FEBS Letters 345 (1994) 203–206

FEBS 14042

Picosecond absorbance difference spectra of the antenna of photosynthetic purple bacteria

The influence of exciton interactions and librations

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Received 4 April 1994

Abstract

The influence of exciton interactions and pseudolocal librational modes on the relaxation of photo-induced absorption changes of the light-harvesting antenna of the photosynthetic purple bacterium, *Rhodospirillum rubrum*, was studied in the ps time domain. A hypothesis is put forward that the long-lived librational modes can occur in the circular aggregate of light-harvesting bacteriochlorophyll molecules of the core antenna. The model spectra obtained quantitatively fit the shape of the photo-induced picosecond absorbance difference spectra. The model predicts the decay of photo-induced spectra with the time constant within tens of picoseconds.

Key words: Exciton interaction; Pseudolocal librational mode; Bacterial photosynthesis; Light-harvesting antenna

1. Introduction

Spectral properties of the light-harvesting antennae of purple photosynthetic bacteria have been studied intensively by absorption and fluorescence picosecond spectroscopy [1–5]. The results obtained are difficult to interpret using traditional models based on the assumption of localized exciton migration. We have proposed an alternative model which takes into account exciton interactions within the circular aggregate of N light-harvesting BChl molecules [6]. This model permits one to explain all experimental facts in a qualitative way. However, a quantitative correlation between the theoretical and experimental spectra had not been obtained because of the lack of information about the vibronic structure of electron (exciton) levels.

It has been shown [7] that energy exchange between a vibronically excited BChl molecule and the protein matrix takes about 20–30 ps in *Rhodospirillum rubrum* chromatophores. Some components with a similar time constant (10–30 ps) were measured in the picosecond fluorescence [5] and absorbance difference spectra [4] of several purple bacteria.

In this work we have proposed that these components are due to the relaxation of librational modes in the BChl circular aggregate of the *R. rubrum* antenna, and studied its influence on the time dependence of the antenna picosecond absorbance difference spectra.

2. Materials and methods

R. rubrum was grown anaerobically in continuous culture as described in [8]. Chromatophores were prepared using a French press and diluted in buffer containing 10 mM Tris (pH 8.0), 5 mM $MgCl_2$, 0.5 M sucrose, 55% (v/v) glycerol. 2 mM $K_3Fe(CN)_6$ was added prior to the measurements, and continuous background illumination was given to keep the primary donor in the oxidized state. The sample absorbance at the excitation wavelength was no more than 0.15.

The picosecond absorbance difference measurements were performed with the apparatus described in [2]. This spectrometer was additionally equipped with an optical parametric oscillator in the pump channel and with an optical multichannel analyzer OMA-2 (PARC, Princeton) in the probe channel. The repetition rate of the pulses was 1 Hz. The pump and probe pulse duration was about 35 ps.

The antenna picosecond absorbance difference spectra obtained by means of OMA contained 426 points with a signal-to-noise ratio of about 10. In an effort to reveal the general form of the spectra, moderate smoothing was used.

3. Model

The light-harvesting complexes of the core antenna have been assumed to form a circular aggregate with C_N symmetry, where N is the number of BChl molecules [6]. Let us suppose that pigment electron excitations can interact with long-lived vibronic excitations, having lifetimes of the order 10–30 ps and frequencies of the order

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Abbreviations: BChl, bacteriochlorophyll; *R. rubrum*, *Rhodospirillum rubrum*.

SSDI 0014-5793(94)00428-X

50–150 cm^{-1}. This assumption is a possible interpretation of dynamic fluorescence and absorption changes in the antenna of purple bacteria in the ps time domain. These vibronic excitations can be local modes (low frequency intramolecular vibrations of the BChl molecule) or pseudolocal librational modes (rotational motions of the BChl molecule relative to the protein matrix) which decay slowly due to inelastic interactions with the protein matrix phonons. It should be noted that librational modes in linear molecular aggregates also have lifetimes of the order 30 ps [9].

In this paper we shall discuss only pseudolocal librational modes, ignoring the contribution of local vibrations which predominantly exist within the region of hundreds·cm^{-1} and have lifetimes shorter than 10 ps.

The interaction of pigment electron excitations with the pseudolocal mode gives rise to new levels in the energy diagram which correspond to vibronic states of the ground level, |g), one-exciton levels |k), two-exciton levels |k',k") etc. The intensities of spectral components corresponding to transitions from level |k;n) to levels |k',k";m) or |g;m), where n and m are the numbers of vibronic states, are equal to:

$$A_{kn}^{k'k''m} = |(k',k'';m|d|k;n)|^2\ \delta(\nu-(E_{k'k''} - E_k)/h - \Omega\ (m - n)) \quad (1)$$

