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Investigation of spatial relationships and energy transfer between complexes B800–850 and B890–RC from *Chromatium minutissimum* reconstituted into liposomes

Andrey A. Moskalenko[a], Olga Toropygina[a], Valentina I. Godik[b], Kou Timpmann[c] and Arvi Freiberg[c]

[a]*Institute of Soil Sciences and Photosynthesis, Russian Academy of Sciences, 142292 Puschino, Russia,* [b]*A.N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, 119899 Moscow, Russia* and [c]*Institute of Physics, Estonian Academy of Sciences, 202400 Tartu, Estonia*

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Spatial relationships between different pigment–protein complexes in the membranes of the purple photosynthetic bacterium, *Chromatium minutissimum*, have been studied. The possibility of restoring the function of efficient excitation energy transfer from bacteriochlorophyll molecules to the reaction centers in the system of soybean liposomes, reconstituted with pigment–protein complexes B800–850 and B890–RC from *C. minutissimum*, has been explored. The chemical cross-linking method, together with stationary and picosecond spectrally resolved fluorescence measurements were employed. It has been shown that after the incorporation of the complexes into the liposome membranes conditions for directed excitation energy transfer from the light-harvesting pigments to the reaction centers are created, which are less optimal, however, than those in the native state. Possible reasons are considered.

Chromatium minutissimum; Pigment–protein complex; Liposome; Chemical cross-linking; Excitation transfer; Picosecond kinetics

1. INTRODUCTION

The photosynthetic apparatus of purple bacteria usually contains two types of light-harvesting bacteriochlorophyll–protein complexes (LHC), B800–850 and B890, and complexes of the reaction centers (RCs). The analysis of the nearest neighbour relationships, chemical cross-linking and immunofractionation studies [1,2] have shown, in agreement with earlier kinetic spectroscopic data [3], that the immediate neighbours of RCs in the photosynthetic membranes are LHC B890. They surround RCs and form strongly coupled assemblies, B890–RC. The latter assemblies in turn are surrounded and interconnected by the 800–850 complexes. Picosecond fluorescence spectrally resolved studies of *Chromatium minutissimum* chromatophores [4] have shown that excitation energy is channeled from B800–850 to B890 and then to RCs, where charge separation occurs. A small (approximately 25%) part of all the B800–850 complexes are suggested to be located peripheral to the rest of the photosynthetic unit. A similar conclusion has also been deduced in [5] from chemical cross-linking studies of intact *C. minutissimum* membranes.

The main task of the present work was to further investigate the spatial relationships between different complexes and to explore the possibility of creating a system which reproduces the conditions for efficient directed energy transfer to the RCs, on the basis of soybean liposomes containing LHC B800–850 and assemblies of B890–RC from *C. minutissimum*.

2. MATERIALS AND METHODS

C. minutissimum chromatophores, LHC B800–850 and assemblies of B890–RC were isolated as described earlier [5,6]. The liposomes were prepared by dispersing 10 mg of soybean lecithin (Calbiochem, Switzerland) in 5 ml of buffer A (10 mM Tris-HCl, 50 mM KCl, 0.5 mM EDTA), followed by vigorous mixing. The non-ionic detergent, octyl-β-D-D-glucopironiside, was added to a final concentration of 1% to clarify the solution. To incorporate the complexes into the liposomes, equal volumes of the liposome and complex suspensions (ratio (B800–850)/(B890–RC) = 2, the total protein concentration, 2 mg/ml) were mixed and sonicated for 1 min at 4°C. The proteoliposomes were incubated for 2 h at 4°C and then diluted by buffer A (1:50 v/v). The detergent was removed by ultrafiltration. The concentrated sample was loaded on a Sepharose 6B column equilibrated with buffer A. The coloured fraction was harvested and proteoliposomes were sedimented at 220,000 × *g* for 3 h and then resuspended in 50 mM Bicin-NaOH buffer. The chemical cross-linking of chromatophores and liposomes with dithiobissuccinimidyl propionate (DSP) was performed as described in [5].

