Volume 261, number 1, 191-195 FEBS 08127 February 1990

Investigation of models for photosynthetic electron acceptors

Infrared spectroelectrochemistry of ubiquinone and its anions

M. Bauscher, E. Nabedryk+, K. Bagley*,[1], J. Breton+ and W. Mäntele

Institut für Biophysik und Strahlenbiologie, Albertstr. 23, 7800 Freiburg FRG, +*Service de Biophysique, Département de Biologie, CEN Saclay, 91191 Gif/Yvette Cédex, France* and **Department of Physics, University of California, San Diego, La Jolla, CA 92093, USA*

Received 10 November 1989; revised version received 22 December 1989

In order to gain more information about the binding and interaction of quinones acting as electron acceptors in photosynthetic reaction centers, infrared spectra of 'model' quinones and their reduced species (semiquinones, quinone dianions and quinols) were recorded in a spectroelectrochemical cell. The frequencies and the extinction coefficients of the quinone C = O and C = C stretching vibrations which are shifted upon reduction were obtained in different solvents. These spectra are used to estimate the contribution of quinone reduction to light-induced infrared difference spectra between the charge-separated and the relaxed state of photosynthetic reaction centers.

Photosynthesis; Electron transfer; Reaction center; Infrared spectroscopy; Electrochemistry; Quinone

1. INTRODUCTION

Recent progress in membrane protein crystallization has allowed the detailed structure of the bacterial photosynthetic reaction center (RC) to be visualized at atomic resolution [1,2]. The arrangement and interactions of the cofactors within the RC suggest structure-function relationships for the processes of the primary act of light-induced charge separation. The protein environment of the primary electron donor P (a bacteriochlorophyll dimer), of the intermediary electron acceptor I (a bacteriopheophytin molecule), and of the quinone electron acceptors Q_A and Q_B has been described [1,2]. Nevertheless, it should be kept in mind that the X-ray structure provides a static picture of the quiescent state of the RC, and that additional information from spectroscopic techniques is needed to understand its function.

In order to obtain information on the molecular processes occurring upon primary charge separation in RC, infrared spectroscopy has been used in previous work to study the charge-separated (P^+Q^-) [3] and the reduced intermediary acceptor (I^-) states [4]. Using Fourier-transform infrared difference spectroscopy, a sensitivity has been obtained which allows individual bonds to be detected against the large protein background. Highly structured and detailed difference spectra were obtained which necessitate infrared spectroscopic model studies of isolated pigments and cofactors. Using a combination of electrochemical and infrared spectroscopic techniques [5], isolated pigments and electrogenerated cation or anion radicals have served as models for P, P^+, I and I^- [6], in different solvents simulating the environment and the interaction within the protein. The comparison of the light-induced infrared difference (P^+Q^-/PQ and I^-/I) spectra with the electrochemically-induced model difference spectra has allowed pigment C=O groups to be assigned.

The quinone electron acceptors Q_A and Q_B can exist in several redox (Q_A, $Q_A^{\cdot -}$, Q_B, $Q_B^{\cdot -}$, Q_B^{2-}) and protonation states ($Q_BH^{\cdot}$, Q_BH^- or Q_BH_2) with different binding properties, some of which are energetically favoured by specific protein environments. Identification of infrared bands of such species in the P^+Q^-/PQ spectra should provide information on these binding properties and allow the interactions to be followed through the processes of primary charge separation in RC. Using an improved infrared spectroelectrochemical cell, we report here on the investigation of model quinones.

Abbreviations: Q_A, primary quinone; Q_B, secondary quinone; $Q^{\cdot -}$, semiquinone anion; Q^{2-}, quinol dianion; P^+Q^-/PQ, difference between the charge-separated (the electron remains at Q_A or Q_B) and the relaxed state; CPE, constant potential electrolysis; CV, cyclic voltammogram; E_m, midpoint potential; RC, reaction center; Q, 2,3-dimethoxy-5-methyl-1,4-benzoquinone; DMF, dimethylformamide; THF, tetrahydrofuran

Correspondence address: M. Bauscher, Institüt für Biophysik und Strahlenbiologie, Alberstr. 23, 7800 Freiburg, FRG

[1] *Present address:* Los Alamos National Laboratory, Isotope and Structural Chemistry Group, Los Alamos, NM 87545, USA

