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ABSTRACT

Measurements of the quantum efficiencies of photochemistry and fluorescence, in photosynthetic reaction centers from Rhodospseudomonas sphaeroides, have been extended to low temperatures. These data have consolidated earlier data on efficiencies for the formation of triplet and biradical transient states in the reaction centers, and have elucidated the nature of the fluorescence. Newly discovered fluorescence transients promise to give more information on energy transfer in reaction centers.

Studies of linear dichroism in oriented fragments of photosynthetic membranes have revealed the orientations of bacteriochlorophyll and other pigments, both in the "antenna" and in the reaction centers. These studies have clarified the nature of certain light-induced absorbance transients and have given further support to the picture of a bacteriochlorophyll dimer serving as electron donor in the reaction center. This view is supported again by observations of some effects of dehydration upon reaction centers.

New measurements of absorbance changes associated with reduced components in reaction centers will hopefully allow us to trace the movement of electrons from primary to secondary acceptors. We are preparing to study the requirements for quinones as electron acceptors in the reaction centers of various species of photosynthetic bacteria.

By reconstituting the transfer of excitation energy in mixtures of antenna pigment-protein complexes and reaction centers, both isolated from photosynthetic membranes, we are learning quantitative aspects of the ability of reaction centers to trap energy from the antenna. At the same time we are attempting to use these materials in the construction of a solar battery.

In collaboration with Dr. Aaron Lewis we have measured the spectrum and quantum efficiency of fluorescence from bacteriorhodopsin; our aim is to

learn more about the excited states of this counterpart to the visual pigment of vertebrates.

INVESTIGATIONS AND RESULTS

1. Fluorescence and photochemical efficiency in reaction centers at low temperatures.

We have measured the quantum efficiency for bacteriochlorophyll (BChl) photo-oxidation in reaction centers (RC's) from Rhodopseudomonas sphaeroides, at temperatures from 300 K to 4 K. The efficiency is greater than 85% at all temperatures tested. Correlation with earlier measurements shows that the quantum efficiencies for formation of triplet BChl and of the state p^f (a transient intermediate in the photochemistry), in RC's at low redox potential, is greater than 85% at low temperature.

The long wave fluorescence (900 - 920 nm) of RC's was found to be independent of temperature down to 40 K, at low potential and also at physiological redox potential. This shows that the increased fluorescence at low redox potential is due to a slower conversion from the excited singlet state to the p^f state, and not to a feedback into the singlet state from the p^f state. This conclusion is valid unless the energy of the p^f state is nearly equal to that of the singlet state: within 0.025%, assuming a precision of 10% in our measurements of the fluorescence versus temperature.

2. Energy transfer and fluorescence in reaction centers.

The long wave (900 nm) fluorescence of RC's corresponds to the 865 nm absorption band of the BChl dimer that functions as photochemical electron donor. When the BChl dimer is photo-oxidized, both the 865 nm absorption and the 900 nm fluorescence disappear. We now find that these reversible light-induced changes are accompanied by other fluorescence changes having the same kinetics: an increase at 810 - 850 nm and a decrease centered at 780 nm. Presumably these changes reflect alterations in the efficiency of energy transfer from shorter-wave components (BChl and bacteriopheophytin) to longer wave components. The increased fluorescence at 810 - 850 nm may

result because the 865 nm component of BChl is no longer present to receive energy from components absorbing at 800-810 nm. With electron donors present to keep the BChl in its reduced form, illumination causes reduction of electron acceptors without bleaching the 865 nm component. In this case the 900 nm fluorescence increases in response to photochemical reduction of the primary electron acceptor, and there are no light-induced changes in fluorescence at shorter wavelengths.

Excitation spectra in the region 500-650 nm, for the 900 nm fluorescence, show that both molecules of bacteriopheophytin in the RC, as well as all four molecules of BChl, sensitize the long wave fluorescence with approximately equal efficiencies.

