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A spectral characterisation of the light-harvesting pigment-protein complexes from *Rhodopseudomonas acidophila*

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Four different antenna-complexes were prepared from two wild-type strains of *Rhodopseudomonas acidophila* and their absorption, fluorescence emission and excitation spectra were recorded as a function of temperature in the range from 280 K to 4 K. In each case as the temperature was lowered the fluorescence yield increased. The efficiency of singlet–singlet energy transfer from the carotenoids to the bacteriochlorophyll *a* within each complex was determined. The efficiency of this process was shown to be temperature independent. Interestingly the relative efficiency of the carotenoid-to-bacteriochlorophyll *a* energy transfer varied markedly, depending upon the type of complex. It is suggested that this variation may, in part, explain why *Rps. acidophila* alters the type of the variable antenna component present in its cells when it is grown at different light intensities.

Introduction

The photosynthetic unit in purple photosynthetic bacteria is composed of discrete reaction centres and antenna complexes [1–3]. However, both the number of photosynthetic units per cell and the composition of these units is very variable [4–6]. Environmental parameters, such as the light intensity at which the cells are grown, exert a strong influence on the cellular pigment content [4].

So far three main types of response of the photosynthetic unit to growth at different light intensities have been described in purple bacteria [4,6,7]. In the first type, typified by *Rhodospirillum rubrum*, lowering the light intensity at which the cells are grown induces the biosynthesis of more photosynthetic units per cell [4]. *R. rubrum* only contains a single antenna type (the B890 complex [1]) and at all light intensities the size of its photosynthetic unit remains constant, at about 25–30 molecules of bacteriochlorophyll *a* per reaction centre. In species such as *Rhodopseudomonas sphaeroides*, which contain two antenna types (the B875 complexes and the B800–850 complexes) a rather more subtle adaption to changing light intensity is seen [1,4]. When *Rps. sphaeroides* is grown at high light intensities the cells are weakly pigmented and contain a small number of photosynthetic units. In this case the photosynthetic units are mainly composed of reaction centres and B875 complexes [4]. However, when cells of *Rps. sphaeroides* are grown at low light-intensities then not only does the number of photosynthetic units per cell increase, as in *R. rubrum*, but also the size of these units gets larger. At all light-intensities the ratio of B875 complexes per reaction centre ap-

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Abbreviation: LDAO, lauryldimethylamine *N*-oxide.

pears to remain constant; the increase in size of the photosynthetic unit is associated with an increase in the number of B800–850 complexes present. The B800–850 complexes act as a variable component of the photosynthetic unit and their synthesis is turned on by lowering the light intensity. A third type of response to growth at different light intensities has been described for species such as *Rhodopseudomonas acidophila* [7]. Strains of *Rps. acidophila* such as 7050 can, depending upon the growth conditions, synthesise four different types of antenna complexes, B880 complexes (similar to the B890 complex from *R. rubrum*), type I B800–850 complexes (similar to the B800–850 complex from *Rps. sphaeroides*), type II B800–850 complexes and B800–820 complexes [1,7]. When *Rps. acidophila* 7050 is grown at high light intensities its photosynthetic unit is mainly composed of reaction centres and B880 complexes. At intermediate light intensities the cells become more pigmented and the photosynthetic units include the type I B800–850 complexes, while when the cells are grown at rather low light intensities the photosynthetic units contain reaction centres, B880 complexes, type II B800–850 complexes and B800–820 complexes. At the lower light-intensities the variable type I B800–850 complexes are replaced by type II B800–850 complexes and B800–820 complexes. In *Rps. acidophila* strain 7050, therefore, the type of variable antenna component, as well as its amount, is influenced by the light intensity at which the cells are grown.

We have previously presented an initial characterisation of the composition of the antenna complexes from *Rps. acidophila* [7]. In this paper we present a detailed description of their absorption, fluorescence emission and excitation spectra. These basic studies are required to provide the background information so that the reasons for the changes in the type of the variable antenna complexes in *Rps. acidophila*, as a function of light intensity, can be investigated.

