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ROTATIONAL MOBILITY OF THE PHOTOREACTION CENTER IN CHROMATOPHORE MEMBRANES OF *RHODOSPIRILLUM RUBRUM*

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We investigated the rotational mobility of the photoreaction center in chromatophores of *Rhodospirillum rubrum* by studying the photoinduced linear dichroism of absorption changes at 865 nm. The study was carried out in suspensions of chromatophores treated with ferricyanide in order to bleach their antenna bacteriochlorophyll and thus minimize depolarization by energy transfer. Very little depolarization of the photoinduced absorbance change at 865 nm was observed at room temperature for chromatophores immersed in a highly viscous medium over the time range 0–10 ms following an exciting light flash. In the light of independent evidence for transmembrane arrangement of the photoreaction center, we conclude that the photoreaction center protein is immobilized in the chromatophore membrane for at least 10 ms.

Introduction

The photoreaction center is a chromoprotein the function of which is to trap light energy captured by antenna pigments and to convert it into an oxidoreduction potential. The photoreaction center of a few photosynthetic bacteria has been isolated and fairly well characterized [1]. In *Rhodospirillum rubrum* and in *Rhodopseudomonas sphaeroides*, the apoprotein has a molecular weight of about 90 000 and is composed of three different subunits [2–5]. The α (H) subunit appears to be exposed to the cytoplasmic side of the membrane whereas the β and γ (M and L) subunits seem to span the whole membrane [6–9]. The available evidence based on proteolytic digestion or labeling with antibodies or with radioactive iodine indicates that the protein does not tumble from one side of the membrane to the other [7–9]. However, it is not known whether the protein can rotate on an axis perpendicular to the plane of the membrane. Such knowledge would help in assessing those models of the electron-transport chain which are based on the mobility of some of the electron or proton carriers.

In the present work, we investigated this problem using the method of photoinduced linear dichroism introduced by Cone [10] to study the mobility of rhodopsin. In this method, a randomly oriented population of chromophores is bleached photochemically with a nonsaturating beam of polarized light while its absorbance is monitored with a weak beam of polarized light orthogonal to the first. For an immobilized population of chromophores, the anisotropy of the absorbance change does not vary with time whereas a relaxation is observed if the chromophores are mobile. This technique is nicely suited to study the bacterial photoreaction center, since when the preparation is immobilized, excitation of its 870 nm band produces a highly polarized ($p = 0.45$) absorbance change at this wavelength [11]. Another favorable circumstance is that the bleached

Abbreviation: FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone.

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state has a long lifetime lasting from about 100 ms to several seconds according to the experimental conditions. However, in intact chromatophores, light would first be absorbed by antenna pigments and be depolarized by energy transfer before exciting the pigments of the photoreaction center. In the present work, we overcame this difficulty by selectively bleaching the antenna bacteriochlorophyll with ferricyanide treatment of the chromatophores [12].

Materials and Methods

Chromatophores of *R. rubrum*, wild-type strain S1 (ATCC No. 1170), were isolated by alumina grinding and differential centrifugation. The isolated chromatophores were suspended in 50 mM Tris-HCl (pH 7.4) containing 15% (w/v) sucrose. To bleach the far-red absorption band of the antenna bacteriochlorophyll molecules, we used the method of Beugeling [12] with some modifications. The isolated chromatophore suspension was dialyzed against 100 mM potassium ferricyanide in water for about 50 h at room temperature under the illumination of a 60 W tungsten light bulb at 50 cm. The bleached green chromatophores were then dialyzed against water at 4°C in the dark for 24 h and centrifuged (4°C) at 36 000 $\times g$ for 45 min. The sediment was resuspended in 10 mM Tris-HCl (pH 7.4) hereafter called buffer.

Chromatophores were suspended in a viscous solution of Ficoll 400 (Pharmacia) and buffer (1 : 4, w/v), 20% gelatin or polyacrylamide gel of different viscosity by mixing various concentrations of acrylamide and bisacrylamide [13]. The sample was polymerized by adding 0.03% (v/v) *N,N,N',N'*-tetramethylenediamine and 0.0002% (w/v) ammonium persulfate and keeping it in the dark at 4°C for 12 h.

