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The construction and properties of a mutant of *Rhodobacter sphaeroides* with the LH1 antenna as the sole pigment protein

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Recent work on the spectroscopic properties of the B875 (LH1) antenna of *Rb. sphaeroides* has been performed on the complex purified from membranes of the wild-type solubilised in lithium dodecyl sulphate. In order to facilitate an examination of the properties of the membrane-bound antenna free from other complexes, mutant M2192 was constructed from mutant M21, which contains reaction centres and LH1 but lacks the B800-850 (LH2) complex. This was accomplished by insertion of transposon Tn5 into the *puf* L gene which encodes the L polypeptide of the reaction centre. This manipulation leaves B875 as the sole pigment protein, which has been confirmed by Southern and Northern hybridisation, gel electrophoresis and fluorescence and absorbance spectroscopy. Evidence from Gaussian deconvolution of the Q_y absorbance region, and from fluorescence polarisation, suggests that the long-wavelength species BChl 896 is present, and may be an inherent property of the LH1 antenna.

Introduction

The photosynthetic apparatus of *Rb. sphaeroides* contains two light-harvesting complexes designated B800-850 (LH2) and B875 (LH1) according to their absorbance maxima in the near infrared. Their function is to absorb light energy and then transfer excitations to the reaction centre. In the model of Vos et al. [1], B875 and reaction centres form core structures which are surrounded and interconnected by B800-850. Levels of B800-850 are regulated by light and oxygen, but whereas the ratio of B875-to-reaction-centres remains constant, B800-850 levels are variable [2]. In this and other respects these two antennae have counterparts in other photosynthetic bacteria and so a more general description of LH2 (B800-850) and LH1 (B875) can also be used. The study of light-harvesting systems is facilitated by detergent solubilisation of photosynthetic membranes, which can yield pure LH2 or LH1 antenna complexes [3,4]; much of the present information on these complexes was obtained from such an approach [5–7]. The recent development of molecular genetic techniques for *Rb. sphaeroides* [8–10] provides an opportunity to study antenna complexes in mutant strains with no complications arising from the solubilisation of the membranes by means of detergents, which may affect the normal lipid environment of the complex and its aggregation properties. Several recent studies have demonstrated the usefulness of light-harvesting mutants [11–14]. The availability of mutant NF57, which contains only LH2, has provided evidence for new spectral forms of bacteriochlorophyll in this antenna [15–17]. Another mutant called M21 contains only LH1 and reaction centres but no LH2 [18]. In many respects, M21 resembles other LH1 + RC systems that exist in nature, such as in the bacterium *Rhodospirillum rubrum* [16,17]. It has been shown that transfer of *puc* genes encoding B800-850 polypeptides to M21 restores the complex, giving a wild-type absorbance spectrum [18].

In this paper we describe the construction of mutant M2192 which contains only LH1; it is derived from mutant M21. The availability of M2192 allows us to examine the biophysical properties of LH1 in situ and a comparison with the parent mutant M21 reveals that the long-wavelength species Bchl 896 [7,19–21] is likely to be an integral part of the LH1 antenna.

Materials and Methods

Bacterial strains and plasmids

pSRCl::Tn5 : 9 contains the Tn5 mutagenised *puf* genes of *Rb. sphaeroides* cloned into the *Bam*HI site of pSUP202 (Amp ^{R}, Tc ^{R}, Cm ^{R}, Ref. 22). The map of

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the plasmid insert is shown in Fig. 1. This construction was transferred to mutant M21 of *Rb. sphaeroides* from *E. coli* strain 17-1 (*thi, pro, hsd*R, *hsd*M, *rec*A, Ref. 22) using a conjugation procedure [8]. M21 lacks LH2 (B800-850) but contains LH1 (B875) and reaction centres [18] and was obtained from the wild-type strain 8253 by chemical mutagenesis. pJW1 contains a 12.2 kb *Bam*HI fragment bearing *puf* genes for reaction centre L,M polypeptides and LH1 α and β polypeptides cloned into pBR322. This was a gift from Dr. Joanne Williams. pKan2 contains the 3.5 kb *Hin*dIII fragment of Tn5 cloned into pBR322.

