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Reaction Centers of Photosynthetic Bacteria

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With 165 Figures

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Contents

PART II **Native Reaction Centers: Electron Transfer Dynamics**

PART III **Modified Reaction Centers: Effects of Mutagenic and Chemical Modifications**

Analysis of Transient Absorption Data from Reaction Centers of Purple Bacteria

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1. Techniques of Transient Spectroscopy

The primary reaction in photosynthetic bacteria is an ultrafast charge separation and electron transfer process which proceeds on the timescale of 10^{-12}s. In order to understand the molecular mechanisms different spectroscopic techniques with very high time resolution are used. The most common spectroscopic method is the so-called "pump and probe" technique. A strong excitation pulse (wavelength favourable in the lowest absorption band of the special pair P) starts the photosynthetic reaction which induces absorption changes in the sample. These changes are monitored by a second, properly delayed weak probing pulse of variable wavelength. The experiment yields the absorbance change ΔA as a function of delay time t_D and probing wavelength λ_{pr}. This two-dimensional information is currently recorded by one of the two following methods:

i) Measurements of $\Delta A(t_D)$ at certain probing wavelengths λ_{pr}: Here the experimental technique, optimized for a single wavelength, allows very high time and good amplitude resolution /1-5/. This single channel time dependent measurement is well suited if the time dependence of ΔA is unknown and if weak kinetic components are present. Different records obtained at various probing wavelengths may be used to get the complete spectral information. However, the combination of these data to transient spectra requires calibration of the different experiments, which may lead to reduced precision of the spectra. In addition this single channel method requires long measuring times.

ii) Spectral multichannel measurements of $\Delta A(\lambda_{pr})$ at certain delay times t_D: This technique supplies the whole absorption spectrum at a specific delay time t_D /3,6/. The overall measuring time is short and a large number of spectral data points is obtained. However, group velocity

dispersion in generation and propagation of the probing light continuum imposes a large uncertainty for the actual delay time setting and strongly limitates the time resolution. In addition the precision of the amplitude determination of ΔA of an multichannel system does not reach the quality obtained by single-channel detection.

2. Theorie of Transient Absorption Changes

The recorded time and wavelength dependent data can be related to the molecular processes within the framework of the theorie of transient spectroscopy. All time constants of the primary ET are known to be longer than 500 fs and therefore they are much longer than oszillation periods of the relevant molecular vibrations. Consequently one may assume that the absorption changes originate from intermediates with spectroscopically well defined properties. Under these conditions only exponential processes are present and the populations of the various intermediates follow a rate equation system /5/:

$$\frac{dN_i(t)}{dt} = -\sum_{j=0}^{n} k_{ij}\, N_j(t) \qquad (1)$$

n: number of intermediate states

$N_i(t)$: population density of intermediate I_i at time t

k_{ij}: rate constant for population transfer from I_j to I_i

The measured quantity is the induced absorption change $\Delta A(\lambda_{pr}, t_D)$. In the case of weak excitation, for short light pulses of duration $t_p \ll 1/k_{ij}$ and delay times $t_D > 0$, ΔA can be written as a sum of exponential functions:

$$\Delta A(\lambda_{pr}, t_D) = \frac{\ell}{\ln(10)} \sum_{j=1}^{n} \left\{ \sum_{i=1}^{n} [\sigma_i(\lambda_{pr}) - (\sigma_0(\lambda_{pr})] N_{ij} \right\} e^{-t_D/\tau_j} \qquad (2)$$

ℓ: pathlength in the sample

τ_j: eigenvalues of the rate constant matrix k_{ij}

$\sigma_i(\lambda_{pr})$: absorption cross-section of the intermediate I_i at λ_{pr}

The matrix N_{ij} depends on the rate constant matrix k_{ij} and the initial conditions generated by the excitation process.

