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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, on Sabbatic Leave, has contributed 100 per cent of his time to work under this contract, and will continue to do so until the end of the Contract Year. Leave was at Univ. of California (9/1 - 5/15) and Univ. of Washington (5/16 - present).

Current Summary of Work Plan

See "Plans for 1974-75" in the Renewal Proposal for September 1, 1974 - August 31, 1975.

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Abstract

These experiments deal with materials from the photosynthetic bacterium Rhodopseudomonas spheroides.

By a new technique of extraction and reconstitution of photosynthetic reaction centers (RC's) we have shown that ubiquinone (UQ) is required for photochemical activity and is probably a component of the primary electron acceptor system.

We have clarified the reaction in which bacteriochlorophyll (BChl) extracted from RC's becomes oxidized in vitro.

We have found that RC's, when unable to perform photochemistry, display two types of light-induced absorbance change, one of which probably reflects formation of the triplet state of BChl and the other a precursor of this state.

Redox titrations of the state of the primary acceptor have shown pH dependence in photosynthetic membranes but not in isolated RC's. This has important implications for the dynamics of protonation attending photochemical electron transfer, and for the meaning of equilibrium redox titrations when applied to dynamic biological systems.

We have studied the absorbance change (X450) that seems to reflect UQ reduction in RC's; in response to flashes of light. This change attends primary photochemistry in dark-adapted preparations, but an alternative photochemical act seems possible when X450 has become reduced. These findings may clarify questions about the relevance of photochemistry in isolated RC's to photochemistry in living cells.

We are developing instruments for low-temperature absorption spectroscopy in the sub-microsecond time range, and are beginning the study of RC's by resonance Raman spectroscopy.

Activities since May, 1973

1. Properties of photosynthetic reaction centers after extraction and replacement of ubiquinone (R. J. Cogdell, D. C. Brune and R. K. Clayton).

It has long been known that hydrocarbon solvents remove from photosynthetic tissues a component of UQ that acts as a secondary electron acceptor. This extraction fails to remove residual UQ (one molecule per RC); we have now found that iso-octane containing a trace of methanol removes the remaining UQ and obliterates the primary photochemistry. Photochemical activity is restored when purified UQ is added back to the RC's. This result is of great importance because it bears directly on the puzzling question of the nature of the primary acceptor, supporting a model in which iron and UQ cooperate as primary acceptor.

2. Spontaneous oxidation of BChl in extracts of illuminated RC's (D. C. Brune). BChl extracted with methanol from RC's that had previously been illuminated becomes oxidized to the extent of about 35 per cent after a lag of several minutes or more. Ultimately the oxidized BChl is converted to a re-reduced but altered form, either spontaneously or (more rapidly) if a reducing agent is added. The reaction obeys kinetics described by

where BChl_{al} is the altered re-reduced form. Oxygen and an amine oxide are needed for the first step. The requirement for $\text{BChl}^{+\cdot}$ to initiate its own formation accounts for the dependence on previous illumination of the RC's.

3. Absorbance changes in RC's that cannot perform photochemistry (R. J. Cogdell and R. K. Clayton in collaboration with W. W. Parson of the Univ. of Washington). At sufficiently low redox potential (such that the primary electron acceptor is reduced) the light-induced absorbance changes

that reflect "normal" photochemistry are absent. In their place we observe changes of two types. One type, with half-life 7 microseconds at room temperature, has a spectrum very like that of BChl in the triplet state. The other is much shorter-lived (less than 10 nsec), has a slightly different spectrum, and could be a precursor of the "triplet-like" one. The short-lived state is formed with quantum efficiency close to 100 per cent; the triplet-like one is generated with lesser efficiency (about 20 per cent). Since these absorbance changes reflect states that accumulate when photochemistry is prevented, they may represent early steps in the photochemical process, between light absorption and electron transfer from BChl to the acceptor. These effects are induced also when the photochemistry is blocked by extracting the UQ (see earlier).

4. Redox titrations of phenomena associated with the primary electron acceptor in photosynthetic bacteria (C. A. Wraight in collaboration with P. L. Dutton and J. S. Leigh, Jr. at the Univ. of Pennsylvania). Both RC's and chromatophores (membrane fragments) of Rps. spheroides were examined at various redox potentials to determine the state of the primary electron acceptor by the following criteria: ability to oxidize BChl or cytochrome in a light-induced reaction, intensity of the ESR signal at $g=1.82$ reflecting the reduced state of a non-heme iron group, and formation of triplet BChl upon illumination. By all these criteria the redox mid-point was found to be -0.05 v at; H 7 in both RC's and chromatophores. The mid-point was independent of pH in RC's, but varied with pH in chromatophores. The difference suggests that protonation of electron-accepting components can play a significant part in determining the redox state, but that the environment

of the RC's is important. It is possible that electron turnover is so rapid in vivo that H^+ binding cannot keep pace, as it does in equilibrium measurements. In that case the "equilibrium" redox titrations do not reflect the actual operative redox properties of the reactants in vivo.