Absorption and steady-state fluorescence spectra were measured by standard means using commercial spectrometers. For the time-re-

Abbreviations: LHC, light-harvesting complex; RC, reaction center; *C., Chromatium*; BChl, bacteriochlorophyll; DSP, dithiobissuccinimidyl propionate.

Correspondence address: V.I. Godik, Department of Photosynthesis, A.N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, 119899 Moscow, Russia.

solved measurements the apparatus described earlier [7] was employed.

3. RESULTS AND DISCUSSION

As shown in Fig. 1, the maximum of the bacteriochlorophyll (BChl) spectral form B850 in *C. minutissimum* chromatophores is at 854 nm and is 5 nm blue shifted in the liposomes, reconstituted with B800–850 and B890–RC. In the latter system relative absorbance at 850 nm is diminished considerably, as compared with that at 800 and 890 nm. Similar absorption changes were described for Triton X-100-treated LHC B800–850 [6], and shown to be reversible after the detergent removal. The presence of a small amount of the detergent was necessary in our measurements to prevent aggregation of the complexes before the reconstitution.

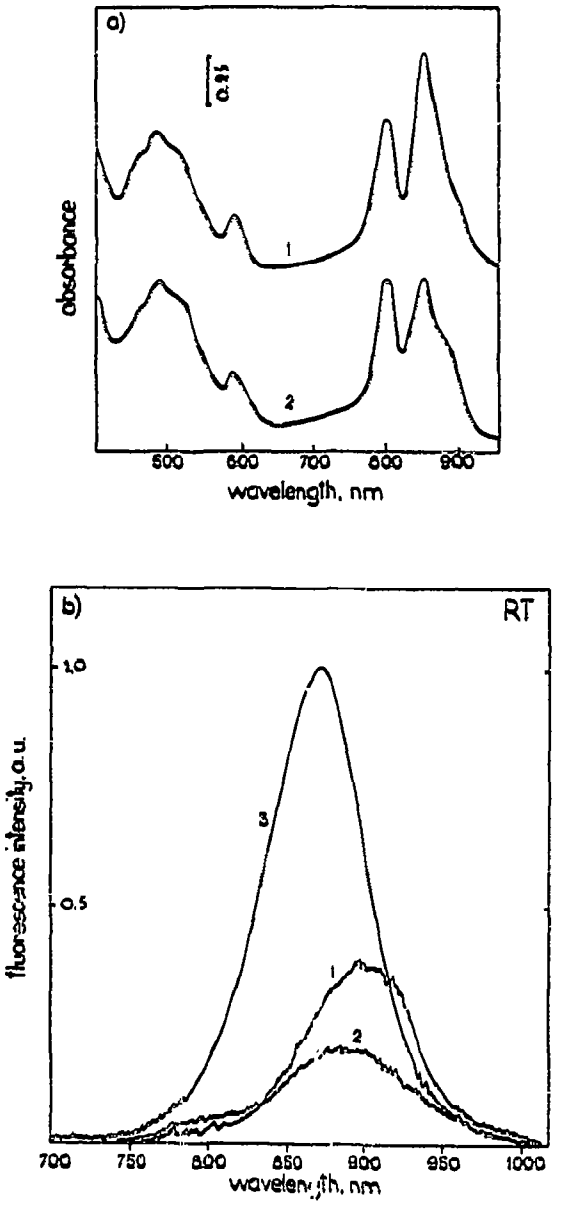

Fig. 1. (a) The absorption spectra of *C. minutissimum* chromatophores (1) and proteoliposomes (2) reconstituted with B800–850 and B890–RC. (b) The steady-state fluorescence spectra of the chromatophores (1), the proteoliposomes (2) and complexes of B800–850 (3). Optical density at the excitation wavelength of 365 nm was 0.2, and the monochromator bandwidth 4 nm.

The maximum of the chromatophore fluorescence at room temperature is at 908 nm, while that of the isolated LHC B800–850 is near 865 nm (Fig. 1b). The fluorescence spectrum of the reconstituted liposomes looks very similar to that of the chromatophores, peaking at about 903 nm. The data of Fig. 1b indicate that in the liposomes B850 fluorescence is quenched considerably, presumably due to the excitation transfer to B890. In the reconstituted system, however, a small increase in the emission at the short-wavelength part of the spectrum is seen, indicating that the efficiency of the energy transfer from B800–850 to B890–RC could be lower, as compared with the native chromatophores.

