Photooxidation of antenna bacteriochlorophyll in chromatophores from carotenoidless mutant \textit{Rhodopseudomonas sphaeroides} and the attendant loss of dimeric exciton interaction

\textit{(photosynthetic bacteria/absorption spectra/monomer absorbance/circular dichroism)}

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ABSTRACT Intense continuous illumination of purified chromatophores from carotenoidless mutant \textit{Rhodopseudomonas sphaeroides} results in progressive photooxidative loss of the near infrared absorption band near 860 nm assigned to antenna bacteriochlorophyll. The quantum yield of this reaction is low, approximately $1.7 \times 10^{-5}$. The loss in near infrared absorption is accompanied by a proportional shift in the absorption maximum to shorter wavelengths. The double circular dichroism feature in the near infrared decreases at a faster rate than does the absorbance. These results are explained by a model in which the antenna bacteriochlorophyll, initially associated as dimers ($\lambda_{\text{max}} = 860.2$ nm), is progressively converted to the monomeric state ($\lambda_{\text{max}} = 851.9$ nm). The wavelength shift is attributed to disruption of exciton coupling in the dimer. Acetone/methanol extraction indicates that the maximum molar extinction coefficients of the dimer and monomer do not differ by more than 4%. The occurrence of an absorption maximum at 852 nm for monomeric bacteriochlorophyll in a protein complex demonstrates that it is not necessary to invoke aggregation of the chromatophores as the origin of the shift from 770 nm in typical organic solvents.

Carotenoidless mutant strains of the photosynthetic bacteria have long been known to have especially susceptible to photooxidative injury (1, 2). We have exploited this susceptibility to examine the states of association of antenna bacteriochlorophyll in carotenoidless mutant \textit{Rhodopseudomonas sphaeroides}. The framework of our analysis was suggested by a recent study by Sauer and Austin (3) on antenna pigment–protein complexes derived from various photosynthetic bacteria. They isolated a complex from carotenoidless mutant \textit{R. sphaeroides} consisting of two bacteriochlorophyll molecules bound noncovalently to a 22-kilodalton lipoprotein. Circular dichroism (CD) spectra of the isolated complex and of chromatophores suggested the presence of exciton coupling characteristic of bacteriochlorophyll dimers.

METHODS AND RESULTS

Photooxidation of Antenna Bacteriochlorophyll in Purified Chromatophores. Purified chromatophores from carotenoidless mutant strain R26 of \textit{R. sphaeroides} were isolated as described (4, 5). They were suspended in 0.01 M Tris-HCl, pH 7.5, and stored at 4°C until use. Chromatophores were illuminated in a cuvette (1-cm path) at 25°C. The actinic light source was a Sylvania 1000-W tungsten iodine lamp. The actinic light passed through glass lenses and 7.0 cm of water but was otherwise unfiltered. The filament image was focused on the cuvette face. The intensity of light at the cuvette was approximately 520 mW/cm². Illumination was interrupted periodically and near infrared absorption spectra were recorded with a Cary 14R spectrophotometer (IR1 mode; weak measuring beam). The spectra obtained for one chromatophore suspension are shown in Fig. 1. In the spectrum of the sample before illumination (0 min), the major band centered at 860.2 nm is primarily due to antenna bacteriochlorophyll; about 5% of the total absorption at 860 nm is due to reaction center bacteriochlorophyll (6). The minor bands near 760 and 800 nm are attributed to reaction center bacteriopheophytin and bacteriochlorophyll, respectively (6). The spectra of the sample taken after different periods of illumination show progressive loss in absorption associated with all three bands, indicating irreversible photooxidation of both antenna bacteriochlorophyll and reaction center pigments. Contributions to the near infrared spectrum from decomposition products were slight under these experimental conditions. Significant accumulation of bacteriopheophytin, indicated by a relative increase in absorption near 760 nm, was observed only when the sample temperature was allowed to rise significantly above 25°C. The losses in absorption of the major band shown in Fig. 1 were accompanied by shifts of the absorption maximum to shorter wavelengths. The band width (width at $A = e^{-1}$ $\lambda_{\text{max}}$) of the major band was relatively unchanged by illumination; it was approximately 37 nm before illumination and 62 nm after 47.9 min of illumination.

