Biochimica et Biophysica Acta, 460 (1977) 393–407

BBA 47307

SINGLET-TRIPLET FUSION IN *RHODOPSEUDOMONAS SPHAEROIDES* CHROMATOPHORES

A PROBE OF THE ORGANIZATION OF THE PHOTOSYNTHETIC APPARATUS

TERESA G. MONGER* and WILLIAM W. PARSON

Department of Biochemistry, University of Washington, Seattle, Wash. 98195 (U.S.A.)

(Received November 22nd, 1976)

SUMMARY

Chromatophores from various strains of *Rhodopseudomonas sphaeroides* were excited with laser flashes lasting about 20 ns. Fluorescence from the antenna bacteriochlorophyll of the photosynthetic apparatus was measured both during the laser flash, and during a weak Xe flash following the laser flash. Strong laser flashes caused severe quenching of the fluorescence, which could be correlated with the formation of triplet states of the antenna pigments. Triplet states of both BChl and carotenoids acted as quenchers, but bacteriochlorophyll triplets were the more effective of the two. In the double-flash experiments, the reciprocal of the fluorescence yield was proportional to the concentration of triplet quenchers remaining at the time of the second flash. This relationship indicates that singlet excitations can migrate over large domains in the antenna, rather than being restricted by boundaries separating individual reaction centers. Comparisons of chromatophores from different strains and from cells grown under different conditions showed that excitations are concentrated rapidly in the antenna complexes with the longest wavelength absorption bands (B870), and that the migration of excitations to trapping sites is relatively insensitive to the amount of antenna bacteriochlorophyll absorbing at shorter wavelengths (B800–B850). This suggests that the B870 complexes are organized in the membrane so as to interconnect many reaction centers, and that the B800–B850 complexes are arranged peripherally.

INTRODUCTION

The light-harvesting antenna system of photosynthetic bacteria consists of

Abbreviations: BChl, BChl*, and BChl^T, bacteriochlorophyll in its ground and excited singlet and triplet states, respectively.

* Present address: Physics Department, City College of New York, New York, N.Y. 10031, U.S.A.

bacteriochlorophyll (BChl) and carotenoids, associated non-covalently with hydrophobic proteins [1–3]. The role of the antenna is to funnel energy to photochemical reaction centers, which trap the energy in an electron transfer reaction, the oxidation of *P*-870 [1, 4]. If the reaction centers are not in a photochemically active state, the energy must be dissipated in other processes, such as fluorescence and the formation of triplet states of the antenna pigments [5, 6].

When chromatophores are excited with a relatively weak flash lasting about 20 ns, the quantum yield of triplet states of the antenna pigments is on the order of 2 % [5]. However, the yield declines severely as one increases the strength of the flash, so that even extremely strong flashes convert only a very small fraction of the antenna pigments into triplet states [5, 6]. Because the yield of fluorescence from the antenna BChl declines similarly, the decrease in the triplet yield appears to result from quenching processes that limit the lifetime of the excited singlet state of BChl (BChl*) [5]. Two quenching processes that could become important at high flash intensities are singlet-singlet fusion ($2\ \text{BChl}^* \rightarrow \text{BChl} + \text{BChl}^*$) and singlet-triplet fusion ($\text{BChl}^* + \text{BChl}^T \rightarrow \text{BChl} + \text{BChl}^T$ or $\text{BChl}^* + \text{carotenoid}^T \rightarrow \text{BChl} + \text{carotenoid}^T$) [7–9]. Quenching by triplets seems likely to outweigh that by singlets under many conditions, because the triplet quenchers have much longer lifetimes. In chromatophores from the carotenoidless strain R-26 of *Rhodopseudomonas sphaeroides*, BChl^T decays with a halftime of approx. 70 μs [5], whereas the lifetime of BChl* is about 300 ps with weak excitation and less than this with strong excitation [10]. On the other hand, the quantum yield of triplets is low. One would expect, therefore, that singlet-singlet fusion might be the predominant quenching mechanism during flashes that are shorter than several hundred ps, but that singlet-triplet fusion would become increasingly important with longer flashes [8, 10].

In the present work, we have investigated the importance of singlet-triplet quenching under two different conditions. We first measured the yield of fluorescence from the antenna BChl during a 20 ns laser flash. Such a flash generates BChl*, BChl^T, and in some cases carotenoid triplets, and any of these species might act to quench fluorescence during the flash. We then examined the fluorescence during a weak Xe flash, several μs after the laser flash. The test flash provides a measurement of the fluorescence yield under essentially steady-state conditions. Excited singlet states generated by the laser flash have decayed in the interval between the flashes, and only triplet states survive to cause quenching during the second flash. Mauzerall [30] has used this technique previously to study fluorescence quenching in *Chlorella pyrenoidosa*. To compare the effectiveness of BChl^T and carotenoid triplets as quenchers, we studied both the carotenoidless strain R-26 of *R. sphaeroides* and strains that contain carotenoids. The results show that both types of triplet do quench fluorescence severely.

Our principal goal in studying singlet-triplet fusion has been to extract information on the movement of singlet excitations within the antenna. If one defines a "domain" in the antenna as a region which is uniform with respect to the transfer of singlet excitations [13], one would like to know, first of all, whether the reaction center can trap excitations only within a relatively small and exclusive domain, or whether the 10–50 reaction centers in a chromatophore compete for a common pool of excitations in an extended domain. These two contrasting views have been called the "puddle" and "lake" models of the photosynthetic apparatus [11]. One can

distinguish between the two models by examining the relationship between the fluorescence yield and the concentration of photochemically active reaction centers [12–14]. If domains in the antenna contain many reaction centers, the yield of fluorescence during steady-state illumination can be written as

$$\phi = \frac{k_f}{k_f + k_c Ra + k_d R(1-a) + k_e} \tag{1}$$

where R is the number of reaction centers per domain, a is the fraction of these that are in a photochemically active state, and k_f, k_c, k_d, and k_e are rate constants for the decay of excitations by fluorescence, photochemical processes in the reaction centers, wasteful quenching by reaction centers that are not in a photochemically active state (for example, reaction centers in which *P*-870 is already oxidized, or the primary electron acceptor already reduced), and other paths. In agreement with Eqn. 1, Vredenberg and Duysens [14] and Clayton [12, 13] have shown that the reciprocal of the fluorescence yield is proportional to a.

