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The light-harvesting complexes of a thermophilic purple sulfur photosynthetic bacterium *Chromatium tepidum*.

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Several biophysical properties (absorption, fluorescence, linear dichroism) are reported for the chromatophore membranes of the thermophilic purple sulfur bacterium, *Chromatium tepidum*. Like the mesophilic strain *Chromatium vinosum*, two types of light-harvesting complex are present: one absorbs at 800 nm and 855 nm; the second complex is equivalent to the B890 complex of *C. vinosum*, but absorbs at 918 nm, i.e., 30 nm higher. This is the highest absorption band observed so far for a light-harvesting complex containing bacteriochlorophyll *a*. In spite of the small overlap between the fluorescence and absorption bands of the two light-harvesting complexes, specially at low temperature, an efficient energy transfer occurs from the high-energy (B800–855) to the low-energy (B920) complexes. The B800–855 complexes have been isolated from the whole membrane by lauryldimethylamine *N*-oxide treatment, whereas only a partial purification was achieved for the B920 complexes.

Introduction

Recently, a thermophilic photosynthetic bacterium containing only BChl *a* has been obtained in pure culture [1]. This bacterium grows photoautotrohically in mineral medium with HCO_3^-/CO_2 as sole carbon sources and sulfide as substrate [2]. That property and several others identify this thermophilic bacterium as a member of the genus *Chromatium* [1,2]. Its optimum temperature of development is 48–50°C and it has been proposed as a new species, *Chromatium tepidum* [2]. This opens the possibility to compare biophysical and biochemical properties of mesophilic and thermophilic strains of a same genus. Several optical properties, absorption [3–6], fluorescence [4–6] and linear dichroism [7] have been reported in large details for the mesophilic strain *Chromatium vinosum*. Three spectrally distinct light-harvesting complexes, B800–820, B800–850 and B890 can be isolated and purified from that species [4,8].

In the present report, we describe different optical properties and a procedure for isolation of the light-harvesting complexes of the thermophilic strain *Chromatium tepidum*.

Material and Methods

Chromatium tepidum was grown at 48°C under light-anaerobic conditions in the medium described by Madigan [1]. The light intensity was 2000 lx. *Chromatium vinosum* was grown at 30°C according to Ref. [9]. Cells were harvested after 4–5 days and stored frozen until used. Chromatophores were prepared by disruption of the cells by

Abbreviations: LDAO, lauryldimethylamine *N*-oxide; BChl, bacteriochlorophyll.

two passages through a French press at 110 MPa. After centrifugation at $15000 \times g$ for 10 min to remove unbroken cells and cells debris, chromatophores were collected by ultracentrifugation at $195000 \times g$ for 90 min, and resuspended in 10 mM Tris (pH 8). Absorption spectra were recorded with a Varian 2300 spectrophotometer equipped with a helium gas cryostat (Meric) for low-temperature measurements. Fluorescence emission spectra were recorded with a home-built fluorimeter. Excitation light was provided by a 800 watt quartz iodine lamp filtered through two blue filters (Corning 4-96). Fluorescence light was focused on the entrance slit of a monochromator (Jobin Yvon H2O) with appropriate lenses, and detected with a photomultiplier (Hamamatsu R316).

Measurements of linear dichroism spectra of chromatophores, oriented in squeezed polyacrylamide gel, were performed as described in Ref. 10.

Results and Discussion

(A) Biophysical properties of the light-harvesting complexes of Chromatium tepidum

(1) Absorption spectra

Fig. 1A shows the room temperature spectrum of a suspension of *C. tepidum* chromatophores. In the near infrared region, three main bands are present at 800, 855 and 918 nm. They can be associated to two different light-harvesting complexes called thereafter B800–855 and 920. For comparison, the absorption spectrum of *C. vinosum* chromatophores is depicted in Fig. 1B (see also Refs. 3–6). The visible parts are very similar for both strains (Fig. 1A–B). Lowering the temperature to that of liquid helium allows the resolution of the 800 nm absorption band into two distinct bands, centered at 795 and 808 nm for both species (Fig. 2A and B) [4,6]. A shoulder at 830 nm is only apparent in the case of *C. vinosum* (Fig. 2B) [4,6]. Low temperature induces also spectral shifts of the long-wavelength absorption band. In case of *C. tepidum* the 855 nm band of the B800–855 complexes shifts to 872 nm at 4.2 K, while the 920 nm band (B920 complex) moves to 943 nm (Fig. 2A). The B800–850 and B890 complexes absorb at 868 nm and 910 nm, respectively, at 4.2 K for *C. vinosum* chromatophores (Fig. 2B) [4,6].