$$A_{kn}^{gm} = |(g;m|d|k;n)|^2\ \delta(\nu - E_k/h - \Omega(n-m)) \quad (2)$$

where d is the operator of the aggregate dipole moment; ν is the frequency of the probing pulse; $E_{k'k''}$ and E_k are the exciton level energies; Ω is the frequency of the pseudolocal mode (we neglect the dependence of Ω on the wave number); $\delta(\nu)$ is the delta function, which can be replaced by line shape function $f(\nu)$ (let everywhere below $f(\nu) = (1/a\cdot\sqrt{\pi})\ \exp(-\nu^2/a^2)$. The absorbance difference spectrum is

$$\Delta A(\nu) = [\sum_{kn}\ \sum_{k'k''m}\ (A_{kn}^{k'k''m} - A_{kn}^{gm})P_{kn}] - [\sum_{knm} A_{gm}^{kn}\ P_{gm}] \quad (3)$$

where P_{kn}, P_{gm} are population probabilities of |k;n) and |g;m) levels; $A_{gm}^{kn} = A_{kn}^{gm}$. In this formula the first term (in square brackets) corresponds to absorption of the excited aggregate (with negative contribution of the stimulated emission) and the second term corresponds to absorption of the non-excited aggregate. Let us assume that the influence of librations on the selection rules for transitions between exciton levels can be ignored. Then one can write:

$$(k',k'';m|d|k;n) = (k',k''|d|k)\ (m|n) \quad (4)$$

$$(g;m|d|k;n) = (g|d|k)\ (m|n) \quad (5)$$

The analytical expression for matrix elements (k',k"|d|k) and (g|d|k) is given in [6]. Values (m|n) are the Frank-Condon integrals depending on m, n and on the non-dimensional value of Stocks losses, S. For example, for the three lowest vibronic levels, $n = 0,1,2$, these integrals are equal to:

$$|(m|0)|^2 = e^{-S}\cdot S^m/m! \quad (6)$$

$$|(m|1)|^2 = e^{-S}\cdot S^{m-1}\ (S-m)^2/m! \quad (7)$$

$$|(m|2)|^2 = e^{-S}\cdot S^{m-2}\cdot(1/2)\ [(S-m)^2-m]^2/m! \quad (8)$$

4. Results and discussion

To estimate the S and $h\Omega$ values, as well as to determine the structure of each vibronic line, there is a need to analyze the low temperature absorption spectra. Two components with a high amplitude can be extracted from these spectra [10]. These components may be ascribed to the two lowest exciton levels, |±1) and |0). The weaker long-wavelength components may be ascribed to the vibronic levels, |±1;1) and |0;1). We obtained similar results by derivative spectroscopy using the absorption spectra of *R. rubrum* chromatophores at 4 K (data not shown). Unfortunately due to the lack of spectral data we can estimate the required values for the B875 complex with only a low accuracy: the energy gap between the lowest exciton levels E_1–E_0 = 100–150 cm^{-1}, $h\Omega$ = 60–100 cm^{-1}, S = 0.3–0.8, a = 110–150 cm^{-1}.

Fig. 1 shows the model and experimental picosecond absorbance difference spectra of *R. rubrum* chromatophores at 4 K (the long-wavelength side excitation). Excitation of level |0;0) only was taken into account when calculating the difference spectrum.

Figs. 2 and 3 show the same spectra but for the short-wavelength side excitation. Model spectra (Fig. 2) were calculated assuming selective excitation of the |±1;1) level with subsequent fast relaxation at the |0;1) level. The relaxation of the |0;1) level to the lowest |0;0) level took place with the time constant of 10–30 ps. Notice that the spectrum for a 45 ps time delay between pump and probe pulses is red shifted relative to the spectrum for a zero time delay. A similar shift is seen from the

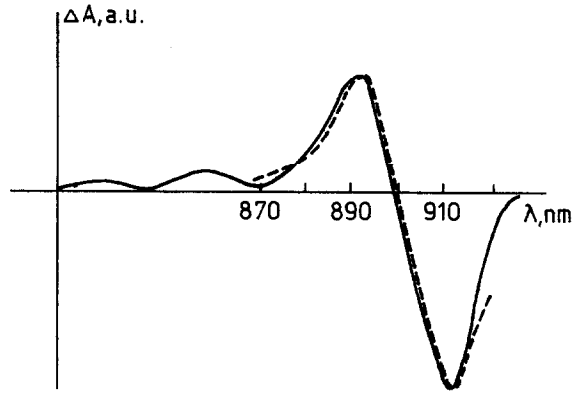

Fig. 1. The experimental (dashed curve) picosecond absorbance difference spectrum of *R. rubrum* chromatophores at 4 K (the long-wavelength side excitation (τ_{exc} = 912 nm)) and the model (solid curve) spectrum calculated for N = 24, $h\Omega/(E_1-E_0)$ = 0.7, $a/(E_1-E_0)$ = 1, S = 0,5, ϕ = 29°, where ϕ is the angle between the dipole moment of the BChl monomer and the circle plane.