One can expand Eqn. 1 to include quenching by other sites or species, such as triplet states, by adding further terms to the denominator. Then

$$\phi = \frac{k_f}{k_0 + k_q RQ} \tag{2}$$

where Q is the number of additional quenchers per reaction center, k_q is a rate constant for quenching, and $k_0 = k_f + k_c\, Ra + k_d\, R\,(1-a) + k_e$. Eqn. 2 can be rewritten:

$$\frac{\phi_0}{\phi} = 1 + \frac{k_q R}{k_0} Q \tag{3}$$

where ϕ_0 is the fluorescence yield when Q is zero.

If the antenna is divided into many small domains, and if the transfer of excitations from one domain to another is slow, Eqn. 3 still will describe the fluorescence yield from domains with a given value of Q. However, the overall yield of fluorescence from a large collection of domains now will be given by

$$\phi = \sum_i b_i \phi_i \tag{4}$$

where b_i is the fraction of the domains that have i quenchers, and ϕ_i is the fluorescence yield from domains in this subset. According to Eqn. 4, a plot of ϕ_0/ϕ vs. Q will not be linear.

We have examined the relationship between the fluorescence yield and the triplet concentration in the double flash experiments and have found Eqn. 3 to apply. Our results support the conclusions of previous workers who have studied the fluorescence quenching by the photochemical reaction centers themselves [12–14, 37]. However, the use of antenna triplet states as quenchers allows one to extend the test of the relationship significantly, because the concentration of triplet quenchers that can be generated by strong flashes is about twice the concentration of reaction centers. In addition, the formation of triplet quenchers causes much larger changes in fluorescence yield than those that result from alterations of the state of the reaction centers. Further, triplet quenchers appear to be free of several complications that have been

bothersome in previous studies [12, 15]. The ability of the reaction center to trap energy depends on the redox states of both *P*-870 and the primary electron acceptor, X. Because measurements of the redox state of X are generally not straightforward, most investigators have monitored only the state of *P*-870; this is simply related to the fluorescence yield only under restricted conditions.

Strains of *R. sphaeroides* that contain carotenoids also contain several different types of bacteriochlorophyll complexes in their antenna. These are conventionally designated "B800, B850, and B870", after the approximate positions of their infrared absorption maxima. B800 and B850 appear to be associated in one pigment-protein complex; B870 appears to be a separate complex, which is linked more closely to the reaction center [16–18]. The antenna of the carotenoidless strain R-26 contains only B870. When both types of complexes are present, the question arises whether they are distributed randomly in the membrane, or whether they are arranged so as to optimize directional flow of energy toward the reaction centers [19, 20]. A study of fluorescence quenching can provide information on this point as well.

MATERIALS AND METHODS

Cultures of *R. sphaeroides* were grown essentially as described previously [5], on media containing yeast extract and casamino acids plus the additional carbon source of succinate for anaerobic photosynthetic growth or malate for semiaerobic growth in the dark. Strain PM-8 dpl, which lacks photosynthetic reaction centers, was grown under the latter conditions; other strains generally were grown photosynthetically. Strain Ga was grown under both conditions to vary the relative amounts of B800–B850 and B870. The absorption spectra of chromatophores prepared from the dark-grown cultures of strain Ga were very similar to those from strain PM-8 dpl. They differed from those of photosynthetically grown cultures of strain Ga in having lower levels of B800–B850, relative to B870 [16, 17]. Chromatophores were prepared by passage of cells through a French pressure cell at 12 000 lb/inch^2 [5]. Reaction center and BChl concentrations were estimated as described previously [5]. The values obtained for BChl concentrations were verified by measuring the concentration of BChl in acetone/methanol extracts of the chromatophores [21].

For study, chromatophores were resuspended in 0.01 M Tris · HCl, 0.1 M KCl, pH 7.6, and bubbled with N_2 to remove O_2. Low redox potentials were achieved by the addition of excess solid $Na_2S_2O_4$. In the figure legends and text, "moderate" redox potential indicates a potential of about +250 mV for chromatophores prepared from cultures grown in the light, and about +350 mV for chromatophores prepared from cultures grown in the dark.

Absorbance changes following laser flash excitation and fluorescence measurements during laser excitation were measured essentially as described previously [5, 22, 23]. The flash wavelength was 694 nm, and the width at half-maximum amplitude was about 20 ns. For the double-flash fluorescence measurements, a Xe lamp was placed 90° to the laser excitation path, 180° to the photomultipler which detected fluorescence. The Xe lamp provided a very weak flash with a width of about 1 μs at half-maximal amplitude. The light pulse passed through a Corning 9782 (blue-green) filter before reaching the sample. Complementary filters and a monochromator beyond the sample prevented light from either of the excitation flashes from reaching

the photomultiplier. To determine whether the intense pulse of fluorescence induced by the laser flash prevented the photomultiplier or oscilloscope from properly registering the weak signal due to the Xe flash, we replaced the chromatophore suspension by an inert, scattering sample (diluted milk). The monochromator and filters were altered to allow the photomultiplier to measure a weak pulse of transmitted infrared light from the Xe flash, and to allow a strong pulse of laser light also to reach the tube. The amount of laser light reaching the photomultiplier was adjusted to make the photomultiplier's response the same as the response to the fluorescence when the chromatophore sample was excited by the laser flash. Artifacts due to the laser flash appeared to be negligible if the interval between the flashes was greater than about 2 μs.

To compare the fluorescence yields in chromatophores from different strains, we used flashes that were very weak, relative to the saturation of photochemistry. The incident irradiance, measured with a ballistic thermophile, was approx. 10^{13} quanta/cm^2. The amount of laser light that the chromatophores absorbed was calculated from the incident irradiance and the absorbance of the sample, as measured with continuous light at the laser's wavelength. Because some of the chromatophore suspensions were slightly turbid, an integrating sphere was used for the latter measurement, and chromatophore suspensions that had been bleached by the addition of a minimal amount of NaOCl were used as blanks. The integrating sphere's photomultiplier was equipped with a fluorescence-blocking filter for measurements of the strongly fluorescent chromatophores from strain PM-8 dpl. The chromatophore concentrations were adjusted so that the samples absorbed between 10 and 20 % of the excitation flash. The ordinate scales in Figs. 1 and 6 indicate the relative values of the apparent fluorescence yields (fluorescence/light absorbed) in the different strains. They are subject to an uncertainty of approx. $\pm$20 %, mainly because of the difficulty of measuring accurately the amounts of light that the chromatophores absorbed. Only fluorescence at wavelengths near 910 nm was included in these measurements, but separate measurements indicated that the emission spectra of the various strains were not significantly different.