Materials and Methods

Growth of the cells and preparation of the antenna complexes

Cells of *Rps. acidophila* strains 7050 and 7750 (the latter strain is able to synthesise neither the type II B800–850 complexes nor the B800–820 complexes, and so is a better source of the type I B800–850 complexes) were grown anaerobically in the light at 30°C in Pfennig's medium [8] with succinate as the main carbon source. Strain 7050 was grown at light intensities of approx. 0.1 $mW \cdot cm^{-2}$, under which conditions the cells were purple and contained B880, type II B800–850 and B800–820 complexes. Strain 7750 was grown at light intensities of approx. 6 $mW \cdot cm^{-2}$, under which conditions the cells were red-brown coloured and contained B880 and type I B800–850 complexes. The cells were grown in 250 cm^3 flat-sided glass bottles and were harvested in log phase.

After growth the cells were harvested, washed and resuspended in 20 mM Tris-HCl (pH 8.0). The cells were then either used immediately or stored at −20°C until they were required. The washed cells were broken by passage through a French pressure cell at 154 MPa in the presence of a little DNAase. The disrupted membranes were collected by centrifugation. The antenna complexes were isolated and purified from membranes solubilised by lauryldimethylamine *N*-oxide (LDAO) as previously described [7]. The B880 and type I B800–850 complexes were prepared from strain 7750 and the type II B800–850 and B800–820 complexes were prepared from strain 7050.

Absorption and fluorescence spectroscopy

The room temperature and low temperature absorption spectra of the antenna complexes were recorded with a Pye-Unicam SP8-250 spectrophotometer using a CF 204 Oxford Instruments helium flow cryostat. The fluorescence emission and excitation spectra of the complexes were determined with a home-built spectrophotometer [9]. The excitation light from a xenon arc lamp (XBO 900 W) was passed through a water filter, dispersed by an $f = 220$ mm double monochromator (Spex Doublemate) and focussed on the sample in the cryostat. A small proportion of the output of the monchromator was diverted by a beam splitter and focussed onto a photodiode. The signal from the photodiode was then used as a reference for the control of the variable slit at the entrance of the monochromator to ensure constant excitation intensity at all wavelengths. The fluorescence emitted by the antenna complexes was detected by

a near-infrared sensitive photomultiplier (S1-photocathode, type EMI 9684) positioned behind a second double monochromator ($f = 220$ nm, Spex Spectromate). In order to compensate for the intensity minima in the spectral output of the xenon lamp, in both the near-infrared and the UV regions, which cannot be adequately regulated by the slit control, the amplified signal from the photomultiplier was divided by the signal from the photodiode. This normalised signal was then digitised and processed by a mini-computer. The computer was also used to control the scanning of the two monochromators. The emission spectra were also corrected for the spectral transmission of the detection path.

The antenna samples were mixed with glycerol in the ratio of 1 :2 (v/v) in order to obtain a clear glass at low temperatures. For the fluorescence measurements the antenna samples were further diluted with 50 mM Tris-HCl (pH 7.9) prior to the addition of glycerol so that the final optical density of the samples at the peak of the major long-wavelength absorption band was 0.05 in a 2 mm cuvette.

The energy-transfer efficiencies of the carotenoid to bacteriochlorophyll *a* singlet–singlet energy transfer were determined by comparing the amplitudes of the carotenoid peaks in the absorption and excitation spectra with those of the Q_x band of bacteriochlorophyll *a*, as described by Van Grondelle et al. [10].

Results and Discussion

The temperature dependence of the absorption spectra of the light-harvesting complexes from Rps. acidophila

The absorption spectra of the four types of antenna complexes prepared from *Rps. acidophila* were recorded at 20 K intervals in the temperature range from 280 to approx. 4 K. The results are presented in Fig. 1a–d and are summarised in Table I. For simplicity, spectra are only shown for three different temperatures.

The absorption spectrum of the B880 complex is shown in Fig. 1a. The peaks at approx. 380 nm, 590 nm and approx. 880 nm arise from the Soret, Q_x and Q_y absorption bands of the bacteriochlorophyll, while the peaks in the 440–570 nm range are due to the carotenoids, which are also present in this antenna complex [7]. The small absorption bands in the near-infrared at 750 and 800 nm show that this preparation also contains a few reaction centres and in this respect is similar to Thornber's *Chromatium* fraction A preparation [11]. As the temperature is lowered the bacteriochlorophyll Q_y absorption band narrows and shifts to the red, so that at 6 K the peak is at 900 nm. At 6 K the reaction centre absorption bands are also much more clearly visible including the shoulder at approx. 870 nm which comes from the reaction centre 'special-pair' of bacteriochlorophylls [12]. As the temperature is lowered, the

TABLE I

THE ABSORPTION AND EMISSION MAXIMA OF THE ANTENNA COMPLEXES FROM *RPS. ACIDOPHILA* IN THE NEAR-INFRARED AT 280 K AND 4 K

The relative fluorescence yields were calculated as described in ref. 10. The values at 4 K are taken as 100%.