Time- and spectrally resolved fluorescence data of Fig. 2 are in general agreement with the steady-state data. The samples were excited by 3 ps pulses of 800 nm wavelength, and emission decay was registered at several indicated wavelengths. The isolated B800–850 complexes are characterized by the longest-lived fluorescence, whose kinetics is best fit by two components with 630 ± 60 and 90 ± 30 ps time constants and amplitude ratio (1.3:1.0), being independent of the emission wavelength. This kinetics is plotted in Fig. 2a for emission wavelength of 880 nm, together with those of the chromatophores and the liposomes. It can be seen that the two latter kinetics are much faster than the first one, and are practically coincident. At 860 nm, however, where B850 fluorescence predominates, the decay is slower in the liposomes than in the chromatophores. This is in accordance with the data of Pennoyer et al. [7], showing that, after fusion of *Rhodobacter sphaeroides* chromatophores with liposomes, fluorescence yield of B850 increases both at room temperature and 77 K, presumably due to a dissociation of a part of B800–850 from B890–RC assemblies. The selective dissociation may be confined to those B800–850 complexes that are located at the periphery of the photosynthetic units.

The dependence of fluorescence kinetics of both the chromatophores and the liposomes on the emission wavelength (Fig. 2) is typical of a directed excitation energy transfer from the peripheral B800–850 antenna to the core LHC B890 and then to the RC, as described in detail in [4]. This dependence is more pronounced at 77 K as compared with that at room temperature (Fig. 2b), but lengthening of the fluorescence kinetics at the short-wavelength part of the liposome spectra becomes much more significant under these conditions. It follows that at 77 K a greater part of the B800–850 complexes is detached from the B890–RC assemblies.

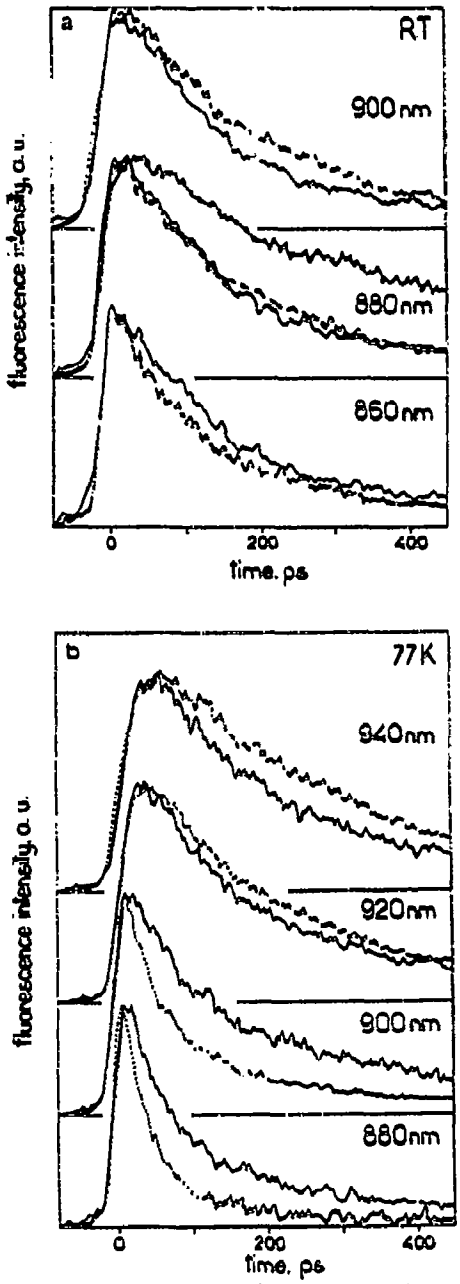

Fig. 2. Picosecond spectrally selective (the monochromator bandwidth, 4 nm) fluorescence kinetics of the chromatophores (solid lines), the proteoliposomes (dashed lines) and complexes of B800–850 (dotted lines) at room temperature and 77 K at different indicated emission wavelengths. Excitation wavelength, 800 nm.