experimental spectra in Fig. 3, but its value is less than for the model spectra. The reason for such discrepancy may be explained in the following way. The experimental spectrum measured at a zero or negative time delay (the probe pulse coincides with or is ahead of the pump one) does not correspond exactly to the spectrum for the |0;1) state of the circular aggregate. First, it is impossible to excite only the |±1;1) level because of line overlapping. The |± 1;0) level and the weakly allowed |±2;0) level will be excited simultaneously with the |±1;1) level. Second, when 35 ps pump and probe pulses are used, the probing reveals a partly relaxed system (to the |0;0) level) even for negative time delay values. That is why experimental spectrum 1 in Fig. 3 corresponds to a linear superposition of the |0;1) and |0;0) states. This decreases the differences between the experimental spectra for $\Delta t = -15$ ps and $\Delta t = 45$ ps.

The proposed model allows the shape of the picosecond absorbance difference spectra of the antenna of purple bacteria for the long-wavelength excitation to be quantitatively described and to obtain its time dependence for the short-wavelength excitation. According to this model the fast (subpicosecond) and slow (10–30 ps) components of these spectra are due to the dephasing of the k = 1 and k = − 1 exciton states and to vibronic relaxation, respectively.

It should be noted that the components described in [11,12] with subpicosecond and 10–30 ps time constants in fluorescence and induced absorption time-resolved measurements, were assigned to the spectral diffusion of localized excitations via a spectrally and spatially disordered antenna.

The model described in [11,12] is, however, in contradiction with the results of hole-burning measurements according to which the inhomogeneous width, Γ_{inh}, is much less than the homogeneous width, Γ_h, even at 4 K for the B800–850 and B875 complexes [13]. Such spectral

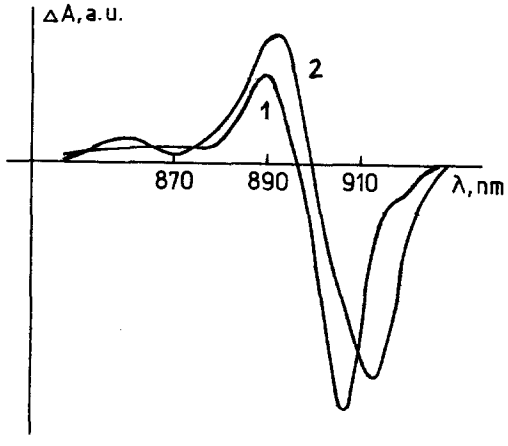

Fig. 2. The model spectra for the short-wavelength side excitation. The time delay is equivalent to 0 ps (curve 1) and to 45 ps (curve 2). It was assumed that the pump and probe pulse duration was much less than the relaxation time equal to 20 ps.

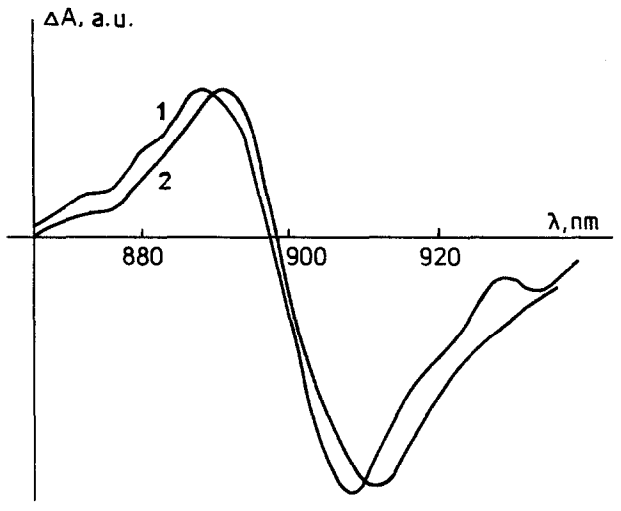

Fig. 3. The experimental spectra of *R. rubrum* chromatophores at 4 K (the short-wavelength side excitation (τ_{exc} = 878 nm)). The time delay was equal to −15 ps (curve 1) and to 45 ps (curve 2).

disorder cannot disrupt the antenna exciton level structure. We think, unlike [10,11], that the antenna is not highly spectrally and spatially disordered. This is indicated by measurements of the Γ_{inh} and Γ_h values and also by the observation of anomalously high absorption changes per absorbed light quantum. The latter fact is direct evidence for excitation delocalization in the antenna [6], which is impossible in a disordered system.

Acknowledgements: The research described in this publication was made possible in part by a grant (Reg. no. Bi.1-3331-0925) from the International Science Foundation and Russian program 'Laser physics and laser systems' (Grant 6.1.2.3). R.D. acknowledges the Netherlands Organization for the Advancement of Pure Research (ZWO) for a visitor's grant. Experimental spectra were measured during R.D.'s post-doctoral training at Department of Biophysics, Huygen's Laboratory of the State University, Leiden, The Netherlands.

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