Annual Progress Report

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Compliance with Terms of Contract

Research has been conducted in full compliance with the terms of Special Research Support Agreement No. At(11-1)-3162. The Principal Investigator has contributed 50% of his time to work under this Contract, and will continue to do so to the end of the Contract Year.

Current Summary of Work Plan

See "Plans for 1973-1974" in the Renewal Proposal for September 1, 1973 - August 31, 1974.

Abstract

Our studies have dealt almost entirely with photochemical reaction centers isolated from the photosynthetic bacterium Rhodospseudomonas spheroides. We have investigated the generation of singlet and triplet excited states in photosynthetic reaction centers, in relation to the photochemical oxidation of bacteriochlorophyll (BChl). We have shown that in reaction centers reduced chemically so as to block the photochemistry, triplet BChl is formed with quantum efficiency close to 100% at liquid helium temperature and at least 20% at room temperature. The singlet excited population, measured by fluorescence, rises fourfold when photochemistry is blocked. In photochemically active reaction centers the quantum efficiency for photochemistry is at least 95%. These results are consistent with the possibility that photochemistry is mediated by the triplet state of BChl. The fluorescence may be taken as an indicator of the state of the reaction centers, but not as part of a simple quantitative theory of photochemical quenching of the excited singlet state.

We have discovered that when reaction centers are extracted with methanol, a delayed oxidation of BChl can occur in the extract. The reaction involves oxygen, and its rate, but not its extent, is governed by prior illumination of the reaction centers. This reaction appears to involve secondary chemical products following the primary photochemistry in reaction centers.

We have found that a major protein of the photosynthetic membrane of Rps. spheroides, of molecular weight 9000, is the bearer of the "B800 + B850" antenna BChl. Mutants lacking this component of BChl lacks this protein also.

Anilino naphthalene sulfonate can replace detergents in maintaining solubility of reaction centers. The fluorescence of this dye, associated with reaction centers, did not change when the reaction centers were illuminated so as to cause photochemistry.

Activities since May, 1972

1. Relations between excited singlet and triplet states of BChl and photochemistry in reaction centers (C. A. Wraight and R. K. Clayton in collaboration with P. L. Dutton, J. S. Leigh, Jr., and W. W. Parson).

We have known for several years (1,2) that when reaction centers are treated so as to reduce the photochemical electron acceptor, and thus to block the photochemistry ("closed"), the fluorescence of BChl rises three-to-fourfold over its level in "open" (photochemically active) reaction centers. In terms of a simple theory in which photochemical quenching of the excited singlet state is in direct competition with fluorescence, and in which the only consequence of closing the reaction center is to eliminate this photochemical quenching, a fourfold rise in fluorescence is consistent with a

photochemical efficiency of 75% in open reaction centers, becoming zero in closed reaction centers. Our redetermination of optical absorption coefficients of reaction centers (3) afforded a more reliable estimate of the photochemical efficiency, and recalculation of earlier data (4) did give efficiencies of 70 to 80%. But with new information (5) about the appearance of triplet BChl in illuminated closed reaction centers, which cast doubt on a simple interpretation of the fluorescence, we were led to redetermine the photochemical efficiency as carefully as possible. Our new measurements were based on the ferrioxalate actinometer (6) as a standard. They provide a datum of unprecedented reliability in this area. The efficiency of BChl oxidation in open reaction centers was found (7) to be $100 \pm 5\%$, which is not consistent with a fourfold rise in fluorescence by the simple theory mentioned above. The rise in fluorescence can be taken as an empirically justified probe of the states of the reaction centers, but not as a quantitative component of a simple theory in which the photochemical route of utilization of singlet excitation energy is eliminated when reaction centers are closed. This is an extremely important finding because the "simple" interpretation of fluorescence rise has played a major part in the study of photosynthesis for the past half-century.