5. Absorbance changes associated with the primary electron acceptor in RC's (R. J. Cogdell, C. A. Wraight, and R. K. Clayton). We have earlier described light-induced absorbance changes associated with primary photochemistry in RC's, suggesting formation of the anionic semiquinone of UQ and featuring a maximum at 450 nm (X450). We have subjected RC's to flash excitation under conditions that allow observation of X450. The magnitude of this change was observed, and also the capacity for photochemistry as revealed by cytochrome oxidation in response to another flash. If X450 represents the reduced state of the primary acceptor, renewed photochemistry should not be possible until this change has decayed after a flash. We found this to be true in RC's devoid of secondary electron acceptors. However, if excess UQ was added as a secondary acceptor, we observed that the recovery of photochemical capacity was much faster than the relaxation of X450; less than 5 sec. for the former and about 3 min. for the latter. One possibility is that the natural photochemical act involves generation of X450 as a sign that the natural primary acceptor is reduced. When the primary acceptor has been reduced, it can be bypassed in a variant photochemical act in which an electron is transferred from BChl directly to the "normally secondary" UQ. The direct reaction with "secondary" UQ might be a property of purified RC's but not of the living system. It is also possible that the reaction that is restored by adding UQ to extracted RC's (see item 1, earlier) falls in this "unnatural" category.

6. Instrumentation and special methods (T. Yamamoto, R. C. Downs and R. K. Clayton in collaboration with A. Lewis of Cornell). We have procured a Q-switched ruby laser and are constructing an absorption spectrometer that will give us access to the sub-microsecond time domain at temperatures down to 4°K. This will afford correlation of ESR signals, variations of fluorescence yield, and optical absorbance changes that reflect formation of oxidized BChl and triplet BChl. We have begun to study RC's and purified BChl by resonance Raman spectroscopy under the supervision of Aaron Lewis; this work remains in a preliminary state.

Publications

1. R. K. Clayton, "Primary processes in bacterial photosynthesis". Ann. Rev. Biophys. Bioeng. 2, 131-156 (1973).
2. S. C. Straley, W. W. Parson, D. C. Mauzerail and R. K. Clayton, "Pigment content and molar extinction coefficients of photochemical reaction centers from Rhodopseudomonas spheroides". Biochim. Biophys. Acta 305, 597-609 (1973).
3. D. C. Brune and R. K. Clayton, "A light initiated, dark oxidation of bacteriochlorophyll from reaction center particles". Biochim. Biophys. Acta 333, 37-43 (1973).
4. C. A. Wraight and R. K. Clayton, "The absolute quantum efficiency of bacteriochlorophyll photooxidation in reaction centres of Rhodopseudomonas spheroides". Biochim. Biophys. Acta 333, 246-260 (1973).
5. P. L. Dutton, J. S. Leigh, Jr., and C. A. Wraight, "Direct measurement of the midpoint potential of the primary electron acceptor in Rps. spheroides in situ and in the isolated state". FEBS Letters 36, 169-173 (1973).

6. R. J. Cogdell, C. A. Wraight, and R. K. Clayton, "Some properties of the primary electron acceptor in reaction centres from Rhodopseudomonas spheroides". Abstr., Ann. Mtg. Biophys. Soc. (1974).
7. C. A. Wraight, J. S. Leigh, Jr., P. L. Dutton, and R. K. Clayton, "The triplet state of reaction centre bacteriochlorophyll: Determination of a relative quantum yield". Biochim. Biophys. Acta, in press, (1974).
8. R. J. Cogdell, D. C. Brune, and R. K. Clayton, "Effects of extraction and replacement of ubiquinone upon the photochemical activity of reaction centers and chromatophores from Rhodopseudomonas spheroides". FEBS Letters, submitted for publication in 1974.
9. R. K. Clayton, "Photosynthesis: how light is converted to chemical energy". Addison Wesley Modular Series, in press, (1974) (51 pp).
10. R. K. Clayton, "The position and prospects of solar energy conversion". To be published as part of the proceedings of a Symposium on Photobiology, Rio de Janeiro, July, 1973; L. R. Caldas and A. Lamola, organizers.