Volume 250, number 2, 183–186 FEB 07166 July 1989

The cooperativity phenomena in a pigment-protein complex of light-harvesting antenna revealed by picosecond absorbance difference spectroscopy

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Received 16 March 1989

A model of the cooperative changes in optical properties of light-harvesting bacteriochlorophyll molecules of complex B890 in response to the absorption of light quanta is proposed. According to the model, each antenna chromophore may persist in either of two optically non-excited states, R and T. The occurrence of at least one excitation per complex causes all optically non-excited chromophores of the complex to be converted from state R to state T. The theory is shown to be in good agreement with experimental 'light curves' (ΔAvs intensity of picosecond excitation pulse) for the 'minor' and 'major' signals of light-harvesting bacteriochlorophylls of complex B890 from *Chromatium minutissimum*.

Light-harvesting antenna; Bacteriochlorophyll-protein complex; Bacterial photosynthesis; Cooperativity

1. INTRODUCTION

The primary processes of photosynthesis occur in the two main subunits of the bacterial photosynthetic apparatus, i.e. the light-harvesting antenna and photochemical reaction centre (RC). Previously, it was assumed that the antenna of some purple bacteria (e.g. *Rhodospirillum rubrum*) contains bacteriochlorophyll (BChl) molecules of only one spectral form [1] and that it should be possible to isolate complexes containing RC and BChl of the long-wavelength form only (i.e. pigment-protein complex B890 from *Chromatium minutissimum* [2]). It was later shown via picosecond absorbance difference spectroscopy that the two types of absorbance signal are connected with the light-harvesting BChl molecules in chromatophores of *R. rubrum* [3–6] and some other preparations (review [7]). Both types of antenna signal may be induced by using picosecond excitation pulses of low and high intensities. In accordance with the first study [3], we shall designate the corresponding absorption changes as the signals of the 'minor' and 'major' forms. Spectral heterogeneity among the light-harvesting antennae of the above-mentioned objects was also revealed by derivative spectroscopy, spectrofluorimetry with laser excitation, fluorescence polarization at 4 K (see [7]), and picosecond fluorescence spectrochronography at 77 K [8]. According to the first [3] and now widespread [6,9] interpretation, the second signal results from the existence of a minor fraction of light-harvesting BChl molecules having an absorption band at longer wavelengths (~20 nm) as compared to the major BChl antenna molecules. Other interpretations of the minor and major signals have also been proposed [5,10,11]. However, in our opinion, each of these models does not provide a sufficient explanation of all experimental results [7]. In particular, no quantitative description is available regarding the absorbance changes of minor and major forms of light-harvesting BChls

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Abbreviations: BChl, bacteriochlorophyll; RC, reaction centre

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

with respect to the intensity of the picosecond excitation pulses ('light curves'), and no explanation is at hand concerning the marked absorption changes in the bleaching band of the minor form with an approximately normal bandwidth (FWHM ~40 nm) per absorbed light quantum ($\epsilon > 6 \times 10^5$) [12].

Here, we propose a model for the cooperative changes in optical properties of light-harvesting BChl molecules induced by optical excitation of the antenna. We demonstrate that the experimental light curves for the major and minor forms and RC photooxidation coincide with the theoretical plots, calculated on the basis of this model for a wide range of excitation intensities.

2. MATERIALS AND METHODS

Pigment-protein complex B890 was isolated from the purple photosynthetic bacterium *C. minutissimum* according to a modification of the method of Moskalenko and Yerokhin [2]. Samples were suspended in 0.05 M Tris-HCl buffer (pH 8.0). A sample of complex B890 in solution consists of individual particles, each containing a single RC and about 30 light-harvesting BChl molecules. All measurements were carried out at room temperature. For calculations it was assumed that $\epsilon_{887} = 126 \times 10^3\ M^{-1} \cdot cm^{-1}$ for antenna BChl molecules, and $\Delta\epsilon_{877} = 114 \times 10^3\ M^{-1} \cdot cm^{-1}$ for RC photooxidation.

Time-resolved measurements were recorded using the picosecond laser pulse absorbance spectrometer described in [3,13].

3. RESULTS AND DISCUSSION

The light minus dark difference spectrum at low pulse intensities does not coincide with the absorption spectrum. Chromophore bleaching alone is insufficient to explain this phenomenon (i.e. spectral incoincidence). It has been shown that excited chromophores absorb over a wide range of wavelengths in the interval from 400 to 1500 nm ($S_1 \longrightarrow S_n$ transitions), and thus at high pulse intensities one can observe diffuse absorption on both sides of the bleaching band [14]. However, the fact that for high excitation intensities the bleaching maximum nearly coincides with that of the absorption spectrum implies a slight dependence of the absorption $\bar{A}(S_1 \longrightarrow S_n)$ on the wavelength of the light (for a different point of view, see [10]).