Type of complex	Absorbance (nm)		Fluorescence (nm)		Relative yield of fluorescence of the major emission band	
	280 K	4 K	280 K	4 K	280 K	4 K
B880	760 [a], 803 [a], 886	760 [a], 801 [a], 900	783 [b,c], 905	783 [b,c], 887 [a,b], 923	≈ 25%	100%
Type I B800–850	802, 861	803, 868	816 [b], 882	793 [b,c], 816 [b], 900	≈ 35%	100%
Type II B800–850	803, 852	804, 823, 861	869	891	≈ 30%	100%
B800–820	804, 829	804, 825, 861	863	791 [b,c], 838 [b], 878	≈ 30%	100%

[a] Reaction centre bands.
[b] Weak emission bands.
[c] Probably due to some free bacteriochlorophyll.

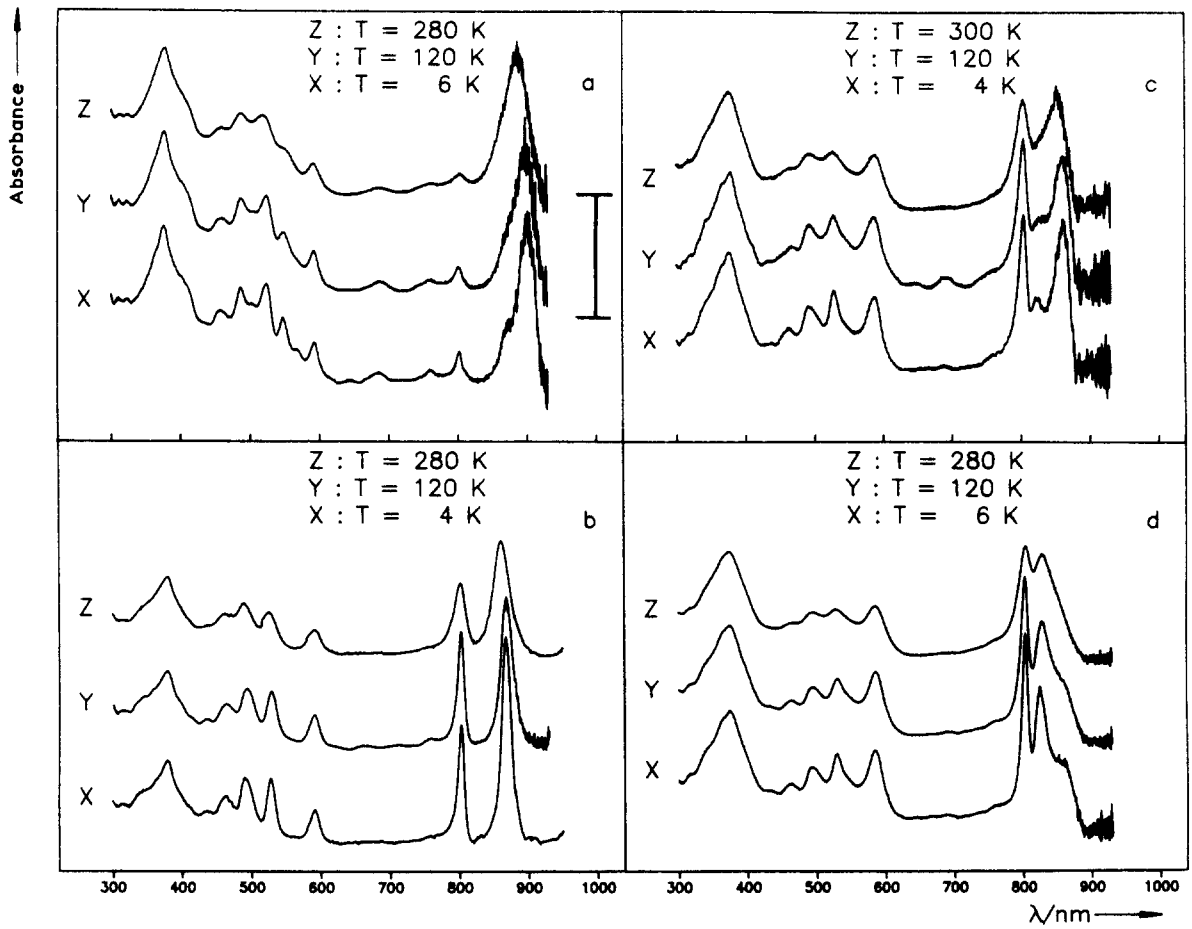


Fig. 1. The effect of temperature upon the absorption spectra of the antenna complexes isolated from *Rps. acidophila*. (a) The B880 complex; (b) the type I B800-850 complex; (c) the type II B800–850 complex; and (d) the B800–820 complex. In each case the antenna samples were diluted with 20 mM Tris-HCl (pH 8.0), so that when they were mixed with glycerol in the ratio of 1:2 (v/v), respectively, the samples had an absorbance of approx. 0.5 in a 2 mm pathlength cuvette at the peak of the strongest near infrared bacteriochlorophyll *a* absorption band at room temperature. For clarity the spectra shown at each temperature have been displaced by 0.35 absorbance units at 300 nm. The vertical bar shown in (a) represents 0.5 absorbance units. In some of the spectra shown it is clear that there was a curved baseline. This was due to scattering induced by 'cracking' in the sample when it was frozen which was very difficult to correct completely with the buffer/glycerol blank.

carotenoid absorption bands also narrow and the multiple types that are present are now clearly visible.

The absorption spectrum of the type I B800–850 complex is shown in Fig. 1b. As the temperature is lowered from 280 K to 4 K, the two near-infrared bacteriochlorophyll absorption bands narrow. The 800 nm absorption band does not shift, but the 861 nm absorption is red shifted to 868 nm at 4 K. Again the carotenoid absorption bands also sharpen markedly at cryogenic temperatures. A similar temperature dependence of the absorption spectra of the B800–850 complexes from *Rhodopseudomonas sphaeroides* and *Rhodopseudomonas capsulata* has been reported [10,13].

The absorption spectrum of the type II B800–850 complex is depicted in Fig. 1c. At 280 K two strong near-infared bacteriochlorophyll absorption bands are seen, one at 800 nm and a second asymmetric one at 852 nm. As the temperature is lowered the 800 nm band narrows and the asymmetric long-wavelength absorption band is

