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# Picosecond dynamics of excitations in light-harvesting complex B800–850 from *Chromatium minutissimum* studied using fluorescence spectrochronography

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The picosecond dynamics of excitations in the isolated B800–850 light-harvesting complex of the purple sulfur bacterium *Chromatium minutissimum* has been studied using picosecond fluorescence spectrochronography. A short-lived component of about 20 ps lifetime has been found at 77K at the short wavelength part of the B850 fluorescence spectrum similar to that previously described for the core antenna bacteriochlorophyll band B880 of *Rhodospirillum rubrum*. Evidence has been presented indicating that this component is likely to reflect excitation energy relaxation step(s) involving both photoexcited bacteriochlorophyll and the protein environment. A new kinetic scheme of excitation transfer from the peripheral antenna to the photoreceptor units in purple bacteria is suggested which takes into account these findings.

*Chromatium minutissimum*, B800–850 complex; Picosecond fluorescence, Excitations dynamics; Protein relaxation

## 1. INTRODUCTION

The photosynthetic apparatus of *Chromatium minutissimum*, as well as of most purple bacteria, consists of three types of pigment–protein complexes: those of the reaction centers (RCs) are surrounded and interconnected by the light-harvesting antenna complexes B890 (B875), thus forming the so-called photoreceptor units, B890–RC. The third type of the complex, B800–850, constitutes the peripheral antenna [1]. Stationary fluorescence polarization as well as picosecond absorption (for review, see [2]) and fluorescence measurements [3,4] unambiguously showed that B890 (B875) BChl bands of the core antenna of *Rhodospirillum rubrum*, *Rhodobacter sphaeroides* and *C. minutissimum* are spectrally inhomogeneous. The peripheral antenna B850 band was also suggested to be nonhomogeneous on the basis of singlet–singlet annihilation studies and deconvolution of the stationary absorption spectrum of *Rb. sphaeroides* mutant NF57, containing only B800–850 [5,6]. These data were recently confirmed by direct low temperature picosecond absorption recovery measurements [7]. At the same time, inhomogeneity of the B850 band has not been found in the detergent-solubilized B800–850 complex [5].

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*Abbreviations* BChl, bacteriochlorophyll; RC, reaction center.

This paper reports data of the spectrally-resolved picosecond fluorescence measurements for isolated B800–850 complexes, clearly demonstrating the nonhomogeneous nature of the B850 band. Evidence is presented for the dynamic nature of this nonhomogeneity, resulting from vibrational relaxation of the photoexcited chromophore–protein system.

## 2. MATERIALS AND METHODS

Cells of purple sulfur bacteria *C. minutissimum*, strain MSU, were grown and the chromatophores isolated as described elsewhere [8]. The B800–850 complexes were prepared by Triton X-100 electrophoresis in 7% polyacrylamide gels [8]. Excess of the detergent was removed by ultrafiltration

Absorption and steady-state absorption and fluorescence spectra were measured by standard means using commercial spectrometers. Spectrally-resolved picosecond fluorescence studies were performed with the instrument described elsewhere [3] employing a mode-locked dye laser, synchronously pumped at 76 MHz by a Nd-YAG laser. Fluorescence was detected with a streak camera via two single-grating monochromators combined in a subtractive-dispersion mount. The instrument response function was 20 ps. The excitation density was held below the onset of the lifetime shortening due to singlet-triplet annihilation, $\leq 0.1$ W/cm$^2$ [3].

## 3. RESULTS

The absorption and fluorescence spectra of the B800–850 complexes obtained from *C. minutissimum* with Triton X-100 were the same as those described elsewhere [8]. In particular, the absorption spectrum was charac-