The goal of the investigations is to understand the microscopic properties of the electron transfer. The primary experimental result concerns the number n of intermediate states involved, which is equal to the number of detected time constants τ_j. This determination requires a very careful decomposition of the time dependence of $\Delta A(\lambda_{pr}, t_D)$ into exponentials:

$$\Delta A(\lambda_{pr}, t_D) = \sum_{j=1}^{n} a_j(\lambda_{pr})\, e^{-t_D/\tau_j} \qquad (3)$$

High precision experimental data at a variety of probing wavelengths are required to obtain the complete set of time constants. Furthermore the data analysis according to eq. (3) supplies the amplitudes $a_j(\lambda_{pr})$ related to the exponential functions. From eq. (2) we learn that these amplitudes contain information on the difference cross-section spectra of the intermediates and the initial ground state in a rather complex form:

$$\sigma_i(\lambda_{pr}) - \sigma_0(\lambda_{pr}) = \frac{\ln(10)}{\ell} \sum_{j=1}^{n} a_i(\lambda_{pr})\, (N_{ij})^{-1} \qquad (4)$$

The difference spectra can only be determined for a known matrix N_{ij}. However, the calculation of N_{ij} requires the complete rate constant matrix k_{ij}. Experimentally we obtain the decay rates, i.e. the eigenvalues of this matrix. The determination of the complete matrix from the eigenvalues is not possible without assumptions on the reaction scheme. The applicability of an assumed reaction scheme can only be tested by its success: Do the spectra fit the assumed reaction model or exist intrinsic contradictions?

3. Analysis of the Experimental Data

As shown before the first step of data analysis is the determination of the number of intermediates, i.e. the number of exponentials needed to fit the absorbance data at all probing wavelengths. As an example Fig.1 shows the time dependence of the absorbance change for RC's from Rhodobacter sphaeroides (λ_{pr} = 785nm). In this curve an initial absorbance increase is followed by a rapid absorption decrease close to t_D= 1ps. In the time range t_D= 2ps - 10ps the absorption rises again. Combining Fig. 1 with a number of other signal curves we found a set of

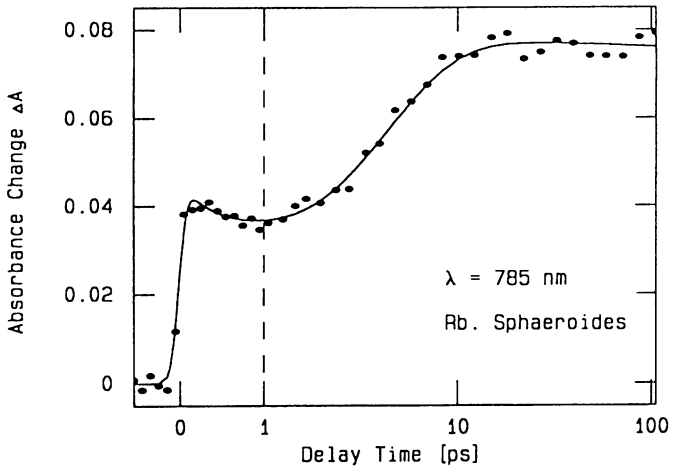


Fig. 1 Kinetics of absorption changes at the probing wavelength λ=785 nm for Rb. sphaeroides. The filled circles represent the experimental data, the solid curve corresponds to a model calculation with decay times given in the text. For delay times > 1 ps the linear time scale is replaced by a logarithmic scale. The excitation wavelength is λ_{ex}= 860 nm.

four time constants (τ_1= 0.9ps, τ_2= 3.5ps, τ_3= 220ps, τ_4= ∞) explaining the experimental data /5/. While the existence of three time constants (τ_1, τ_3 and τ_4) is unchallenged one may try to question the need of two short time constants. Data at various probing wavelengths (e.g. Fig. 1) prove that both (τ_1 and τ_2) are necessary : In Fig. 1 the absorbance decease at t_D= 1ps and the absorbance increase at t_D= 3ps - 10ps cannot be explained by using only one time constant. This demonstrates that quite accurate absorption data measured over a wide range of delay times are required to reveal the existence of two short time constants. The reduced accuracy and the often limited number of delay time settings in transient multichannel experiments may prevent detection of weak components. As an example we show in Fig. 2 transient spectra $\Delta A(\lambda_{pr})$ for delay-times t_D of 200fs, 1ps, 5ps, 10ps and 500ps. They were calculated from a series of time dependent absorption measurements at various probing wavelenths. Even in this case where the data-precision was high enough to resolve the 0.9ps-kinetic in the time courses (see Fig. 1), it is nearly impossible to obtain even an indication for the short kinetic in the transient spectra. The mixture of the various intermediates leading to complex spectral changes hide the weak 0.9ps-component.