Media and antibiotics

E. coli strain 17-1 containing pSRC1::Tn5 : 9 was grown on LB medium supplemented with 50 $\mu g \cdot ml^{-1}$ ampicillin and 30 $\mu g \cdot ml^{-1}$ neomycin. M21 was grown on M22 + medium [8], supplemented with 0.1% casamino acids; for M2192, this medium was supplemented with 20 $\mu g \cdot ml^{-1}$ neomycin.

Preparation and analysis of DNA and RNA

Total DNA and RNA were prepared and analysed according to procedures described in Ref. 8. Radiolabelling was performed as in Ref. 23.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis

This was carried out using the buffer system of Laemmli [24] and a 12–20% gradient of acrylamide.

Low temperature fluorescence and absorbance spectroscopy; fluorescence polarisation

The apparatus for this work was described in Ref. 25.

Results

Fig. 1 shows the map of the construction used to produce the reaction-centre-less derivative of M21, M2192. The transposon Tn5 is inserted in the *puf* L gene and is therefore flanked by the genes *puf* B, A and M. Transfer of this 18 kb length of DNA into a strain carrying wild-type *puf* genes is accomplished using a 'suicide' vector unable to replicate in *Rb. sphaeroides* [9,22]. Two possibilities are single or double recombination events promoted by the homology between the plasmid-borne and genomic sequences. In the first case, vector and insert will form a cointegrate with the genome in which wild-type and Tn5 mutated *puf* genes lie side by side. In the second case, the plasmid-borne insert will exchange for the wild-type sequence, yielding a mutant unable to synthesise reaction centres. DNA sequence analysis (data not shown) demonstrates that *puf* A and B in M21 (parent strain 8283) and M2192 (containing *puf* A and B from strain 2.4.1) are identical. These events have been analysed quantitatively

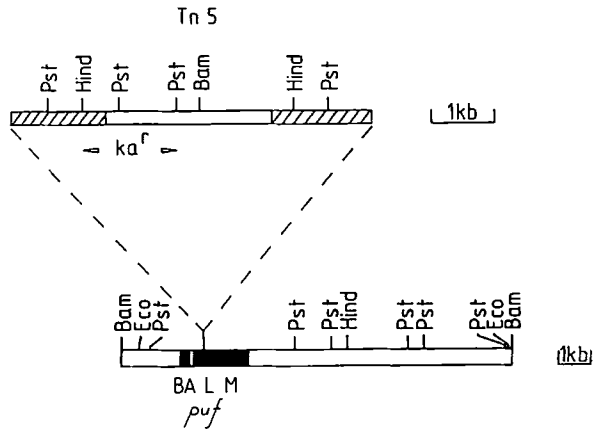

Fig. 1. Physical map of the construction used for Tn5 mutagenesis of *puf* L encoding the reaction centre L subunit. The transposon Tn5 (5.7 kb) is inserted into the gene encoding the L subunit of the reaction centre, *puf* L, and is flanked by other genes in the *puf* operon, borne on a 12.2 kb *Bam*HI fragment cloned in plasmid vector pSUP202 (see Materials and Methods). This construction is designated pSRC1::Tn5 : 9.

elsewhere [9] and it has been shown that transfer of pSRC1::Tn5 : 9 to the wild-type produces a non-photosynthetic mutant which possesses LH2 and LH1 but no reaction centres [9].

Transfer of pSRC1::Tn5 : 9 to M21 produces colonies with enhanced fluorescence and an inability to grow photosynthetically. Hybridization analysis with radiolabelled pSUP202 confirmed that the vector bearing the mutagenised *puf* genes was not present (data not shown). Fig. 2 shows the results of probing genomic DNA from one clone, M2192, with radiolabelled Tn5, that is, the 3.5 kb *Hin*dIII fragment of Tn5 cloned into pBR322, giving pKan2. Tn5 is only present in M2192, and not in the wild-type or mutant M21. pJW1, a 12.2 kb *Bam*HI fragment bearing *puf* genes cloned into pBR322, was used here to establish that the 11.2 kb *Eco*RI fragment seen in tracks WT and M21 is altered through Tn5 insertion (5.7 kb; Ref. 25) into a 16.7 kb fragment. Additionally, *Bam*HI and *Hin*dIII digests establish the location of Tn5 in M2192, because sites for these restriction enzymes are present in Tn5 [26].

