenna levels is $\exp(-\omega^2/\omega_A^2)$ with $\omega_A = 1.3(\omega_1 - \omega_0)$; for RC is $\exp(-\omega^2/\omega_R^2)$ with $\omega_R = 1.2\omega_A$.

We supposed that $\overline{e}_p$ and $\overline{e}$ are correlated in the measurements due to the zero delay between pump and probe and non-correlated in the ΔA measurements due to the excitation energy migration between antenna aggregates during the pump-probe delay (with the exception of the monocentral B890 complex).

4. Discussion

Notice that A_{01}, A_{12} and A_{10} values are proportional to Nd^2 whereas A_{01}^R is proportional to d_R^2. Thus the maximum allowable ξ value is $(A_{01} + A_{10})/A_{01}^R = 2Nd^2/d_R^2$, i.e. 24 in our DCA model. This maximum value is reached when the excited state absorption spectrum, A_{12}, does not overlap with the linear absorption band. Really the contribution from A_{12} results in decreasing of ξ from 24 to 10–12. The relative intensities of the spectral components (as well as ξ) also depend on excitation and probing conditions. In the case when pump and probe pulses interact only with the long-wavelength transition, ξ is equal to 6–8.

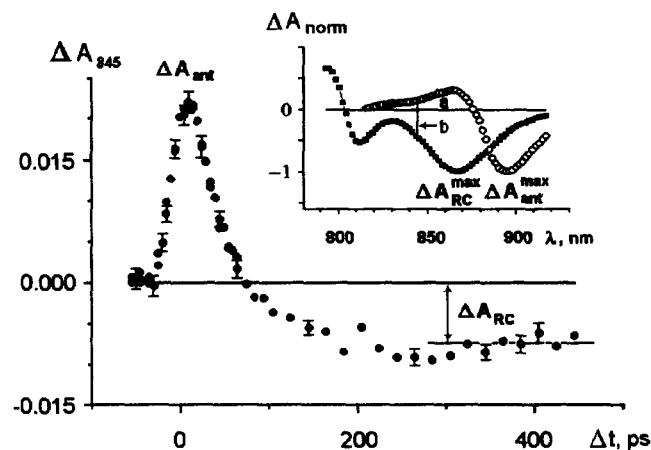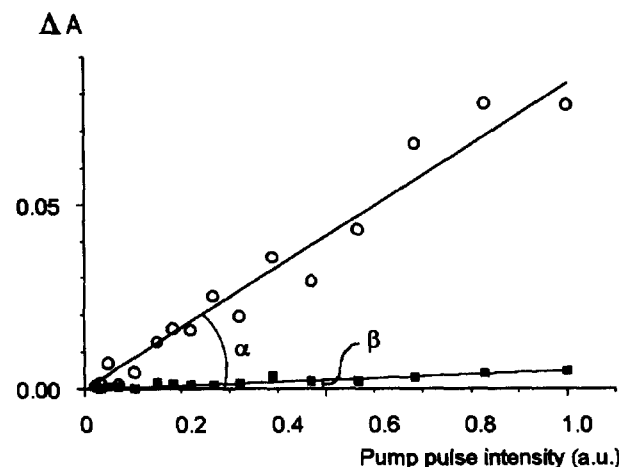For the LE model the maximum ξ value is 2; for the model

Fig. 1. The kinetic curve of the absorbance difference changes at 845 nm in chromatophores from *R. rubrum* induced by 940 nm pump pulse. The angle between pump and probe pulse polarization was equal to 54.7°; the intensity of the pump pulse corresponded to the absorption of <0.2 hv per RC. Insert: The absorbance difference spectra of the excited antenna (opened circles; the time delay between pump and probe pulses, Δt, is equal to zero) and of the photooxidized RCs (closed squares; $\Delta t \sim 1000$ ps) in the *R. rubrum* chromatophores. $\Delta A_{ant}^{max}/\Delta A_{RC}^{max}$ ratio can be calculated as $[\Delta A_{ant}(\Delta A_{ant}^{max}/a)]/[\Delta A_{RC}(\Delta A_{RC}^{max}/b)]$.