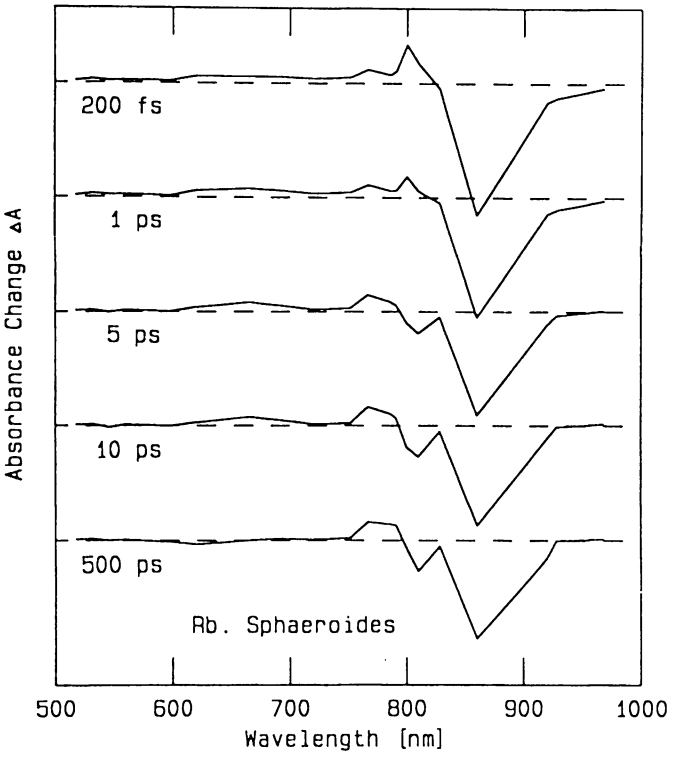


Fig.2 Transient spectra for delay times of 200 fs, 1 ps, 5 ps, 10 ps and 500 ps. The spectra are calculated from kinetic traces taken at about twenty different probing wavelengths.

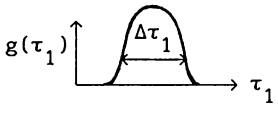
4. Different Reaction Models

The most straigthforward reaction model containing four intermediates is the linear sequential one. In this case each measured time constant gives directly the decay time of one intermediate. Thus only the order of the intermediates in the reaction scheme requires further informations. We have discussed the two possible arrangements of the 0.9ps- and the 3.5ps-kinetic as well as a branched reaction scheme elsewhere /6,7/. Here we present the specific case where the 3.5ps-kinetic precedes the 0.9ps-kinetic. The main purpose is to show that the absorption data can really decide between a scheme with four intermediate states of well defined time constants (model I) and one with only three intermediates

(model II). The same number of free parameters is used in both models since in model I the decay of intermediate I_1 is described by a distribution of time constants with a given width $\Delta\tau_1$. Such a distribution may be caused by a sample inhomogeniety.

$$P \xrightarrow{h\nu} I_1 \underset{3.5ps}{\xrightarrow{\tau_1}} I_2 \underset{0.9ps}{\xrightarrow{\tau_2}} I_3 \underset{220ps}{\xrightarrow{\tau_3}} I_4 \underset{\infty}{\xrightarrow{\tau_4}} \ldots \qquad (I)$$

$$P \xrightarrow{h\nu} I_1 \underset{1ps\ to\ 4ps}{\xrightarrow{\tau_1}} I_2 \underset{220ps}{\xrightarrow{\tau_2}} I_3 \underset{\infty}{\xrightarrow{\tau_3}} \ldots \qquad (II)$$



A dependence of the absorption cross-sections on the inhomogeniety would strongly complicate the microscopic nature of the reaction and is not discussed here. A suitable distribution of time constants $g(\tau_1)$ may readily fit a biexponential behaviour of a signal curve at early delay times if the amplitudes of both exponentials have the same sign. However, the signal curve in Fig.1 exhibits a local extremum between 100 fs and 10 ps. It is not possible to reproduce this extremum by a distribution of time constants $g(\tau_1)$ which by definition does not change its sign. As a consequence one has to employ model 1 which is able to fit the data quite well (solid line in Fig. 1).

5. Spectra of the Intermediates and Transient Absorption Spectra

The *DIFFERENCE CROSS-SECTION SPECTRA* are due to the absorption difference of the respective intermediates and the unexcited ground-state RC. Note that the spectra are calculated from transient absorption data according reaction model I. In Fig. 3 the features of the respective intermediates show up. For example the spectrum of I_2 shows all features expected for an intermediate P^+B^- where one monomeric bacteriochlorophyll is reduced. The spectrum of I_3 shows the properties of intermediate $P^+H_A^-$ with the anion absorption band of the pheophythin H^- around 665 nm.