Northern blot analysis was performed to establish the effect of Tn5 insertion on the two *puf* transcripts identified by Zhu and Kaplan [27]. It can be seen in Fig. 3 that only one transcript of approx. 0.5 kb is detectable in M2192, confirming that insertion of Tn5 into *puf* L results in no detectable 2.6 kb transcript which encodes *puf* B,A,L,M [9,27].

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of membranes from the wild-type, M21 and M2192 (Fig. 4) shows that as a result of Tn5 insertion, only two antenna polypeptides of approx. 7 and 3 kDa remain in M2192, which must be the α and β polypeptides of B875 first identified by Broglie et al.

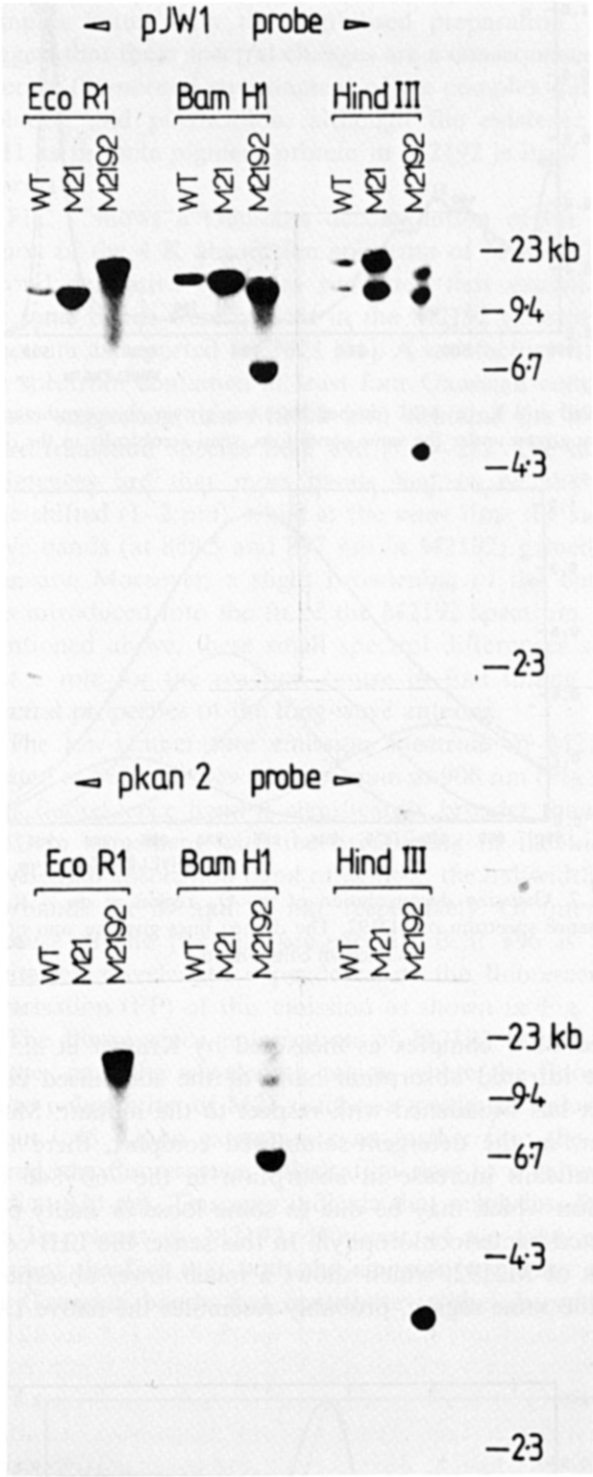

Fig. 2. Southern blot analysis of transposon Tn5 insertion into *puf* L. Genomic DNA (10 μg) from the wild-type (WT) and mutants M21 and M2192 was restricted using enzymes *Eco*RI, *Bam*HI and *Hin*dIII as indicated. The pJW1 probe was used to demonstrate the location of Tn5 in the genomic DNA from M2192. pKan2 was used to demonstrate the presence of Tn5 in M2192.