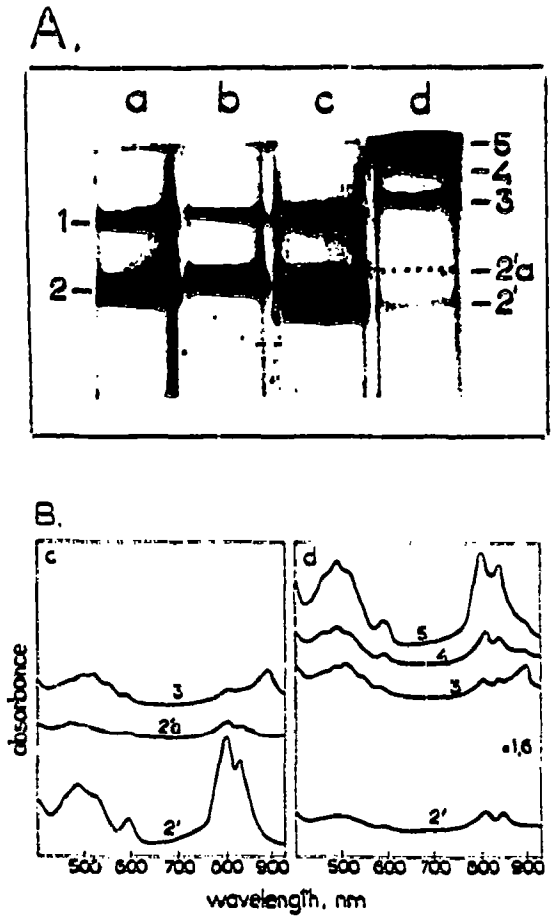

Fig. 3. (A) Electrophoresis with Triton X-100: lane a, proteoliposomes; lane b, chromatophores; lane c, proteoliposomes treated with DSP; lane d, chromatophores treated with DSP. 1 and 2, bands corresponding to B890–RC and B800–850, respectively. From untreated proteoliposomes (a) or chromatophores (b), 2',2a',3–5, the bands from DSP-treated proteoliposomes (c) or chromatophores (d), corresponding to B800–850 (2',2a') or associations of B800–850/B890–RC (3–5). (B) The absorption spectra of the coloured bands (numbers as in (A)) from the electrophoresis of the proteoliposomes (c) and the chromatophores (d) both treated with DSP.

Spatial relationships between the complexes in the liposomes and the chromatophores have been studied using bifunctional cross-linker DSP. The DSP and Triton X-100-treated proteoliposomes yield pigment bands very similar to those for untreated liposomes and chromatophores (Fig. 3A, a and c). The only difference is a new weak band 2a', belonging to B800–850 dimers, judging by its electrophoretic mobility and absorption spectrum (Fig. 3B). Band 2' belongs to B800–850 monomers with increased electrophoretic mobility caused by interaction of the cross-linker with the polypeptide charged groups [8,9]. Band 3 corresponds to B800–850/B890–RC associations with a low content of B800–850.

Quite a different picture is observed for the chromatophores treated with DSP, where the majority of the pigment–protein complexes are cross-linked into these associations with a high content of B800–850 (a band at the top of the gel) and only a small part of B800–850 moves as a monomer (Fig. 3A, d).

The practical absence of the cross-links between B800–850 and B890–RC in the proteoliposomes is a result of either a modification of the protein amino groups during electrophoresis, or different lateral topography of the LHC B800–850 and the assemblies of

B890–RC in the chromatophore and liposome membranes. The yield of the cross-links in the isolated complexes is high, which is not consistent with the first suggestion. However, the phospholipids in the liposomes could possess specific reactive sites for the cross-linking in great abundance. On the other hand, the above presented stationary and time-resolved data suggest that the main features of the lateral organization of the complexes, necessary to provide the conditions for the directed excitation energy transfer, are observed in the liposome membranes. The differences in the picosecond fluorescence kinetics indicate, however, that these conditions are modified by the presence of lipid in a way quite similar to that observed for intact chromatophores fused with liposomes [7].

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