The dependence of the wavelength of maximum absorption of the major band ($\lambda_{\text{max}}$) on the fraction of antenna bacteriochlorophyll that survived strong illumination\textsuperscript{1} was determined for four different chromatophore samples. The combined data are shown in Fig. 2. A straight line was fitted to the data by the method of least squares. The fit was excellent (correlation coefficient = 0.997), demonstrating a precise linear dependence of the parameters. The fitted straight line gives the best values of $\lambda_{\text{max}}$ for two limiting conditions: when the surviving fraction of bacteriochlorophyll is 1.0, $\lambda_{\text{max}} = 860.2$ nm and when the surviving fraction of bacteriochlorophyll approaches zero, $\lambda_{\text{max}} = 851.9$ nm.

Relative Maximum Extinction Coefficients of Antenna Bacteriochlorophyll in Chromatophores before and after Strong Illumination. An aliquot of a chromatophore sample was illuminated until the maximum absorbance near 860 nm

Abbreviation: CD, circular dichroism.

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3 The surviving fraction of antenna bacteriochlorophyll was calculated as the ratio of maximum sample absorbance (after an illumination interval) to the maximum absorbance before illumination, $A_{\text{max}}(t)/A_{\text{max}}(0)$. To be valid, this method requires that the maximum extinction coefficient of the surviving bacteriochlorophyll is unaffected by the extent of photooxidation. This assumption was verified as described in the text.

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Fig. 1. Effect of intense illumination on near infrared absorption spectrum of purified chromatophores from carotenoidless mutant *R. sphaeroides*. Experimental details are given in the text.

was decreased to 23% of its original value. The concentrations of surviving bacteriochlorophyll were determined for this aliquot and the original sample by extracting the samples with acetone/methanol (7:2, vol/vol) and measuring the maximum absorbance at 770 nm of the extracts (7). By using the maximum absorbance values near 860 nm before extraction and the concentrations of bacteriochlorophyll found in the extracts, we calculated the maximum extinction coefficients of the band near 860 nm for the two samples. Based on the reproducibility of measurements and the presence of additional trace amounts of bacteriopheophytin in the illuminated aliquot (which bias estimates of surviving bacteriochlorophyll concentrations), we found that the maximum extinction coefficient of bacteriochlorophyll surviving extensive photooxidation was within 4% of that of the native antenna bacteriochlorophyll.

**Quantum Efficiency of Antenna Bacteriochlorophyll Photooxidation.** The general method used for estimating quantum efficiency is given elsewhere (8). In the present procedure, the slope of the absorbance change at 860 nm associated with photooxidation was measured with the Cary 14R spectrophotometer operated in the IR2 mode (intense measuring beam). An interference filter (d_{\text{max}} = 860 \text{ nm}, \text{ half bandwidth} = 10 \text{ nm}) was positioned between the unfiltered tungsten light source and the sample. After passage through the interference filter, the measuring beam was still sufficiently intense to promote measurable photooxidation of antenna bacteriochlorophyll. Light intensities were measured with a Yellow Springs Instrument-Kettering model 65 radiometer. The quantum efficiency was found to be $1.7 \pm 0.4 \times 10^{-5}$. The error limits are based on the reproducibility of experimental data (SD) and estimates of the absolute reliability of the method by using the reversible photooxidation reaction of reaction center bacteriochlorophyll as a control (9).

Fig. 2. Dependence of wavelength of maximum absorption on fraction of original antenna bacteriochlorophyll surviving intense illumination in chromatophores from carotenoidless mutant *R. sphaeroides*. Data were taken from spectra similar to those shown in Fig. 1. Assumptions involved in calculating the surviving fraction of bacteriochlorophyll and the significance of the right-hand ordinate are discussed in the text.

