COO-3162-36

Annual Progress Report

ERDA Contract E(11-1)-3162



For period September 1, 1976 - November 30, 1977

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## ABSTRACT

We have extended our studies of the orientations of chromophores in photosynthetic membranes and reaction centers, using linear dichroism. Reaction centers could be oriented in stretched films of gelatin or polyvinyl alchohol, giving linear dichroic effects stronger than in dried films of membrane fragments, and different in the two types of film. These different preparations allow us to construct three-dimensional models of the orientations of bacteriochlorophyll and other chromophores in reaction centers and in relation to the membrane. Such modeling is under way for two distinctive types of photosynthetic bacteria: Rhodopseudomonas sphaeroides and R. viridis. We have improved our ability to resolve optically those components of bacteriochlorophyll and bacteriopheophytin that engage in photochemical electron transfer and those that do not.

We have begun to elucidate the cycle of one-electron and two-electron redox states of quinones engaged in photosynthetic electron transfer, by measuring optical absorbance changes that signal these reactions. Light flashes, each promoting one turnover (photochemical electron transfer) in every reaction center, were applied to isolated reaction centers and to membrane fragments. Consecutive flashes caused alternately the reduction of quinone to semiquinone and the reduction of semiquinone to the fully reduced form. Protons are bound only by the fully reduced form. The conversion of semiquinone to fully reduced quinone happens in secondary quinones and not in the primary quinone of the reaction center. Primary semiquinone and secondary semiquinone can be distinguished because each induces its own characteristic spectrum of band shifts of bacteriochlorophyll and bacteriopheophytin. The half-time for electron transfer from primary to secondary

quinone is 0.2 msec. We have evidence suggesting that carotenoid pigments affect the mobilities of quinones in the membrane.

We have purified and characterized reaction centers from Rhodopseudomonas gelatinosa. They are optically and photochemically similar to those from other species, but their protein composition is quite different. We have also improved the purification and optical characterization of reaction centers from R. viridis.

Properties of the fluorescence of bacteriorhodopsin have given us new insights into excited states and photochemical transformations of this visual pigment analog. These studies, made in collaboration with Prof. Aaron Lewis, are being extended to squid rhodopsin.

## INVESTIGATIONS AND RESULTS

1. Orientations of chromophores in the photosynthetic membrane.

When photosynthetic membrane fragments are dried on a glass plate they become oriented, presumably because the membranes lie flat on the plate. This is manifested by optical linear dichroism: at some wavelengths polarized light is absorbed more strongly when the electric vector is perpendicular to the plate; at other wavelengths when it is in the plane of the plate. This shows the orientation of the dipole transition moment of each chromophore in each of its absorption bands. The information (see Publication No. 1) is incomplete, partly because the orientation is not perfect and also because the orientation in the plane of the plate is random. We have now developed a technique, using isolated reaction centers (RC's) rather than membrane fragments, that adds to this information. The RC's are dissolved in a solution of gelatin and dried. The resulting gelatin films, wich resemble Kodak-Wratten optical filters, are perfectly uniform and optically clear,

even at low temperatures (35K). In this respect they are superior for optical investigations of any such materials at low temperature. If these gelatin films are exposed to high humidity they can be stretched about threefold; they retain their new shape when returned to low humidity. These stretched films of RC's are strongly dichroic, with the long-wave ($Q_y$) transition moment of the photochemically active bacteriochlorophyll (bchl) aligned $40^o$ from the axis of stretching. Similar stretched films made with polyvinyl alcohol instead of gelatin show orientation but are less uniform. Because the angles and symmetries of orientation in the three kinds of preparation (films dried on glass, gelatin films and polyvinyl alcohol films) are different, we have a basis for constructing a three-dimensional model showing orientations of the various chromophores in relation to the photosynthetic membrane. We are engaged in solving this geometric puzzle. In each RC, two of the four molecules of bchl and one of the two of bacteriopheophytin (bph) engage in photochemical electron transfer. Measurements of linear dichroism and of absorption spectra and light-induced absorbance changes at low temperature have enabled us to distinguish the photochemically active molecules from the others, and to describe the orientation of each type. We still do not know the functions of the "inactive" chromophores. We are indebted to Dr. W. W. Parson, who suggested stretching as a way to render the films anisotropic.

We have begun to learn techniques of photoselection, in which entities having a given orientation are selected with polarized exciting light. This will allow studies of liquid samples and hence of reactions that depend on diffusion or rotation.