The apparatus used for the photodichroism measurement in the short time range is similar in principle to that of Junge and DeVault [14]. The sample was excited by a 2–3 ms flash of linearly polarized light from a strobe flash (General Radio Co. Type 1539A). The light flash was filtered with a broad-band interference filter (Baird-Atomic) with central wavelength at 930 nm and half bandwidth of 178 nm in order to excite only the 870 nm absorption band. The absorbance changes were measured with a continuous beam of linearly polarized light. The wavelength (865 nm) of this light was selected by a pair of monochromators placed before and after the sample chamber. Both the excitation and measuring light beams were polarized by Polaroid HR 2.8 sheet polarizers especially selected for their high degree of polarization. Each measurement was the average of 32 signals accumulated with a Biomation 102S signal averager. Measurements at different polarizations were analyzed by calculating the absorption anisotropy $r(t)$ given by

$$r(t) = \frac{\Delta A_{vv}(t) - \Delta A_{hv}(t)(\Delta A_{vh}(t)/\Delta A_{hh}(t))}{\Delta A_{vv}(t) + 2\Delta A_{hv}(t)(\Delta A_{vh}(t)/\Delta A_{hh}(t))} \quad (1)$$

where ΔA_{vv} and ΔA_{hv} are the absorbance changes when the measuring light is polarized parallel or perpendicular, respectively, to the plane of polarization of the exciting flash which is oriented perpendicular to the propagation vector of the measuring beam. With the plane of polarization of the exciting flash oriented parallel to the propagation vector of the measuring beam, ΔA_{hh} and ΔA_{vh} are the absorbance changes when the measuring light is polarized parallel and perpendicular, respectively, to the direction of propagation of the exciting light. Low-temperature measurements were done with a Cary 14R spectrophotometer as described previously [15]. Photodichroic measurements in the seconds range were done in a similar manner with a Cary 14R spectrophotometer. The exciting light pulse of 0.125 s was controlled by a mechanical shutter.

The light-induced pH changes were measured with a glass electrode (GK 2302C, Radiometer) connected to a pH meter (22, Radiometer Copenhagen). Actinic light of saturating intensity was provided by a tungsten lamp and a Schott RG10 filter.

Results and Discussion

Preliminary experiments with untreated chromatophores suspended in 1 : 4 (w/w) Ficoll/buffer gave a low anisotropy value measured 200 μs after the exciting light flash. This value, 0.014, is in agreement with the low polarization of fluorescence found on exciting the 880 nm absorbance band of similar preparations [16]. Such low anisotropy, as will be substantiated below, is attributable to energy transfer among the many randomly oriented bacteriochlorophyll molecules of the antenna.

Fig. 1 shows the absorption spectrum of ferri-

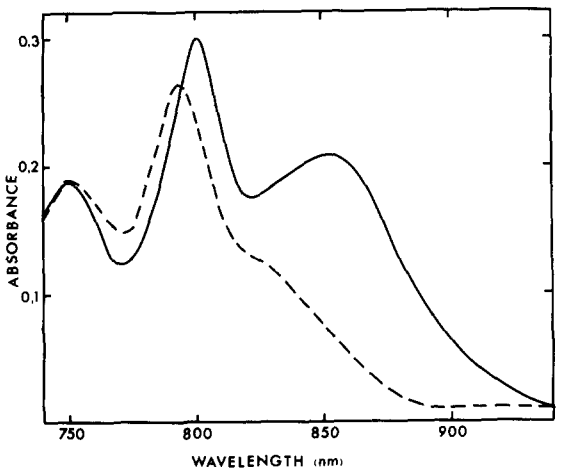

Fig. 1. Absorbance spectra of ferricyanide-treated chromatophores from *R. rubrum*. Spectrum of the reduced (———) and of the oxidized (- - - - -) state.

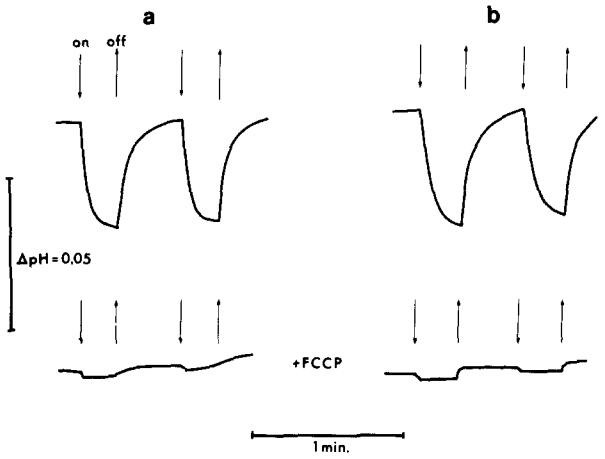

Fig. 2. Light-induced increase in pH in suspensions of normal (a) and of ferricyanide-treated chromatophores (b) with and without the addition of the uncoupler FCCP. The suspending medium (pH 6.5) contained 3 mM glycylglycine, 200 mM KCl, 100 μM 3,6-diaminodurene and 200 μM sodium ascorbate. Protein concentration was 1.1 and 1.3 mg/ml for normal and for ferricyanide-treated chromatophores, respectively.