Fig. 3. CD in near infrared absorption spectrum of purified chromatophores before and during photooxidation. The fraction of bacteriochlorophyll remaining was 1.0 (curve a), 0.62 (curve b), and 0.26 (curve c). For this particular culture of *R. sphaeroides* R26 (grown in Berkeley), the absorption shift occurred between 863.5 and 855.3 nm. The absorption maximum of antenna bacteriochlorophyll in strain R26 has been found to vary slightly among different subcultures.
Circular Dichroism Changes Accompanying Bacteriochlorophyll Photooxidation. CD spectra were recorded by using a spectrometer described elsewhere (10). Representative CD spectra of chromatophores in the near infrared region recorded at progressive stages of photobleaching are shown in Fig. 3. The major feature is a double CD with peaks at 878 nm (negative) and 852 nm (positive) attributed to the exciton-coupled bacteriochlorophyll dimer of the antenna complex (3). Reaction centers contribute to the spectrum near 805 nm, where another double CD is centered. Progressive bleaching of the chromatophore absorption is accompanied by bleaching of the CD spectrum; however, the reaction center CD decays less rapidly than does that of the antenna bacteriochlorophyll. In Fig. 4 the antenna CD amplitude is plotted as a function of the surviving bacteriochlorophyll.

ANALYSIS AND DISCUSSION

The wavelength shift of the major near infrared absorption band upon photooxidation of antenna bacteriochlorophyll can be explained by a simple model. Sauer and Austin (3) have shown that two bacteriochlorophyll molecules are bound to each antenna protein complex in the chromatophore membrane of carotenoidless mutant R. sphaeroides. Circular dichroism spectra show adjacent positive and negative bands in the neighborhood of the major absorption band, indicating dimeric exciton interaction. As antenna bacteriochlorophyll is photooxidized, some antenna protein complexes will be occupied by only one reduced bacteriochlorophyll molecule and the absorption should reflect the appearance of monomeric bacteriochlorophyll. We therefore attribute the wavelength shift depicted in Figs. 1 and 2 to loss in exciton coupling induced by photooxidation. The absorption spectrum of monomeric antenna bacteriochlorophyll should be seen as we approach complete photooxidation. Extrapolation of the data shown in Fig. 2 gives 851.9 nm as the \( \lambda_{\text{max}} \) of monomeric antenna bacteriochlorophyll. The maximum extinction coefficients and the bandwidths are similar for both the dimeric and monomeric forms, consistent with the small difference in peak wavelengths.

A linear relationship between \( \lambda_{\text{max}} \) of the photooxidized samples and the surviving fraction of bacteriochlorophyll (Fig. 2) is also predicted by this model. Initially, all antenna bacteriochlorophyll molecules are reduced and in the dimeric form (B-B). The number of dimers (fully occupied antenna protein complexes) is \( N_q/2 \) where \( N_q \) is the number of bacteriochlorophyll molecules in the sample. After partial photooxidation, the individual antenna pigment–protein complexes exist in three forms: those with the dimeric bacteriochlorophyll intact (B-B), those with one bacteriochlorophyll intact but in a monomeric state (B-B\(_0\)), and those with both bacteriochlorophylls oxidized (B\(_0\)-B\(_0\)). Assuming that the optical cross-section for photooxidation is the same for bacteriochlorophyll in dimers and as monomers, we write \( p \) for the probability that a molecule has been photooxidized after a given exposure to light, and \( q = 1 - p \) for the probability that the molecule has survived the exposure. Then the binomial statistical distribution gives \( q^2 \) for the probability that a dimer (B-B) has survived, \( 2q(1-q) \) for the probability that a monomer (B-B\(_0\)) has been formed, and \( (1-q)^2 \) for the probability that an antenna complex has lost both bacteriochlorophyll molecules (B\(_0\)-B\(_0\)). The surviving number of bacteriochlorophylls existing as dimer is \( N_q/2 \). The surviving number in the monomeric state equals the total number of antenna complexes, \( N_0/2 \), times \( 2q(1-q) \), or \( N_q(1-q) \). The sum of these numbers, the total surviving bacteriochlorophyll, equals \( N_q^2 + 2N_q(1-q) \) or \( N_q \), as should it. Of this residual bacteriochlorophyll, the fraction that exists in a dimeric form is \( N_q^2/N_q \), or simply \( q \). Thus, the proportion of residual bacteriochlorophyll in a dimeric state is equal to the fraction \( q \) of bacteriochlorophyll that has survived photooxidation.