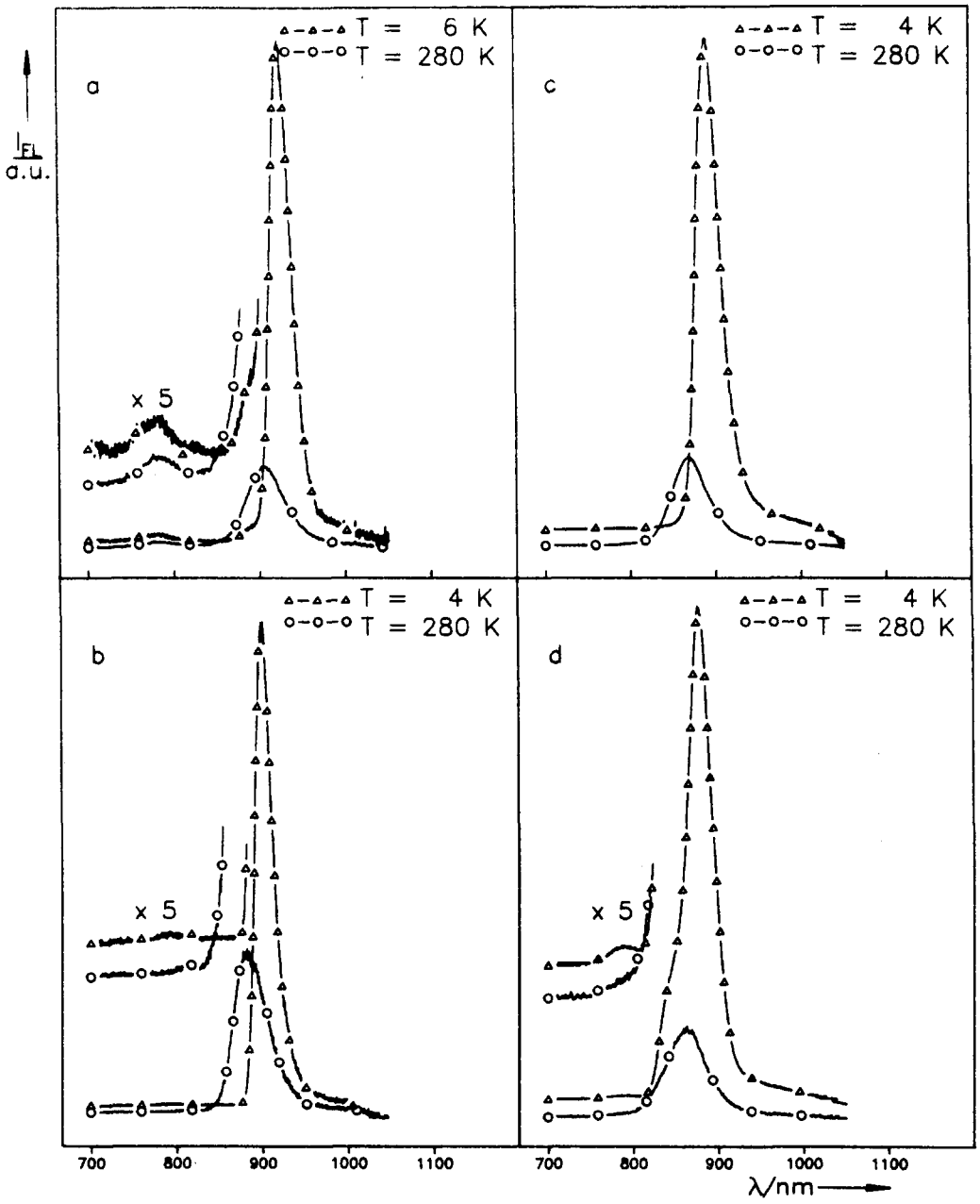


Fig. 2. The emission spectra of the antenna complexes isolated from *Rps. acidophila* recorded at 280 K and liquid helium temperatures. (a) The B880 complex; (b) the type I B800–850 complex; (c) the type II B800–850-complex; and (d) the B800–820-complex. In each case the vertical scale of intensity of fluorescence is in arbitrary units. The following excitation wavelengths were used: (a) 592 nm, (b) 592 nm, (c) 587 nm, (d) 584 nm. Each sample had an absorbance of 0.05 in the 2 mm cuvette at the long-wavelength bacteriochlorophyll *a* absorption band.

resolved into two components. The major component is red shifted to 861 nm and the minor component is seen as a small peak at 823 nm. This most probably represents a small contamination with B800–820 complexes. In our previous study [7] we described an apparent spectral variation in the position of the long-wavelength bacteriochlorophyll absorption band in different preparations

of the type II B800–850 complex. The data in Fig. 1c suggest that this variation may have been due to contamination with different amounts of the B800–820 complex, which at room temperature is not spectrally resolved. One striking feature of these absorption spectra shown in Fig. 1c is the high intensity of the bacteriochlorophyll Q_x band relative to the Q_y bands. In both the B880 complex and the type I B800–850 complex at 280 K the ratio of the intensity of the Q_x band to the longest-wavelength Q_y band is approx. 0.25:1. This ratio is approx. 0.5:1 at 280 K for the type II B800–850 complex (the same is true for the B800–820 complex, see below). The reason for this is not clear. Again the absorption bands due to the carotenoids are better resolved at cryogenic temperatures.

Fig. 1d shows the effect of temperature on the absorption spectrum of the B800–820 complex. At 280 K the 820 nm absorption band is very broad. As the temperature is lowered and the bacteriochlorophyll Q_y bands become better resolved, a clear shoulder at 861 nm develops. This undoubtedly represents a small contamination with type II B800–850 complexes. At cryogenic temperatures the 800 and 820 nm absorption bands narrow and become more intense, but the positions of their maxima do not shift significantly.

The relationship between the type II B800–850 complex and the B800–820 complex is still not clear. They could even be two different spectral forms of the same basic complex. We are currently studying, in detail, their respective polypeptide compositions to see whether they are indeed two separate complexes, since in all but the positions of their bacteriochlorophyll Q_y bands they are spectrally very similar.

The temperature dependence of the fluorescence emission and excitation spectra of the antenna complexes from Rps. acidophila

The emission spectra of the antenna complexes are shown in Fig. 2a–d and summarised in Table I.

