The antenna system of *Rhodospirillum rubrum*: Radical formation upon dark oxidation of bulk bacteriochlorophyll

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1. INTRODUCTION

Dark oxidation of light-harvesting (antenna) bacteriochlorophyll in *Rhodospirillum rubrum* membranes elicits a decrease of the optical absorption around 880 nm and the appearance of a new band at 1230 nm [1]. Such near infrared changes are very similar to those observed upon light-induced or dark oxidation of photoreaction center bacteriochlorophyll (the primary donor of bacterial photosynthesis), i.e., a bleaching at 865 nm and increase in optical density at 1245 nm [2]. Besides, the oxidized primary donor exhibits an ESR signal with a Gaussian lineshape, a g-value of 2.0025 and a peak-to-peak derivative linewidth of 9.5 G [3]. In view of the similarities existing between the near infrared changes that follow oxidation of antenna and photoreaction center bacteriochlorophylls, it seemed interesting to investigate whether the oxidized antenna pigment is a paramagnetic species which can also be detected by ESR spectroscopy. As reported here, a nearly Gaussian ESR signal with a g-value of 2.0025 and a linewidth of 3.8 G appears to be due to the oxidized bacteriochlorophyll constituent which exhibits the 1230-nm band. Such a constituent seems to account only for $\sim$1/3rd of total antenna bacteriochlorophyll and has an apparent midpoint redox potential of $\sim$555 mV (pH 8.0). A preliminary report of this work has been presented in [4].

2. METHODS

Two *Rds. rubrum* strains were used, wild-type strain T and its non-phototrophic mutant derivative T102 [5]. The mutant lacks photoreaction center but contains unaltered bulk pigments. Cells were grown in the dark under low oxygen tension and the chromatophore preparation were obtained as in [6]. Photoreaction centers were solubilized from the chromatophores and purified as in [7]. Controlled amount of potassium ferricyanide were added to chromatophore suspensions in 50 mM potassium Tricine (pH 8.0). Spectra were recorded within 5 min of ferricyanide addition and the redox potential of each particular sample was measured afterwards.

Near infrared spectra were obtained at room temperature in a double beam spectrophotometer (Hitachi 330) with automatic baseline subtraction. ESR spectra were recorded, also at room temperature, with a Varian E-12 X-band spectrometer, using a modulation frequency of the magnetic field of 100 kHz and a non-saturating microwave power of 4 mW. The highest modulation amplitude consistent with no distortion of the lineshape was chosen. The g factors were calculated by using a solution containing 1 mM Na_2CO_3 and 5 mM potassium peroxylamine sulfonate as a standard whose magnetic parameters are $g = 2.00559$ [8] and $a_N(G) = 13.09$ [9]. For samples exhibiting signals

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of the same g-value and recorded with the same instrument settings, the relative concentrations of paramagnetic centers are given by the relative areas under the respective absorption curves [10]. Such areas were assumed to be directly related to the vertical amplitude and to the square of the peak-to-peak line width in the first derivative spectrum.

3. RESULTS

Fig.1 shows the ESR signals with a g-value of 2.0025 which were induced in suspensions of wild-type (strain T) chromatophores by the addition of potassium ferricyanide in the dark. At 410 mV (trace a) the signal was Gaussian and had a peak-to-peak linewidth of 9 G, properties which agree well with those reported for the signal of the oxidized primary electron donor [3]. When the redox potential was further raised to 450 mV, the chromatophore signal increased in amplitude and changed its shape (trace b). Such alterations seemed to be caused by the appearance of an additional and narrower spectral constituent, as it became more evident at 590 mV (trace c) where the narrower constituent predominated and completely concealed that of the oxidized primary donor. The additional, high potential signal was also nearly Gaussian and had a peak-to-peak derivative linewidth of 3.8 G. It is probably due to some oxidized chromatophore component because it disappeared completely when the redox potential of the sample was brought back to < 400 mV by the addition of potassium ferricyanide (not shown).

