

Electric field effect on absorption spectra of reaction centers of *Rb. sphaeroides* and *Rps. viridis*

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Comparison of the Stark effect on the Q_y transitions of the pigments in reaction centers of *Rb. sphaeroides* and *Rps. viridis* at 77 K shows great similarity in the long-wavelength absorption of the bacteriochlorophyll dimer but differs significantly in the absorption region of the accessory bacteriochlorophylls and bacterio-pheophytins.

Stark effect; Absorption spectrum; Reaction center; (*Rb. sphaeroides*, *Rps. viridis*)

1. INTRODUCTION

Of considerable interest for the understanding of the primary charge separation in reaction centers (RCs) of purple bacteria are electronic excitations which do not have their parentage in the excitation of a single pigment constituent of the electron-transfer chain. Such excitations involve charge-transfer states with an electron and a hole residing on different molecules. Optical spectroscopy in external electric fields (Stark spectroscopy) provides an optimal tool for the identification of such charge-transfer states which, due to their small oscillatory strength, escape from direct observation in absorption or emission. A change of the absorption spectrum of the individual pigments upon application of an electric field is expected if either (i) the electric field favours mixing of electronic states, (ii) the polarizabilities and/or (iii) the permanent dipole moments $|\mu|$ of the ground and excited states are different. If one of these possible origins of the Stark effect predominates, characteristic elec-

trochromic changes are observed which may either follow the absorption spectrum, its first or second derivatives corresponding to the cases (i)–(iii), respectively.

The measurement of the Stark effect on the absorption spectrum of RCs of *Rb. sphaeroides* R-26 [1,2] embedded in a rigid polymer matrix showed a large Stark effect on the Q_y absorption band of the primary donor, the bacteriochlorophyll dimer (BC_P), which considerably exceeded the effect observed on the Q_y transitions of the accessory bacteriochlorophylls (BC) and the bacterio-pheophytins (BP).

The Stark spectrum of the Q_y transitions of BC_P , BC and BP in *Rb. sphaeroides* resembles crudely the second derivative of the absorption band profiles, thus supporting the view that effect (iii) is the predominating one. This implies that the excitation of the dimer is accompanied by a much larger value $|\Delta\mu|$ as compared to the monomer pigments BC and BP indicating that the permanent dipole moment associated with the ground state of the dimer differs significantly from that of the electronically excited state. In the simplest picture this could be due to the heavy mixing of charge-transfer states with the neutral electronic excitation of the dimer. Two types of charge-transfer states

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can be considered in this context:

(A) *Dimer charge-transfer states*,

$$\alpha|(BC_{PL}^+ BC_{PM}^-) > + \beta|(BC_{PL}^- BC_{PM}^+) >$$

where $(BC_{PL} BC_{PM})$ corresponds to the two dimer components. Provided that the local environment of the two dimer components is different, these dimer-internal charge-transfer states are expected to contribute to the Stark effect due to their finite permanent dipole moment.

(B) *Dimer-monomer charge-transfer states*. These states of the type

$$\alpha|(BC_{PL}^+ BC_{PM}) BC^- > + \beta|(BC_{PL} BC_{PM}^+) BC^- >$$

are characterized by a large permanent dipole moment. The transition moment from the electronic ground state to these charge-transfer states is very small, so that their mixing with the lowest singlet neutral dimer excitation does not contribute to the integrated intensity of the dimer band but rather appreciably to the Stark effect.

On the basis of the Stark spectrum [1] the change in dipole moment associated with the Q_y transition of the dimer in RCs of *Rb. sphaeroides* has been calculated resulting in $|\Delta\mu| \approx 7$ Debye [3] and the discussion of both types of charge-transfer admixtures (A) and (B) to 1BC‡ . This value is also confirmed in more recent polarized Stark spectroscopy $|\mu| = (8.6 \pm 0.6)$ Debye [2]. It is larger by a factor of approx. 3.4 and approx. 2 than $|\mu|$ for the accessory BCs and BPs, respectively. The measured value of $|\Delta\mu|$ together with the measured angle between $\Delta\mu$ and the transition dipole moment for excitation of the Q_y state of BC_P suggested a model in which the state 1BC‡ has substantial charge-transfer character [2].

This interpretation of the large Stark effect on the dimer band is also supported by recent MO calculations [4,5] which indicate that deviation of the dimer structure from C_2 symmetry induces a considerable charge asymmetry in the excited singlet state of the dimer. According to these calculations on RCs of *Rps. viridis*, the static dipole moment of 1BC‡ is larger than that of the corresponding singlet ground state, and approximately antiparallel to the latter. According to these calculations, the essential charge-transfer contribution to the dimer absorption is expected to originate from the dimer charge-transfer state (A).

