Discussion Letter

Minor component of the difference absorption spectra of photosynthetic bacteria chromatophores and nonlinear effects during excitation

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Received 11 September 1985

Photosynthesis *Primary process* *Difference absorption spectrum* *Singlet-singlet annihilation*
(Rhodospirillum rubrum)

1. INTRODUCTION

Of late, interest has been increasingly focused on the primary processes of photosynthesis, i.e. the stages from light quantum absorption to the formation of the state of separate charges in the reaction centre (RC). It has been basically connected with the introduction of new devices for picosecond spectroscopy (as well as new isolation procedures of pigment-protein complexes) which facilitated direct analysis of the sequence of the spectral forms of pigment molecules in the light-harvesting antenna (LHA) during the process of the energy migration. Findings of the stationary absorption spectrum [1,2] revealed the presence of at least two spectral forms in the long-wave absorption of the LHA of the purple bacterium *Rhodospirillum rubrum*. The nonhomogeneity of the infrared absorption of complexes B875 in *Rhodopseudomonas sphaeroides* and complexes B880 in *R. rubrum* was shown by low-temperature excitation spectra of fluorescence polarization and by circular dichroism spectra [3]. Picosecond difference absorption spectroscopy of *R. rubrum* chromatophores [4–11] carried out at room temperature detected a bleaching band (the minor component) which is slightly red-shifted in comparison with the principal absorption B880. All the above-mentioned spectroscopic data are in full accord with the model [12] which postulates the coupling pigment-protein complex mediating excitation energy transfer between LHA and RC.

However, the data of the difference absorption spectra kinetics [4–11] are unexpected. First, according to Borisov et al. [4,5] the excitation of LHA concentrates on the minor component of the LHA bacteriochlorophyll within a very short period (<5 ps), and then decays within about 30 ps, which is half the time established by the fluorescence decay kinetics of LHA [13]. It has to be pointed out that the minor spectral component is red-shifted by 30 nm as compared to the accepting photodonor band of RC, although the transfer of the excitation energy to RC takes place through the minor component [5,7,10]. Second, the LHA excitation decay kinetics does not depend on the RC redox state [6,8,9] which does not conform with the well-known Vredenberg-Duysens model [14]. Thus, the interpretation of the minor spectral component proposed in [4–11] is in contradiction to the generally accepted concepts of the primary processes of the photosynthesis [15]. We consider, therefore, that a re-evaluation of the

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Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

causes of these contradictions is of paramount importance.

In the present paper we proffer an interpretation of the data of picosecond difference absorption spectroscopy which makes it possible to eliminate all the above-mentioned discrepancies. In the course of our discussion we shall constantly refer to the specific parameters of the purple bacterium *R. rubrum*. We shall also make use of the generally accepted concept of the photosynthetic unit (PSU) determining the RC and its surrounding LHA as well as that of the domain involving a set of PSU within which excitation random walk occurs.

2. INTENSITY OF THE EXCITING LIGHT PULSES

Measurements of the difference absorption of the chromatophores [4–11] indicate different temporal courses as well as light dependencies of the absorbance of the sample at low ($J_0 \simeq 10^{14}$ $h\nu/cm^2$) and high ($J_0 \geq 10^{16}$ $h\nu/cm^2$) excitation intensities (normalization of the intensity per single pulse is used everywhere in the text). The main argument that the kinetics of the spectral changes in the case of $J_0 \simeq 10^{14}$ $h\nu/cm^2$ reflects the picture of natural photosynthesis lies in the fact that the quantum yield of the charge separation in RC is more than 0.5 [8–10]. The given intensity for 1 mm samples with an absorbance of about unity at 880 nm when 50 bacteriochlorophylls are related to 1 RC corresponds to $N_{PSU} \simeq 1$, the latter being the average number of absorbed photons per PSU in the whole sample. Detailed analysis of the fluorescence quantum yield [16,17] shows that the process of singlet-singlet annihilation becomes rather important at such intensities.

When chromatophores are subjected to excitation, the occupation of the first excited singlet state (S_1) of the antenna bacteriochlorophyll takes place immediately. The process of annihilation sets the transfer of excitation from one excited molecule to another thus promoting the latter to a higher excited state (the Soret band). This high-energy state gives rise to rapid internal excitation conversion to the S_2 state, the lifetime of which was found to be relatively long [18,19]. Therefore, when analysing the difference absorption results [4–11] in the whole region of excitation intensities, attention should be paid to the influence of non-linear phenomena.