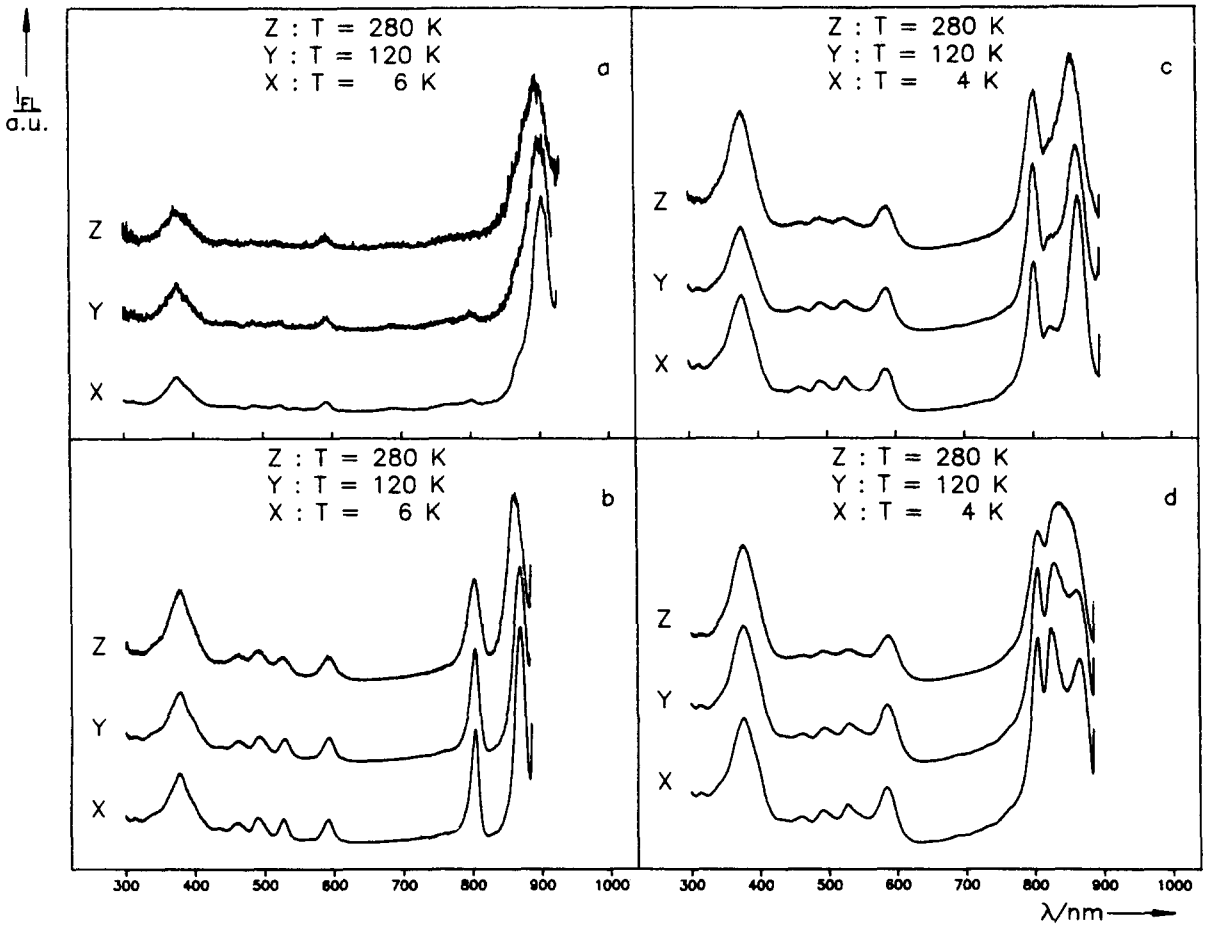
At 280 K the emission spectrum of the B880 complex shows a single major emission band centred at 905 nm. There is also a small emission centred at 783 nm, which probably arises from a low level of contaminating 'free bacteriochlorophyll', which because of its high fluorescence yield as compared with 'bound bacteriochlorophyll' shows up much more clearly in the fluorescence emission spectrum than in the absorption spectrum (compare Fig. 2a with Fig. 1a). As the temperature is lowered the major emission band shifts to the red and its relative intensity increases (Table I). At lower temperatures small emissions can also be seen emanating from the reaction centres which are present in this complex.

The type I B800–850 complex shows a single strong emission band which is centred at 882 nm at 280 K (Fig. 2b). A small emission with much lower yield is also seen at 816 nm. The 883 nm emission comes from the 850 nm absorption band, while the 816 nm emission represents a low level of fluorescence from the 800 nm absorption band. As the temperature is lowered the major emission band is red-shifted to 900 nm at 5 K and its yield increases by a factor of approx. 3× (Table I). Similar results have been reported for the B800–850 complexes from *Rps. sphaeroides* and *Rps. capsulata* [10].