Excitation with the strongest flashes appeared not to cause irreversible changes in any of the chromatophores. The results were independent of whether the flash intensity was progressively increased, decreased, or changed randomly over the course of the experiment.

RESULTS AND DISCUSSION

Fig. 1 shows the relative quantum yield of fluorescence from the antenna BChl of strain R-26 chromatophores, as a function of the strength of the excitation flash. Single laser flashes were used for these measurements. The open circles represent chromatophore suspensions at moderate redox potentials, under optimal conditions for photochemical activity at the reaction center. With weak illumination, the reaction centers trap excitations very efficiently, and the absolute quantum yield of fluorescence is only about 2 % [24]. The fluorescence yield is greater if the primary electron acceptor is chemically reduced by lowering the redox potential (Fig. 1, filled circles). The reaction centers evidently trap energy less efficiently at low redox potentials, even though the initial steps of the photochemical electron transfer reaction still

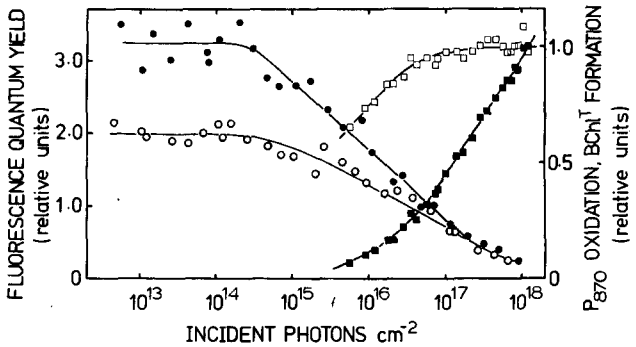

Fig. 1. Fluorescence, *P*-870 oxidation, and $BChl^T$ formation in *R. sphaeroides* R-26 chromatophores excited by laser flashes, as a function of the flash intensity. Samples contained 26 μM BChl and absorbed about 37 % of the incident laser light. ○, relative yield of fluorescence at 910 nm (fluorescence intensity/flash intensity) measured at moderate redox potential; ●, same as ○, except at low redox potential; □, *P*-870 photooxidation, measured from absorbance changes at 510 nm at moderate potential; 1.0 (relative unit) is a ΔA of 0.0037; ■, $BChl^T$ formation, measured from absorbance changes at 510 nm at moderate potential; 1.0 (relative unit) is a ΔA of 0.0107. Absorbance changes due to *P*-870 and $BChl^T$ were distinguished by their different decay kinetics, as described previously [5].

occur [23, 25] (see below). At both moderate and low redox potentials, the yield of fluorescence declines severely as the strength of the laser flash increases (Fig. 1). The filled squares in Fig. 1 show that the formation of $BChl^T$ accompanies the decrease in the fluorescence yield. The open squares show that the photooxidation of *P*-870 in the reaction centers is about 60 % saturated when the formation of $BChl^T$ and the decline

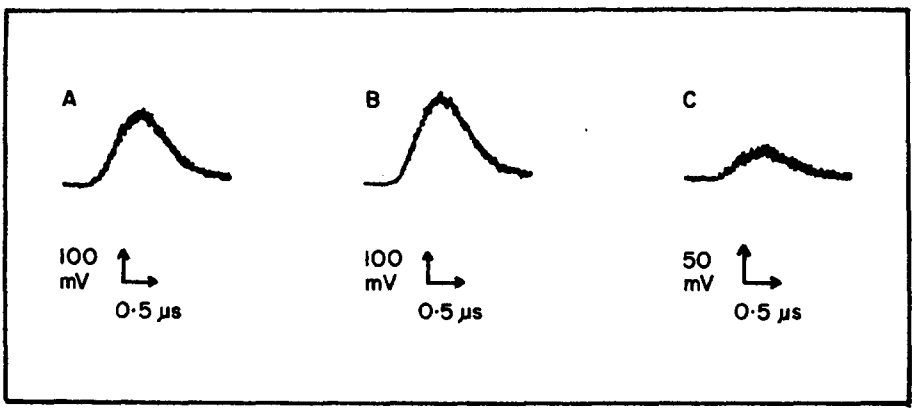

Fig. 2. Fluorescence induced by weak Xe flash excitation of chromatophores of strain R-26. (A) No laser flash prior to the Xe flash. (B) Sample exposed to strong laser flash (approx. 10^{18} photons/cm^2 incident intensity) 503 μs before the Xe flash. (C) Same as B, except laser flash 5.7 μs before Xe flash; note expanded vertical scale. For all measurements, the sample contained 14 μM bacteriochlorophyll, was at moderate potential, and absorbed about 20 % of the laser flash. The Xe flash was weak enough not to cause significant photooxidation of *P*-870. The intervals between the laser and Xe flashes were measured separately, with slower oscilloscope sweeps, and are expressed relative to the peaks of the Xe flashes.

in the fluorescence yield begin to be significant. One would expect an increase in the fluorescence yield to accompany the saturation of the photochemical system [12], but this rise apparently is masked by the competing fluorescence quenching process.

The data in Fig. 1 suggest that the quenching of fluorescence which occurs with strong laser flashes could result largely from singlet-triplet fusion. To obtain more direct information on the importance of singlet-triplet fusion, we examined the fluorescence that resulted from a weak Xe test flash, several μs after the chromatophores had been exposed to the laser flash. The delay between the two flashes assured that all BChl* generated by the laser flash would have decayed by the time of the test flash. Fig. 2 shows typical measurements of the fluorescence on the second flash, for several different flash spacings. If the flashes are spaced about 500 μs apart (Trace B), the fluorescence yield on the second flash is higher than the yield that is observed with a single, isolated flash (Trace A). The increased fluorescence presumably reflects the photooxidation of *P*-870 by the first flash. If the spacing between the flashes is decreased to about 6 μs, fluorescence on the second flash is severely quenched (Trace C). We interpret the quenching as being due to singlet-triplet fusion.