[4]. Although there is no transcript encoding *puf* L or M detected (Fig. 3), antisera would be needed to demonstrate the absence of reaction centre polypeptides from M2192.

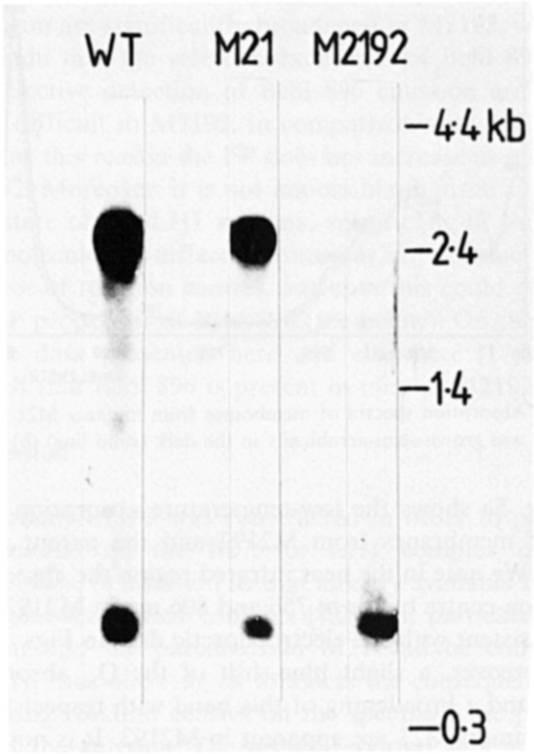

Fig. 3. Northern blot analysis of *puf* transcripts in mutants M21 and M2192. Northern blots (10 μg total RNA) were probed with the 7.2 kb *Bam*HI-*Hin*dIII fragment of pJW1 bearing the *puf* operon. The figures on the right refer to the molecular weights of the Bethesda Research Laboratories RNA ladder.

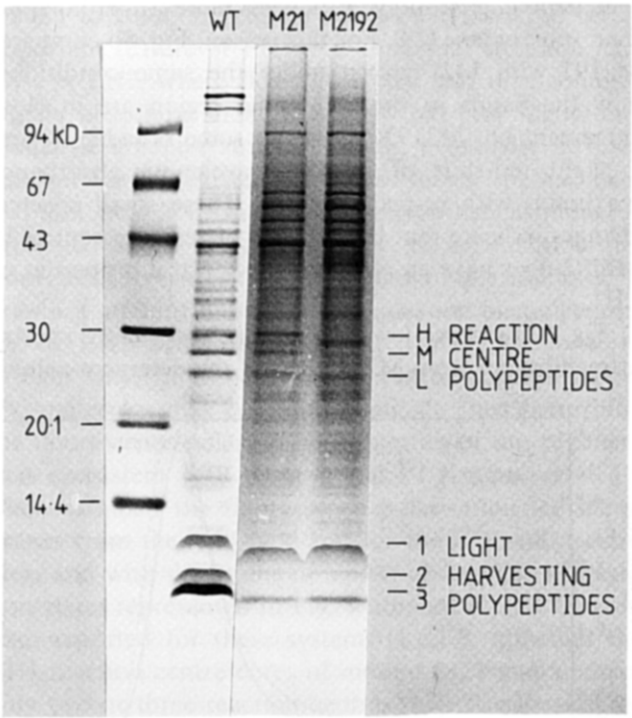

Fig. 4. SDS-polyacrylamide gel electrophoresis of membranes from wild type and mutant strains. 40 μg protein was used per gel lane. The regions of the gel that contain reaction centre and light-harvesting polypeptides are indicated. The figures on the right refer to the apparent molecular weights of the protein standards used.

