ONE- AND TWO-PHOTON PICOSECOND PROCESSES OF ELECTRON TRANSFER AMONG THE PORPHYRIN MOLECULES IN BACTERIAL REACTION CENTERS

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Received 1 April 1980

1. Introduction

Picosecond (ps) spectroscopy of bacterial reaction centers (RC) has shown that charge separation between bacteriochlorophyll (BChl) and bacteriopheophytin (Bph) molecules is the primary photoprocess in RC [1,2]. But ps laser techniques, although providing high time resolution, can cause serious artifacts if applied to photosynthetic objects [3]. Photosynthetic systems are characterized by an efficient migration of excitations in antenna chlorophyll and BChl, which focuses the excitations in the RC and their vicinity. This favours involvement of nonlinear excitation processes that may greatly obscure the kinetics of the primary photosynthetic processes. To minimize such artifacts, we have used tunable parametric oscillators in order to obtain selective excitation of the RC pigments studied [4] (near the A_{870} peak, instead of at 530 nm as had been done). The sensitivity of the instrument has been increased greatly, so that the quantity of photons in each pulse could be reduced to the level $\lesssim 1$ photon/RC [5]. In this work one- and two-photon A_{880} has been shown to occur in prereduced *Rhodopseudomonas sphaeroides* R-26 RC, in agreement with [6,7]. One-photon absorption results in charge separation with formation of P-870$^{\cdot+}$ and P-800$^{\cdot-}$ in <10 ps. Then fast electron exchange ($\leqslant$10 ps) between P-800 and Bph is observed. This state has some absorption near 880 nm and thus can absorb a second photon. This causes the localization of an electron on the Bph molecule first and then within several ns on the BChl-800 molecule during recombination with P-870$^{\cdot+}$.

2. Materials and methods

RC were isolated from the carotenoidless *Rhodopseudomonas sphaeroides* R-26 as in [8]. To reduce the Fe–quinone complex (X), 5 mM ascorbate and 0.1 mM *o*-phenantroline were added and the sample was illuminated by continuous light during the experiment. The measurements were done in a 2 mm sample cell. The A_{870} of the sample was 0.4.

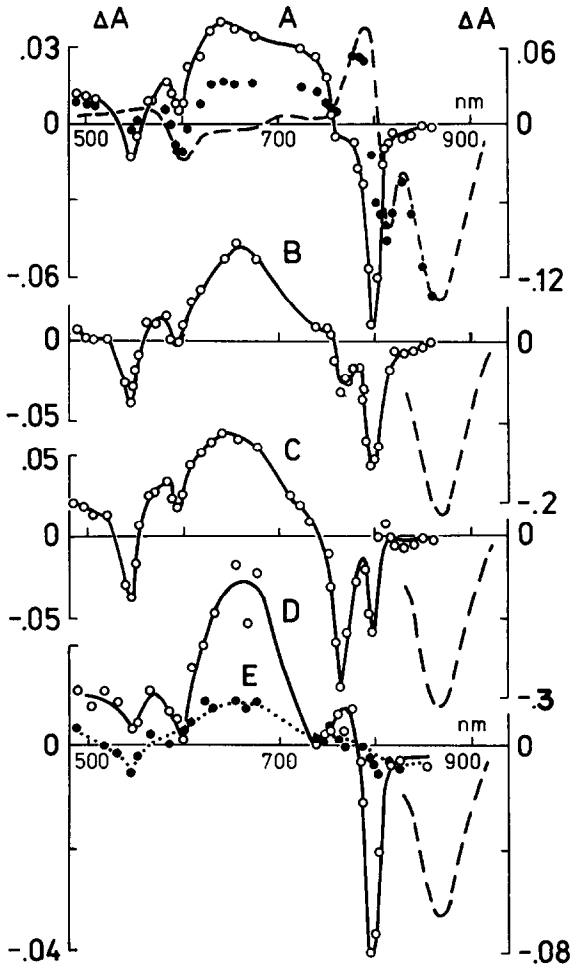
For the ps measurements, the ps parametric oscillators in [5,6] were used. The RC were excited at 800 nm by ~30 ps single pulses with maximal energy about 3×10^{17} photons/cm^2. For probing, weak pulses ($\leqslant 10^{13}$ photons/cm^2, $\leqslant$30 ps) from a parametric oscillator were used; they were tunable over 480–1300 nm [5].

