Biochimica et Biophysica Acta, 504 (1978) 255—264

BBA 47584

EFFECTS OF DEHYDRATION ON REACTION CENTERS FROM *RHODOPSEUDOMONAS SPHAEROIDES*

RODERICK K. CLAYTON

Section of Botany, Genetics and Development, Cornell University, Ithaca, N.Y. 14853 (U.S.A.)

(Received February 13th, 1978)

Summary

Air-dried films of reaction centers from *Rhodopseudomonas sphaeroides* were found to resemble aqueous suspensions of these reaction centers in their optical and photochemical properties, except that the long wave absorption band of bacteriochlorophyll was shifted from about 860 to 845 nm. The quantum efficiency of the photochemical reaction that produces oxidized bacteriochlorophyll and reduced ubiquinone was >0.75 in the films, using 800-nm actinic light. Dehydration of the films caused further blue shifts of all the absorption bands between 500 and 900 nm, and some loss (about 20%) of intensity of the long wave band, coupled to a gain of absorbance centered at 795 nm. The latter changes were not accompanied by an increase at 1250 nm, ruling out the oxidation of bacteriochlorophyll as a cause of these changes. Dehydration caused about 50% of the reaction centers to become photochemically inactive. For the component that remained active, the photochemical quantum efficiency was about 0.5 at room temperature, rising to 0.7 or more as the temperature was brought below 250 K. The yield of 900 nm fluorescence was approximately the same in air-dried films as in aqueous suspensions; dehydration of a film raised the fluorescence yield about 3-fold. The yield of this fluorescence in a dehydrated film was independent of temperature (±10%) between 300 and 70 K. The back reaction after illumination, return of electrons from reduced ubiquinone to oxidized bacteriochlorophyll, has kinetics in which a most rapid first-order component is mixed with slower components. The fastest component, identified with the return of electrons from 'primary' ubiquinone to oxidized bacteriochlorophyll, was predominant in the materials tested here. Its half time in an aqueous suspension of reaction centers fell from 86 ms at 300 K to 33 ms at 200 K and declined more gradually to 15 ms at 50 K. An air-dried film showed similar behavior. The dramatic change of half-time between 300 and 200 K can be ascribed to one or more phase transitions involving water; in a dehydrated film the half-time of the fastest decay component was about 22 ± 5 ms, independent of the temperature between 300 and

70 K. The original properties of an air-dried film were restored fully, after dehydration, by exposure to either H_2O or ^2H_2O vapor. There was no significant difference, in these measurements, between H_2O-restored and ^2H_2O-restored films.

Introduction

Films of chromatophores (intracytoplasmic membrane fragments) and photochemical reaction centers from photosynthetic bacteria can be prepared by allowing aqueous suspensions to dry onto glass or quartz plates. Such air-dried films, made with materials from *Rhodopseudomonas sphaeroides*, show essentially the same photochemical properties as do the aqueous suspensions [1]. When these films are dehydrated, either by pumping away the surrounding air or by exposing them to a dessicant such as $CaSO_4$ or P_2O_5, their photochemical activity (photo-oxidation of bacteriochlorophyll in the reaction centers) declines and the yield of their fluorescence increases [2—4]. These effects are reversible; exposure of the films to water vapor restores their original properties.

I shall describe here a more detailed study of some effects of dehydration on reaction centers from *Rps. sphaeroides*. We shall see that dehydration of reaction centers alters their absorption spectra, photochemical efficiency, fluorescence yield and reaction kinetics (back reaction; return of electrons from reduced ubiquinone to oxidized bacteriochlorophyll). After dehydration the properties of air-dried films are restored by exposure to either H_2O or ^2H_2O. Apparently water helps to preserve an optimal configuration for high photochemical efficiency and for stability of the photoproducts in reaction centers.