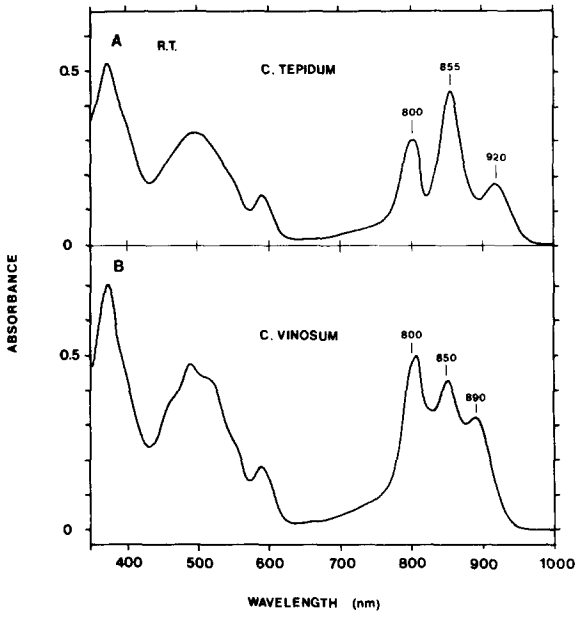

Fig. 1. (A) Absorption spectrum of a suspension of *C. tepidum* chromatophores suspended in 10 mM Tris (pH 8) at room temperature (RT). (B) Same as (A), but for *C. vinosum* chromatophores.

In the visible part, two absorption bands are resolved at 4.2 K for the BChl Q_x transitions, at 590 nm and around 610 nm for both species (Fig. 2A and B). At least, five components are resolved in the carotenoid region in each case (Fig. 2A and B). Three main differences between the absorption spectra of the two species are obvious. (1) The ratio between the different bands in the 440–560 nm region depends on the species (Fig. 2A–B). This certainly reflects their different composition in carotenoids [2]. (2) The ratio between the B800–850 and B890 complexes is higher in the case of *C. tepidum* than for *C. vinosum*. (3) The wavelength position of one of the light-harvesting complexes is higher in the case of *C. tepidum* (920 nm at room temperature and 943 nm at 4.2 K) than for *C. vinosum* (890 nm at room temperature and 910 nm, 4.2 K) (Fig. 2A, B) [4,6]. This light-harvesting complex of *C. tepidum* possesses the highest wavelength position found so far for a photosynthetic bacteria containing BChl *a*.

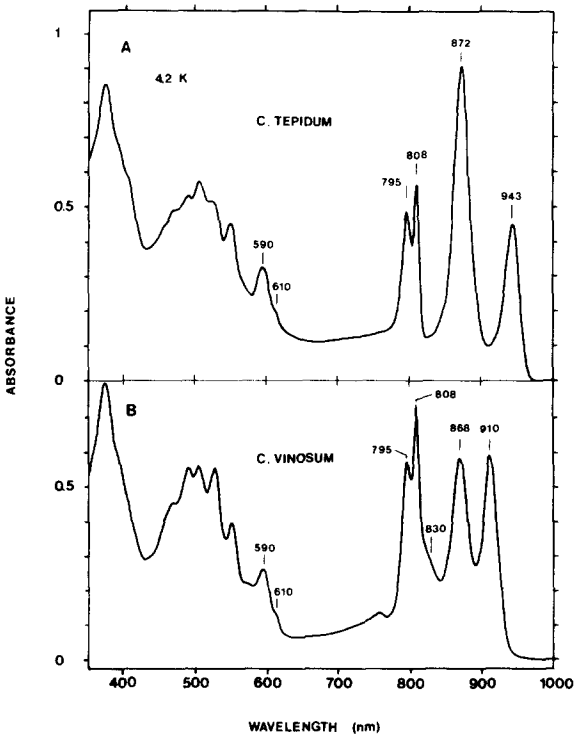

Fig. 2. (A) Absorption spectrum of a suspension of *C. tepidum* chromatophores suspended in 10 mM Tris (pH 8) and glycerol (60% v/v), recorded at 4.2 K. (B) Same as (A), but for *C. vinosum* chromatophores.