The emission spectrum of the type II B800–850 complex is shown in Fig. 2c. At 280 K the emission band is centred at 869 nm. This emission undoubtedly arises from the 850 nm absorption band. As the temperature is lowered the major band shifts to the red to 891 nm and its yield increases (Table I).

At 280 K the emission spectrum of the B800–820 complex (Fig. 2d) is centred at 863 nm. As the temperature is lowered the main peak of the emission band shifts to the red to 878 nm and increases in intensity. At the lower temperatures a smaller underlying emission band centred at 838 nm begins to be resolved. The origin of these two emission bands is not yet completely clear cut because of the presence of a small amount of contamination with the type II B800–850 complexes (cf. Fig. 1d).

The fluorescence yield of each complex rises as the temperature is lowered (Table I). In each case the rise is gradual throughout the temperature range studied and there are no sudden breaks in the curve (data not shown) of yield vs. temperature. A similar temperature dependence of the fluorescence yield has been described [10] for the B800–850 complex from *Rps. sphaeroides*.



FDig. 3. The fluorescence excitation spectra of the antenna complexes isolated from *Rps. acidophila* recorded as a function of temperature. (a) The B880 complex; the detection wavelength at which the fluorescence intensity was recorded was 945 nm; (b) The type I B800–850 complex; the detection wavelength at which the fluorescence intensity was recorded was 900 nm; (c) The type II B800–850 complex; the fluorescence intensity was recorded at 909 nm; (d) The B800–820 complex; the fluorescence intensity was recorded at 895 nm. In each case the vertical scale of fluorescence intensity is in arbitrary units. The excitation spectra have been displaced to avoid confusing overlap and have been normalised to the same height in the bacteriochlorophyll *a* Q_x absorption band in the 590 nm region. In each case the samples had an absorption of 0.05 in a 2 mm cuvette at the long-wavelength absorption band of the bacteriochlorophyll.

The fluorescence excitation spectra were recorded for each complex as a function of temperature between 280 K and 4 K. Also for each complex the excitation spectra were determined while monitoring the fluorescence at various wavelengths across the major emission band. In every case the excitation spectra showed no significant changes dependent upon the detection wavelength. For convenience here, therefore, data will only be presented for each complex at a single emission wavelength.

The fluorescence excitation spectrum at 6 K of the B880 complex is shown in Fig. 3a. The excitation spectrum is very similar to the absorption spectrum (cf. Fig. 1a), except that the contribution from the carotenoids is relatively small (see Fig. 4 below). A small contribution from the reaction centres is also apparent. The carotenoids are more prominent in the fluorescence excitation spectrum of the type I B800–850 complex (Fig. 3b). Again in this case all the bacteriochlorophyll *a* absorption bands seen in the absorption spectrum (cf. Fig. 1b) contribute to the fluorescence excitation spectrum. The involvement of the carotenoids in

the fluorescence excitation spectrum is also clear in the case of the type II B800–850 complex and the B800–820 complex (Fig. 3c and d, respectively). Figs. 3c and d show that there is some cross-contamination of these two antenna complexes and that there is energy transfer between them. This is especially marked in the case of the B800–820 complex, where in absorption (Fig. 1d) the level of contamination with the type II B800–850 complex is low, but in the excitation spectrum it is a very prominent feature. If for both the type II B800–850 complex and the B800–820 complex the ratio of the intensities of the Q_y bacteriochlorophyll *a* absorption bands at 800 nm, 825 nm and 861 nm are compared with the relative heights of these bands in the fluorescence excitation spectra, it is quite clear that the efficiency of energy transfer between the 800 nm absorbing bacteriochlorophylls and the 825 or 861 nm absorbing ones is considerably less than 100%. We estimate that at 4 K the efficiency of energy transfer from the short-wavelength absorbing bacteriochlorophylls (at 800 nm) to the long-wavelength absorbing ones is only about 60%.