Here, we compare the electric field-modulated

absorption spectra for the Q_y region of *Rps. viridis* and *Rb. sphaeroides* RCs. In order to improve the spectral resolution as well as to increase the amplitude of the electrochromic effect through narrowing of the absorption bands the measurements are performed at 77 K.

2. MATERIALS AND METHODS

2.1. Samples

RCs of *Rb. sphaeroides* and *Rps. viridis* were prepared by standard methods in the laboratories of J.B. and H.M., respectively. At a concentration of typically $\approx 10^{-4}$ mol/l these were embedded in polyvinyl alcohol films (PVA; Wacker; M_r 40 000) with a final thickness of ≈ 0.15 mm. Only the optically isotropic central patch of the cast film was used which was tested with respect to its mechanical homogeneity and also to its optical isotropy. When a stress-induced birefringence was observed (occurring predominantly at the rim of the film), an additional electro-optic modulation was found to be superimposed on the quadratic Stark effect. The film was sandwiched between two glass slides coated with SnO_2 (surface resistance $100 \Omega/cm^2$) allowing for $\approx 90\%$ transmission in the visible and near-infrared range. In order to prevent plasma discharge the air gap between sample and electrode (of the order of μm) was filled with silicone grease.

Measurements applying this Stark capacitor method might suffer from dielectric losses that may occur within the PVA film due to the presence of polar contaminants. These may lower the local electric field strength acting on the RC. Thus, for the quantitative treatment of Stark spectra an internal standardization procedure is required, e.g. by incorporating carotinoids.

2.2. Apparatus

The sample was mounted in a bath cryostat and probed with light from a 250 W tungsten lamp which had passed a monochromator (25 cm, resolution 10 nm). The intensity of the probing light was typically $1-2 \mu W/cm^2$. A sinusoidal voltage (0-6 kV_{eff}) with a frequency of 4.7 kHz and low harmonic distortion was applied to the conducting glass slides. The modulation of the transmitted light intensity was detected with a cooled germanium diode. The photocurrent was measured with a lock-in amplifier (SR 530) and the two or-

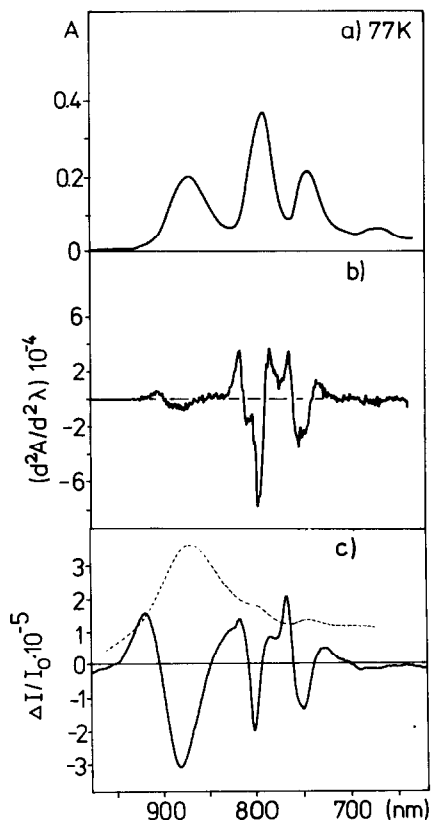


Fig.1. Absorption (a), second derivative (b) and Stark spectra (c) for the Q_y region of *Rb. sphaeroides* RCs in PVA film at 77 K ($F_{\text{ext}} = 60$ kV/cm).

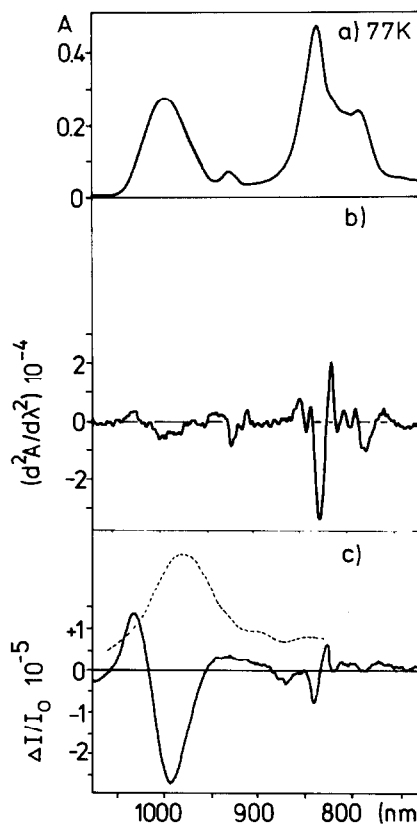


Fig.2. Absorption (a), second derivative (b) and Stark spectra (c) for the Q_y region of *Rb. viridis* RCs in PVA film at 77 K (F_{ext} as in fig.1).

thogonal components of the Stark signal appearing at twice the frequency of the external voltage were recorded together with the unmodulated transmitted light intensity I_0 . The absorption spectra were measured in the Stark capacitor using a blank PVA film as reference.