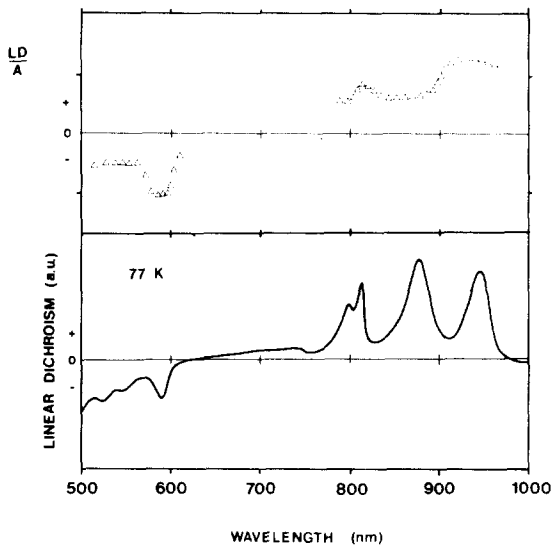

Fig. 3. Linear dichroism spectrum recorded at 77 K of a suspension of *C. tepidum* chromatophores oriented in a squeezed polyacrylamid gel. This spectrum corresponds to the difference between the absorption occurring parallel ($A_{\parallel}$) and perpendicular ($A_{\perp}$) to the membrane plane. The values of the reduced dichroism LD/A are also plotted vs. the wavelength.

(2) Linear-dichroism spectrum

To investigate the orientation properties of the B800–855 and B920 complexes of *C. tepidum*, we have recorded the linear dichroism spectrum, at 77 K (Fig. 3) of a suspension of chromatophores oriented in a squeezed polyacrylamide gel [10]. The BChl *a* Q_y transitions (800, 870 and 937 nm at 77 K) are oriented mainly parallel to the membrane as seen by the positive value of $LD = A_{\parallel} - A_{\perp}$. The BChl *a* Q_x transitions (approx. 590 nm) and the carotenoids transitions (500, 520, 545 nm) are oriented more or less perpendicular to the membrane plane (LD < 0). This result is very similar to what is encountered in other photosynthetic bacteria membrane [11]. One characteristic is, however, that the long-wavelength component (937 nm) is oriented more parallel to the membrane plane than the 870 nm component, as shown by the higher LD/A value measured at the longest wavelength (Fig. 3). This is in contrast to what has been observed for *C. vinosum* chromatophores where both types of light-harvesting complexes, B800–850 and B890, present the same orientation with the membrane plane [7].

(3) Fluorescence emission spectrum

The chromatophore fluorescence of *C. tepidum* (Fig. 4) is emitted preferentially by the long wavelength light-harvesting complexes as shown by the wavelength position of the maxima (935 nm and 965 nm at room temperature and 77 K, respectively). Some fluorescence of the B800–855 is seen as a shoulder at 875 nm at room temperature, and a small peak at 900 nm at 77 K. These results imply a good energy transfer between the B800–855 and B920 complexes, even at low temperature, although the overlap between their fluorescence and absorption bands (Fig. 1A) is less pronounced than, for example, in the case of *C. vinosum* (Fig. 1B).