Materials and Methods

Rps. sphaeroides, carotenoidless mutant strain R-26, was grown anaerobically in the light, and reaction centers were isolated from these bacteria, as described elsewhere [5,6]. Reaction centers were dialyzed overnight against 0.01 M Tris-HCl, pH 7.5 and then allowed to dry on quartz plates to form films.

For optical measurements at low temperatures the samples were mounted on the cold tip of a closed cycle helium refrigerator [7].

Absorption spectra were measured in a Cary 14R Spectrophotometer fitted for cross-illumination. The No. 2 infrared mode of this instrument yielded spectra of samples exposed to strong light (up to 200 mW/cm^2) during measurement. Light-induced absorbance changes were measured with a split-beam absorption spectrometer using unmodulated light and providing an output voltage proportional to absorbance [8]. Fluorescent measurements were made as described elsewhere [7]. The output of each of these instruments could be stored in a signal averager (Tracor-Northern TN-1500) capable of such manipulations as differentiation and conversion to the logarithm. Logarithmic conversion was checked and calibrated by using a capacitor discharge (series resistance-capacitance circuit) as the input.

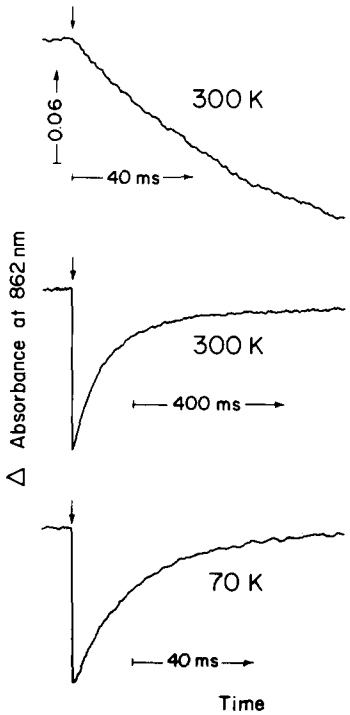

Fig. 1. Light-induced absorbance changes in an air-dried film of reaction centers from *Rps. sphaeroides*, measured at 862 nm. Application of 800-nm actinic light is shown by the arrows. Upper trace, continuous actinic light. Lower traces, submillisecond xenon flash. Each trace shows the average of eight replicate measurements.

Light-induced absorbance changes were measured at 862 nm, with actinic light at 800 nm provided by either a sub-millisecond xenon flash or a tungsten-iodine (Sylvania Sun Gun) continuous lamp in conjunction with an 800 nm interference filter. An 862-nm interference filter in the measuring path prevented scattered actinic light from reaching the detector. The xenon flash lamp was used to examine kinetics of the back reaction following photochemistry; continuous actinic light was used in determining the initial rate of the forward reaction and thus measuring photochemical quantum efficiency. The intensity of the 800-nm continuous light incident on the sample was 5 mW/cm^2. For each condition of measurement, 8—16 replicate measurements were summed in the signal averager. Representative traces are shown in Fig. 1. The decay curves (back reaction) were converted to semilogarithmic plots for identification of first order components.

Details concerning the computation of quantum efficiency from the rate of the light-induced absorbance change at 862 nm have been given elsewhere [9]. The computation required two convolutions. The absorption spectrum of the sample (fraction absorbed; not absorbance) was convolved with the spectrum of actinic light transmitted by the 800 nm filter, to estimate the rate at which the sample absorbed actinic light. Also the absorbance spectrum of the sample was convolved with the spectrum of the measuring light as trans-

mitted by the combination of monochromator and 862-nm interference filter. This was done in order to determine an effective differential extinction coefficient for the reaction, a bleaching of the long wave absorption band of bacteriochlorophyll in consequence of its photo-oxidation.

Quantum efficiencies were normalized to an arbitrary value of 1.00 for reaction centers in an air-dried film at room temperature (300 K). From comparisons with aqueous suspensions, the quantum efficiency of an air-dried film of reaction centers appeared to be within 20% of the value, 0.95, established [10] for aqueous suspensions with 800-nm actinic light. A more accurate comparison was difficult because the films were not perfectly uniform. However, the non-uniformities apparent in any one film did not change upon dehydrating or lowering the temperature.