Fig. 3 shows the yield of fluorescence measured on the test flash, as a function of the strength of the laser flash. For these measurements, the delay between the flashes was 8 μs. Strong laser flashes cause a quenching of fluorescence similar to the quenching that occurred in the single-flash experiments of Fig. 1, as expected if the quenching during the single flashes also is due mainly to singlet-triplet fusion. In the double-flash experiments, the 10 % decay of $BChl^T$ that occurs during the 8 μs between the flashes reduces the masking of the fluorescence rise due to the photooxidation of *P*-870. Flashes of intermediate strength therefore cause a small increase in the fluorescence yield.

To investigate the relationship between the concentration of $BChl^T$ and the extent of the fluorescence quenching, we varied the time between the two flashes, keeping the laser flash at maximal intensity. The closed circles in Fig. 4A show measurements of the amount of $BChl^T$ remaining as a function of time after the laser flash, and

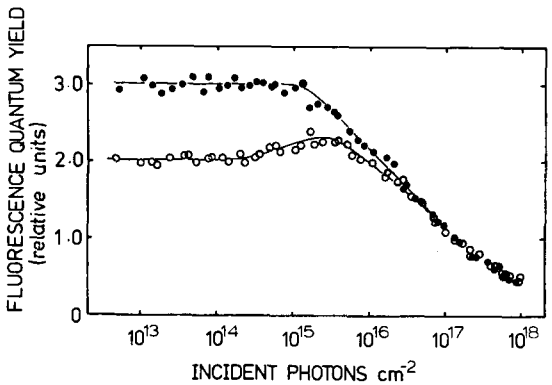

Fig. 3. Relative yield of fluorescence excited by the Xe flash, 8 μs after exposure of the chromatophores to a laser flash, as a function of the strength of the laser flash. Strain R-26 chromatophores; 26 μM bacteriochlorophyll; about 37 % of the laser flash absorbed. ○ and ●, as in Fig. 1. The 8-μs interval between the two flashes allowed about 10 % decay of the $BChl^T$ generated by the laser flash (Fig. 4).

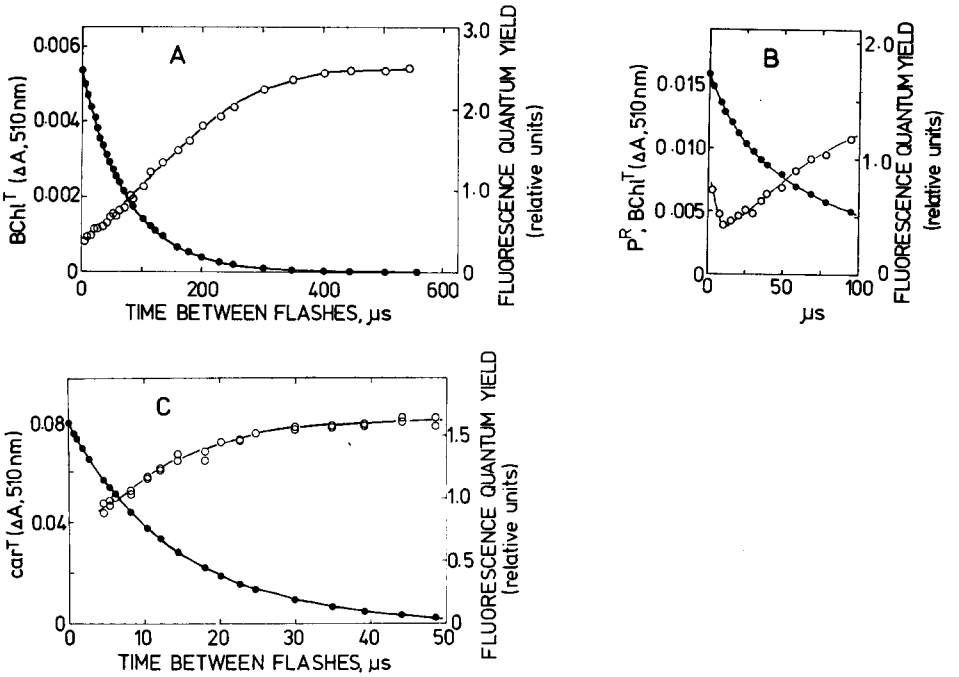

Fig. 4. Yield of bacteriochlorophyll fluorescence excited by the Xe flash, and amounts of BChl^T, P^R, and car^T remaining at the time of the flash, as a function of the time between the laser and Xe flashes. Incident laser flash intensity, about 10^{18} photons/cm^2. (A) Strain R-26 chromatophores at moderate redox potential; 14 μM BChl; about 20 % of the laser flash absorbed. ○, relative yield of fluorescence; ●, absorbance changes at 510 nm reflecting the amount of BChl^T remaining at the time of the fluorescence measurement. (B) Same as A, except R-26 chromatophores at low redox potential; 26 μM BChl; about 37 % of the laser flash absorbed. At low potential, the absorbance changes at 510 nm are due to both P^R and BChl^T; P^R decays more rapidly than BChl^T [5, 23]. (C) Same as A, except chromatophores from photosynthetically grown strain Ga; 61 μM BChl; about 46 % of the laser flash absorbed; moderate redox potential. The absorbance changes at 510 nm reflect the amount of carotenoid triplet (car^T) remaining at the time of the Xe flash.

the open circles show measurements of the relative fluorescence yield. The BChl^T population decays exponentially with a halftime of 55 μs. The fluorescence quenching decays approximately, but not exactly, in parallel. Separate measurements showed that only negligible changes in the redox state of *P*-870 occurred on the time scale of this experiment. In Fig. 5 (open circles) the reciprocal of the fluorescence yield is plotted as a function of the concentration of BChl^T remaining at the time of the Xe flash. The data provide a good fit to the straight line that is predicted by the lake model of the photosynthetic apparatus. Note that the data extend from a quencher concentration of zero to about 2 per reaction center, twice the range that is accessible in experiments that use the reaction center itself as the quencher. The dashed line in Fig. 5 illustrates the curvature that would be expected in the plot if the puddle model were correct. Although the amount of curvature that one expects depends to some extent on assumptions that one must make about the distribution of quenchers among the different domains in the sample, it is clear that the puddle model is unsatisfactory. However, the observation of a reciprocal relationship between fluorescence and