3. Results

At $E_h \simeq +250$ mV the A_{545} decrease after a ps excitation pulse at 880 nm relaxes in ~200 ps, while in the presence of 5 mM ascorbate and 0.1 mM *o*-phenantroline and preillumination (to reduce X) the A_{545} decrease is stable for >500 ps (fig.2A) as in [1]. In agreement with [9], the excitation of prereduced RC at 880 nm induces bleaching of the 870 and 600 nm bands, a blue shift of the 800 nm band and the development of a 650 nm band (fig.1A) in <10 ps. It is apparent that all the spectra measured reflect the formation of P870$^{\cdot+}$ plus BChl-800 and/or Bph radical anions. Therefore it is useful to subtract from the experimental spectra the one for P-870$^{\cdot+}$ forma-

tion normalized in its main peak near 880 nm. This allows us to obtain information about the radical anions involved. The results of such a subtraction are presented in fig.1. The subtraction of the spectrum of P-870$^{\cdot+}$ formation as measured upon continuous illumination (dashed curve) from the experimental spectrum (filled circles in fig.1A) shows that there is an additional bleaching of the 800 nm band (solid curve). The bleaching of the 545 nm band has a very short, but reliable delay in time with respect to the bleaching of the 600 nm band (fig.2B). The bleaching of the band at 750 nm after 100 ps is only half as large as that at 545 nm, if the no. exciting photons/RC (PRC) is ~ 1 (fig.1B), as in [6,7].

An increase of the PRC value up to 20–50 favours second photon absorption during the excitation pulse, as was demonstrated in [5,7], and causes additional ΔA near 760 and 800 nm. The spectrum measured at



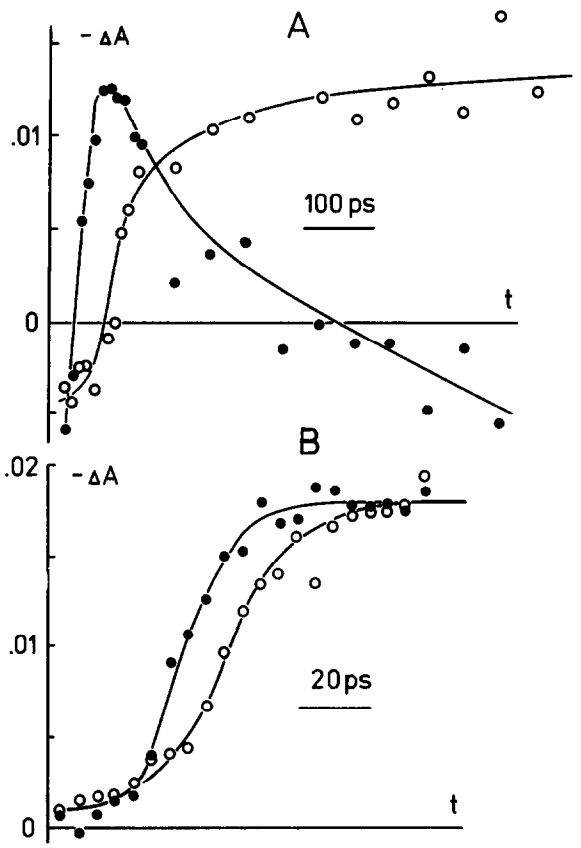


Fig.2A. The kinetics of ΔA_{545} at $E_h \simeq +250$ mV (•——•) and in the presence of 5 mM ascorbate and 0.1 mM *o*-phenantroline after continuous illumination to reduce X (○——○).

Fig.2B. The kinetics of ΔA_{545} and ΔA_{600} when X is in the reduced state and PRC ≃ 1 (○ and •, respectively).

◂

Fig.1.(A) Difference absorption spectrum as measured at 0 time after exciting light pulse at 880 nm (duration ~30 ps, PRC (photons/reaction center) ~40) in *Rhodopseudomonas sphaeroides* R-26 reaction centers in the presence of 5 mM ascorbate and 0.1 mM *o*-phenantroline after continuous illumination to reduce X (•–•). The bleaching of the 870 nm band, which is ~1/3 of maximal (fig.1C), shows that during this time <1 photon is absorbed/RC. The dashed curve shows the spectrum of P-870^{+} formation as measured upon continuous light illumination at $E_h \simeq +250$ mV. The solid curve (○–○) shows the result of subtraction of the P-870^{+} spectrum from the experimental one, after normalization at 870 nm. The right ordinate is for the dashed curve and experimental points (•–•); the left one is for the solid curve.