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

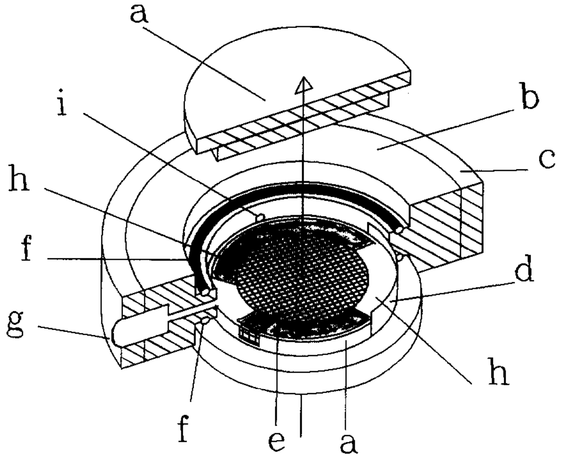

Fig.1. Spectroelectrochemical cell: a, CaF_2-windows; b, teflon body; c, steel ring; d, Pt-counter electrode; e, Au-working electrode; f, O-ring; g, filling port; h, teflon spacer (50 μm); i, salt bridge to the reference electrode.

2. EXPERIMENTAL

A spectroelectrochemical cell was constructed (fig.1) for the in situ electrochemical generation and simultaneous spectroscopic detection. It consists of CaF_2-windows (high spectral transmission between 200 nm and 10 000 nm) which were fixed in a teflon body and sealed with *O*-rings. A small optical path-length, achieved with 50 μm teflon spacers, resulted in a fast equilibration of the volume in the measuring beam with the electrode surface (thin-layer conditions). The two filling ports as well as the two holes to connect the electrodes were sealed with septa to ensure strictly anaerobic conditions. External reference electrodes were used and contacted through a small hole, filled with the respective electrolyte solution (salt bridge). The reference electrode for methanol (CH_3OH) was an AgCl coated Ag-wire in the appropriate solution which was saturated with LiCl (Ag/AgCl satd LiCl). For acetonitrile (CH_3CN), an Ag-wire in the appropriate solution which contained 10 mM $AgNO_3$ (Ag/Ag^+) was used as a reference electrode producing a stable and well-defined potential.

CH_3OH, deuterated methanol ($C^2H_3O^2H$), CH_3CN, deuterated acetonitrile (C^2H_3CN), together with the dissolved supporting electrolyte, were dried by passing through a column of activated Al_2O_3 in an inert atmosphere. Tetrahydrofuran (THF) and CCl_4 were dried over activated Al_2O_3. 2,3-Dimethoxy-5-methyl-1,4-benzoquinone (Q) obtained from Sigma was used without further purification. Tetrabutylammonium hexafluorophosphate, used as supporting electrolyte, was purified by recrystallization from CH_3OH.

Ultraviolet/visible spectra were obtained with a Perkin Elmer Lambda 17 spectrophotometer. Constant potential electrolysis (CPE) and cyclic voltammetry were performed at 298 K, with a computer-controlled potentiostat built in our laboratory. Fourier transform infrared spectra were obtained on Nicolet 60 SX- or Bruker IFS 25 spectrophotometers. 64 interferograms were co-added at a resolution of 4 cm^{-1}.

For CPE the applied potentials were 150 mV more positive (negative) for oxidation (reduction) than the respective midpoint potentials (E_m). The equilibrium Q (oxidized) ↔ Q (reduced) was thus changed from 99.7% oxidized to 99.7% reduced (according to the Nernst-equation).

3. RESULTS AND DISCUSSION

3.1. *Ultraviolet/visible spectra*

Ultraviolet/visible spectra of Q in CH_3CN (fig.2a) demonstrated, by the shift of the $\pi\rightarrow\pi^*$ transition of Q (262 nm), the formation of $Q^{\cdot -}$ (peaks at 321 nm, 413 nm and 439 nm) at −1.1 V and the appearance of Q^{2-} (peak at 327 nm) at −1.6 V [7] (for band assignment see table 1). Stable isosbestic points showed that no irreversible side reaction had occurred.