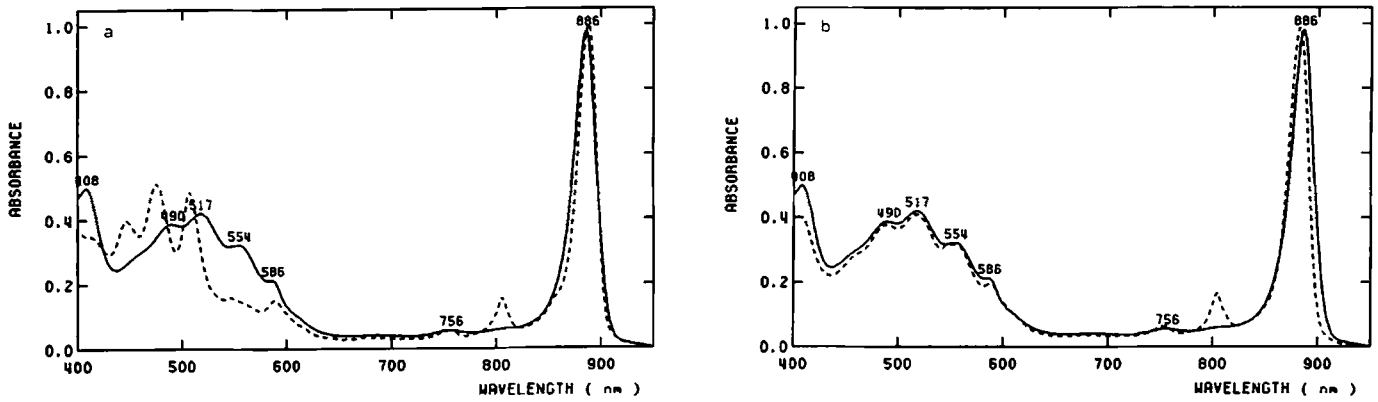

Fig. 5. Absorption spectra of membranes from mutants M21 and M2192 recorded at 4 K. (a) M21 (dashed line) was grown photosynthetically; M2192 was grown semi-aerobically in the dark (solid line) (b) both mutants were grown under the same conditions, semi-aerobically in the dark.

Fig. 5a shows the low-temperature absorption spectra of membranes from M2192 and the parent strain M21. We note in the near infrared region the absence of reaction-centre bands at 756 and 806 nm in M2192; this is consistent with the electrophoretic data in Figs. 3 and 4. Moreover, a slight blue-shift of the Q_y absorption band and a broadening of this band with respect to the spectrum of M21 are apparent in M2192. It is not clear what causes these subtle spectral effects but they are also manifested in the fluorescence emission spectra and the excitation wavelength dependence of the fluorescence polarisation in M2192 as compared to M21. In Fig. 5a, in the blue region, different contributions of carotenoid are observed for M2192 because growth in semiaerobic conditions produces more spheroidenone than spheroidene [28]. For this reason, Fig. 5b compares M2192 with M21 grown under the same conditions; now the bands in the carotenoid region are in close agreement but M2192 still shows some broadening and a slight red-shift of its long-wavelength absorbance maximum with respect to M21. These small spectral changes indicate that the absence of reaction centres in M2192 does have an effect on the spectral properties of LHI.

Fig. 6 compares low-temperature absorbance spectra of membranes from M2192 and of the detergent-solubilised B875 complex as measured by Kramer et al. [7]. The infrared absorption band of the solubilised complex has broadened with respect to the mutant. Moreover, in the detergent-solubilised complex, there is a significant increase in absorption in the 760–840 nm region which may be due to some loose or badly connected bacteriochlorophyll. In this sense, the LHI complex of M2192, which shows a much lower absorption in the same region, probably resembles the native LH1

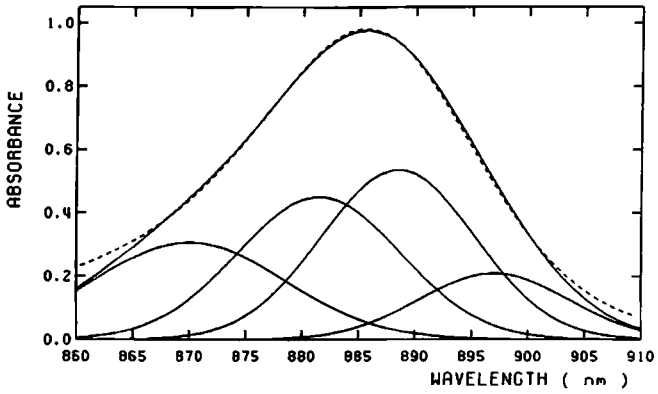

Fig. 7. Gaussian deconvolution of the Q_y region of the 4 K absorbance spectrum of M2192. The dashed lines give the sum of the Gaussian components.