Relative quantum yields of fluorescence were determined from the integrated area of the 900-nm fluorescence band. In films any contamination by shorter wave fluorescence [8] was negligible.

Results and Discussion

Absorption spectra

The absorption spectrum of an air-dried film of reaction centers at 300 K is shown as the solid curve ('hydrated') in Fig. 2. This spectrum is like that of an aqueous suspension of reaction centers, except that the long wave absorption maximum is near 850 nm rather than 860 nm. In an aqueous suspension this absorption band becomes blue-shifted in the presence of high concentrations of the detergent lauryl dimethyl amine oxide that is used to maintain solubility of the reaction centers. The suspensions used in making films contained residual lauryl dimethyl amine oxide even after dialysis against Tris buffer, and this

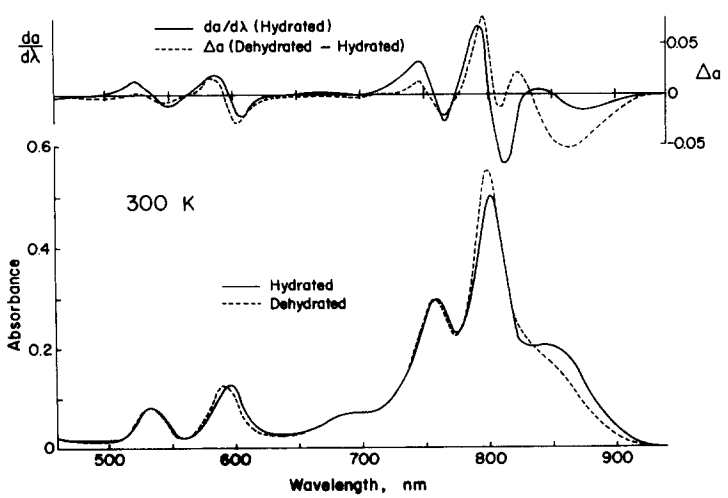

Fig. 2. Absorption spectra of an air-dried film of reaction centers from *Rps. sphaeroides* at 300 K. Solid curve ('hydrated'), air-dried film before dehydration. Dashed curve, the same film after dehydration. The upper curves show the first derivative of the absorption spectrum of the hydrated film (solid curve) and the change induced by dehydration (dashed curve).

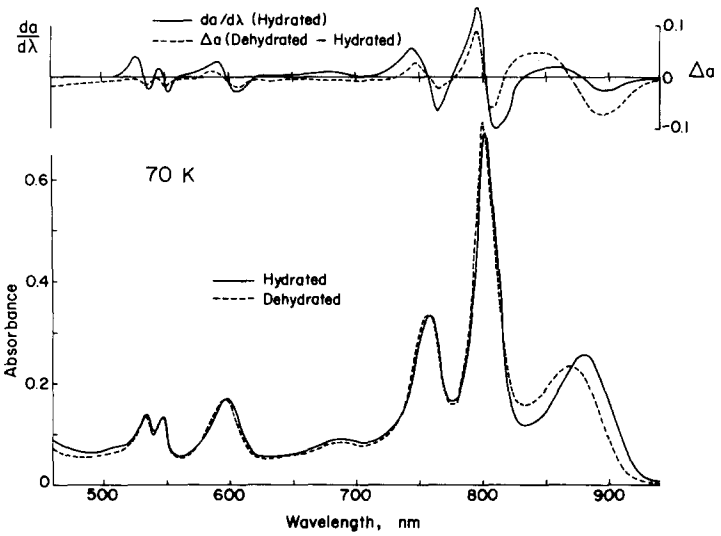

Fig. 3. Same as Fig. 2, measured at 70 K.