In our further analysis we shall use parameters obtained from the fluorescence data. Referring to the chromatophore fluorescence quantum yield measurements [16,17], the annihilation rate of two excitations in the domain γ_2 is approximately equal to 0.006 ps^{-1}. Assuming that the excited molecules are homogeneously distributed throughout the sample the mean time of the annihilation process can be estimated by the formula

$$\tau_a^{-1} \simeq \gamma_2 n_d v/2 = \gamma_2 \lambda N_{PSU}/2 \quad (1)$$

n_d denoting the excitation density in the domain, v the volume of the domain, and λ the number of PSU in the domain. When $\lambda = 16$ [17] and $N_{PSU} = 1$, by use of eqn 1 we obtain $\tau_a \simeq 22$ ps.

3. EXCITATION DECAY KINETICS IN THE LHA

The foregoing estimate points out a way by which the difference absorption kinetics obtained and interpreted in the study [4–11] can be explained otherwise. As observed at extremely weak excitation intensities the fluorescence decay time τ_0 in the case of open RC approximately equals 60 ps [13]. Therefore, even if all the RCs are open, the initial excitation decay kinetics is mainly determined by the singlet-singlet annihilation process because $\tau_a < \tau_0$. In the course of time the contribution of annihilation decreases whereas the relative contribution of the excitation quenching by open RCs increases. At a later time the closed RCs start influencing the excitation decay process (the average time of the fluorescence quenching by means of closed RCs, τ_c equals 200 ps [13]). Thus, for example, it is easy to demonstrate numerically that the nonexponential experimental kinetic curve for $N_{PSU} = 1$ [9] can be adequately approximated by the sum of three exponential terms with the decay indexes $\tau_a \simeq 22$ ps, $\tau_0 \simeq 60$ ps and $\tau_c \simeq 200$ ps. We have found the preexponential factor of the 'rapid' exponent (with index τ_a) to be essential (≥ 0.5) even in this case while the calculation of the quantum yield of the charge separation resulted in $\mu \simeq 0.5$.

The presented three-exponential approximation of the LHA excitation decay kinetics enables us easily to understand its independence of the redox

state of the RC [6,8,9]. If part of the RCs is closed (by photo-oxidation or chemically) the excitation decay kinetics will vary only slightly over the region of the time τ_0. If all the RCs are closed the rapid (annihilation) and 'slow' (excitation quenching by the closed RCs) exponent contributions to the kinetics will be preserved whereas the contribution of the 'moderate' one (excitation quenching by the open RCs) will vanish. Consequently, within the limits of the time resolution restricted by the duration of the probing pulse ($\tau \simeq$ 30 ps) the kinetics for the open and the closed RCs will only be distinguished by relative contributions of the slow exponent, which is manifestly proved in the experiment [6,9].

4. ORIGINS OF THE MINOR SPECTRAL FORM

The differential absorption spectrum of chromatophores containing the minor spectral form can be defined by the following formula [20]:

$$\Delta A(\lambda_{pr},t) = \alpha[n_0(t)\sigma_0(\lambda_{pr}) + n_1(t)\sigma_1(\lambda_{pr}) + n_2(t)\sigma_2(\lambda_{pr}) - \sigma_0(\lambda_{pr})] \quad (2)$$

in which α denotes the numerical coefficient; n_i, the occupation of the ground state ($i = 0$) and the excited singlet states: the first ($i = 1$) and the second ($i = 2$); $\sigma_i(\lambda_{pr})$, the cross-section of the light absorption (and the stimulated emission in the case when $i \neq 0$) of the i-th state at the wavelength λ_{pr} of the probing pulse. The temporal dependence of ΔA is determined by the state occupations n_i, whose precise values can be estimated by way of solving the corresponding kinetic equations.

When the intensities of the exciting light pulse are low, $n_2 \ll 1$, hence $n_0 + n_1 \simeq 1$. Then from eqn 2 it follows that

$$\Delta A(\lambda_{pr},t) \simeq \alpha n_1(t)[\sigma_1(\lambda_{pr}) - \sigma_0(\lambda_{pr})] \quad (3)$$