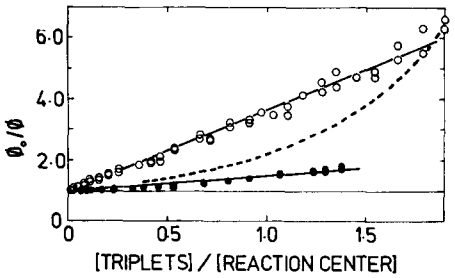

Fig. 5. Data of Figs. 4A and 4C replotted according to the equation $\phi_0/\phi = 1+K\,[Q]$, where K is a constant; ϕ is the relative fluorescence yield measured at time t after the laser flash; $[Q]$ is the concentration of triplet quenchers remaining at time t; ϕ_0 is the asymptote of ϕ for large t, when $[Q] \to 0$ ($t \approx 500$ μs for $BChl^T$ or 50 μs for car^T). ○, $BChl^T$ data from Fig. 4A; ●, carotenoid triplet data from Fig. 4C. Concentrations of $BChl^T$ and carotenoid triplet were calculated from the absorbance changes at 510 nm, using extinction coefficients of 11 and 80 $mM^{-1} \cdot cm^{-1}$, respectively [5]. To normalize the $BChl^T$ and carotenoid triplet concentrations, reaction center concentrations were calculated from measurements of *P*-870 photooxidation. The dashed curve is predicted for the $BChl^T$ data (open circles) by a puddle model, in which the chromatophores are composed of independent photosynthetic units, each containing one reaction center plus an antenna. The curve is given by the equation $\phi/\phi_0 = C+B[\phi_1/\phi_0]+A[\phi_2/\phi_0]$, where A, B, and C are the fractions of the units with two, one, and no triplet quenchers and ϕ_1 and ϕ_2 are the relative fluorescence yields for units with one and two triplet quenchers. The assumption is made that the laser flash generates two triplet quenchers in all units at time $t = 0$. This is a simplifying approximation; in fact, a small fraction of the units will have one triplet, and a small fraction will have three. However, the increasing quenching rate causes the quantum yield for triplet formation to decrease successively with each triplet that is added, so that essentially all of the units will have two triplets before the fraction with three becomes appreciable. The measured ratio of triplets to reaction centers was slightly greater than 2 at the time of the laser flash. Following the flash, the triplets are assumed to decay independently with first-order kinetics. These assumptions give $A = e^{-kt}$, $B = kte^{-kt}$, and $C = 1-(1+kt)e^{-kt}$, where k is the first-order decay rate constant. ϕ_2 is obtained from the intercept at time $t = 0$ in Fig. 4A; $\phi_2/\phi_0 = 0.14$. ϕ_1 is determined by assuming that within each unit, the quenching rate is proportional to quencher concentration; this assumption gives $\phi_1/\phi_0 = 2/(1+\phi_0/\phi_2) = 0.25$.

quenchers does not allow one to be very specific about the number of reaction centers that share each domain. The relationship will be essentially invariant for any value of this number greater than about 7 (ref. 13).

The situation is somewhat more complicated at low redox potentials, when the reaction centers start out in the form *P*-870-X^-. In addition to generating triplet states of the antenna BChl, excitation under these conditions also can generate a triplet state of the reaction center, called state P^R [5]. State P^R forms by way of a biradical state called P^F, which has a lifetime of about 10 ns [23, 26]. In double-flash experiments, Holmes et al. [27] have shown that the formation of state P^R causes an increase in the yield of fluorescence from the antenna BChl. Their measurements were made at 77 °K, where P^R has a high quantum yield and a lifetime of about 110 μs. At room temperature, P^R is formed in lower yield and decays with a halftime of approx. 6 μs [5, 23], making measurements of its effect on fluorescence more difficult. Fig. 4B shows measurements of the fluorescence yield at low redox potentials at room temperature. The data at very early times are consistent with the idea that the fluorescence yield is enhanced by the conversion of the reaction centers into state P^R. This effect is superimposed on the strong quenching due to the longer lived antenna $BChl^T$.