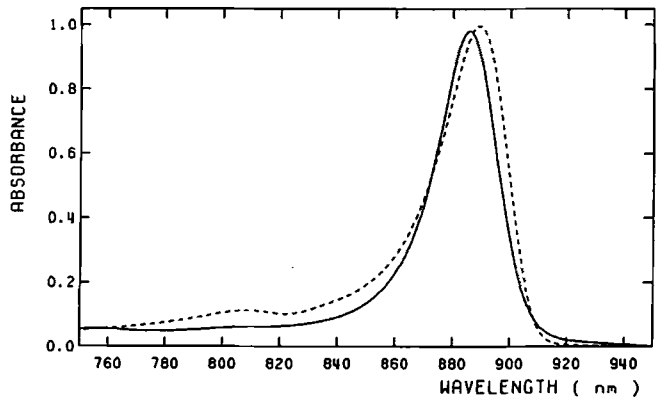

Fig. 6. Q_y region of the 4 K absorption spectrum for the LDS-B875 complex (Ref. 7; dashed line) and mutant M2192 (solid line).

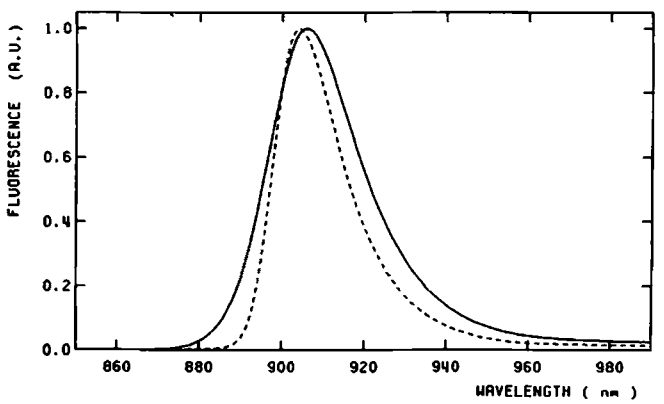

Fig. 8. Emission spectra of M21 (dashed line) and M2192 (solid line) measured at 4 K and excited at 590 nm.

complex better than the solubilised preparation. We suggest that these spectral changes are a consequence of altering the normal environment of the complex during isolation and purification, although the existence of LH1 as the sole pigment protein in M2192 is itself not 'normal'.

Fig. 7 shows a Gaussian deconvolution of the Q_y region of the 4 K absorption spectrum of M2192. The second derivative spectrum suggested that essentially the same bands were present in the M2192 absorption spectrum as reported for M21 [16]. A satisfactory fit of the spectrum contained at least four Gaussian components, suggesting that M2192 also contains the long-wave transition species Bchl 896 [7,19–21]. The main differences are that most bands had to be slightly blue-shifted (1–2 nm), while at the same time the long-wave bands (at 888.5 and 897 nm in M2192) gained in intensity. Moreover, a slight broadening of the bands was introduced into the fit of the M2192 spectrum. As mentioned above, these small spectral differences suggest a role for the reaction centre in fine tuning the spectral properties of the long-wave antenna.

The low-temperature emission spectrum of M2192 excited at 585 nm shows a maximum at 906 nm (Fig. 8). This fluorescence band is significantly broader than in M21, in agreement with the broadening of the long-wavelength absorption band of M2192; the halfwidth of the bands are 27 and 20 nm, respectively. Of interest because of the possible presence of Bchl 896 is the excitation wavelength dependence of the fluorescence polarisation (FP) of this emission as shown in Fig. 9.

The fluorescence polarisation of M2192 hardly increases over the wavelength region where the fluorescence polarisation of M21 reaches a maximum value of about 0.45. Upon excitation even further into the infrared, the fluorescence polarisation rises to a value of 0.25 at 920 nm. This may indicate that much less Bchl 896 is present in M2192. However, if we take into account the fact that both the emission spectrum and the Gaussian bands that contribute to the absorption spectrum are significantly broadened in M2192, we may conclude that the selective excitation of Bchl 896 and the selective detection of Bchl 896 emission are much more difficult in M2192, in comparison with M21, and that for this reason the FP does not increase as much in M2192. Moreover, it is not impossible that the aggregation state of the LH1 antenna, specifically of the Bchl 896 molecules, is different from that in M21 due to the absence of reaction centres, and that this could perturb the FP properties of Bchl 896 (see below). On the basis of the data presented here and elsewhere [17,21] we suggest that Bchl 896 is present in mutant M2192.