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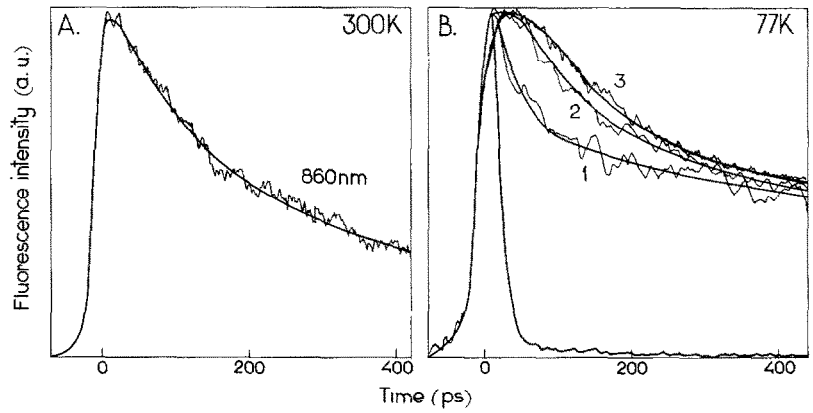



Fig. 1. (A) Experimental kinetics of the B850 fluorescence decay (noisy curve) measured at room temperature for a registration wavelength of 860 nm. The continuous curve represents a result of computer-simulation of the experiment with a sum of two exponentials with $\tau_1$ = 685 ps, $\tau_2$ = 110 ps and the amplitude ratio $A_1/A_2$ = 1.0:0.8. (B) The same, except that the measurements were performed at 77K for registration wavelengths 850 (1), 900 (2) and 930 (3) nm. The continuous curves correspond at 850 nm, a three-exponential fit with $\tau_1$ = 18 ps, $\tau_2$ = 1,200 ps, $\tau_3$ = 72 ps and $A_1/A_2/A_3$ = 10:3:1, at 900 nm, a two-exponential fit with $\tau_1$ = 1,010 ps, $\tau_2$ = 68 ps and $A_1/A_2$ = 1.0:0.8; at 930 nm, a three-exponential fit with $\tau_1$ = 12 ps, $\tau_2$ = 1,150 ps, $\tau_3$ = 68 ps and $A_1/A_2/A_3$ = −1, 78·1.0:0.78. Excitation wavelength, 790 nm. Spectral resolution, 8 nm. The real apparatus response function, shown in part B, was taken into account in the above approximations.

terized by an increased ratio of B800 to B850 and a blue-shifted position of the 850 band maximum as compared to the native state. These absorption changes are reversible after detergent removal and, according to the measurements of linear dichroism spectra in polyvinyl alcohol films, they are not accompanied by changes in the mutual orientation of the transition dipole moments of BChl molecules, constituting the B850 pair [9].

The stationary fluorescence spectrum of the complexes at room temperature peaked at 865 nm [8]. The kinetics of the fluorescence decay measured at 860 nm are shown in Fig. 1A. It best fitted a sum of two exponentials with 650 ± 60 ps and 100 ± 20 ps time constants and the amplitude ratio 1:0.8, with both parameters being independent of the registration wavelength. This is in agreement with the data of Bergstrøm et al. [10] who connected the presence of the second shorter-lived component in the fluorescence decay of the B800–850 complexes with the excitation quenching by BChl photodestruction products. The deconvoluted value of the time constant of the main component was about 20% shorter than that measured earlier for the excitation decay in isolated B800–850 complexes from *Rb. sphaeroides* and *Rb. acidophila* [10,11].

The kinetics of the B850 fluorescence decay at 77K shown in Fig. 1B were found to be dependent on the registration wavelength. As compared with room temperature, the duration of the main component increased to 1,000 ± 100 ps and that of the shorter-lived component decreased to 70 ± 10 ps; also, at the blue part of the spectrum a short-lived component with about 20 ps lifetime appeared (Fig. 1B).