3. Linear dichroism in oriented membrane fragments.

By drying photosynthetic membrane fragments (chromatophores) onto glass plates, we achieve a high degree of orientation, with the plane of the membrane presumably parallel to the plate. This is shown by strongly dichroic absorption, with orthogonal polarizations of the analyzing beam giving up to twofold differences in the intensities of absorption bands. We have measured dichroism in the bulk antenna pigments and in the RC chromophores, the latter by two methods: First, the antenna pigments could be selectively and irreversibly bleached with chloroaurate, leaving membrane fragments with the absorption spectrum of RC's. Second, the reversible light-induced absorbance changes, reflecting changes of the RC chromophores, could be examined. These measurements have led to the following conclusions: (1) The long wave (Q_y) transition moments of both antenna and RC BChls lie in the plane of the membrane. The Q_x transition at 600 nm is perpendicular to that plane. Therefore the tetrapyrrole planes of the antenna BChl, and also of the electron-donor BChl dimer in the RC, are perpendicular to the plane of the membrane. The bacteriopheophytin of the RC and the transition moments of carotenoid pigments in the antenna are also perpendicular

to the plane of the membrane.

As an unexpected by-product of these studies we found that the light-induced absorbance decrease at 810 nm and the increase at 790 nm do not have the same orientations; they should if they represent the two limbs in the difference spectrum of a band shift centered near 800 nm. We therefore dismiss the traditional view that these changes reflect blue-shift of a BChl component absorbing at 803 nm. Instead we infer that the decreases at 810 and 865 nm show the bleaching of both components of the absorption spectrum of the BChl dimer, the "special pair" that functions as photochemical electron donor in the RC. When this dimer is singly oxidized, $(B)_2 \rightarrow (B)^+_{2\cdot}$, its absorption spectrum acquires some properties of monomeric BChl, accounting for the new band at 790 nm. The absorbance changes associated with the absorption band of bacteriopheophytin at 760 nm remain consistent with the interpretation of a red-shift of that band.

These studies have shown that polarization measurements can give fresh insights into the interpretation of absorbance changes as well as revealing the orientations of chromophores.

We are now preparing to see whether purified RC's become oriented while they are diffusing through polyacrylamide in an electric field. If so, these studies of RC's can be continued with improved signal/noise ratio.

4. Effects of dehydration upon reaction centers.

The removal of water from air-dried films of RC's leads to decreased absorbance at 865 and 810 nm and increased absorbance at about 830 and 790 nm. Measurements of linear dichroism in oriented membrane fragments oxidized with chloroiridate so as to expose the RC chromophores, indicate that these changes are due to a shift of the spectrum of the electron donating BChl dimer, from 865 - 810 nm to 830 - 790 nm. Further progress on this problem will await the measurement of circular dichroism and fluores-

cence in dehydrated preparations, and the substitution of D_2O or other substances for H_2O .

5. Absorbance changes associated with reduction of primary and secondary electron acceptors.

Earlier measurements on RC's illuminated in the presence of electron donors have revealed absorbance changes associated with the reduction of electron acceptors. These include an increase at 450 nm, attributed to the reduction of ubiquinone to the anion radical $UQ^{\cdot-}$, and red-shifts of the absorption bands of bacteriopheophytin. The effects of added ubiquinone, serving as a secondary or alternative electron acceptor, and of orthophenanthroline, which blocks electron transfer from primary to secondary acceptors, were anomalous. Also if RC's were illuminated in the presence of electron donors and then frozen, the bacteriopheophytin showed a blue-shift at low temperature, rather than a red-shift. We now find that under different conditions we can observe at room temperature either the blue-shift or the red-shift of bacteriopheophytin, or neither, together with varying amplitudes of the 450 nm absorbance change. We are trying to sort out the conditions that give these various effects, so that the absorbance changes will tell us reliably whether the primary electron acceptor, or alternative primary acceptor (added ubiquinone), or a secondary electron acceptor has been reduced.

6. The participation of quinones as primary electron acceptors in various photosynthetic bacteria.

Recent studies in our own and other laboratories have established an obligate role for ubiquinone as primary electron acceptor in Rhodopseudomonas sphaeroides, and for menaquinone in Chromatium. A requirement for ubiquinone in Rhodospirillum rubrum remains unsettled. The method in these studies has been to extract quinones from RC's and to add them back while

monitoring photochemical activity. We wish to examine other species of photosynthetic bacteria in this regard, and recognizing that carotenoidless mutants of photosynthetic bacteria are the best sources of purified RC's, we have newly isolated four such strains of Rps. capsulata and four of Rps. gelatinosa. The isolation and testing of RC's from these species is in progress.