Table 1
Experimental and theoretical values of ratio $\xi = \Delta A_{ant}^{max}/\Delta A_{RC}^{max}$ for several preparations from purple bacteria

Sample	λ_{ex}	α	ξ_{exp}	ξ_{calc}
R. rubrum chromatophores	930 nm	0°	4.5	6.7
R. rubrum chromatophores	930 nm	54.7°	10.0	10.2
C. minutissimum complex B890*	930 nm	0°	4.2	9.6
C. minutissimum complex B890	930 nm	54.7°	15.0	10.8
R. viridis membrane thylakoid fraction	1064 nm	54.7°	15.8	10.2
C. vinosum chromatophores**	532 nm	54.7°	8.0	11.1
C. tepidum chromatophores**	532 nm	54.7°	16.0	11.1

Here λ_{ex} is the excitation wavelength, α is the angle between vectors $\overline{e}_p$ and $\overline{e}$, ξ_{exp} and ξ_{calc} are experimental and theoretical ξ values; * experimental data from [9]; ** experimental data from [10].

of exciton delocalization over the antenna globule, containing two dimers ($N = 2$), the maximum ξ value is 4 (the real ξ values would be <2 or 4). The experimental ξ values are not consistent with these models. On the other hand they agree well with the calculation according to the DCA model with 12 strongly coupled dimers or 24 monomers.

The variation of the calculated ξ values with ψ, ψ_R, ω_R and ω_A is not critical. But ξ essentially depends on d/d_R. For example the decrease in the oscillator strength of the special pair due to interactions with other RC pigments may account for the increase in ξ. This is one of the reasons why ξ differs by a factor of 2 for different bacteria under the same experimental conditions.

References

[1] Pullerits, T., Visscher, K.J., Hess, S., Sundstrom, V., Freiberg, A., Timpmann, K. and van Grondelle, R. (1994) Biophys. J. 66, 236–248.

[2] Novoderezhkin, V.I. and Razjivin, A.P. (1993) FEBS Lett. 330, 5–7.

[3] Novoderezhkin, V.I. and Razjivin, A.P. (1994) FEBS Lett. 345, 203–206.

[4] Novoderezhkin, V.I. and Razjivin, A.P. (1994) Photosynthesis Res. 42, 9–15.

[5] Novoderezhkin, V.I. and Razjivin, A.P. (1995) Biophys. J. 68, 1089–1100.

[6] McDermott, G., Prince, S.M., Freer, A.A., Hawthornthwaite-Lawless, A.M., Papiz, M.Z., Cogdell, R.J. and Isaacs, N.W. (1995) Nature 374, 517–521.

[7] Karrasch, S., Bullough, A. and Ghosh, R. (1995) EMBO J. 14, 631–638.

[8] Spano, F.C. and Mulkamel, S. (1989) Phys. Rev. A 40, 5783–5801.

[9] Abdourakhmanov, I.A., Danielius, R., Razjivin, A.P. and Rotomskis, R. (1986) in: Poster Abstracts 7th Int. Congr. Photosynthesis, pp. 303–043, Providence.

[10] Kennis, J.T.M., Aartsma, T.J. and Amesz, J. (1994) Biochim. Biophys. Acta 1188, 278–286.

[11] Moskalenko, A.A. and Erokhin, Yu.E. (1974) Stud. Biophys. 44, 17–32. (in Russian.)

[12] Isaev, P.I., Liberman, E.A., Samuiliv, V.D., Skulachev, V.P. and Tsofina, L.M. (1970) Biochim. Biophys. Acta 216, 22–29.

[13] Borisov, A.Yu., Razjivin, A.P., Danielius, R.V. and Rotomskis, R.I. (1986) Laser Chem. 6, 291–305.