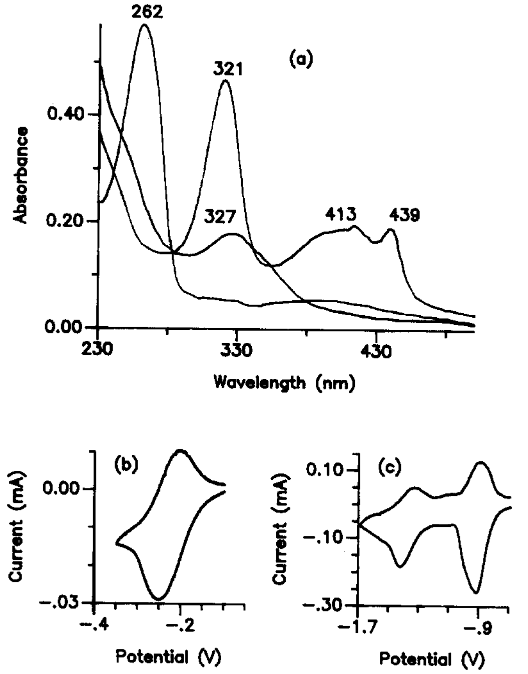

Fig.2. (a) Ultraviolet/visible spectra of Q in CH_3CN during a CPE at −1.1 V ($Q^{\cdot -}$) and at −1.6 V (Q^{2-}). CV of Q in CH_3OH (b) and in CH_3CN (c). See text for details.

3.2. *Electrochemistry*

The cyclic voltammogram (CV) of Q in CH_3OH (fig.2b) showed one ($n=2$) wave resulting from the Q/QH_2 redox couple and in CH_3CN (fig.2c) two ($n=1$) waves resulting from $Q/Q^{\cdot -}$ and the $Q^{\cdot -}/Q^{2-}$ redox couples. The situation in CH_3OH can be explained by assuming the following reaction scheme: $Q\rightarrow Q^{\cdot -}\rightarrow QH^{\cdot}\rightarrow QH^{-}\rightarrow QH_2$. A protonation of 0.9% of $Q^{\cdot -}$ is reasonable because its p*K* value is about 6.45 [8] and the CH_3OH used exhibits a pH-value of 8.5. Finally $QH^{\cdot}$ becomes reduced and protonated its E_m is more positive than that of $Q^{\cdot -}$ [9]. To compare E_m data obtained with different reference electrode systems, it is useful to refer the E_m values to the solvent-independent

Table 1

Band assignment of the ultraviolet/visible peaks of Q

Wavelength (nm)	Transition	Species (assignment according to [7])
262	$\pi\rightarrow\pi^*$	Q
380	$n\rightarrow\pi^*$	Q
321	$\pi\rightarrow\pi^*$	$Q^{\cdot -}$
413	$n\rightarrow\pi^*$	$Q^{\cdot -}$
439	$n\rightarrow\pi^*$	$Q^{\cdot -}$
327	$\pi\rightarrow\pi^*$	Q^{2-}

Table 2

E_m values against the appropriate reference electrodes, obtained in different solvents: $E_{r1} = Ag/Ag^+$ in CH_3CN; $E_{r2} = Ag/AgCl$ satd LiCl in CH_3OH, E_{sce} = satd calomel electrode, values from [11], $E_{fc} = E_m$ against the ferrocene/ferricinium redox couple

	DMF (V)	CH_3CN (V)	CH_3OH (V)
First wave	−0.539 (E_{sce})	−0.911 (E_{r1})	−0.224 (E_{r2})
Second wave	−1.25 (E_{sce})	−1.344 (E_{r1})	
E_{fc} (first wave)	−1.065	−0.993	−0.682
E_{fc} (second wave)	−1.776	−1.87	

potential of the ferrocene/ferricinium redox couple (E_{fc}, see table 2) [10]. Prince et al. [11] reported E_m values for $Q/Q^{\cdot -}$ and $Q^{\cdot -}/Q^{2-}$ in the aprotic solvent dimethylformamide (DMF) (−1.065 V and −1.776 V, respectively) which are in good agreement with those presented here for Q in CH_3CN (−0.993 V and −1.87 V). The value for Q/QH_2 in CH_3OH (−0.682 V) is more positive, attributable to the protic environment.