2. Light-initiated electron transfer and proton binding involving quinones, and the involvement of carotenoid pigments.

The stable oxidized and reduced forms of quinones differ by two electrons, whereas those of chlorophylls and cytochromes differ by one electron. In bacterial photosynthetic membranes, electrons move through a cycle from bchl to bph (light-driven step), thence to a single quinone and on to a larger pool of quinone, then to cytochromes and back to bchl. We have new information on the way that the "two-electron" chemistry of quinones is coupled to the "one-electron" chemistry of the other components. RC's isolated from Rhodopseudomonas sphaeroides contain one molecule of firmly bound "primary" ubiquinone (UQ) and one of weakly bound "secondary" UQ, the latter able to interact with an external pool of added UQ. The conversion of UQ to the one-electron reduced semiquinone, $UQ^{-}\cdot$, and to the fully reduced $UQ^{=}$ or $UQH_2$ can be detected by characteristic optical absorbance changes. Also the semireduced primary and secondary quinones of the RC can be distinguished because each is attended by characteristic shifts of the absorption bands of bchl and bph in the RC. We have found that a single flash of light drives an electron from bchl to primary UQ. The electron moves on to the secondary UQ with half-time 0.2 msec, and the resulting secondary $UQ^{-}\cdot$ is stable for many seconds if the oxidized bchl has been neutralized by receiving an electron from an external donor such as cytochrome. A second flash of light delivers another electron to the primary UQ and on to the secondary $UQ^{-}\cdot$, converting the latter to $UQ^{=}$. Only then are protons bound ($UQ^{=} + 2H^{+} \rightarrow UQH_2$), as detected by a change in the pH of the solution. The fully reduced state can be transferred to an external pool of UQ, perhaps by a process in which the reduced secondary UQ comes off the RC and a new molecule of UQ comes onto the binding site.

Having seen this pattern of electron transfer and $H^+$ binding in isolated RC's, we sought the same pattern in chromatophores (vesicular fragments of the photosynthetic membrane), and found it in chromatophores of the carotenoidless mutant strain R-26 of Rp. sphaeroides: formation of $UQ^-\cdot$ on odd-numbered flashes; disappearance of $UQ^-\cdot$ and binding of $H^+$ after even-numbered flashes. Chromatophores from strains containing carotenoid pigments did not show such a pattern; in them $UQ^-\cdot$ was formed and then decayed rapidly after each flash, and protons were bound after each flash. Perhaps the presence of carotenoids facilitates the migration of $UQ^-\cdot$ in the membrane, so that molecules of $UQ^-\cdot$ formed at different RC's can encounter each other and perform a dismutation reaction: $2UQ^-\cdot \rightarrow UQ + UQ^= \xrightarrow{2H^+} UQ + UQH_2$. Alternatively, in those strains with carotenoids the $UQ^-\cdot$ can interact with the aqueous environment and become protonated: $UQ^-\cdot + H^+ \rightarrow UQH\bullet$. We shall explore these possibilities using mutant strains that are transitional between the carotenoidless and the normal carotenoid-containing forms.

RC's isolated from wild type Rp. sphaeroides have one molecule of the carotenoid sphaeroidene bound to each RC. The absorption bands of this carotenoid shift to the red when bchl in the RC is oxidized, either chemically or through the normal photochemistry. The presence of negative charge on the quinone has no effect on the carotenoid, but it does cause band shifts of the bph and, to a lesser degree, of the bchl. The shifts are of different magnitudes and in some cases of opposite sign, depending on whether the negative charge is on the primary or the secondary quinone. The RC chromophores thus act as intrinsic probes for the presence of both positive and negative charge at different locations in the RC. We have exploited these phenomena to monitor the migration of electrons from primary to secondary UQ in RC's.

3. New reaction center preparations from Rhodopseudomonas gelatinosa and viridis.

We have developed a simple and effective method for isolating RC's from photosynthetic bacteria, and have applied it to obtain an improved RC preparation from Rp. viridis and to isolate for the first time RC's from Rp. gelatinosa. The method involves exposure of membrane fragments to a high concentration (3-5%) of the detergent lauryl dimethyl amine oxide, followed by chromatography on hydroxylapatite.

The RC from Rp. viridis has been characterized with respect to molar extinction coefficients of the chromophores, molecular weight and protein components. The RC from Rp. gelatinosa has been characterized in these ways, and the electron paramagnetic resonance (EPR) spectra of photochemical products have been measured. The optical and EPR properties of RC's from Rp. gelatinosa are closely similar to their counterparts in RC's from other photosynthetic bacteria (Rp. sphaeroides and capasulata, Rhodospirillum rubrum and Chromatium vinosum). The EPR parameters for Rp. gelatinosa show differences between RC's in situ and in isolation; these may be related to the extreme lability of the isolated RC's. The most notable differences are in protein composition. RC's from Rp. sphaeroides and capsulata, Rs. rubrum and Chr. vinosum all contain a characteristic triad of subunits in the range 20 - 30 kilodalton. The RC's from Rp. gelatinosa and viridis contain fewer and heavier subunits. Thus there is variability in the protein configuration that can support the chromophores in a photochemically effective arrangement, and in the detailed manner in which the RC is integrated into the membrane.

