000--3162-25
CONF-740946-13
Proc. 3d Int. Cong. on Photosynth.
Rehovot, Israel, Sept. 1974

EXCITED STATES IN THE PHOTOCHEMISTRY
OF BACTERIAL PHOTOSYNTHESIS

Roderick K. Clayton
Section of Genetics, Development and Physiology
Cornell University

and

William W. Parson
Department of Biochemistry
University of Washington

MASTER

Reaction center (RC) preparations from Rhodopseudomonas spheroides strain R-26 show the following light-induced phenomena:

1. Optical absorbance changes¹ and an electron paramagnetic resonance (EPR) signal² indicating oxidation of the "P865" component of bacteriochlorophyll (BChl). The quantum efficiency for BChl oxidation is 1.02 ± 0.04 at room temperature³. At temperatures down to that of liquid helium the efficiency does not fall by a large factor⁴; it might be the same as at room temperature. The capacity for BChl oxidation is lost when the redox potential is brought below a mid-point of -0.05 v, presumably because the photochemical electron acceptor is reduced⁵.

2. BChl fluorescence (maximum at 900 nm, corresponding to the 865 nm absorption band) with quantum yield 3.5×10^{-4} at room temperature in "open" RC's⁶. As the redox potential is lowered the fluorescence yield rises to about 10^{-3} in "closed" RC's⁷. Taking the intrinsic fluorescence lifetime to be 2×10^{-8} sec (from the area of the absorption band), the lifetime of the excited singlet state is $3.5 \times 10^{-4} \times 2 \times 10^{-8}$ or 7 psec in open RC's, and 20 psec in closed RC's. The fluorescence yield has not been measured at lower temperatures.

3. An EPR signal that suggests formation of a triplet state of BChl, observed below about 20°K in closed RC's but not in open RC's^{8,9}. The signal decays with half-time about 5 μ sec at 10°K; this decay time might reflect equilibration among spin states rather than quenching of the triplet. The quantum efficiency for formation of this EPR signal in closed RC's equals that for BChl oxidation in open RC's at the same temperature.

4. Two types of optical absorbance change (Δa) observed in closed RC's (at low redox potential), studied by ourselves in collaboration with R. Cogdell; unpublished. A "slow" change has the spectrum of triplet BChl¹⁰ and a rise time less than 50 nsec at temperatures down to about 20°K. The decay half-time is

NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Atomic Energy Commission, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights

DISTRIBUTION OF THIS DOCUMENT UNLIMITED

gy

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

Excited states in photosynthesis

about 7 μ sec at room temperature; it slows to about 100 μ sec as the temperature is lowered to 100°K and remains so at temperatures down to about 20°K. The quantum efficiency for generation of this "slow" change is about 0.2 at room temperature. Judging from comparative measurements of signal amplitude versus exciting flash energy the efficiency rises, perhaps to a value near unity, as the temperature is lowered to about 20°K. Also at low redox potential, a "fast" Δa can be distinguished from the foregoing. The "fast" change has a rise time less than 5 nsec; the decay time is about 15 nsec (partly instrument-limited as measured) at room temperature and about 40 nsec at temperatures near 20°K. The spectrum of this change is similar but not identical to that of triplet BChl; the quantum efficiency for its formation appears to be about unity at both room and low temperatures.

We speculate that both the EPR signal and the "slow" Δa seen in RC's at low redox potential reflect a triplet-like state of BChl, and that the "fast" Δa indicates a precursor of this state. The lifetime of the "fast" Δa is far too great to correspond to the excited singlet state that gives rise to fluorescence. These metastable states may have the nature of charge-transfer states, with centers of positive and negative charge displaced relative to the neutral ground state of BChl. Observations thus far do not show whether these states, seen only in closed RC's, are intermediates in the photochemical process of open RC's.

From the high efficiency of BChl oxidation in open RC's at room temperature, we should expect a rise in fluorescence far greater than the threefold increase that is observed when the photochemistry is prevented by lowering the redox potential. This anomaly awaits clarification. If we assume that the singlet excited state of BChl is quenched by competing first-order processes, we can write, for open RC's,

$$-dN/dt = (k_f + k_d + k_p)N$$

where N is the number of molecules in the excited state, k_f is a rate constant for spontaneous radiative de-excitation (fluorescence), k_d is a constant for radiationless de-excitation, and k_p is a constant for the first step leading to BChl oxidation. In closed RC's the sum of k_d and k_p is replaced by a quenching constant k_q . We do not know to what extent the processes represented by k_d , k_p , and k_q involve the formation of triplet BChl, but we can recapitulate the following quantitative data, with values of k taken as reciprocals of characteristic lifetimes mentioned earlier, and quantum efficiencies denoted ϕ . The value of k_f is estimated from the area under the long wave absorption band.⁶ Values of k_p , k_d , and k_q have been inferred from the efficiencies of fluorescence and photochemistry³.

Excited states in photosynthesis

Table 1

Rate constants and quantum efficiencies for light-induced processes in photochemical reaction centers from Rps. spheroides. Symbols are explained in the text.

	OPEN REACTION CENTERS	CLOSED REACTION CENTERS
Room temperature	$k_f = 5 \times 10^7 \text{ sec}^{-1}$ $k_d \ll 10^{11} \text{ sec}^{-1}$ $k_p = 1.4 \times 10^{11} \text{ sec}^{-1}$ ϕ_p (BChl oxid.) = 1.0	$k_f = 5 \times 10^7 \text{ sec}^{-1}$ $k_q = 0.5 \times 10^{11} \text{ sec}^{-1}$ $k_p = 0$ $\phi_T = 0.2$ ("slow" Δa) ϕ ("fast" Δa) ≈ 1
Temperature $\lesssim 20^\circ\text{K}$	$k_f \approx 5 \times 10^7 \text{ sec}^{-1}$ $\phi_p \approx 1$	$k_f \approx 5 \times 10^7 \text{ sec}^{-1}$ $\phi_T \approx 1$ (triplet-like EPR signal and "slow" Δa) ϕ ("fast" Δa) ≈ 1

References

1. Clayton, R.K., 1962, Photochem. Photobiol. 1, 201.
2. McElroy, J.D., Feher, G., and Mauzerall, D.C., 1969, Biochim. Biophys. Acta 172, 180.
3. Wraight, C.A., and Clayton, R.K., 1974, Biochim. Biophys. Acta 333, 246.
4. Clayton, R.K., 1962, Photochem. Photobiol. 1, 305.
5. Dutton, P.L., Leigh, J.S., and Wraight, C.A., 1973, FEBS Letters 36, 169.
6. Zankel, K.L., Reed, D.W., and Clayton, R.K., 1968, Proc. Natl. Acad. Sci. U.S. 61, 1243.
7. Clayton, R.K., Fleming, H., and Szuts, E.Z., 1972, Biophys. J. 12, 46.
8. Wraight, C.A., Leigh, J.S., Dutton, P.L., and Clayton, R.K., 1974, Biochim. Biophys. Acta 333, 401.
9. Dutton, P.L., Leigh, J.S., and Seibert, M., 1972, Biochem. Biophys. Res. Commun. 46, 406.
10. Connolly, J.S., Gorman, D.S., and Seely, G.R., 1973, Ann. New York Acad. Sci. 206, 650.