3. RESULTS AND DISCUSSION

The Stark spectra of the RC of *Rb. sphaeroides* and *Rps. viridis* measured at 77 K are shown together with their absorption spectra and the second derivative thereof in figs 1 and 2, respectively.

The characteristics of low-temperature absorption spectra (e.g. the red shift and narrowing of the dimer band) of both RCs in glassy aqueous solutions [6,7], polyacrylamide gel [8] and dried films

[9] persist in the PVA matrix (the small absorption peak at 950 nm in the absorption spectrum of *Rps. viridis* is a degradation product depending on age and the preparation procedure).

Comparing the previous room-temperature Stark spectra [1,2] of *Rb. sphaeroides* RCs with the Stark spectrum in fig.1, the most prominent feature is conserved at 77 K: the larger amplitude of the electrochromic effect in the dimer band relative to that observed in the absorption region of the monomer pigments, BC and BP. As also observed at room temperature, the Stark spectrum at 77 K (although showing a small red shift of ≈ 5 nm) follows crudely the second derivative of the absorption spectrum. This indicates again a change in the transition dipole moment as the dominant effect of the external field. The overall ratio of the integrated Stark effect on dimer and

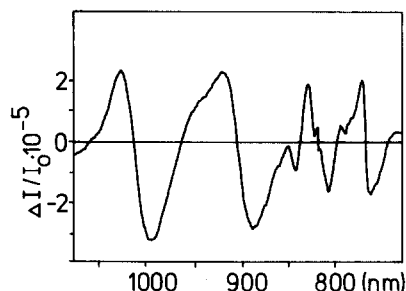


Fig.3. Comparison of Stark spectra of *Rb. viridis* and *Rb. sphaeroides* RCs at 77 K by mounting two PVA films containing either RCs in series within the Stark cell.

monomer absorptions is similar to that at room temperature. We observed that the ratio of the Stark modulation ΔI vs the dimer absorption may change with external conditions of the sample, e.g. age, dehydration, aggregation, partial orientation, etc. Another possible artifact consists of the field-dependent recombination of the oxidized dimer and the reduced ubiquinone (Q^-) under the condition of the PVA film as reported in the literature [10]. Apart from recombination studies on RCs in lipid bilayers [11], the prompt appearance of the Stark modulation at an operating frequency of ≈ 10 kHz, however, excludes the contribution of the slow recombination $P^+Q^- \rightarrow PQ$ on a time scale exceeding 100 ms.

In a qualitative comparison of the Stark spectra of *Rb. sphaeroides* and *Rps. viridis* in figs 1 and 2, the most striking features are:

(1) Stark effect on dimer absorption

The amplitude and characteristics of the Stark effect seem to be similar for the two RCs. In order to exclude differences in the local electric field strength, two equally prepared films containing either RCs of *Rb. sphaeroides* and *Rps. viridis* were mounted in series in the same Stark cell. The electrochromic spectrum thus obtained (fig.3), shows the equivalency of the magnitude of the Stark effect on the dimer band in both RCs. A similarly equivalent Stark effect is measured when both RCs are mixed (1:1) within the same film.

As observed for RCs of *Rb. sphaeroides*, the Stark spectrum of *Rps. viridis* resembles the second derivative of the absorption spectrum, indicating that the change in the charge distribution

following excitation is the same in both RCs. Since the rates of the primary electron transfer $^1BC\dot{\Phi}-BC-BP \rightarrow BC\dot{\Phi}^+-BC-BP^-$ are also the same [12,13] and both rates are activationless, the electronic coupling should be the same. Then the structure of the two RCs should be very similar as indeed has been found [14–16] and a similar magnitude of $\Delta\mu$ in both RCs should be expected.

Surprisingly, in both RCs the electrochromic effect at the long-wavelength absorption of the dimer crosses the zero baseline. This could in principle point to a lower-lying charge-transfer state characterized by small oscillatory strength and therefore not detectable in direct absorption. Although these findings are preliminary, they focus on the inherent strength of Stark spectroscopy being able to resolve extremely weak optical transitions provided that the change in either the permanent dipole moment and/or polarizability is sufficiently large.