4. Construction of a solar battery.

We attempted to make a solar battery by sandwiching RC's and UQ between conducting glass plates. This cell did indeed generate several millivolts and microamperes when illuminated, but its action did not require the presence of RC's and the only effective light was that absorbed by the UQ. Our provisional interpretation is that the distribution of light was unequal at the two metal electrodes, so the photo-induced injection of charge from UQ to the electrode was correspondingly unequal and a voltage difference was thereby developed (the "Denber effect"). Uniform illumination from both sides of the sandwich did not elicit a voltage. The system thus owes nothing to photosynthesis, but might nevertheless be developable into a useful device.

5. Fluorescence of bacteriorhodopsin.

In collaboration with Prof. Aaron Lewis we have measured the emission spectrum, excitation spectrum, quantum yield and temperature dependence of bacteriorhodopsin fluorescence. This information, combined with other information on fluorescence lifetime and photochemical reaction time, is consistent with the following picture: Excited bacteriorhodopsin is converted with high efficiency and within one picosecond to batho-bacteriorhodopsin. A side-reaction with very low efficiency (about $10^{-4}$) yields a metastable excited state, possibly a two-electron state having the same symmetry as the ground state. This metastable state is the source of the fluorescence. We are extending these measurements to invertebrate (squid) rhodopsin.

# PUBLICATION

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2. P. Heathcote and R. K. Clayton, "Reconstituted electron transfer from antenna pigment-protein to reaction centers isolated from Rhodopseudomonas sphaeroides". Biochim. Biophys. Acta 459 (1977) 506-515.

3. R. K. Clayton, "Fluorescence of photosynthetic reaction centers at low temperatures". Photosynthetic Organelles, Special Issue No. 3 of Plant and Cell Physiology (Japan), pp 87-96 (1977).

4. A. Vermeglio, "Secondary electron transfer in reaction centers of Rhodopseudomonas sphaeroides: Out-of-phase periodicity of two for the formation of ubisemiquinone and fully reduced ubiquinone". Biochim. Biophys. Acta 459 (1977) 516-524.

5. R. K. Clayton, "What can investigators of photosynthesis and vision learn from each other?" Biophys. of Struc. and Mech. 3 (1977) 107-116.

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7. A. Vermeglio and R. K. Clayton, "Kinetics of electron transfer between the primary and the secondary electron acceptor in reaction centers from Rhodopseudomonas sphaeroides". Biochim. Biophys. Acta 461 (1977) 159-165.

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9. B. J. Clayton and R. K. Clayton, "Properties of photochemical reaction centers purified from Rhodopseudomonas gelatinosa". Submitted to Biochim. Biophys. Acta.

10. R. K. Clayton and B. J. Clayton, "Molar extinction coefficients and other properties of an improved reaction center preparation from Rhodopseudomonas viridis". Submitted to Biochim. Biophys. Acta.

11. Y. Barouch and R. K. Clayton, "Ubiquinone reduction and proton uptake by chromatophores of Rhodopseudomonas sphaeroides R-26: Periodicity of two in consecutive light flashes". Submitted to Biochim. Biophys. Acta.

12. J. P. Spoonhower, M. A. Marcus, A. Lewis and R. K. Clayton, "The excited states of bacteriorhodopsin: Temperature and environmental effects on the emission spectrum". In preparation for Photochem. Photobiol.

13. R. C. Prince, P. L. Dutton, B. J. Clayton and R. K. Clayton, "EPR properties of reaction centers of Rhodopseudomonas gelatinosa, in situ and in detergent-solubilized form". In preparation for Biochim. Biophys. Acta.

14. C. N. Rafferty and R. K. Clayton, "Linear dichroism of photosynthetic reaction centers in stretched gelatin films". In preparation for Photochem. Photobiol.

15. R. K. Clayton and W. R. Sistrom (eds.), "The Photosynthetic Bacteria". Approximately 50 chapters by various contributors. Plenum Publishing Corp. (1977-78) in press. Chapter contributed by R.K.C. entitled "Physico-chemical mechanisms in reaction centers of photosynthetic bacteria".

## APPENDIX

Reprints and preprints of publications 1 - 11 and 15 are appended to this report.

Reprints + Preprints Removed