On the other hand, at low pulse intensities the bleaching maximum does not coincide with the absorption peak (fig.1). A decrease in number of excited chromophores alone would not shift the spectral extremum. Therefore, it is logical to suggest that the excitation of some part of the antenna chromophores could induce spectral changes in other, non-excited ones. In other words, the light minus darkness difference spectrum must arise from at least two types of optically non-excited chromophores, and the relative proportions of both types should depend on the intensity of the excitation pulse.

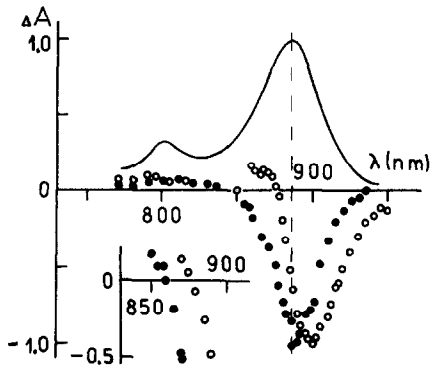

Fig.1. Normalized difference spectra of photo-induced absorption changes in the B890 complex from *Chromatium minutissimum* at high (•) and low (○) intensities of picosecond excitation (λ = 840 nm) pulses (>0.3 and <0.03 photons absorbed per BChl molecule, respectively). The probe pulse was synchronized with that for excitation. The absorption spectrum for B890 complex is shown by the continuous line. Absorbance of samples at 887 nm in a 1 mm cell was ~1.5 *A* unit. (Inset) Sections of the same spctra for long-wavelength excitation (at 920 nm).

In order to support this statement, a firm basis must be established to explain the dependence between the two spectral forms as a function of pulse intensity, with evaluation of the experimental data being carried out accordingly.

In a recent paper [15], we described the curves of RC photooxidation vs pulse intensity in terms of the statistics for the photon distribution in a set of monocentral antenna domains. Also, it was noted previously [4] that the light curve for RC photooxidation closely resembles that of the minor form of antenna signal (fig.2). This remarkable similarity led us to assume that: (i) each BChl chromophore of the light-harvesting antenna may persist in either of two optically non-excited states, R and T; (ii) the monocentral domain (complex B890), when optical excitation is not performed, consists of chromophores of the R state; (iii) the ap-

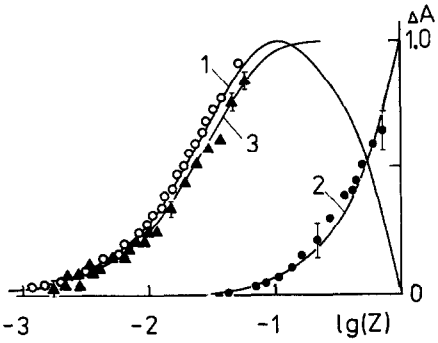

Fig.2. Normalized dependences of absorption changes of B890 complex on intensity of the excitation pulses (light curves). Curves 1 (○—○) and 2 (●—●) were measured for minor and major BChl signals at ~860 and ~880 nm, respectively, curve 3 (▲—▲) being recorded at ~890 nm for RC photooxidation; the probe pulse was synchronized with the 930 nm excitation pulse for first two curves and had a 500 ps delay for the third curve. Theoretical curves 1 and 2 were calculated according to eqns 5 and 6, respectively, curve 3 being obtained according to a modified eqn 4 from [15]. Curves 2 and 3 were normalized according to $\Delta A_{Bo}(880)/A(880)$ and $\Delta A_{RC}(890)/\Delta A^{ox}_{RC}(890)$, respectively, where $A(880)$ denotes the absorbance of B890 complex at ~880 nm and $\Delta A^{ox}_{RC}(890)$ represents the change in absorbance due to complete RC oxidation under conditions of continuous light. The normalization coefficient for curve 2 was obtained by fitting the experimental point at $\log(Z) = -1.40$ to curve 1. Absorbance of the B890 complex in a 1 mm cell at 887 nm was ~1.6 A unit. Intensity of excitation pulses is represented as the number of photons absorbed per number of BChl molecules (Z).

pearance of at least one excitation per B890 complex causes all optically non-excited chromophores of this complex to be converted into state T.

Here, the term 'chromophore' denotes one or more BChl molecules bleached per absorbed photon.