To compare this prediction with the results shown in Fig. 2, we need to know how the experimental \( \lambda_{\text{max}} \) of partially photooxidized samples is related to the fraction of residual bacteriochlorophyll as dimer. The near infrared absorption bands of the bacteriochlorophyll dimer\(^1\) and monomer were approximated by two Gaussian functions \( [G_1(\lambda) \text{ and } G_2(\lambda)] \), respectively, of equal magnitude and band width (1580 cm\(^{-1}\)) but possessing \( \lambda_{\text{max}} \) = 860.2 and 851.9 nm, respectively. Linear combination of these two Gaussians were computed for different fractions, \( f \), of bacteriochlorophyll in the dimeric form:

\[ fG_1(\lambda) + (1-f)G_2(\lambda) \]

For each linear combination computed for \( f = 1 \) to 0, a single \( \lambda_{\text{max}} \) was found in the interval 860.2 to 851.9 nm, and a precise linear correlation was obtained between the computed \( \lambda_{\text{max}} \) and trial values of \( f \). Based on this theoretical result and the linear relationship of the data shown in Fig. 2, we conclude that the fraction of residual bacteriochlorophyll as dimer shows a linear dependence on the surviving fraction of bacteriochlorophyll. Thus, the experimental result agrees with that predicted by the model.

Conversion of dimeric to monomeric bacteriochlorophyll results in a loss of the exciton-induced CD. When the CD amplitudes plotted in Fig. 4 are normalized to the fraction of bacteriochlorophyll remaining, a linear dependence on the surviving bacteriochlorophyll is found. This confirms the conclusion that the fraction of residual bacteriochlorophyll as dimer shows a linear dependence on the surviving fraction of bacteriochlorophyll. In principle, the CD of monomeric bacteriochlorophyll within the pigment–protein antenna complex could be deduced by extrapolation of plots similar to Fig. 4. However, the presence of reaction centers, which are more resistant to irreversible photooxidation than the antenna bacteriochlorophylls (see Fig. 3), complicates this extrapolation.

\(^1\) In general, an absorption band originating from exciton-coupled transitions in a dimer can be resolved into two orthogonally polarized components. The long wavelength absorption of the antenna bacteriochlorophyll complex from R. sphaeroides B36 has been resolved into two components based on linear dichroism studies (11). However, because the difference in \( \lambda_{\text{max}} \) of the two components is small compared with the absorption bandwidth, the apparent bandwidths of the dimer and monomer are similar. A single Gaussian should adequately approximate either spectrum.
Disruption of dimeric exciton coupling upon partial photo-oxidation of antenna bacteriochlorophyll in chromatophores from carotenoidless mutant R. sphaeroides accounts fully for the observed wavelength shift and CD changes of the major near infrared band. Additional strong electronic interactions between pigments of neighboring antenna protein complexes need not be invoked in accounting for our data.

The existence of a strongly red-shifted monomeric bacteriochlorophyll a, as implied by our findings, is surprising in view of the absence of comparably strong "solvent-induced" absorption band shifts for bacteriochlorophyll a in vitro. Perhaps the strong shift results, in our case, from the proximity of a tetrapyrrole framework: the second bacteriochlorophyll in the dimer and the "ghost" of the photooxidized bacteriochlorophyll in the monomeric case. This explanation would not pertain, however, to a similar example observed recently involving an analogue of chlorophyll. A complex between monomeric chlorophyllin and protein (apomyoglobin) exhibits a strong red shift of the long wavelength absorption band relative to that in organic solvents (R. C. Davis and R. M. Pearlstein, personal communication).

Other examples of strong electronic interaction between intrinsic membrane chromoproteins have been studied in analogous ways. A notable example is bacteriorhodopsin, which is clustered in trimers in the purple membrane of Halobacterium halobium. Graded treatment of purple membranes with detergent results in progressive disruption of exciton coupling of the retinal Schiff base $\pi \rightarrow \pi^*$ transitions as observed by CD and absorption (12-14).

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