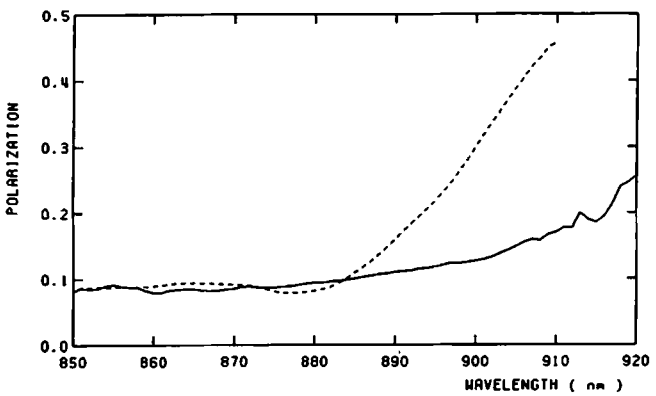

Fig. 9. Fluorescence polarisation spectra of M21 (dashed line) and M2192 (solid line) detected at 940 nm.

Discussion

Mutant M2192 was constructed in order to provide information on the B875 or LH1 complex of *Rb. sphaeroides*, in addition to that already available for the detergent-solubilised complex [7,29]. In particular, this mutant and the parent-strain M21 can be compared directly, thus allowing us to assess the consequences of removing reaction centres on the spectroscopic properties of the antenna. The results reported here are consistent with Bchl 896 being a property of LH1 rather than a consequence of binding to a reaction centre although the spectral properties of this pigment have been slightly altered. Bchl 896, which was also found in the detergent-solubilised complex [7], may serve as the final donor of excitation energy from the whole antenna system to reaction centres, and would therefore be an important feature of LH1. An analogous situation may exist in LH2, where Bchl 870 forms part of a complex largely absorbing at 800 and 850 nm [16]. Thus, any model of antenna structure would eventually have to account for the existence of these minor species, Bchl 896 and Bchl 870. In Fig. 10, a hypothetical model is presented which may account for the annihilation and fluorescence polarisation properties of the mutants. It does not attempt to address the question of the geometry of LH1 complexes, only to provide a model to explain why, in the absence of reaction centres, Bchl 896 molecules may come into contact, thus perturbing the fluorescence polarisation properties of this pigment. It is consistent with observations of Kramer et al. [7] who examined the fluorescence polarisation for membranes from the wild-type and for the LDS-B875 complex, and with the model of Vos et al. [1]. The aggregation states represented in Fig. 9 account for the domain sizes reported for these systems [1,6,17], although the LH1-reaction centre cores of mutant M21 may contain only two or three reaction centres [17]. The low degree of fluorescence polarisation is observed only for M2192; this is the only case where a polarised excited state within the antenna species Bchl 896 can be depolarised further by energy transfer among other similar species. In wild-type and M21 strains the excitation is trapped