Let us now consider the light minus dark difference spectrum which would be observed for a system described by postulates (i)–(iii). First of all, in the case of a B890 complex which absorbs no photon, the complex is obviously not manifested in the difference spectrum. For the B890 complex absorbing one photon, the complex contains, in accordance with (iii), $(n-1)$ chromophores in the T-state (n, number of chromophores per B890 complex) and one bleached chromophore. Thus, in the case of a single optical excitation, the contribution from a number, K_1, of B890 complexes to the difference absorption has the following value:

$$\Delta A_1 = K_1[(n-1)A_T + \tilde{A} - nA_R] \quad (1)$$

where A_T and A_R represent the absorption at any given wavelength per chromophore in the T- and R-state, respectively, $\tilde{A}$ denoting that of the excited chromophore at the same wavelength.

Similar reasoning leads to the following expression for the contribution to the difference spectrum arising from a number, K_i, of B890 complexes, each absorbing $i (i \leqslant n)$ photons:

$$\Delta A_i = K_i[(n-i)A_T + i\tilde{A} - nA_R] \quad (2)$$

We have recently shown [15] that the value of K_i is described well by the binomial distribution:

$$K_i = K\binom{n}{i}(1-Z)^{n-i}Z^i \quad (3)$$

where $\binom{n}{i}$ designates the binomial coefficients, K is the total number of B890 complexes in the sample, and Z denotes the mole fraction of excited chromophores, i.e. the ratio of the number of absorbed photons vs the total number of chromophores, N.

Since $K = N/n$, substitution of eqn 3 into eqn 2 can be carried out, yielding:

$$\Delta A = (A_T - A_R)[1-(1-Z)^n] + (\tilde{A} - A_T)Z \quad (4)$$

Therefore, if postulates (i)–(iii) are valid, eqn 4 must describe the dependence of the light minus dark difference spectrum on the intensity of the excitation pulse at any wavelength of the light. In principle, in fitting A values at various wavelengths, we could obtain the 'pure' absorption spectra of the R-, T- and $\tilde{A}$ states of chromophores. However, we shall restrict ourselves in the present paper to checking the validity of eqn 4 at a number of wavelengths.

The most convenient way of doing so is for the wavelength where $\tilde{A} = A_R$, i.e. when chromophores in the ground and excited states absorb equally. Eqn 4 then takes the form

$$\Delta A_{Bm} = (A_T - A_R)[1 - Z - (1-Z)^n], \quad (5)$$

where Bm indicates the minor form. For the pigment-protein complex B890 the wavelength will be near 860 nm (long-wavelength excitation; see fig.1, inset).

Fig.2 shows experimental points obtained at this wavelength (open circles). Good agreement is evident with the theoretical curve 1 described by eqn 5 with $n = 30$. The closed circles in fig.2 display the variation in amplitude of the difference spectrum at $\lambda \sim 880$ nm. This wavelength corresponds to

zero absorption difference at low intensities when the presence of B890 complexes with more than one excitation may be neglected.

We may therefore regard this value as being the wavelength where an excited chromophore, together with $n-1$ non-excited ones, in a given B8980 complex absorbs equally as n chromophores of a totally non-excited B890 complex: $\tilde{A} + (n-1)A_T = nA_R$. This leads to transformation of eqn 4 into the following form:

$$\Delta A_{Bo} = (A_T - \tilde{A})[nZ - 1 + (1-Z)^n], \quad (6)$$

where Bo indicates the major form. Curve 2 in fig.2 is plotted according to eqn 6, with $n = 30$. Good agreement is observed with the experimental points.

When $Z \ll 1$, i.e. for low intensities of the excitation pulses, eqn 5 becomes

$$\Delta A_{Bm} = (A_T - A_R)(n-1)Z \quad (7)$$

Thus, the amplitude of the difference spectrum is proportional to $n-1$, where n is the number of chromophores participating in the cooperative interaction in a B890 complex. This explains why the bleaching per light quantum in the light minus dark difference spectrum may exceed the absolute absorption of one chromophore, provided n is sufficiently large.

The mechanism of antenna cooperativity is not clear. However, in the most general terms, one could assume the occurrence of conformational changes in the protein environment of chromophores induced by optical excitation of neighbouring chromophores. Such changes in conformation involve characteristic time for nuclear relaxation of at least several picoseconds. Hence, femtosecond measurements appear to be necessary in order to gain some insight into the process.

Acknowledgements: We are grateful to Dr I.A. Abdourakhmanov for the samples of B890 complex. We would like to express our thanks to Professors V.P. Skulachev, A.Yu. Borisov, L.A. Drachev and A.S. Piskarskas for encouragement.

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