3.3. *Infrared spectroscopy of model compounds*

The infrared spectra of Q in a variety of solvents bear a close similarity. The positions of the C=O, and those of the C=C bands, respectively, in $C^2H_3O^2H$ (fig.3), CCl_4, THF and C^2H_3CN (data not shown) agree within 3 cm^{-1}. In case of C^2H_3CN and THF a shoulder, which is better resolved in CCl_4, appears at the high frequency side of the C=O band. In $C^2H_3O^2H$ only very small shoulders are detectable on both sides as seen by forming the second derivation. The molecular environment of Q in these solvents thus seems to be similar, although $C^2H_3O^2H$ is able to form hydrogen (^2H) bonds to carbonyl groups, whereas C^2H_3CN cannot.

NMR studies of quinhydrone complexes [12] have indicated that hydrogen bonding from quinone to quinol is weak and plays only a minor role in complex stabilisation. Slifkin et al. [13] found an infrared shift of the C=O band of quinones upon quinhydrone complex formation. These experiments, however, were performed on KBr discs and may be of less relevance to Q in solution. The strength of a hydrogen bond depends strongly on the degree of delocalization of π-electron density of the carbonyl group. For 1,4-benzoquinone a π-bond order of 0.795 for the C=O bond and of 0.889 for the C=C band has been reported [14]. The colour of Q (orange) indicates a larger delocalization than that present in 1,4-benzoquinone (yellow), thus we expect weaker hydrogen bonds to be present in Q.

Pronounced differences, however, appear in the case of anion formation. The anion-minus-neutral difference spectra are shown in fig.4, with bands of the neutral species appearing negative and bands of the anions positive. When $Q^{\cdot -}$ is formed, the C=O bands (1660 cm^{-1} in C^2H_3CN and 1659 cm^{-1} in $C^2H_3O^2H$) and the C=C bands of Q (1608 cm^{-1} in C^2H_3CN and 1606 cm^{-1} in $C^2H_3O^2H$) disappear completely and bands at 1500 cm^{-1} and 1490 cm^{-1} ($C\overset{..}{-}O$ vibrations of $Q^{\cdot -}$ in C^2H_3CN and $C^2H_3O^2H$, resp.) appear. The difference in frequency of the $C\overset{..}{-}O$ band of $Q^{\cdot -}$ in these two solvents can be attributed to hydrogen-bonding in case of $C^2H_3O^2H$ which is now more likely because of an increased electron density at the carbonyl oxygen. These assignments are based on earlier infrared spectra [15,16], on model calculations [17] and ab initio calculations [18] for different quinones. The anion-minus-neutral infrared difference spectra presented here agree well with previous infrared data from Clark

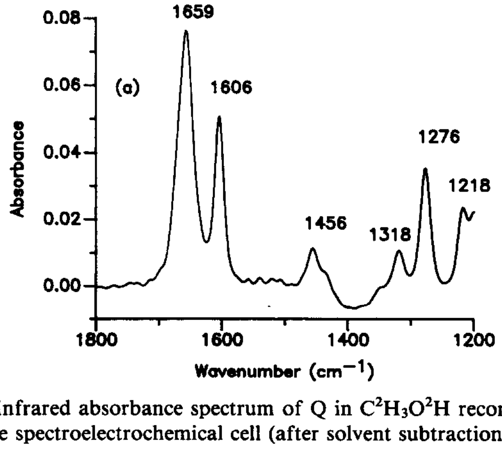

Fig.3. Infrared absorbance spectrum of Q in $C^2H_3O^2H$ recorded in the spectroelectrochemical cell (after solvent subtraction).