cyanide-treated chromatophores (hereafter called bleached chromatophores) from *R. rubrum* in the oxidized and in the reduced state. The spectrum resembles that of the isolated photoreaction center [3], indicating that almost all the antenna bacteriochlorophyll has been bleached and that the ferricyanide treatment causes relatively little damage to the photoreaction center chromophores. However, the ratio A_{800}/A_{870} of 1.88 and the shoulder at 825 nm in the oxidized form indicate that an oxidation product of antenna bacteriochlorophyll is still present in small amounts.

A most important point for the significance of this work is that the ferricyanide treatment should not significantly alter the functional and structural integrity of the membrane which serves as a host to the photoreaction center. This was checked by measuring light-induced pH changes in chromatophores treated with or without ferricyanide. Fig. 2 shows that the pH changes induced by light of saturating intensity have comparable amplitude and kinetics. The sensitivity to the uncoupler FCCP is also similar. This indicates that the treatment does not impair the electron-transport chain or the integrity of the membrane.

The ΔA_{865} anisotropy values were much higher in bleached than in untreated chromatophores. The initial values recorded 200 μs after the exciting flash varied somewhat from one preparation to the next, the observed range being between 0.264 and 0.323. This variability is attributed to energy-transfer depolarization in nonreproducible amounts of residual antenna bacteriochlorophyll.

A relaxation of ΔA_{865} anisotropy could reflect the motion of the *P*-870 chromophore within the membrane and/or the motion of the chromatophore membrane vesicles in the suspension. Of these two types of motion, only the second one is expected to be affected by the viscosity of the suspending medium. In order to distinguish between them, we suspended the chromatophores in buffer alone, in a 1 : 4 (w/v) Ficoll/buffer mixture and in polyacrylamide or gelatin gels of varying water contents.

Fig. 3 shows how the anisotropy varies with time in four of these media. Curves A and B were obtained with a single preparation of bleached chromatophores suspended either in buffer (A) or in 1 : 4 (w/v) Ficoll/buffer (B). Curves C and D were obtained with a different preparation embedded either in 30% polyacrylamide (C) or in 20% gelatin (D). In the case of curves A and B, a relaxation of anisotropy in apparent. Note also that the initial values measured

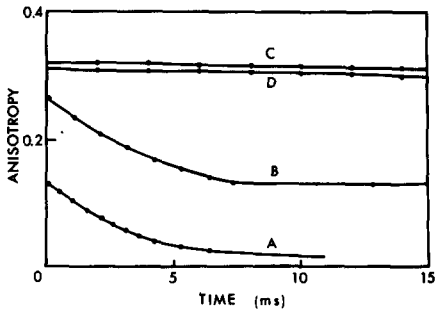

Fig. 3. Time-dependent anisotropy of the absorbance change at 865 nm in bleached chromatophores. Suspending media: curve A, 10 mM Tris-HCl (pH 7.4); curve B, Ficoll/buffer (1 : 4, w/v); curve C, polyacrylamide gel containing 30% acrylamide and 2.4% bisacrylamide in buffer; curve D, 20% gelatin in buffer. A 2–3 μs light flash was used.

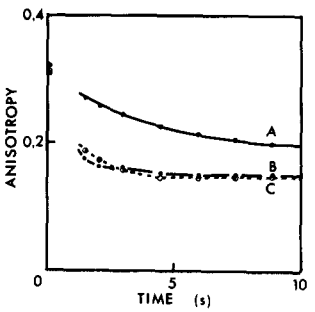

Fig. 4. Time-dependent anisotropy of the absorbance change at 865 nm in bleached chromatophores. Suspending media: curve A, polyacrylamide gel containing 30% acrylamide and 2.4% bisacrylamide in buffer; curve B, polyacrylamide gel containing 20% acrylamide and 1.6% bisacrylamide in buffer; curve C (- - - - -), 20% gelatin in buffer. A 125 ms light flash was used.