P. L. Dutton and collaborators (5) reported an electron spin resonance (ESR) signal that corresponds to the formation of triplet BChl in illuminated closed reaction centers. In illuminated open reaction centers the "triplet" signal was absent and the signal due to oxidized BChl was seen instead. For unexplained reasons the "triplet" signal could be seen only below about 10°K . We undertook to determine the quantum efficiency for formation of triplet BChl in closed reaction centers by comparing characteristics of

this reaction with those of the photochemical oxidation of BChl in open reaction centers. Both reactions could be measured in a single preparation poised at the redox mid-point potential of the primary acceptor, since half the reaction centers were closed and half were open at this potential. In collaboration with P. L. Dutton and J. S. Leigh, Jr. at the University of Pennsylvania, we measured (8,9) the amplitude of the signal in response to brief flashes of light of different intensities, covering the intensity at which on the average each reaction center absorbed a single quantum, and going higher into the region of light saturation. The light-saturation curves for the "triplet" and "oxidized BChl" signals were superimposable, showing that the efficiency for triplet formation in closed reaction centers equals that for BChl oxidation in open reaction centers; i.e., close to 100%. These results are consistent with either of two views: the triplet state of BChl is an intermediate in the photochemical process, and can accumulate when its photochemical utilization is cut off, or else the triplet and the photochemical reactions are competitive ways of leaving the excited singlet state, with photochemistry favored in open reaction centers and triplet formation favored in closed ones. In either case the fourfold rise in fluorescence requires an ad hoc explanation.

Conversion of BChl to the triplet state should be attended by loss of the normal absorption bands of ground state BChl, and the appearance of new bands for triplet-triplet transitions. Such absorbance changes have been confirmed in measurements made at room temperature in collaboration with W. W. Parson (10). Using the flash-saturation technique described above, we concluded that the quantum efficiency for generation of these changes (triplet formation?) in closed reaction centers is about 1/5 that for BChl

oxidation in open reaction centers; i.e., about 20%. We do not have an explanation for the discrepancy between this result and the one based on ESR at low temperature. Obviously we need data on the absorbance changes at low temperature.

The lifetime of triplet BChl in closed reaction centers is about 7 microsec, both at low temperature (ESR) and at room temperature (absorbance change). The absorbance change at room temperature could represent a new state that is reached from the triplet in a time much shorter than 7 microsec.

These results may lead to major revisions in our conception of the dynamics and mechanism of the conversion of singlet excitation energy to chemical potential (redox energy) in reaction centers.

2. Chemical reactions in methanol extracts of reaction centers (D. C. Brune, R. K. Clayton). We reported earlier (11,12) that methanolic extracts of illuminated reaction centers contain some oxidized BChl that can be reduced with ascorbate. We supposed that this was BChl that had been oxidized photochemically in the reaction centers, and was extracted in the oxidized state. We have now found that the explanation is entirely different, and have uncovered a remarkable delayed oxidation of BChl in the methanol extract. If illuminated reaction centers are extracted with methanol, the extract initially contains as much reduced BChl as does a comparable extract of nonilluminated reaction centers, and it contains no oxidized BChl. But if the extract is monitored for several minutes, about 35% of the BChl can be seen to become oxidized, as signaled by loss of absorbance at 770 nm. The 770 nm band is restored to its original height if ascorbate or Fe^{++} is added. This oxidation does not occur in the absence of O_2 . It occurs, to the extent of about 35%, even when the reaction centers are allowed to rest in the dark