(4) Absorption changes linked to the photooxydation of the primary electron donor

The long-wavelength absorption band of one of the light-harvesting complexes, B920, leads us to seek for the wavelength position of the long-wave-

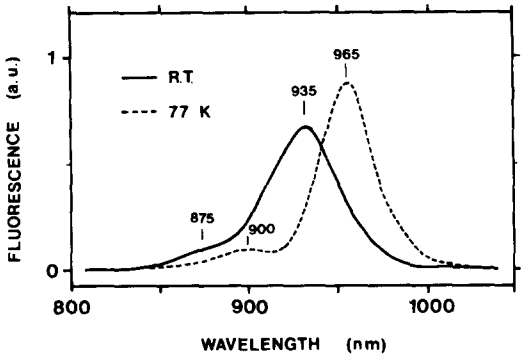

Fig. 4. Fluorescence emission spectrum of a suspension of *C. tepidum* chromatophores suspended in 10 mM Tris (pH 8), glycerol (60% v/v). The spectra were recorded at room temperature (R.T.) (————) and 77 K (- - - - - -).

length absorption band of the primary electron donor of the reaction center, since the efficiency of the photochemistry depends on the overlap between these two bands. Fig. 5 shows the difference absorption spectrum, measured point by point, induced by 5 s of continuous illumination on a suspension of *C. tepidum* chromatophores. The light-minus-dark difference spectrum observed upon excitation with a laser flash is identical (data not shown). The light-induced difference spectrum (Fig. 5) is very similar to the one reported for the mesophilic strain *C. vinosum* [4,12,13] or other photosynthetic bacteria species, with both positive and negative changes around 800 nm and the bleaching of a long-wavelength component. The main difference is the higher

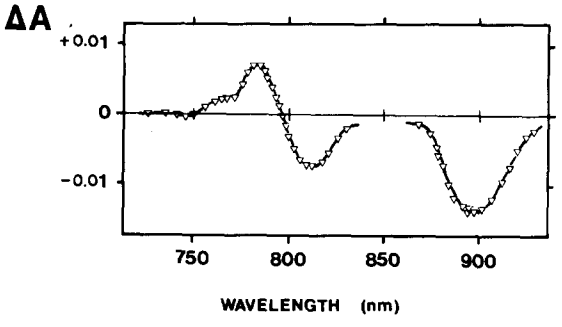

Fig. 5. Light-induced difference spectrum measured for a suspension of *C. tepidum*, adjusted at 0.5 absorbance at 920 nm. The suspension was excited with 5 s of blue light.

position in wavelength observed for that bleaching, 900 nm in the case of *C. tepidum*, compared to 880 nm for *C. vinosum* [4,12,13].

(B) Isolation of light-harvesting complexes from C. tepidum

B800–855 complexes have been isolated from the whole chromatophore membrane by the following procedure. Chromatophores, adjusted at 50 absorbance/cm at 855 nm, were incubated for 10 min in the dark at room temperature in presence of 0.5% LDAO. The chromatophore suspension was then deposited on a linear gradient of sucrose (0.1 M–0.6 M) and centrifuged at $90000 \times g$ for 16 h. This allows a good separation between B800–855 complexes, which stay on the top of the gradient, from large pieces of chromatophores, enriched in B920 complexes which sediment between 0.3 and 0.4 M sucrose. The B800–855 complexes were further purified by ammonium sulfate precipitation (between 18% and 25%). No photo-

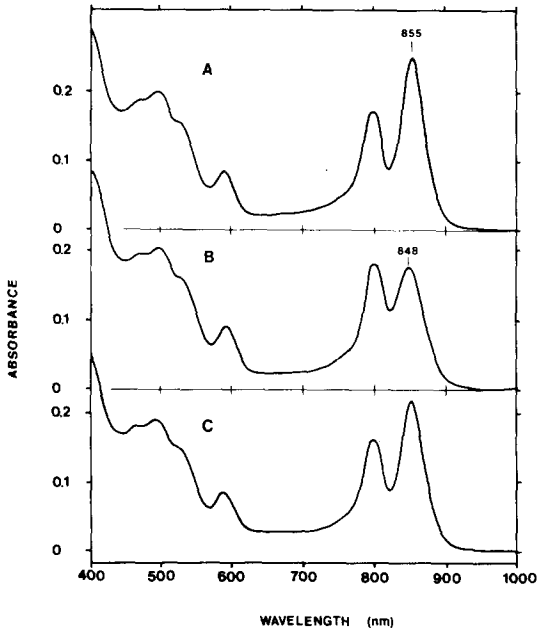

Fig. 6. Absorption spectrum of isolated B800–855 complexes. (A) Suspended in 10 mM Tris (pH = 8), 0.001% LDAO; (B) Same as A after addition of 0.01% LDAO; (C) Same as B after dilution by a factor 10 (the optical pathlength was multiplied by the same factor).