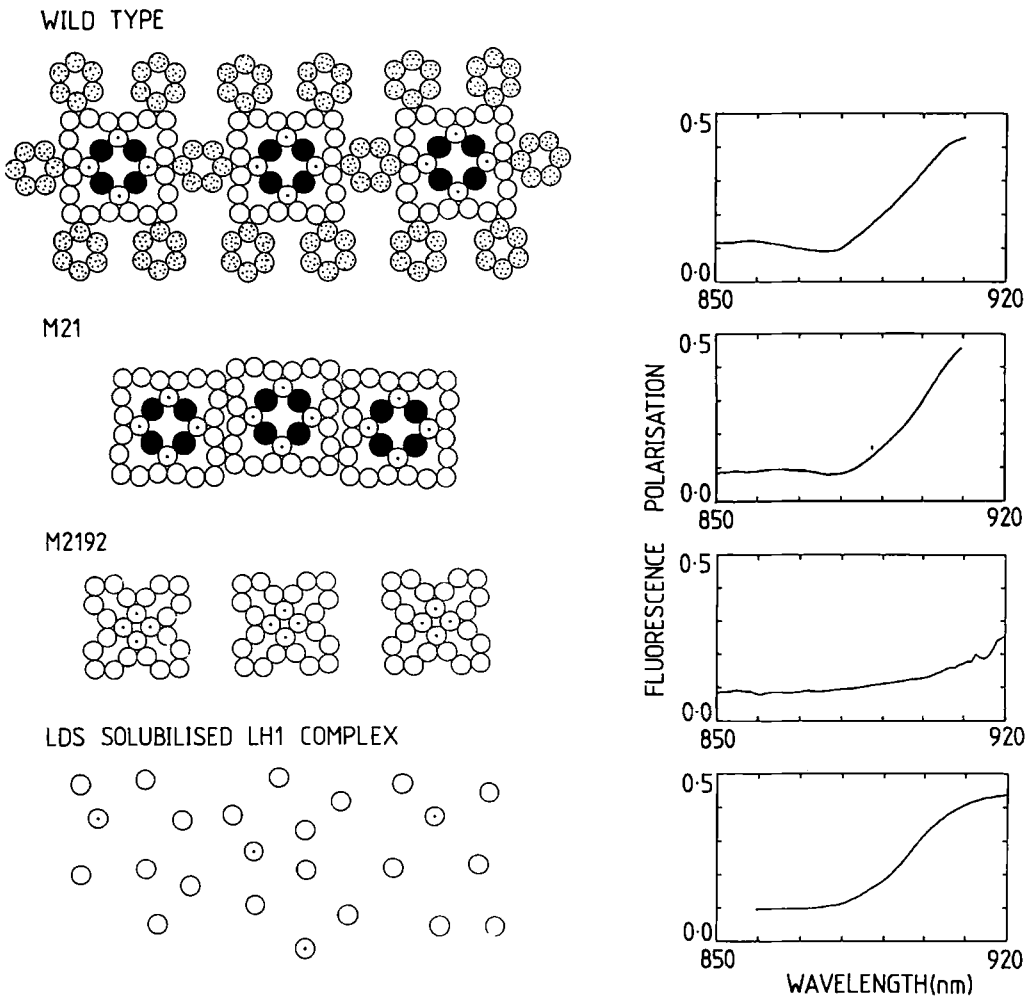

Fig. 10. Model for the arrangement of antenna and reaction-centre complexes in wild-type and mutant strains, and the LDS-LH1 complex. The fluorescence polarisation spectra are taken from this work and Ref. 7. ○ LH2, 6 Bchl 850, 3 Bchl 800; ○ LH1, 6 Bchl 875; ⊙ LH1, 6 Bchl 896; ● reaction centre, 4 Bchl.

by the reaction centre; in the LDS complex the domains (approx. six to eight Bchl *a*; Ref. 6) are sufficiently small that energy transfer between these 'minimal' units does not occur. This model implies that Bchl 896 could exist as aggregates of approx. six Bchl *a* and that this species may be an inherent feature of LH1.

The exciton annihilation properties of M2192 and M21 show that the effect of the removal of reaction centres is to decrease the size of the energy-transfer domain measured at room temperature [17]. It appears that reaction centres play a role in modifying the aggregation properties of LH1 in the membrane and that there may be attendant effects on the spectroscopic properties. The results of Vos et al. [17] are consistent with the presence of Bchl 896 in M2192; a comparison of the fluorescence-emission properties was conducted at low and high laser pulse intensities, and showed that some annihilation within Bchl 896 was occurring, with a consequent blue shift in the emission maximum.

Further studies on M21 and M2192, using time-resolved fluorescence and absorption anisotropy measurements [20] are in progress and are providing us with more information on the role of Bchl 896 within LH1-reaction centre complexes (Bergström, H., Sundström, V., Gillbro, T., Van Grondelle, R. and Hunter, C.N., unpublished data). They are in agreement with the existence of Bchl 896 in the solubilised and purified LH1 complex [30].

Acknowledgements

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