The dependence of σ_1 on λ_{pr} is unknown. However, it should be pointed out that the transition from the first singlet state to the Soret band is evidently blue-shifted in comparison with the maximum (λ_m = 880 nm) of the LHA absorption from the ground state to S_1. The maximum of a stimulated emission from S_1 is at $\lambda \approx 900$ nm. Bearing in mind that σ_1 in order of magnitude equals σ_0 [18], it follows directly that the minor spectral form is due to the difference between the two absorption spectra: $\sigma_1(\lambda_{pr}) - \sigma_0(\lambda_{pr})$. [The nature of $\sigma_0(\lambda)$ has not been made clear so far since the bacteriochlorophyll absorption in chromatophores is considerably red-shifted compared to that in the solution. There is no complete agreement on this point. Some authors attribute the nature of the red-shift to the pigment-protein interaction while others argue that it is due to the pigment-pigment interaction (exciton interaction). The foregoing explanation of the minor spectral form is based on the former viewpoint, although a similar explanation can also be given on the basis of the latter. According to the globular PSU model [21,22] several close bacteriochlorophylls together with proteins can form structural units, viz. pigment-protein complexes (e.g. bacteriochlorophyll dimers are believed to be present in *R. rubrum*). Hence, on account of the resonance interaction, for one light quantum absorbed per dimer the optical transition to the lowest singlet state is red-shifted as compared to that in the monomer. However, the resonance interaction does not manifest itself in the case of two absorbed light quanta and consequently the difference absorption spectrum of the dimer (as well as that of the oligomer) will have the minor red-shifted component.]

On this basis the light curves (e.g. fig.2 in [4] and fig.3 in [5]) also become clear. With the growth of the excitation intensity the process of singlet-singlet annihilation becomes more extensive leading to a nonlinear n_1 dependence on J_0. Besides, at very high intensities, when $N_{PSU} \gg 10$, one more nonlinear process manifests itself, i.e. absorption of the second light quantum with transition from the first singlet state to a higher one as well as to the ground state during the action of the exciting pulse takes place. Both nonlinear processes tend to diminish the difference between the occupations of the ground and first singlet states. At very high intensities, owing to the relatively long lifetime of excitation in the second singlet state (see, e.g. [18,19] for the chlorophylls), considerable occupation of the state S_2 occurs and this is responsible for the observed change of the difference absorption in the main band [4,5]. Again, the lifetime of the state S_2 determines the delay time of the appearance of the minor component in the difference absorption spectrum.

Analytical solution of the kinetic equations for

the occupations of the singlet states of LHA over the range of excitation intensities 10^{14}–10^{16} $h\nu/cm^2$ when the exciting light pulse shape is approximated by a right-angled function of 25 ps width results in the dependencies $n_1^{max} \sim \sqrt{J_0}$ and $n_2^{max} \sim J_0$. Thus the rise of the excitation intensity slackens augmentation of n_1 (and the signal of the minor spectral component) and enhances the occupation number n_2. In the relevant region of the spectrum (880 nm) the absorption cross-section σ_2 apparently does not possess resonances and therefore the signal of the main component of the difference absorption spectrum increases, thus qualitatively reproducing the experimental light curves.

We must point out some factors which can have a decisive effect on the quantitative agreement between the model calculations and experimental data. First, account should be taken of the duration and shape of the exciting and probing pulses. Second, the annihilation process which we treat as being bimolecular introducing only one rate constant γ_2 in fact is a more complex many-body process and hence correlations among the distributions of the excited pigments should be taken into consideration. Third, in experiments [4–11] the absorbance of the sample was significant (e.g. $A \approx 1$). Therefore, the nonhomogeneous excitation distribution on the pigments in the sample must be taken into account. Also, corrections might be achieved by including the statistics of the initial distribution of the excited pigments [16,23] as well as fluctuations of the energy and the duration of the exciting pulse. We believe, nevertheless, that these shortcomings will not change qualitatively the overall picture.

5. CONCLUSION

The proffered analysis shows that in experiments of picosecond difference absorption spectroscopy, the excitation conditions are far from those arising in natural photosynthesis and thus the nonlinear processes whose interpretation turns out to be a nontrivial problem play a decisive role. We assume that the minor component of the difference absorption spectrum of the chromatophores detected in [4–11] is brought about by optical transitions from the higher singlet states. Consequently, at the stage of the intensities employed the kinetics of the excitation decay in the LHA are chiefly determined by nonlinear processes, singlet-singlet annihilation and two-photon absorption.

The foregoing explanations are provable by the kinetics of the transitions $S_0 - S_2$, $S_1 - S_2$, S_2 – Soret band which manifest themselves in the fluorescence and difference absorption spectra within the regions of 600, 2000, and 1240 nm, respectively. Analysis of these kinetics brought about over a wide range of excitation intensities as well as the use of double-pulse excitation might adequately highlight the validity of the present interpretation. It is also worthy of note that the conclusions of this paper do not contradict the existence of the infrared absorption heterogeneity observed in [1–3]. On the contrary, these infrared sub-bands evidently may also be detected through picosecond difference absorption spectroscopy. However, in order for this to happen it is essential to eliminate (or properly evaluate) the effects of nonlinear phenomena, i.e. the optical transitions from the higher singlet states, singlet-singlet annihilation and two-photon absorption, on the difference absorption spectra and kinetics as well as to make use of probing light pulses of a shorter duration.

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