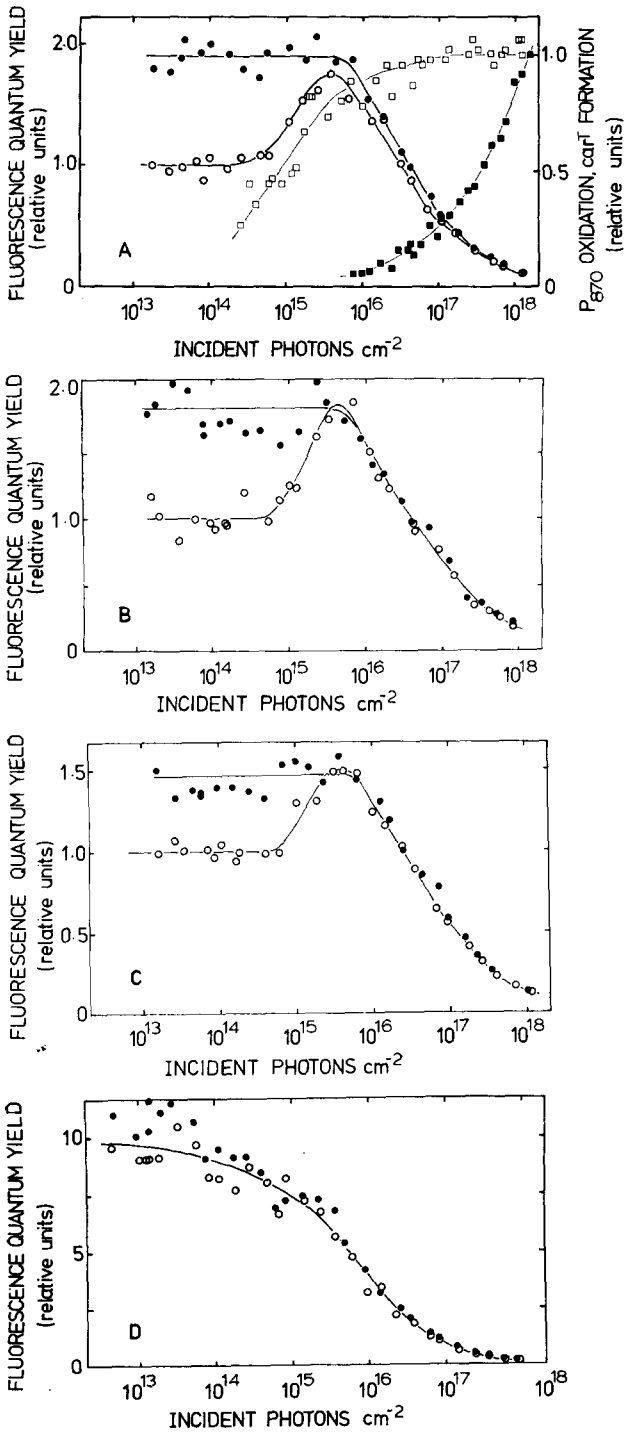

Fig. 6. See opposite page for legend.

Chromatophores from strains that contain carotenoids introduce the additional complexity that triplet states of their antenna BChl do not live long enough to be readily detectable. Instead, they transfer their energy to carotenoids, with half-times on the order of 20 ns [5]. Nonetheless, chromatophores from carotenoid-concontaining strains exhibit fluorescence quenching similar to the quenching that is described above for the carotenoidless strain R-26. Fig. 6 shows plots of the relative yield of fluorescence as a function of the strength of the laser flash for chromatophores prepared from three different strains that contain carotenoids; all show severe fluorescence quenching at high flash intensities. In all cases, the decrease in fluorescence yield occurs approximately in parallel with the formation of carotenoid triplets [5]. This is illustrated for strain 2.4.1 by the filled squares in Fig. 6A.

The question that arises next is whether fluorescence quenching in the carotenoid-containing strains is due solely to the activity of BChl triplet states prior to their decay, or whether carotenoid triplets also can quench BChl fluorescence. We addressed this question with double-flash experiments similar to those shown in Fig. 4A for strain R-26. Fig. 4C shows measurements for chromatophores prepared from strain Ga. The open circles give the relative fluorescence yield on the test flash, and the filled circles, the amount of carotenoid triplets remaining at the time of the flash. Clearly, carotenoid triplets are capable of quenching BChl fluorescence. This supports the suggestion of previous workers that carotenoid triplets quench chlorophyll fluorescence in *C. pyrenoidosa* and spinach chloroplasts [28–30]. As was the case with $BChl^T$, the population of carotenoid triplets and the extent of fluorescence quenching decay approximately but not exactly in parallel. The closed circles in Fig. 5 show the reciprocal of the fluorescence yield, plotted as a function of the concentration of carotenoid triplets. The data provide a reasonably good fit to the lake model, although there is some curvature at triplet concentrations greater than about one per reaction center. The interval between the two flashes is short for these measurements, and it is possible that artifacts due to the laser flash perturb the measurements. In addition, the small magnitude of the quenching by carotenoid triplets makes the distinction between the lake and the puddle models less clear than it is in the carotenoidless strain R-26. Using the reaction center as the quencher, Clayton [12] has obtained agreement with the lake model relationship for both carotenoidless and carotenoid-containing strains.

Fig. 6. Fluorescence, *P*-870 oxidation, and carotenoid triplet formation in carotenoid-containing chromatophores excited by laser flashes, as functions of the flash intensity. (A) Strain 2.4.1 chromatophores. ○, relative yield of fluorescence at 910 nm (fluorescence intensity/flash intensity), measured at moderate redox potential. 45 μM BChl; about 36 % of the laser flash absorbed. ●, same as ○, except at low potential. ■, carotenoid triplet formation, measured from absorbance changes at 535 nm at moderate potential; other conditions as for ○. 1.0 (relative unit) is a ΔA of 0.087. □, *P*-870 photooxidation, measured from absorbance changes at 535 nm at moderate redox potential. (These absorbance changes reflect mainly the bathychromic shift of a carotenoid absorption band, resulting from charge separation in the reaction center [35, 36]. They were distinguished from absorbance changes due to the formation of carotenoid triplets by their very slow decay kinetics [5].) 1.0 (relative unit) is a ΔA of 0.0045. 21 μM BChl; about 19 % of the laser light absorbed. (B) Same as A, except chromatophores from photosynthetically grown strain Ga; 41 μM bacteriochlorophyll; about 31 % of the laser flash absorbed. (C) Same as A, except chromatophores from aerobically grown strain Ga; 25 μM bacteriochlorophyll; about 31 % of the laser flash absorbed. (D) Same as A, except strain PM-8 dpl; 14 μM BChl; about 30 % of the laser flash absorbed.

According to Eqn. 3, the slopes of the lines in Fig. 5 give the ratio k_qR/k_0, where k_qR is an effective rate constant for singlet-triplet quenching, and k_0 is the sum of the rate constants for all other decay paths for BChl*. Because the first flash converts the reaction centers to the photochemically inactive state $P\text{-}870^+\text{-}X^-$, the other non-radiative decay processes are mainly internal conversion, intersystem crossing, and non-photochemical quenching by the reaction centers. In Fig. 5, the slope is about five times greater for quenching by $BChl^T$ in the carotenoidless strain R-26 than it is for quenching by carotenoid triplets in strain Ga. Because the extent of the fluorescence quenching observed during strong laser flashes is similar in the two strains (Figs. 1 and 6B), it seems likely that $BChl^T$ is the major quencher during the laser flash, even when carotenoids are present. This is consistent with our finding that the formation of carotenoid triplets is relatively slow, compared to the width of the flash [5].