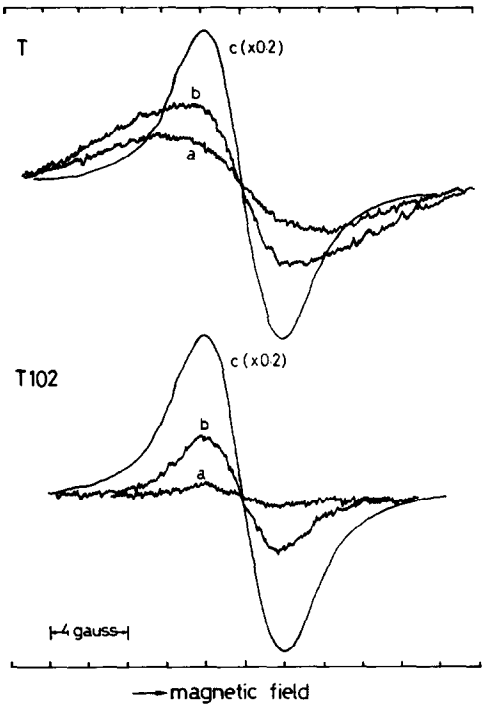

Fig.1. ESR signals of chromatophores from strains T and T102 at different redox potentials: (a) 410 mV; (b) 450 mV; (c) 590 mV.

Parallel spectra of chromatophores isolated from the photoreaction centerless mutant (strain T102) exhibited only the 3.8-G signal which, in this case, could be detected even at 410 mV because of the absence of the overlapping primary donor signal (fig.1). Thus, it may be concluded that the narrower signal cannot be due to a constituent of the photoreaction center.

The g-value of the narrow ESR signal was identical, within experimental error, to that of oxidized photoreaction center bacteriochlorophyll and of oxidized bacteriochlorophyll in solution [3]. This suggests that oxidized antenna bacteriochlorophyll is responsible for the signal. To check this hypothesis, we compared the redox dependence of the ESR signal with that of the 1230-nm band of oxidized antenna bacteriochlorophyll [1]. Chromatophores of strain T102 were used in those experiments to avoid the interference of the corresponding signals of the oxidized primary donor, which was particularly severe at the lower redox potentials. Due to technical reasons it was not feasible to perform simultaneously optical and ESR measurements in the same sample. Still, both potentiometric titrations showed the same apparent midpoint redox potential of ~555 mV (pH 8.0) and had slopes which corresponded closely to one-electron transitions (fig.2). At potentials > 600 mV, both parameters decreased (not shown), what probably reflected the unstability of the responsible chromophore(s) under strong oxidizing conditions. Such parallel behaviour reinforces the conclusion that both spectral features arise from the same chromatophore constituent, presumably the oxidized antenna bacteriochlorophyll.

The data presented above can be used to calculate the concentration of the paramagnetic species

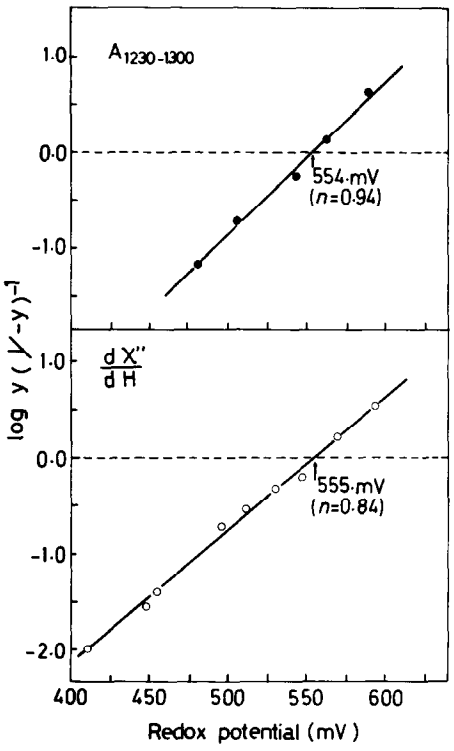

Fig.2. Potentiometric titrations of the 1230-nm absorption band and the 3.8-G ESR signal. Bacteriochlorophyll was 400 μM (top) and 327 μM (bottom). The asymptotic values, *Y*, ($A_{1230-1300}$ = 0.35 and ESR signal corresponding to 36 μM paramagnetic centers) were chosen so as to yield the best correlation. The least square method was used to adjust the experimental points to a straight line (correlation coefficients: 1230-nm band, 0.999; ESR signal, 0.998).