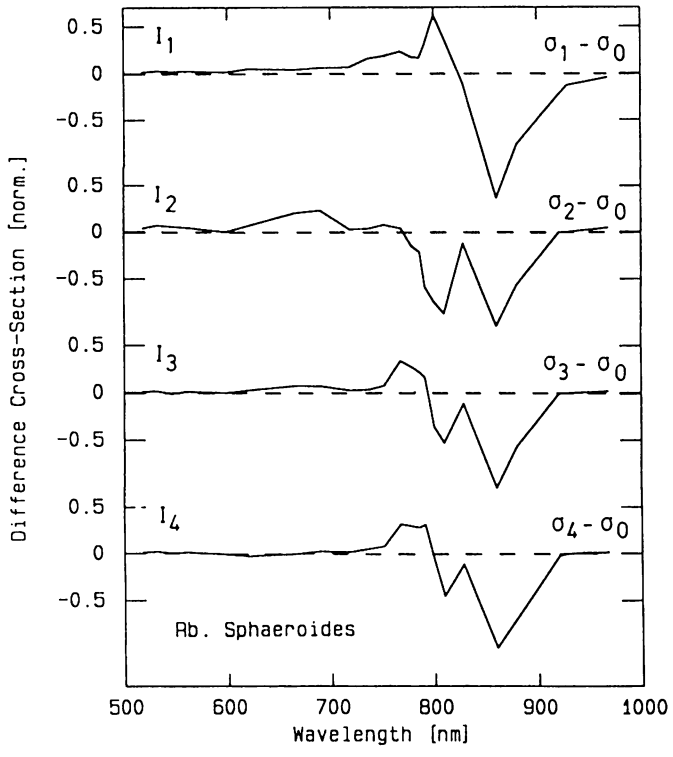


Fig.3 Difference spectra of the cross-sections ($\sigma_i - \sigma_0$) of the four intermediates I_i (σ_i) relative to the cross-section of the unexcited ground state (σ_0). The spectra are calculated from the amplitudes of a series of signal courses assuming model I.

One should now compare these *DIFFERENCE CROSS-SECTION SPECTRA* of the respective intermediates (Fig.3) with the *TRANSIENT ABSORPTION SPECTRA* of Fig. 2, which reflect the actual absorption of the sample at a certain delay time t_D after excitation. According to eq. (2) the *TRANSIENT ABSORPTION SPECTRA* do not characterize the single intermediates, but show a mixture of the absorption properties of the intermediates populated at the specific delay time t_D. As a consequence the mixture of states may hide a weakly populated intermediate, especially if the absorption of this intermediate overlaps with the bands of other intermediates populated at the same time.

In conclusion we have discussed several aspects of transient absorption spectroscopy related to the complicated case where several intermediate states with similar time constants occur and where the reaction model is not known in advance. We have shown for the primary electron transfer of the RC's from Rhodobacter sphaeroides that four time constants and four intermediate states are required to explain the transient absorption data.

References

1 J.L. Martin, J. Breton, A.J. Hoff, A. Migus and A. Antonetti: Proc. Natl. Acad. Sci. USA 83, 957 (1986)

2 J. Breton, J.L. Martin, A. Migus A. Antonetti and A. Orszag: Proc. Natl. Acad. Sci. USA 83, 5121 (1986)

3 N.W. Woodbury, M. Becker, D. Middendorf and W.W. Parson: Biochemistry 24, 7516 (1985)

4 W. Holzapfel, U. Finkele, W. Kaiser, D. Oesterhelt, H. Scheer, H.U. Stilz and W. Zinth: Chem. Phys. Lett. 160, 1 (1989)

5 W. Holzapfel, U. Finkele, W. Kaiser, D. Oesterhelt, H. Scheer, H.U. Stilz and W. Zinth: Proc. Natl. Acad. Sci. USA, 87, 5168 (1990)

6 C. Kirmaier, D. Holten and W.W. Parson: Biochim. Biophys. Acta 810, 33 (1985)

7 K. Dressler, U. Finkele, C. Lauterwasser, P. Hamm, W. Holzapfel, S. Buchanan, W. Kaiser, H. Michel, D. Oesterhelt, H. Scheer, H.U. Stilz, and W. Zinth: this volume