In chromatophores of strain 2.4.1, the photooxidation of *P*-870 is over 80 % saturated before the formation of triplet states and the decline in the fluorescence yield begin to be significant (Fig. 6A). The formation of triplets thus appears to compete less effectively with the trapping of excitations at the reaction center than it does in strain R-26 (Fig. 1). The rise in fluorescence yield due to the saturation of photochemistry is not masked by fluorescence quenching, and flashes of intermediate intensity cause a pronounced increase in fluorescence yield (Fig. 6A). Chromatophores from strain Ga are similar to those from strain 2.4.1. in this regard (Figs. 6B and 6C). As expected, the rise in fluorescence yield at intermediate intensities does not occur in strain PM-8 dpl, which lacks reaction centers (Fig. 6D).

These differences in the relative efficiencies of triplet formation are reflected in differences in the quantum yields of fluorescence when the chromatophores are excited with weak flashes. In strain R-26, the fluorescence yield is approximately twice as great as it is in strains 2.4.1 and Ga, and in strain PM-8 dpl the yield is 9–10 times greater than it is in 2.4.1. and Ga (Figs. 1 and 6). The fluorescence lifetimes measured after excitation with single flashes lasting about 10 ps have approximately the same ratios [10]. Apparently the trapping of excitations at the reaction centers occurs about twice as rapidly in strains 2.4.1 and Ga as it does in strain R-26. The long lifetime of BChl* in strain PM-8 dpl explains why quenching by triplet states becomes important with comparatively weak flashes (Fig. 6D).

At low redox potentials, laser flashes of intermediate intensities do not result in an increase in the fluorescence yield in any of the strains (Figs. 1 and 6). Although the formation of state P^R tends to increase the fluorescence yield in strain R-26 at low potentials (Fig. 4B), P^R forms relatively slowly from the intermediate state P^F, compared to the width of the laser flash [23, 26]. In addition, the quantum yield of P^R is not much greater than that of the antenna $BChl^T$, and the quenching effects of the antenna triplets evidently predominate. One would not expect the formation of state P^R to have much influence on the fluorescence in the carotenoid-containing strains, because in these P^R decays rapidly by transferring energy to a carotenoid that is associated with the reaction center [5, 26, 31]. State P^F probably forms rapidly and with high quantum yield in all of the strains except PM-8 dpl [26], but the data shown in Figs. 1 and 6 indicate that this does not cause a major change in ability of the reduced reaction center to trap excitations.

Let us now return to the question of how the reaction centers can trap singlet

excitations from the antenna more rapidly in strains 2.4.1 and Ga than they do in strain R-26. One might suppose that this difference reflects an advantage to having the B800–B850 components as well as B870 in the antenna. The situation is paradoxical, however, because B870 appears to be linked more directly to the reaction center than are the B800–B850 complexes [16–18]. Unless the different complexes are arranged properly in the membrane, enlarging the antenna by the addition of B800–B850 would only delay the arrival of excitations at the reaction centers. However, it has long been known that energy is transferred very rapidly from the B800–B850 complexes to B870. Duysen's [32] original studies of the antenna complexes in *Chromatium vinosum* showed that essentially all of the fluorescence emanates from the complex with the longest wavelength absorption band, and the same is true in *R. sphaeroides* [18, 33].

If excitations are concentrated quickly in the B870 complexes, the formation of triplet states and the quenching of fluorescence should occur primarily in this part of the antenna. To test this point, we grew cells of strain Ga so as to vary the ratio of B800–B850 to B870. Growing the cells aerobically instead of photosynthetically causes a substantial decrease in the amount of B800–B850 that they contain, without significantly altering the ratio of B870 to the reaction centers [16]. Fig. 7 shows measurements of the amounts of carotenoid triplet that were formed by flash excitation in two samples of chromatophores having the same concentration of B870 but markedly different amounts of B800–B850. The amounts of carotenoid triplet that were formed in the two samples were very similar, except at the highest flash intensities (Fig. 7). The triplet states apparently do arise mainly in the B870 complexes, until the density of excitations in the B800–B850 complexes becomes significant at very high flash intensities. Comparison of Figs. 6B and 6C indicates that the fluorescence yields were essentially the same in chromatophores from the two different cultures, and that the quenching of fluorescence by the triplets also was largely independent of the amount of B800–B850.

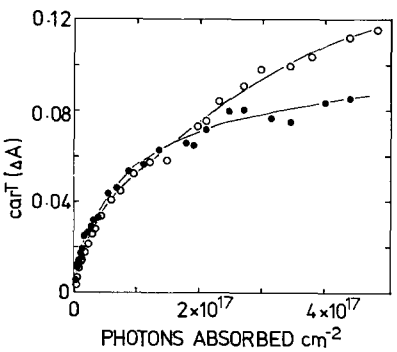

Fig. 7. Formation of carotenoid triplet in strain Ga chromatophores at moderate redox potential, as a function of the strength of the laser flash excitation. ○, chromatophores from photosynthetically grown cultures; 78 μM bacteriochlorophyll ;about 56 % of the laser flash absorbed; ●, chromatophores from aerobically grown cultures; 42 μM bacteriochlorophyll; about 51 % of the laser flash absorbed. The reaction center (*P*-870) concentration was 0.64 μM for both ○ and ●. Assuming that the ratio of B870 to *P*-870 was approx. 30 : 1 in both cultures (ref. 16), the ratio of (B800+B850) to B870 was approx. 3.0 in the photosynthetically grown cells and 1.2 in the aerobically grown cells. Carotenoid triplet formation was measured at the wavelengths giving the largest absorbance changes (510 nm for ○ and 508 nm for ●).

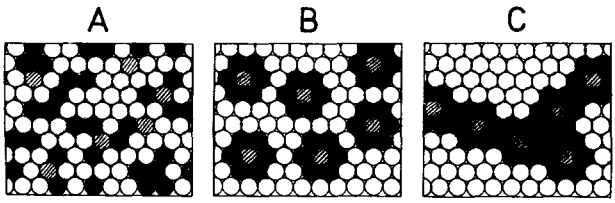

Fig. 8. Variants of the lake model of the photosynthetic apparatus. Striped circle, reaction center; ●, B870; ○, B800–B850. See the text for details.