The efficiency of the carotenoid to bacteriochlorophyll singlet–singlet energy transfer has been determined for each complex as a function of temperature (Fig. 4). The data were collected for

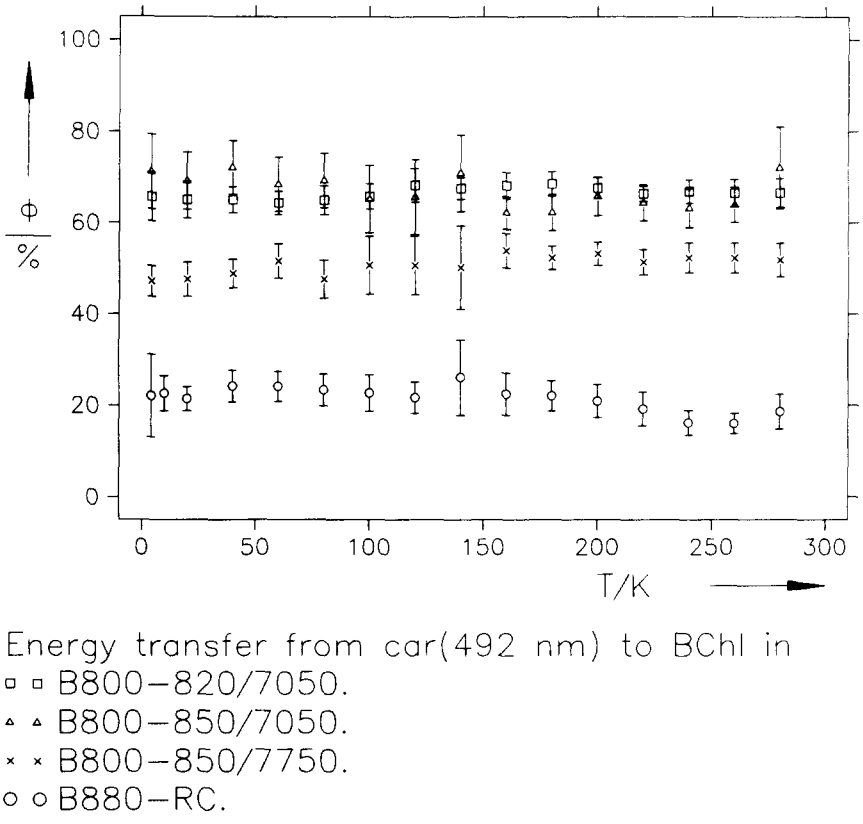


Fig. 4. The temperature dependence of the efficiency of singlet–singlet energy transfer from the carotenoid to the bacteriochlorophyll in the antenna complexes from *Rps. acidophila*. ○, B880 complex; ×, Type I B800–850 complex; □, B800–820 complex; △, Type II B800–850 complex. The excitation wavelength was 492 nm and the fluorescence was detected, for each complex, at the long wavelength peak of its fluorescence emission spectrum (see Fig. 3). The efficiency of energy transfer was calculated as described by Van Grondelle et al. [10] by comparison with the intensity of fluorescence produced when the bacteriochlorophyll was excited directly into the Q_x absorption band. Some of the absorption spectra presented in Fig. 1 show a 'curved baseline' at the lower wavelengths, probably due to incomplete correction of the light scattering. If a 'curved baseline' is taken account of in the energy transfer calculations the quoted values change by 3–5%, which is well within the limits of our experiment values and so we do not consider that this is a significant problem.

each complex at three different excitation wavelengths in the region of the carotenoid absorptions. Since identical values were obtained at each wavelength the data presented in Fig. 4 are only for excitation at 492 nm. It is interesting to note, however, that even though each type of antenna complex studied here has more than one type of carotenoid associated with it [7], no significant variations in energy-transfer efficiency were detected at the different excitation wavelengths across the carotenoid absorption bands. This suggests that within a given antenna type the energy transfer efficiency is rather insensitive to carotenoid type. The efficiency of this energy transfer is essentially independent of temperature. However, the relative efficiencies for each complex are quite different. The average values are 25% for the B880 complex, 50–55% for the type I B800–850 complex and 70–75% for the type II B800–850 complex and the B800–820 complex.

This is an interesting finding, since it may provide a reason for the change in type of antenna complex that occurs with growth at different light-intensities. In very high light where the B880 complex predominates, there is plenty of light and the efficiency of the carotenoid-to-bacteriochlorophyll energy transfer is low. At intermediate intensities where the type I B800–850 complex is synthesised to expand the photosynthetic unit is carotenoid is more efficient as a light harvester. While at very low light intensities, where the type II B800–850 complex and the B800–820 complex are synthesised, the carotenoid-to-bacteriochlorophyll singlet–singlet energy transfer is at its most efficient.

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