200 μs after the exciting flash as well as the shapes of curves A and B are different. This means that the rate of relaxation is viscosity dependent. This is shown even more clearly by experiments in which the chromatophores were embedded in highly viscous media such as polyacrylamide and gelatin (curves C and D) where no relaxation of anisotropy could be observed within 10 ms of the exciting flash.

We designed the next experiment to ascertain whether the *P*-870 chromophore undergoes any significant rotation in the membrane matrix within the first 200 μs after the exciting flash. This experiment was performed with the same bleached chromatophore preparation that was used to obtain curves A and B except that is was suspended in 1 : 1 (v/v) glycerol/buffer and its ΔA_{865} anisotropy value was measured at 132 K. At this temperature, the photoreaction center protein is completely immobilized [11]. The anisotropy values measured in the steady state at 132 K (0.267) and 200 μs after the flash in the Ficoll/buffer suspension at room temperature (0.264) were very close. We conclude that the *P*-870 chromophore is immobile in the membrane within the time range considered.

Instability in the baseline of our single-beam flash photolysis instrument prevented us from studying the relaxation kinetics at times between 10 ms and 1 s. We therefore turned our attention to the seconds range which is accessible to a double-beam recording spectrophotometer such as the Cary 14. Fig. 4 shows that some relaxation is observed in the seconds range. But again the relaxation rate is viscosity dependent. Compare, for instance, curve A obtained with chromatophores embedded in a 30% polyacrylamide gel and curve B obtained with the same preparation in 20% polyacrylamide. From these results, we cannot exclude the possibility of a hypothetical minor component of the relaxation between 10 ms and 1 s which might be due to molecular motion within the membrane. However, from curve A we can estimate a minimal correlation time of 2 s for a minor component of this motion, assuming that none of the relaxation is due to a motion of the membrane vesicles. A similar experiment was also carried out with chromatophores embedded in a 20% (w/v) gelatin/buffer matrix. Relaxation kinetics similar to those with chromatophores in 20% polyacrylamide were observed (cf. Fig. 4, curves B and C). This indicates that the reactants that compose the polyacrylamide gel did not interact with the membrane in such a way as to cause a slow rate of anisotropy relaxation.

Our experimental results show no evidence of a ΔA_{865} anisotropy relaxation in 10 ms at room temperature. In other words, we have no evidence that the *P*-870 chromophore undergoes any movement during that time. However, our results alone cannot be taken as showing complete immobilization of the chromophore on that time scale, since rotation of the chromophore along the axis of its absorption

dipole would not be detected by this technique. Additional evidence that this is highly unlikely comes from absorption dichroism measurements by Vermeglio and Clayton [17] which show that the *P*-870 absorption dipole is nearly parallel to the plane of the chromatophore membrane. The only rotational motion that the photoreaction center protein could possibly have, then, is a tumbling motion across the membrane. But thermodynamic considerations as well as recent studies on the localization of the photoreaction center protein in the chromatophore membrane appear to exclude the possibility of such a tumbling motion [6–9].

Assuming, as was discussed above, a minimal correlation time of 2 s for the major part of the photoreaction center embedded in the membrane, we can calculate the microviscosity of the medium surrounding the protein by using the equation of Perrin [18]. Taking the Stokes' radius of the isolated photoreaction center as 42 Å [5], we calculate a microviscosity of $2.6 \cdot 10^5$ P around the protein at 293 K. This is very much higher than the microviscosity of 0.8 and 6 P found for the hydrocarbon region of lecithin and sphingomyelin liposomes, respectively [19]. In fact, a spherical particle with a Stokes' radius of 42 Å would have a rotational correlation time of 76 μs in a medium with a viscosity of 10 P. This would fall well within the range of 10–400 μs reported for the anisotropy relaxation time of most membrane proteins [20]. (The calculated correlation time of 76 μs is not very accurate, since it is based on a value of the Stokes' radius which strictly applies to a protein-detergent complex. However, such an inaccuracy is at least two orders of magnitude smaller than the observed rotational correlation time). Since the photoreaction center is not immobilized by lipid-protein interactions, we assume that its immunobilization is the result of protein-protein interaction within the membrane. A likely candidate for such an interaction might be the light-harvesting protein which may represent up to 50% of the chromatophore membrane protein [21].

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