Fig.1.(B–E) are the same as fig.1(A) except for the time delays between the excitation and measurement and the PRC values, which were 100 ps and 1 (B), 100 ps and 40 (C), 6.7 ns and 40 (D), 6.7 ns and 1 (E), respectively.

100 ps after absorption of the second photon (fig.1C) is characterized by considerable bleaching at 760 and 545 nm, while the bleaching at 600 and 800 nm is smaller than that in fig.1A,B.

The ΔA_{860} measured at 6.7 ns after excitation are ~3-fold less than those at 100 ps. The spectra measured at 6.7 ns for PRC $\simeq$ 1 and PRC $\simeq$ 40 are considerably different. When PRC $\simeq$ 1 this spectrum mainly resembles that for P-870$^{\cdot+}$ formation, since the bleaching of 545 and 800 nm bands and the development of 650 nm band are considerably decreased relative to the bleaching at 860 nm (fig.1E). When PRC $\simeq$ 40 the spectrum at 6.7 ns is similar to that measured at time 0 after excitation (see fig.1A) and is characterized by bleaching of the 870, 800 and 600 nm bands, and development of the 650 nm band (fig.1D). The bleaching of Bph bands at 760 and 545 nm is considerably decreased relative to the spectrum at 100 ps (see fig.1C,D). An A_{760} increase is even observed in this case.

4. Discussion

The above results can be probably interpreted in the following way. When X is in the reduced state, the first photon induces charge separation between P-870 and P-800 within <10 ps, as suggested [9]. In this case, either the bleaching of Bph bands at 760 and 545 nm is not observed at all [9] or the bleaching at 545 nm has a delay in time (fig.2B), while the bleaching at 800 nm is considerable (see [9] and fig.1A). The bleaching of the 545 nm band occuring within next 10 ps (fig.1B), can be attributed to the fast ($\simeq$10 ps) exchange of an electron between P-800 and Bph, because the bands at 545, 600, 800 nm are bleached in this case, but the band at 760 nm is only slightly bleached (fig.1B). The redistribution of the dipole strengths in the near infrared between interacting Bph and P-800 [10] can induce bleaching at 760 nm when Bph is reduced (fig.1C) and an A_{760} increase when P-800 is reduced (fig.1D). The sum of these effects can result in small ΔA_{760} when electron exchange between P-800 and Bph occurs (fig.1B).

The P-870$^{\cdot+}$ (P-800 Bph)$^{\cdot-}$ state seems to be an intermediate in the electron transfer to X. The electron exchange could create an additional entropy barrier to the reversal of the initial charge separation.

The second photon changes the ΔA spectrum in such a way that the bleaching of the Bph bands at 545 and 760 nm is increased while bleaching of the BChl band at 800 nm is decreased (fig.1C). These ΔA plus the simultaneous bleaching at 870 nm and development of the 650 nm band show the appearance of the ion-radical pair P-870$^{\cdot+}$ Bph$^{\cdot-}$. Thus the second photon absorption probably changes the exchange of the electron between P-800 and Bph and almost all the electron density is shifted to the Bph molecule.

After one-photon absorption, the ΔA spectrum measured after 6.7 ns shows (fig.1E) mainly P-870$^{\cdot+}$ formation. The electron appears to have transferred to the prereduced non-porphyrine acceptors. The ΔA_{545} and ΔA_{650} measured at 100 ps and 6.7 ns give a preliminary approximation for the lifetime of radical anion to be ~3–6 ns. After two-photon absorption the ΔA spectrum measured at 6.7 ns indicates (fig.1D) that the electron density is shifted to P-800 (state P-870$^{\cdot+}$ P-800$^{\cdot-}$). The lifetime of the recombination of P-870^{+} and the electron is ~7 ns as estimated from ΔA_{860} and ΔA_{650} measurements.

We suggest that the second photon is absorbed by the anion radical and changes the interaction between P-870, P-800 and Bph in the RC. Firstly, it is accompanied by the localization of an electron on Bph. Then, within several ns, the electron density is shifted to P-800, which is probably intercalated between P-870 and Bph [10]. The long lifetimes of the above-mentioned states, and the considerable A_{650} increase after second photon absorption indicate the formation of isolated radical anions, rather than excited singlet states of Bph and BChl-800 generated by the second photon at 880 nm. The possibility of the formation of triplet ion-radical pairs in this case may need to be taken into account.

Acknowledgements

We are grateful to Dr L. G. Erokhina for the help in RC preparation, to Professor A. A. Krasnovsky and Dr Ju. E. Erokhin for their interest in the work and to Professor W. W. Parson for carefully reading and commenting on this paper.

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