lauryl dimethyl amine oxide would become concentrated as the suspensions dried into films, accounting for the band shift. Blue shift of the long wave absorption band in films, as compared with aqueous suspensions, is reflected also in the absorption spectrum at 70 K (solid curve, Fig. 3) and in the light-dark difference spectra (solid curves, Fig. 4). For comparison with aqueous

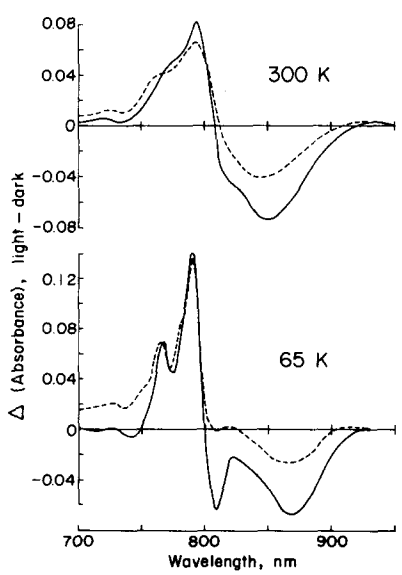

Fig. 4. Light-induced difference spectra (absorbance) of an air-dried film of reaction centers from *Rps. sphaeroides*, before and after dehydration (solid and dashed curves respectively). The spectra were measured with a Cary 14R Spectrophotometer using cross-illumination with blue light (tungsten-iodine lamp; Corning No. CS4-96 filter) at an intensity below saturation.

suspensions the reader is referred to Figs. 1 and 3 of ref. 11 and Figs. 10 and 11 of ref. 12.

Dehydration of an air-dried film caused further absorbance changes as indicated by the dashed curves in Figs. 2—4. Comparison with the first derivative spectra of the hydrated film (upper curves in Figs. 2 and 3) shows that all of the absorption bands were blue-shifted to varying degrees by dehydration. In addition, dehydration caused a decrease of intensity of the long wave band and an increase near 795 nm. This effect was more pronounced at 300 K than at low temperature. Associating the long wave band with the 'special pair' of bacteriochlorophyll molecules that acts as photochemical electron donor [13], some of this special pair appears to have been altered so as to give a monomer-like spectrum with an absorption maximum at 795 nm. The special pair has an absorption band near 805 nm, companion to the long wave band as appropriate for the spectrum of a dimer [12]. This 805-nm band is obscured by the much larger absorption band at 800 nm, and it is difficult to see in Figs. 2 and 3 whether dehydration attenuated the 805-nm band as well as the long-wave band.

The loss of absorption in the long-wave band and the gain near 795 nm is similar to the change induced by oxidation of the special pair, and one could speculate that dehydration has driven some of the special pair bacteriochlorophyll into its oxidized state. If so, dehydration should cause an increase of absorbance at 1250 nm characteristic of the oxidized state [14]. In fact, any change at 1250 nm was less than 10% of that anticipated if the decrease of the long wave band is due to oxidation of the special pair. We are left with the interpretation that dehydration has weakened the dimeric interaction of the special pair bacteriochlorophyll molecules.

Photochemical efficiency

Dehydration of an air-dried reaction center film allowed approximately half of the reaction centers to remain photochemically active, judging from the extent to which the residual long wave absorption band could be bleached by saturating illumination. This was tested by recording absorption spectra with the No. 2 infrared mode of the Cary Spectrophotometer, using neutral filters to attenuate the strong white measuring beam impinging on the sample by factors of 2, 4, and 8. Saturation of the light-induced bleaching of the long wave band was achieved with the beam attenuated fourfold (about 50 mW/cm^2). The inactive category included reaction centers that had lost their long wave absorption as a result of dehydration, and also those for which the long wave band was not bleached under saturating illumination. In air-dried (hydrated) films fewer than 10% of the reaction centers were inactive at temperatures above 200 K; 20% were inactive at 70 K.