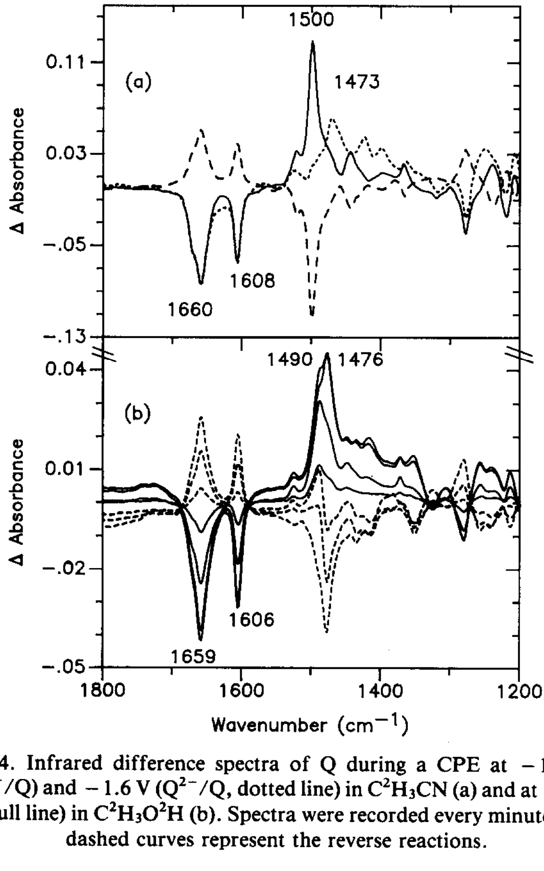

Fig.4. Infrared difference spectra of Q during a CPE at −1.1 V ($Q^{\cdot -}/Q$) and −1.6 V (Q^{2-}/Q, dotted line) in C^2H_3CN (a) and at −0.4 V (full line) in $C^2H_3O^2H$ (b). Spectra were recorded every minute; the dashed curves represent the reverse reactions.

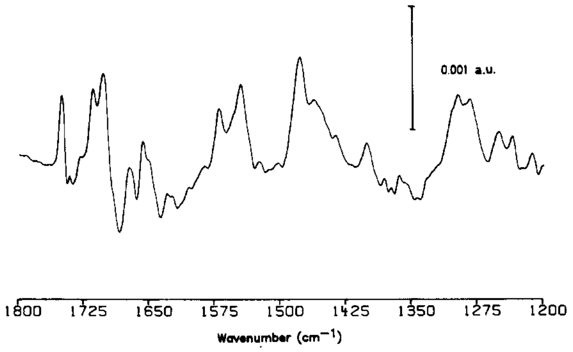

Fig.5. Light-induced $P^+Q_B^-/PQ_B$ infrared difference spectrum of *Rhodobacter sphaeroides* reaction centers, taken from [21].

et al. [16] obtained on 1,4-benzoquinone in dimethylsulfoxide using flow-cell electrolysis [16]. The spectrum of Q^{2-}/Q in C^2H_3CN shows 3 small peaks between 1473 cm^{-1} and 1400 cm^{-1}. The situation in $C^2H_3O^2H$ is different in that $Q^{\cdot -}$ and Q^2H_2 are formed subsequently at the same potential. This is consistent with CV data (fig.2b,c) and ultraviolet/visible spectra (data not shown). At first a band at 1490 cm^{-1} appeared and then a new band at 1476 cm^{-1} (Q^2H_2) increased while the other one disappeared.

3.4. *Infrared spectroscopy of reaction centers*

The majority of the bands from 1750 cm^{-1} to 1665 cm^{-1} of the P^+Q^-/PQ infrared difference spectrum from *Rhodobacter sphaeroids* RC (fig.5) have been assigned to pigment C=O groups using bacteriochlorophyll model spectra [6]. For BChl in vitro, the infrared extinction coefficient (ϵ) of the 9-keto C=O group (1100 ± 100 M$^{-1}\cdot$cm^{-1}; M. Bauscher and M. Leonhard, unpublished data) which shifts from 1683 cm^{-1} to 1715 cm^{-1} upon cation formation, is about twice that of the quinone C=O (500 ± 50 M$^{-1}\cdot$cm^{-1}) determined in this study. The infrared spectra presented in fig.3 and fig.4 imply that negative bands arising from quinone reduction should be found around 1660 cm^{-1} and 1610 cm^{-1} (C=O and C=C stretching mode of Q, respectively) in the P^+Q^-/PQ spectrum. In the spectral region between 1500 cm^{-1} and 1450 cm^{-1}, positive bands arising from the $C\overset{\cdot\cdot}{-}O$ and $C\overset{\cdot\cdot}{-}C$ vibrations of $Q^{\cdot -}$, Q^{2-} or QH_2 should be located (for band assignment see table 3). In this respect, the positive 1478 cm^{-1} band in the P^+Q^- spectra has been tentatively assigned to a semiquinone band [6,19,20]. However, light-induced infrared difference spectra ($P^+Q_A^-/PQ_A$ and $P^+Q_B^-/PQ_B$) or RC reconstituted with ^{13}C or ^{18}O isotope-labelled quinones [21], if compared to the P^+Q^-/PQ spectra of native RC, show only very small but highly reproducible features in the 1600 cm^{-1} to 1650 cm^{-1} and the 1400 cm^{-1} to 1450 cm^{-1} spectral region. This observation shows that the quinone C=O mode cannot be unambiguously identified in the $P^+Q_A^-/PQ_A$ and $P^+Q_B^-/PQ_B$ spectra and only part of the 1478 cm^{-1} band can be assigned to quinone radical modes. A small band at 1604 cm^{-1}, close coinciding with the C=C mode in Q and shifting upon ^{13}C-substitution, was assigned to the C=C absorption of Q_A [21]. In addition, the comparison of $P^+Q_A^-/PQ_A$ and $P^+Q_B^-/PQ_B$ infrared difference spectra for native RC shows no band which can be assigned to Q_A or Q_B C=O vibration in either *Rhodobacter sphaeroides* [21] or *Rhodopseudomonas viridis* [20] RC. The surprising absence of a strong quinone C=O difference band in the P^+Q^-/PQ spectra can be explained in several ways.