7. Reconstitution of energy transfer in mixtures of purified antenna pigment-protein complexes and reaction centers from Rhodopseudomonas sphaeroides.

We showed last year that efficient transfer of excitation energy from antenna pigments of the photosynthetic membrane to RC's could be established in mixtures of the purified components. This was evidenced both by the efficient sensitization of RC photochemistry by the antenna chromophores, and by the way that fluorescence from the antenna varies depending upon the state of the RC's. Quantitative analysis of these phenomena has shown that "closed" (photochemically inactive) RC's are able to quench excitation energy from the antenna, as could be expected from the short lifetime of the excited state of BChl in isolated RC's. This was confirmed by measuring the yield of fluorescence from the antenna BChl in intact membranes from two strains of Rps. sphaeroides: In the non-photosynthetic strain PM8, which lacks RC's, the yield is threefold greater than in the parent strain Ga in which the RC's have been closed by photochemical conversion.

The lifetime of the excited singlet state of BChl in RC's has been estimated from the yield of long wave (900 nm) fluorescence, both in photochemically active preparations and in RC's at low redox potential, which are closed because the primary electron acceptor has been reduced. If RC's are closed by oxidizing the electron donor (the dimeric BChl), the 900 nm fluorescence is absent and the excited singlet lifetime cannot be estimated by this method. But by observing the ability of RC's to quench excitation

energy from the antenna pigment-protein, in a mixture of these components, we have been able to conclude that the excited state lifetime is approximately the same, 20 picrosec, in photo-oxidized RC's as in chemically reduced RC's.

8. Construction of a solar battery from photosynthetic materials.

Our approach to constructing a solar cell from photosynthetic materials is to make a sandwich of the following layers: A metal-coated semitransparent glass electrode, RC's with or without admixture of antenna pigment-protein, ubiquinone as a secondary electron acceptor (to impart polarity to the sandwich), and another metal-coated glass electrode. We are experimenting with various metals in an effort to secure effective conductivity for electrons or holes between the electrodes and the organic constituents.

9. Fluorescence of bacteriorhodopsin.

The detection of fluorescence of bacteriorhodopsin by Dr. Aaron Lewis of Cornell last year marked the first successful attempt to see fluorescence from any form of the visual pigment. In collaboration with Dr. Lewis we have measured the spectrum and the efficiency of this fluorescence. Combining the measured quantum efficiency, about 10^{-4} , with a measured lifetime of about 20 psec, we estimate an intrinsic lifetime of the order of 0.2 μ sec for the emitting state. This would be unusually long for the dipole-allowed excited state, and perhaps points to a metastable state as the source of the fluorescence. Further experiments of this kind may tell us more about the excited states involved in the photochemistry of vision.

All the foregoing experiments have been facilitated greatly by the acquisition of a new laser system and a computer-based signal averager. New instruments built around these components have given us indispensable new capabilities in measuring difference spectra for absorption and fluorescence, and in retrieving small optical transients from noise.

PUBLICATIONS

1. W.W. Parson, R.K. Clayton and R.J. Cogdell, "Excited states of photosynthetic reaction centers at low redox potentials". Biochim. Biophys. Acta 387 (1975) 265-278.
2. C.A. Wraight, R.J. Cogdell and R.K. Clayton, "Some experiments on the primary electron acceptor in reaction centers from Rhodopseudomonas sphaeroides". Biochim. Biophys. Acta 396 (1975) 242-249.
3. R.K. Clayton and T. Yamamoto, "Photochemical quantum efficiency and absorption spectra of reaction centers from Rhodopseudomonas sphaeroides at low temperature". Photochem. Photobiol. 23 (1976) xxx-xxx.
4. A. Vermeglio and R.K. Clayton, "Orientation of chromophores in reaction centers of Rhodopseudomonas sphaeroides: Evidence for two absorption bands of the dimeric primary electron donor". Submitted to Biochim. Biophys. Acta.
5. R.K. Clayton, "Fixed and variable fluorescence from reaction centers of Rhodopseudomonas sphaeroides at temperatures from 300 K to 40 K". Manuscript in preparation.
6. P. Heathcote and R.K. Clayton, "Reconstituted energy transfer from a purified antenna pigment-protein complex to reaction centers of Rhodopseudomonas sphaeroides". Manuscript in preparation.

APPENDIX

Reprints and preprints of publications No. 1-4 are appended to this report.

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