I have assumed that for the active reaction centers, the differential extinction coefficient for bacteriochlorophyll photooxidation, measured at the peak of the long wave band, remained the same in films (either hydrated of dehydrated) as in aqueous suspensions: $\Delta\epsilon$ (reduced minus oxidized) = 112 $mM^{-1} \cdot cm^{-1}$ [15]. In computing relative quantum efficiencies, actinic light absorbed by the inactive component was discounted.

The photochemical quantum efficiency of the active component of reaction

TABLE I

RELATIVE PHOTOCHEMICAL QUANTUM EFFICIENCIES OF REACTION CENTERS FROM *RPS. SPHAEROIDES* IN DRIED FILMS

Hydrated refers to air-dried-films; dehydrated refers to the same films after pumping to remove water. The active fraction was defined by the maximum attainable light-inducing bleaching at the long-wave absorption maximum (840—850 nm); see the text. Quantum efficiency has been normalized to 1.00 for the hydrated film at 300 K. The lower part of the Table shows the results of four consecutive cycles of dehydration followed by exposure to either ^2H_2O or H_2O vapor. Precision of measuremnt ±15%.

Temperature (K)	% active		Quantum efficiency (active fraction)	
	Hydrated	Dehydrated	Hydrated	Dehydrated
300	95	57	1.00	0.46
250	97	50		0.86
200	90	48		0.86
150	88	53		0.95
70	80	53	1.06	0.94

300 K; air-dried film;

Treatment	Quantum efficiency
Before dehydration	1.00
Dehydrated, then exposed to ^2H_2O vapor	1.00
Dehydrated again, then exposed to H_2O vapor	0.96
Dehydrated again, then exposed to ^2H_2O vapor	0.94
Dehydrated again, then exposed to H_2O vapor	0.92

centers in hydrated films was normalized to unity at 300 K; it remained close to 1.0 at 70 K. In dehydrated films the quantum efficiency of the active component was approximately 0.5 at 300 K, rising to values near 1.0 as the temperature was brought below 250 K. These results are summarized in Table I. The precision of these measurements was estimated to be ±15%. While the quantum efficiency was set arbitrarily at 1.00 for the hydrated film at 300 K, the absolute quantum efficiency for this case was probably greater than 0.75, as discussed in Materials and Methods.

Table I (lower part) also shows that H_2O and ^2H_2O were equally effective in restoring the photochemical efficiency of reaction centers in films after dehydration.

Fluorescence

Aqueous suspensions and air-dried films of reaction centers have fluorescence spectra showing a band centered near 900 nm, corresponding to the long-wave absorption band of bacteriochlorophyll. The quantum yield of this fluorescence is roughly the same in aqueous suspensions and in films, but a careful comparison is difficult because of non-uniformity of the films. Contaminating fluorescence at shorter wavelengths is far less in films than in aqueous suspensions [7].

In the fluorescence measurements to be reported here the monochromatic light (600 nm) was weak enough that a negligible proportion of the reaction centers had been driven photochemically to a 'closed' state; this was verified by

TABLE II

FLUORESCENCE YIELD AND WAVELENGTH OF THE FLUORESCENCE MAXIMUM IN A FILM OF REACTION CENTERS FROM *RPS. SPHAEROIDES*

The yield is expressed in arbitrary units proportional to the integrated band area when plotted on a frequency scale. Successive treatments applied to a single film are listed under "treatment". The temperature was 300 K until cooling was intitiated.

Treatment	Relative fluorescence yield	Wavelength at fluorescence maximum
Air-dried	300	905
Dehydrated by pumping 30 min	780	895
Exposed to H_2O vapor	270	910
Dehydrated by pumping overnight	960	885
Exposed to water vapor	240	900
Dehydrated by pumping 2 h	710	885
Cooled to 200 K	810	895
Cooled to 70 K	810	905
Returned to 300 K	750	885

showing that the fluorescence yield was invariant with exciting light intensities in the range employed.