(a) The C=O band cannot be detected in the P^+Q^-/PQ spectrum due to extreme broadening. This is the case if the C=O bonds of the quinones exhibit a range of hydrogen-bonding interactions in vivo. Indeed, a low steric specifity of the quinone binding sites [22,23] was reported, possibly allowing several positions for Q_A (see also [2] or Q_B in their respective site.

(b) The unique heterogenous protein environment of the RC, which differs considerably from the homogenous environment in solution, perturbs the normal modes of the quinone in a way that substantially reduces the infrared intensity of the C=O group of the quinone. For example, depending on the directions of the interacting dipole moments for the two C=O groups, a slight bend of the C=O groups out of the plane of the quinone ring might result in significant reduction of the infrared inten-

Table 3

Band assignment of the infrared peaks of Q and its reduction products in $C^2H_3O^2H$ and C^2H_3CN

Wavenumber (cm^{-1})	Solvent	Vibration	Species [ref.]	ϵ (M^{-1} cm^{-1})	
1660	C^2H_3CN	C=O stretch	Q	[15,17]	450
1659	$C^2H_3O^2H$	C=O stretch	Q	[15,17]	550
1608	C^2H_3CN	C=C stretch	Q	[15]	379
1606	$C^2H_3O^2H$	C=C stretch	Q	[15]	430
1500	C^2H_3CN	$C\overset{\cdot\cdot}{-}O$ stretch	$Q^{\cdot -}$	[16,18]	600
1490	$C^2H_3O^2H$	$C\overset{\cdot\cdot}{-}O$ stretch	$Q^{\cdot -}$	[16,18]	350
1473	C^2H_3CN	$C\overset{\cdot\cdot}{-}C$ stretch	Q^{2-}	*	250
1476	$C^2H_3O^2H$	$C\overset{\cdot\cdot}{-}C$ stretch	QH_2	*	350

*Tentative assignment taken from cresolate (F. Siebert, private communication) and cresol spectra [26] sity for the normal mode associated with the C=O stretches.

In order to locate the quinone C=O infrared bands in vivo and to discriminate between these possibilities, spectroelectrochemical studies of isotopically-labelled quinone model compounds will be performed. In addition, time resolved infrared studies in the millisecond [24] and microsecond range [25] are necessary in order to determine the contributions from Q_A and Q_B in the light-induced infrared difference spectra.

Acknowledgements: We would like to thank Profs W. Kreutz, G. Feher and M. Okamura for active support and Prof. J. Heinze for helpful discussions. The help of Dr D.A. Moss and S. Grzybek, who wrote an excellent computer program for spectroelectrochemical measurements and of K.O. Lorenz, who provided us with purified tetrabutylammonium-hexafluorophosphate, is gratefully acknowledged. Part of this work was funded by the Deutsche Forschungsgemeinschaft (Ma 1054/2-1) and by the European Community (contract no. ST2J-0118-2-D). K.B. gratefully acknowledges NSF for a postdoctoral fellowship in plant biology.

REFERENCES

[1] Michel, H., Epp, O. and Deisenhofer, J. (1986) EMBO J. 5, 2445–2451.