These observations indicate that increasing the amount of B800–B850 in the chromatophore membrane does not significantly affect the rate of trapping. Because the B800–B850 complexes appear to transfer energy to the reaction centers by way of B870, rather than directly [18, 32], this conclusion implies that the two types of complexes are not distributed randomly in the membrane. To illustrate this point, Fig. 8 shows three speculative models of the photosynthetic apparatus. All three are variants of the lake model, with many reaction centers sharing a common pool of antenna complexes. In Model A, the B800–B850 and B870 complexes are arranged at random. Such an arrangement creates pockets and dead-end paths consisting of B870 complexes surrounded by B800–B850. These would interfere with the movement of excitations to the reaction centers, because the uphill transfer of energy from B870 to B800–B850 is less favorable than transfer in the opposite direction [18–20, 34]. With a random distribution of the complexes, increasing the amount of B800–B850 in the membrane would increase the number of pockets [34]. Model A is therefore inconsistent with the observation that variations in the B800–B850 content have little effect on the rate of trapping. One can argue against models like that of Fig. 8B for similar reasons: separate rings of B800–B850 surrounding the reaction centers would act as barriers to the migration of excitations from one reaction center to another. The reciprocal relationship between fluorescence and quenchers indicates that migration of this type must occur. In model C, B870 complexes surround the reaction centers, but they also form channels interconnecting them; this model appears to be consistent with all of our observations. To facilitate energy transfer, one could embellish model C by the addition of fingers of B870 reaching into the B800–B850 pool. The model falls short of explaining why trapping is slower in strain R-26 than it is in strains 2.4.1 and Ga, and additional information will be necessary to answer this question. Among the possibilities are differences between the strains in the structure of B870 or in the ratio of B870 to the reaction centers.

ACKNOWLEDGEMENTS

We are indebted to Drs. A. J. Campillo, S. L. Shapiro, and R. E. Blankenship for much helpful discussion, and to National Science Foundation Grant number GMS74-19852 AO1 for financial support.

REFERENCES

1 Sauer, K. (1974) in Bioenergetics of Photosynthesis (Govindjee, ed.), pp. 115–181, Academic Press, New York

2 Parson, W. W. (1974) Ann. Rev. Microbiol. 28, 41–59
3 Thornber, J. P., Trosper, T. L. and Strouse, C. E. (1977) in The Photosynthetic Bacteria (Clayton, R. K. and Sistrom, W. R., eds.), Plenum Press, New York, in the press
4 Parson, W. W. and Cogdell, R. J. (1975) Biochim. Biophys. Acta 416, 105–149
5 Monger, T. G., Cogdell, R. J. and Parson, W. W. (1976) Biochim. Biophys. Acta 449, 136–153
6 Kung, M. C. and DeVault, D. (1976) Photochem. Photobiol. 24, 87–91
7 Mauzerall, D. (1976) Biophys. J. 16, 87–91
8 Campillo, A. J., Kollman, V. H. and Shapiro, S. L. (1976) Biophys. J. 16, 93–97
9 Rahman, T. S. and Knox, R. S. (1973) Phys. Stat. Sol. (b) 58, 715–720
10 Campillo, A. J., Hyer, R. C., Monger, T. G., Parson, W. W. and Shapiro, S. L. (1977) Proc. Natl. Acad. Sci. U.S., in the press
11 Knox, R. S. (1974) in Bioenergetics of Photosynthesis (Govindjee, ed.), pp. 183–221, Academic Press, New York
12 Clayton, R. K. (1966) Photochem. Photobiol. 5, 807–821
13 Clayton, R. K. (1967) J. Theor. Biol. 14, 173–186
14 Vredenberg, W. J. and Duysens, L. N. M. (1963) Nature 197, 355–357
15 Malkin, S. and Silberstein, B. (1972) Biochim. Biophys. Acta 275, 369–382
16 Aagard, J. and Sistrom, W. R. (1972) Photochem. Photobiol. 15, 209–225
17 Niederman, R. A., Mallon, D. E. and Langan, J. J. (1976) Biochim. Biophys. Acta 440, 429–447
18 Zankel, K. L. and Clayton, R. K. (1967) Photochem. Photobiol. 9, 7–15
19 Paillotin, G. (1976) J. Theor. Biol. 58, 219–235
20 Seely, G. R. (1973) J. Theor. Biol. 40, 173–187
21 Clayton, R. K. (1963) in Bacterial Photosynthesis (Gest, H., San Pietro, A. and Vernon, L. P., eds.), pp. 495–500, Antioch Press, Yellow Springs, Ohio
22 Parson, W. W. (1969) Biochim. Biophys. Acta 189, 384–396
23 Parson, W. W., Clayton, R. K. and Cogdell, R. J. (1975) Biochim. Biophys. Acta 387, 265–278
24 Wang, R. T. and Clayton, R. K. (1971) Photochem. Photobiol. 13, 215–224
25 Rockley, M. G., Windsor, M. W., Cogdell, R. J. and Parson, W. W. (1975) Proc. Natl. Acad. Sci. U.S. 72, 2251–2255
26 Cogdell, R. J., Monger, T. G. and Parson, W. W. (1975) Biochim. Biophys. Acta 408, 189–199
27 Holmes, N. G., van Grondelle, R., Hoff, A. J. and Duysens, L. N. M. (1976) FEBS Lett. 70, 185–190
28 Zankel, K. L. (1973) Biochim. Biophys. Acta 325, 138–148
29 Den Haan, G. A., Duysens, L. N. M. and Egberts, D. J. N. (1974) Biochim. Biophys. Acta 368, 409–421
30 Mauzerall, D. (1972) Proc. Natl. Acad. Sci. U.S. 69, 1358–1362
31 Parson, W. W. and Monger, T. G. (1977) Brookhaven Symp. Biol. 28, in the press
32 Duysens, L. N. M. (1951) Nature 168, 548–550
33 Carithers, R. P. and Parson, W. W. (1976) Biochim. Biophys. Acta 440, 215–232
34 Swenberg, C. E., Dominijanni, R. and Geacintov, N. E. (1976) Photochem. Photobiol. 24, 601–604
35 Jackson, J. B. and Crofts, A. R. (1971) Eur. J. Biochem. 18, 120–130
36 Jackson, J. B. and Dutton, P. L. (1973) Biochim. Biophys. Acta 325, 102–113
37 Barskii, E. L., Borisov, A. Y., Il'ina, M. D., Samuilov, V. D. and Fetisov, Z. G. (1975) Molek. Biol. 9, 275–282