Effects of dehydration and of lowering the temperature, on the 900-nm fluorescence of reaction centers in films, are shown in Table II. Dehydration increased the yield of this fluorescence approx. 3-fold. Lowering the temperature of the dehydrated film did not change the yield significantly within the precision of measurement. The wavelength of the fluorescence maximum shifted in ways consistent with the shifts of the long wave absorption band under dehydration and cooling. There was no evidence that dehydration introduced other fluorescence bands between 800 and 900 nm.

I do not know how the increased fluorescence induced by dehydration should be partitioned between reaction centers that had been rendered inactive and those that retained photochemical activity.

Back reaction kinetics

Following illumination of reaction centers, the decay of the light-induced absorbance change near 860 nm reflects a back reaction in which electrons return from reduced ubiquinone to oxidized bacteriochlorophyll. The fastest component of decay corresponds to return of electrons from the 'primary' ubiquinone which has accepted an electron from bacteriochlorophyll via bacteriopheophytin in the photochemical reaction. Slower decay components are associated with the participation of other ubiquinone molecules acting as secondary electron acceptors [16]. The involvement of secondary ubiquinone is prevented by orthophenanthroline [17] and by lowering the temperature to 200 K or less [16]. In earlier studies the most rapid decay component has shown first order kinetics with half-time in the range 50—100 ms at room temperature and about 20 ms at temperatures below 200 K [16,18].

For the reaction centers used in this study, the most rapid first order component comprised the following proportions of the total decay under various conditions: Aqueous suspension with orthophenanthroline (see the legend of Fig.

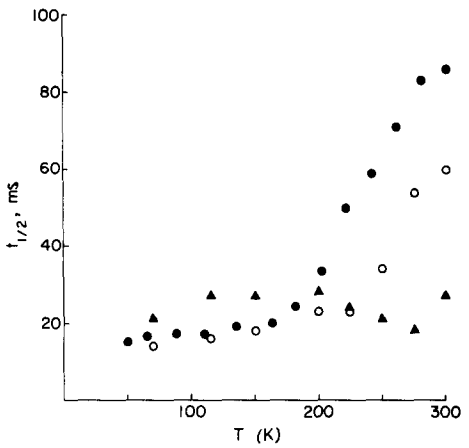

Fig. 5. Half-time of the most rapid first order component of the back reaction after illumination of reaction centers from *Rps. sphaeroides*. This component, monitored by the absorbance change at 862 nm (see Fig. 1), is taken to reflect the return of electrons from 'primary' ubiquinone to bacteriochlorophyll in the reaction centers. Filled circles, aqueous suspension of reaction centers: Glycerol/H_2O, 80 : 20 (v/v); reaction centers, 16 μM; Tris-HCl, 0.1 M, pH 7.5; lauryl dimethyl amine oxide, 1% (v/v); orthophenanthroline, 1 mM. Open circles, air-dried reaction center film. Triangles, the same film after dehydration.

5 for composition of the mixture), more than 85% at 300 K and more than 95% below 200 K. Air-dried film, about 50% at 300 K, rising to 70% at 225 K and more than 80% below 200 K; dehydrated film, 60—80% between 300 and 70 K, showing no systematic variation with temperature. I estimate that the accuracy of these quantifications of the fastest first order component was better than ±10%. The slower decay components observed with films at low temperature might correspond to back reactions from the 'primary' ubiquinone, but in reaction centers that have been altered physically as a result of drying. If this back reaction involves electron tunneling, its rate should be sensitive to minute changes in the configuration of the reaction center.

For the three cases just described, the half-time of the most rapid first-order component is shown as a function of temperature in Fig. 5. With the aqueous suspension the half-time declined rapidly from 86 ms at 300 K to 33 ms at 200 K, and then more slowly, reaching 15 ms at 50 K. The air-dried film showed substantially the same behavior: a rapid decline of the half-time from 60 ms at 300 K to 23 ms at 225 K, and then a slower decline to 14 ms at 70 K. For the hydrated film the half-time was in the range 18—28 ms at all temperatures from 300 to 70 K.