chemical activity could be detected in such a preparation. The absorption spectrum of B800–855 complexes prepared in such a way depends strongly on the amount of detergent present. Fig. 6A shows the absorption spectrum of B800–855 complexes suspended in 10 mM Tris pH 8 and 0.001% LDAO. Addition of 0.01% LDAO induces a large decrease of the 855 nm band with a small shift to the blue (7 nm), while the 800 nm band slightly increases (Fig. 6B). These effects are reversible upon dialysis or dilution of the detergent (Fig. 6C). Similar observations have been reported for B800–850 complexes isolated from *C. vinosum* in the presence of several different nonionic and cationic detergents (3, 4, 5, 8, 14). Incubation of purified B800–855 complexes with LDAO concentration higher than 0.15% induced similar absorption changes, but in this case reversibility is not observed (not shown).

(C) Isolation of B920 complexes

The enriched fraction in B920 complexes obtained after addition of 0.5% LDAO were concentrated and subjected twice to incubation with LDAO 1% followed by ultracentrifugation on a sucrose gradient. The lowest colored fraction, around 0.35 M, is highly enriched in B920 complexes (Fig. 7), although some B800–855 complex is still present as shown by the shoulder at 850 nm

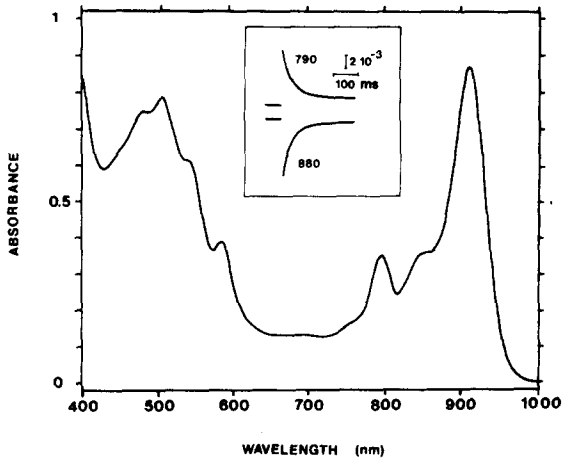

Fig. 7. Absorption spectrum of isolated B910 complexes suspended in 10 mM Tris (pH = 8), 0.01% LDAO. The inset shows the absorbance changes induced by a laser flash observed at 880 nm and 790 nm for such a preparation.

and the absorption band at 800 nm. During the preparation the long-wavelength band shifts from 920 nm, in the native membrane (Fig. 1A) to 910 nm in the enriched fraction (Fig. 7). This fraction retains the photochemical reaction centers as demonstrated by the reversible light-induced absorbance changes detected (inset Fig. 7). The fast decay time observed (Fig. 7) for the back reaction suggests that the secondary acceptor has been removed by the LDAO treatment. The light-induced difference spectrum obtained (not shown) with these B910 fractions is identical to the one observed in the intact membrane (Fig. 5).

Comparison of the absorption spectra of B800–855 and B910 complexes (Fig. 6A and Fig. 7) in the visible shows that the carotenoids absorption bands are shifted by 10 nm between the two complexes. This may result in differences in the chemical nature or the proteinic environment of the carotenoid molecules bound on the two complexes. The superposition of these two sets of carotenoid absorption explains the featureless shape observed for the whole membrane at room temperature (Fig. 1A) in this spectral region.

In conclusion, the pigment organization of the thermophilic photosynthetic bacterium *C. tepidum* is very similar to the one observed for the mesophilic strain *C. vinosum*. In both cases two types of light-harvesting complexes, belonging to two different classes [14,15] are present. The main difference is the high wavelength position of one of the light-harvesting complex observed for the thermophilic strain, 918 nm, i.e., 30 nm upper than the mesophilic component. This is the longest wavelength observed so far for a light-harvesting complex of a BChl *a* containing photosynthetic microorganism.

Further comparison of biochemical (polypeptide composition and protein sequence) and biophysical (Raman spectroscopy) properties will certainly allow a better understanding of the thermophilic character of *C. tepidum*.

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