for several minutes after illumination and prior to extraction. A few sec of darkness, sufficient to allow spontaneous re-reduction of the photochemically oxidized BChl in the reaction centers, does not alter the rapidity or the extent of the later oxidation of BChl in the extract, when compared with reaction centers extracted while they are being illuminated. With longer dark times between illumination of reaction centers and their extraction, there is an ever greater lag before the oxidation begins in the extract, but once the reaction starts, both its rate (about 4 min. for completion) and its extent (about 35% of the BChl) are constant. With reaction centers that had been kept in the dark for about an hour prior to extraction, the lag was about 30 min. Illumination of the reaction centers thus alters their state so that a certain reaction in the methanolic extract is initiated (triggered?) earlier. The altered state persists for many minutes, in contrast to the rapid recovery of the primary electron donor, BChl. The extent of the reaction is not altered by adding purified BChl to the extract; it remains 35% of the BChl that came from the reaction centers. If illuminated and non-illuminated reaction centers are mixed and then extracted, 35% of the total BChl becomes oxidized, in a monophasic reaction with a lag intermediate between that for the two reaction center samples treated separately. Thus the lag appears to be set by the concentration of a secondary product of photochemistry in the reaction centers.

Neither reduced nor oxidized ubiquinone affects the reaction. *o*e and orthophenanthroline have strong effects (the latter prevents the reaction) that are being scrutinized. We are also studying the effects of single and multiple brief strong flashes of conditioning illumination.

We hope that this reaction will tell us more about secondary products of the primary photochemistry in reaction centers.

3. Raman spectroscopy of reaction centers (R. K. Clayton and R. C. Downs in collaboration with Aaron Lewis). We are planning the redesign of a laser Raman spectrometer for excitation at wavelengths useful for the study of photosynthetic reaction centers. A single reaction center contains two molecules of bacteriopheophytin and four of BChl (3). Two of the BChls, responsible for an absorption peak at 865 nm, share a single + charge in the photochemically oxidized state (see 13); the oxidized BChl absorbs from about 980 to 1300 nm. An absorption peak at 800 nm is associated with the two BChls that do not become oxidized. All four BChls share in a peak at 600 nm, and the pheophytin absorbs at 535 and 760 nm. Thus by suitable choices of exciting wavelengths one can, with the Raman technique (inelastic photon scattering), explore vibrational energy states of the different chromophores. This can be done with "open" and "closed" (chemically reduced) reaction centers to look for changes that attend photochemistry. Signal retrieval can be enhanced by exciting Raman spectra beyond 980 nm (oxidized BChl only) and generating oxidized BChl periodically with modulated light of shorter wavelength.

4. Relations between pigments and proteins in the photosynthetic membranes of Rps. spheroides (R. K. Clayton, B. J. Clayton and R. M. Sheetz). We found (14,15) that in wild type Rps. spheroides a protein of molecular weight 9000 bears the antenna BChl system B800 + B850. This accessory pigment system appears to give Rps. spheroides an extra dimension (16) in its ability to adapt to its environment, with relatively more pigment in cells grown under weak light. The carotenoidless mutant of Rps. spheroides lacks B800 and B850, has less ability to alter its density of pigmentation in response to changing illumination, and lacks the special protein (mol. wt. 9000) that bears B800

and B850 in the wild type. By a variety of fractionating procedures we are trying to determine to which proteins the remaining antenna BChl, B870, is preferentially bound. Our aim is to generate a picture of the assembly plan of the pigment and protein components of the photosynthetic membrane, and to understand the consequences for membrane structure and function when one or more of the major proteins (B800 + B850 bearer, reaction center protein, etc.) are missing.

We are sending cells of the reaction center-less mutant of Rps. spheroides to Professor Britton Chance at the University of Pennsylvania for study of the responses of optical probes of electrochemical gradients.

5. Interaction of reaction centers with fluorescent probes (R. K. Clayton and L. Honig, undergraduate honors student). We attempted to bind the well known fluorescent probes auramine o and anilino naphthalene sulfonate (ANS) hydrophobically to reaction centers to see whether the fluorescence might change in response to photochemistry in the reaction centers. The results in this respect were negative, but we did find that the reaction centers remained soluble when the detergent lauryl dimethyl amine oxide (LDAO) was replaced by ANS through dialysis. This offers at least a technical advantage in experiments where the presence of LDAO is objectionable, as in the study of reaction centers during dehydration.

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