The dramatic change of reaction kinetics between 300 and 200 K in hydrated reaction centers can be attributed to one or more phase transitions involving water. This interpretation is supported by the absence of such a change in dehydrated films of reaction centers.

General

Some of these and other data have been discussed in the context of electron tunneling [19]. Concrete conclusions in this regard are premature, pending refinement of experimental criteria for various theoretical formulations of tunneling.

For all of the phenomena reported here, the properties of an air-dried film were restored, after dehydration, by exposure to either H_2O or ^2H_2O vapor. No significant difference was observed in the behavior of H_2O-restored and ^2H_2O-restored films.

Dehydration changes reaction centers in the direction of diminished photochemical efficiency and decreased stability of the photoproducts (faster back reaction). It may be that the removal of superficially bound water changes the locations and/or orientations of the components of the reaction centers and thus induces sub-optimal behavior. This possibility is being tested with measurements of linear dichroism of the chromophores, in order to detect changes in their respective orientations.

Acknowedgement

This work was supported by Contract No. EY-76-S-02-3162 with the U.S. Department of Energy and by Grant No. PCM76-10556 from the National Science Foundation.

References

1 Arnold, W. and Clayton, R.K. (1960) Proc. Natl. Acad. Sci. U.S. 46, 769—776
2 Clayton, R.K. (1966) Brookhaven Symp. Biol. 19, 62—70
3 Clayton, R.K. (1962) Photochem. Photobiol. 1, 313—323
4 Clayton, R.K. (1966) Photochem. Photobiol. 5, 679—688
5 Clayton, R.K. (1960) Biochim. Biophys. Acta 37, 503—512
6 Clayton, R.K. and Wang, R.T. (1971) Methods Enzymol. 23, 696—704
7 Clayton, R.K. (1977) in Photosynthetic Organelles (Miyachi, S., Katoh, S., Fujita, Y. and Shibata, K., eds.), Special Issue No. 3 of Plant Cell Physiol. (Jap.), pp. 87—96, Center for Academic Publications, Japan
8 Clayton, R.K. (1971) Light and Living Matter Vol. 2, Appendix C, McGraw-Hill, New York
9 Clayton, R.K. and Yamamoto, T.Y. (1976) Photochem. Photobiol. 24, 67—70
10 Wraight, C.A. and Clayton, R.K. (1974) Biochim. Biophys. Acta 33, 246—260
11 Feher, G. (1971) Photochem. Photobiol. 14, 373—387
12 Vermeglio, A. and Clayton, R.K. (1976) Biochim. Biophys. Acta 449, 500—515
13 Norris, J.R. and Katz, J.J. (1978) in The Photosynthetic Bacteria (Clayton, R.K. and Sistrom, W.R., eds.), Chapter 21, Plenum Publishing Corp., New York, in the press
14 Clayton, R.K. (1962) Photochem. Photobiol. 1, 201—210
15 Straley, S.C., Parson, W.W., Mauzerall, D.C. and Clayton, R.K. (1973) Biochim. Biophys. Acta 305, 597—609
16 Clayton, R.K. and Yau, H.F. (1972) Biophys. J. 12, 867—881
17 Parson, W.W. and Case, G.D. (1970) Biochim. Biophys. Acta 205, 232—245
18 McElroy, J.D., Mauzerall, D.C. and Feher, G. (1974) Biochim. Biophys. Acta 333, 261—278
19 Clayton, R.K. (1978) Some Data of possible Relevance to Tunneling in Photosynthetic Reaction Centers, Proceedings of the 1st International Congress on Tunneling in Chemical and Biological Systems. Philadelphia, Pa., in the press