[2] Allen, J.P., Feher, G., Yeates, T.O., Komiya, H. and Rees, D.C. (1988) Proc. Natl. Acad. Sci. USA 85, 8487–8491.

[3] Mäntele, W., Nabedryk, E., Tavitian, B.A., Kreutz, W. and Breton, J. (1985) FEBS Lett. 187, 227–232.

[4] Nabedryk, E., Mäntele, W., Tavitian, B.A. and Breton, J. (1986) Photochem. Photobiol. 43, 461–465.

[5] Mäntele, W., Wollenweber, A., Rashwan, F., Heinze, J., Nabedryk, E., Berger G. and Breton, J. (1988) Photochem. Photobiol. 47, 451–455.

[6] Mäntele, W., Wollenweber, A., Nabedryk, E. and Breton, J. (1988) Proc. Natl. Acad. Sci. USA 85, 8468–8472.

[7] Morrison, L.E., Schelhorn, J.E., Cotton, T.M., Bering, C.L. and Loach, P.A. (1982) in: Function of Quinones in Energy Conserving Systems (Trumpower, B.L. ed.) pp. 35–58, Academic Press, New York.

[8] Swallow, A.J. (1982) in: Function of Quinones in Energy Conserving Systems (Trumpower, B.L. ed.) pp. 59–72, Academic Press, New York.

[9] Rich, P.R. (1984) Biochim. Biophys. Acta 768, 53–79.

[10] Koepp, H.M., Wendt, H. and Strehlow, H. (1960) Z. Elektrochem. 64, 483–491.

[11] Prince, R.C., Dutton, P.L. and Bruce, J.M. (1983) FEBS Lett. 160, 273–276.

[12] Perkampus, H.H. and Krüger, U. (1966) Z. Phys. Chem. 48, 379–382.

[13] Slifkin, M.A. and Walmsley, R.H. (1970) Spectrochim. Acta 26A, 1237–1242.

[14] Coulson, C.A. (1946) Trans. Faraday Soc. 42, 106–112.

[15] Flaig, W. and Salfeld, J.C. (1959) Ann. Chem. 626, 215–225.

[16] Clark, B.R. and Evans, D.H. (1976) J. Electroanal. Chem. 69, 181–194.

[17] Meyerson, M.L. (1985) Spectrochim. Acta 41, 1263–1267.

[18] Chipman, D.M. and Prebenda, M.F. (1986) J. Phys. Chem. 90, 5557–5561.

[19] Gerwert, K., Hess, B., Michel, H. and Buchanan, S. (1988) FEBS Lett. 232, 303–307.

[20] Buchanan, S., Michel, H. and Gerwert, K. (1989) in: Proceedings of the VIIIth International Congress on Photosynthesis (Baltscheffsky, M. ed.) Kluwer Academic Publishers, Dordrecht, in press.

[21] Bagley, K., Abresch, E., Okamura, M.Y., Feher, G., Bauscher, M., Mäntele, W., Nabedryk, E. and Breton, J. (1989) in: Proceedings of the VIIIth International Congress on Photosynthesis (Baltscheffsky, M. ed.) Kluwer Academic Publishers, Dordrecht, in press.

[22] Giangiacomo, K.M. and Dutton, P.L. (1989) Proc. Natl. Acad. Sci. USA 86, 2658–2662.

[23] Gunner, M.R., Braun, B.S., Bruce, J.M. and Dutton, P.L. (1985) in: Antennas and Reaction Centers of Photosynthetic Bacteria (Michel-Beyerle, M.E. ed.) pp. 298–304, Springer Verlag, Berlin.

[24] Thibodeau, D.L., Breton, J., Nabedryk, E. and Mäntele, W. (1989) in: Proceedings of the 4th International Conference on Time-Resolved Vibrational Spectroscopy (Spiro, T.G. and Czernuszewics, R.S. eds) p. 17, Princeton.

[25] Hienerwadel, R., Kreutz, W. and Mäntele, W. (1989) in: Spectroscopy of Biological Molecules – State of the Art (Bertoluzza, A., Fagnano, C. and Monti, P. eds) pp. 315–316, Societa Editrice Esculapio, Bologna.

[26] Jakobsen, R.J. (1965) Spectrochim. Acta 21, 433–442.