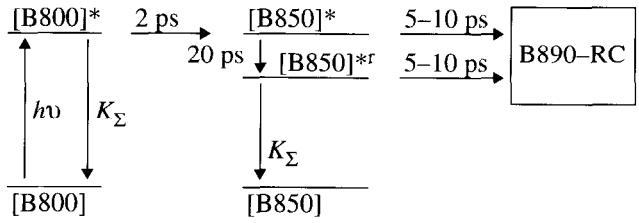
## 4. DISCUSSION

In our earlier work, the B850 fluorescence decay in intact membrane and chromatophores of *C. minutissimum* and *Rb. sphaeroides* was shown to best fit two components of 5–10 ps and 40–50 ps duration [3]. The spectrum of the latter component was 5–10 nm-shifted to longer wavelengths as compared to that of the former. This fluorescence heterogeneity was attributed to the presence of two B800–850 pools, one tightly and the other loosely bound with the photoreceptor units. The fact that kinetic and spectral inhomogeneity of the B850 fluorescence is a peculiar feature also of isolated B800–850 complexes (this work) and of mutant strain of *Rb. sphaeroides* NF57, lacking photoreceptor units [7], prompted us to reconsider the above interpretation.

Two possible views on the nature of the BChl spectral bands of the nonhomogeneity are currently under consideration. According to the first one, minor long wavelength components exist in the main B850 and B890 (B875) bands serving as intermediates in the excitation flow to the RCs [2,5,6]. Such a suggestion is in contradiction with our data [3], according to which the time constant of the longer wavelength component of the B850 fluorescence decay in *Rb. sphaeroides* and *C. minutissimum* is 5–10 times larger than the rise time of the B890 fluorescence. Besides, the idea of the static nature of the B850 and B890 bands nonhomogeneity as a source of the 15–30 ps decay components is in contradiction with recent hole-burning studies [12], where the homogeneous width of the B850 and B890 bands was found to be larger than 200 $cm^{-1}$, several times larger than $kT$ at 77K. Thus, BChl bands inhomogeneity revealed, in particular, in the picosecond fluorescence spectrochronography studies ([3,4] and this work) is likely to be of dynamic nature, caused by vibronic relaxation of the protein environment following the formation of the B850 (B890) singlet excited states.

The kinetic scheme of the excitation dynamics in iso-

lated B800–850 complexes at 77K (up-hill excitation transfers are neglected), which takes into account the above mentioned findings, is given below:




Scheme 1

The main part (70–75%) of the excitations is transferred to the B890–RC complexes in 5–10 ps before the global protein relaxation is accomplished. The latter process occurring with a time constant of 20–30 ps traps part (about 25–30%) of the excitations in the B800–850 complexes, forming the relaxed [B850]*r state. Excitation transfer from the [B850]*r to the B890–RC proceeds also in about 5–10 ps, since Förster's overlapping integral is approximately equal for the relaxed and unrelaxed states. The kinetic treatment of this scheme with its approach based on the balance equations is in agreement with existing experimental data.

In addition, this scheme makes it possible to reconcile the discrepancy between the published values of excitation transfer time from B850 to B890. It was concluded from the steady-state fluorescence data [13] that (i) the B850 → B890 excitation transfer in *Rb. sphaeroides* occurred with approximately the same time constant as excitation trapping by the RCs (which is 50–60 ps [1–4]) and (ii) the thermodynamic equilibrium between the above two BChl forms was not reached when the B850 was selectively excited. In support of this suggestion, the value of 40 ps was published for the B850 → 890 transfer time on the basis of the picosecond absorption recovery studies [14]. On the other hand, according to fluorescence measurements with picosecond resolution [11,15], this value is shorter than 6 ps. In this latter case, the thermodynamic equilibrium should be established during the excitation lifetime, contrary to the published data [13,16].

According to Scheme 1, the dominant contribution to the steady-state fluorescence spectrum from the B850 band is made by the longer-lived component involving the [B850]*r state. The contribution of this component would be greatly diminished when the B890, but not the B850 is selectively excited. This seems to be the reason for differences in the fluorescence spectra which were observed for *Rb. sphaeroides* chromatophores under similar conditions [13,16].

The relaxation steps of 15–30 ps duration, extensively studied for heme proteins [17], have recently been described for the excitation decay of a large variety of different pigment–protein complexes from photosynthetic organisms, including reaction center preparations from purple bacteria [18] and chlorosomal light-harvesting antenna of green bacteria [19]. Thus, such picosecond relaxation steps seem to be a common feature of the photoexcited chromophore–protein systems.

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