which shows the 3.8-G ESR signal. Taking the asymptotic value of the ESR signal from fig.2 and using purified *Rds. rubrum* photoreaction centers as a standard, we estimate that there is ~0.1 paramagnetic center/bacteriochlorophyll molecule in strain T102 chromatophores. This value is 3–4-times higher than the photoreaction center to bacteriochlorophyll ratio and wild-type chromatophores [11–13]. A similar value was obtained when the ESR spectra of wild-type samples (fig.1) was analyzed by a numerical method to estimate the relative contributions of the 3.8-G and the 9-G signals. From the computed concentration of the paramagnetic species and from the intensity of the near infrared band (fig.2), an approximate extinction coefficient of 8 $mM^{-1} \cdot cm^{-1}$ at 1230 minus 1300 nm is obtained.

4. DISCUSSION

In [14] an oxidant-induced signal was described which, because of its high amplitude, its *g*-value and its apparent midpoint redox potential, was probably identical to the 3.8-G signal that has been ascribed here to antenna bacteriochlorophyll. However, no mention was made of its narrow linewidth [14], perhaps its most striking differential feature. In [15] potassium ferricyanide was reported to elicit an anomalous ESR signal in chromatophores isolated from a photoreaction center mutant of *Rds. rubrum*. The signal was also in the $g = 2.00$ region and had a linewidth of 12 G and an amplitude similar to that of the primary donor signal. It was attributed to the monomer radical of bacteriochlorophyll or to the primary donor in a very distorted environment. Such an ESR signal is not present in our mutant strain T102, which only displays the narrower, more intense one of antenna bacteriochlorophyll that is also present in wild-type *Rds. rubrum*. It remains to be tested whether the antenna signal is also present in the mutant, since the oxidizing conditions required to observe it have not been tried [15]. In this regard, it should be mentioned that their strain exhibits the 1230 nm band [16] which has been shown here to accompany the antenna ESR signal.

The data in fig.2 indicate that the magnitude of the 3.8-G ESR signal at 594 mV reaches 78% of its asymptotic value. At the same potential, only 25% of total bacteriochlorophyll seems to be oxidized, as estimated from the extent of the bleaching of the 880 nm band (not shown; cf [1]). It follows from this that the bacteriochlorophyll constituent which exhibits such an ESR signal cannot account, when completely reduced, for > 32% of the total absorbance at 880 nm. Thus, unless it is assumed that the oxidized product has an absorption band in the 880-nm range, other antenna bacteriochlorophyll types must constitute ~2/3rds of the bulk pigment complement in *Rds. rubrum* chromatophores. This interpretation is in accordance with a recent report on fourth derivative analysis of the 880-nm band [17], which has led to the conclusion that two or more kinds of antenna complexes contribute to the overall band.

The narrowing of the oxidized primary donor ESR signal by a factor of $\sqrt{2}$ with respect to that of monomeric oxidized bacteriochlorophyll in solution has been interpreted [18] as the result of unpaired spin delocalization over an entity containing 2 bacteriochlorophylls linked by a water molecule (special pair bacteriochlorophyll). If a similar mechanism was assumed to account for the 3.8-G linewidth of the antenna bacteriochlorophyll signal, aggregates of 10–12 molecules would share the unpaired electron. Since we estimated above a paramagnetic center to bacteriochlorophyll ratio of 0.1, such a large aggregate would require the participation of all antenna bacteriochlorophyll in the radical, which is in disagreement with the conclusion of the preceding paragraph. Therefore, other mechanisms should also contribute (or lead) to the narrowing. Thus, while new low-temperature ESR data seem to confirm the special-pair hypothesis in the case of bacterial preparations, other results do not seem to support it in the case of plant photosystem I primary donor [19]. Besides, recent ENDOR experiments indicate a situation more complex than the special pair for the pigments involved in primary electron donation of bacterial photoreaction centers at room temperature [20]. Therefore, the nature of the molecular interactions which cause the narrowing of the bacteriochlorophyll signals in the membrane cannot be unequivocally established with the available information. Pigment–protein interaction should not be discarded since it appears to be the major factor in causing another marked spectroscopic effect, i.e., the large infrared shifts which are exhibited by the long wavelength band of bacteriochlorophyll in vivo [21].

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