(2) Stark effect on the 800 nm and 830 nm Q_y absorption bands of BC and BP [8,17]

The relative Stark effect in this region of the spectrum is significantly smaller in RCs of *Rps. viridis* as compared to *Rb. sphaeroides*. In principle, this can be due to different effects:

(a) In the RC of *Rps. viridis*, the overlap of the BP and BC Q_y absorption bands is much larger than in *Rb. sphaeroides*. Thus, the smaller magnitude of the Stark effect in this region for *Rps. viridis* compared to *Rb. sphaeroides* could be due to some cancellation effects due to the overlap of positive and negative contributions from the Stark spectra of the different transitions in the former. In addition, the upper excitonic band of the dimer transition could in a different way be superimposed on the absorptions of the two RCs.

(b) An enhanced mixing between $^1BC\dot{\Phi}$ and the excitation of BC and BP. $^1BC\dot{\Phi}$ also includes the higher exciton component and the dimer-internal charge-transfer state A ($BC\dot{\Phi}^+-BC\dot{\Phi}^-$). Due to the closer energetic proximity of the dimer and the monomer excitation this mechanism should be favoured in *Rb. sphaeroides* as compared to *Rps. viridis*.

(c) The admixture of dimer-monomer charge-

transfer states B (BCP^+-BC^-) to the excitation of BC.

A distinction between the mechanisms (b) and (c) can be made on the basis of the direction of the field-dependent change of the dipole moment. For interpretation (b) to apply, the directions of $\Delta\mu$ should be the same, and for (c), they should be different in the two RCs. If mechanism (a) is the predominant one, the Stark effect in this region should respond strongly to the removal of one of the accessory BCs [18].

In summary, the comparison of the Stark effect on the Q_y transitions of the pigments in RCs of *Rb. sphaeroides* and *Rps. viridis* at 77 K shows great similarity in the long-wavelength absorption of the bacteriochlorophyll dimer but differs significantly in the absorption region of the accessory bacteriochlorophylls and the -pheophytins.

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REFERENCES

- [1] De Leuw, D., Malley, M., Buttermann, G., Okamura, M.Y. and Feher, G. (1982) *Biophys. J.* 37, 111a (*Biophys. Soc. Abstr.*).
- [2] Lockhart, D.J. and Boxer, S. (1987) *Biochemistry* 26, 664-668.
- [3] Scherer, P.O.J. and Fischer, S.F. (1986) *Chem. Phys. Lett.* 131, 153-159.
- [4] Lubitz, W., Lendzian, F., Plato, M., Tränkle, E. and Möbius, K. (1986) *Proc. Coll. Ampere XXIII*, Rome, p. 486-487.
- [5] Plato, M. et al. (1987) to be published.
- [6] Rafferty, C.N. and Clayton, R.K. (1978) *Biochim. Biophys. Acta* 502, 51-60.
- [7] Maslov, V.G., Klevanik, A.V., Ismailov, M.A. and Shuvalov, V.A. (1983) *Dokl. Akad. Nauk SSSR* 269, 1217-1221.
- [8] Breton, J. (1985) *Biochim. Biophys. Acta* 810
- [9] Rafferty, C.N. and Clayton, R.K. (1979) *Biochim. Biophys. Acta* 545, 106-121.
- [10] Popovich, Z.D., Kovacs, G.J., Vincett, P.S., Alegria, G. and Dutton, P.L. (1986) *Biochim. Biophys. Acta* 851, 38-48.
- [11] Gopher, A., Blatt, Y., Schönfeld, M., Okamura, M.Y., Feher, G. and Montal, M. (1985) *Biophys. J.* 48, 311-320.
- [12] Martin, J.-L., Breton, J., Hoff, A.J., Migus, A. and Antonetti, A. (1986) *Proc. Natl. Acad. Sci. USA* 83, 957-961.
- [13] Breton, J., Martin, J.-L., Migus, A., Antonetti, A. and Orszag, A. (1986) *Proc. Natl. Acad. Sci. USA* 83, 5121-5125.
- [14] Deisenhofer, J., Epp, O., Miki, K., Huber, R. and Michel, H. (1984) *J. Mol. Biol.* 180, 385-398.
- [15] Chang et al. (1985) *J. Mol. Biol.* 186, 201.
- [16] Allen, J.P., Feher, G., Yeates, T.O., Ress, D.C., Deisenhofer, J., Michel, H. and Huber, R. (1986) *Proc. Natl. Acad. Sci. USA* 83, 8586-8593.
- [17] Meech, S.R., Hoff, A.J. and Wiersma, D.A. (1986) *Proc. Natl. Acad. Sci. USA* 83, 9464-9468.
- [18] Ditson, S.L., Davis, R.C. and Learlstein, R.M. (1984) *Biochim. Biophys. Acta* 766, 623-629.