FEBS Letters 582 (2008) 238–242

Proton transfer in the photosynthetic reaction center of *Blastochloris viridis*

Maria A. Kozlova[a,b], Hanno D. Juhnke[c], Dmitry A. Cherepanov[d], C. Roy D. Lancaster[c], Armen Y. Mulkidjanian[a,b,*]

[a] *A.N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, 119991 Moscow, Russia*
[b] *School of Physics, University of Osnabrück, 49069 Osnabrück, Germany*
[c] *Department of Molecular Membrane Biology, Max Planck Institute of Biophysics, 60438 Frankfurt am Main, Germany*
[d] *A.N. Frumkin Institute of Physical Chemistry and Electrochemistry, Russian Academy of Sciences, Moscow, Russia*

Received 30 October 2007; revised 27 November 2007; accepted 5 December 2007

Available online 18 December 2007

Edited by Peter Brzezinski

Abstract Photosynthetic reaction centers of *Blastochloris viridis* require two quanta of light to catalyse a two-step reduction of their secondary ubiquinone Q_B to ubiquinol. We employed capacitive potentiometry to follow the voltage changes that were caused by the accompanying transmembrane proton displacements. At pH 7.5 and 20 °C, the Q_B-related voltage generation after the first flash was contributed by a fast, temperature-independent component with a time constant of ~30 μs and a slower component of ~200 μs with activation energy (E_a) of 50 kJ/mol. The kinetics after the second flash featured temperature-independent components of 5 μs and 200 μs followed by a component of 600 μs with $E_a \sim 60$ kJ/mol.

Keywords: Photosynthetic reaction center; Ubiquinone; Proton transfer; Capacitive potentiometry; *Blastochloris viridis*; *Rhodobacter sphaeroides*

1. Introduction

The photosynthetic reaction center (RC) is a pigment–protein complex that converts the energy of light into electrochemical energy (for reviews see [1–3]). As shown in Fig. 1A, the RC of the purple α-proteobacterium *Blastochloris viridis* (former *Rhodopseudomonas viridis*), the first membrane protein for which the X-ray structure has been obtained, is formed by two membrane subunits (L and M) being flanked by the H subunit from the cytoplasmic side of the membrane and by a tetraheme *c*-type cytochrome from the periplasmic side [4,5]. The excitation of the *Bl. viridis* RC by a flash of light triggers a charge separation followed by a picosecond electron transfer (ET) across the membrane, from the bacteriochlorophyll dimer *P* to a bound primary quinone Q_A (menaquinone-9). While the oxidized P is reduced by cytochrome *c*, the electron is transferred along the membrane plane to a loosely bound secondary quinone Q_B (ubiquinone-9, see Fig. 1B), and reduces it to a tightly bound semiquinone anion $Q_B^{\bullet-}$. The reduction of $Q_B^{\bullet-}$ by the next electron – e.g. after the second flash of light – yields a ubiquinol Q_BH_2 [6–8]. By using capacitive potentiometry, Dracheva and co-workers followed the electrogenic proton transfer (PT) that accompanied the reduction of Q_B to Q_BH_2 in the RCs of *Bl. viridis* incorporated into proteoliposmes [9]. They have reported a Q_B-related voltage generation with an apparent time constant (τ) of ~400 μs only in response to the second flash. Further detailed studies of the same reactions in *Rhodobacter sphaeroides* have shown, however, that already the $Q_B^{\bullet-}$ formation after the first flash is coupled with electrogenic proton transfer from the surface [10]. As well, the transfers of the second electron and the first proton to $Q_B^{\bullet-}$ after the second flash were found to be kinetically coupled in *Rb. sphaeroides* [11]. In *Bl. viridis,* however, the voltage generation with τ of 400 μs [9] has seemed to be slower than the corresponding ET with τ of 50 μs [7,8]. Here we revisited the problem of voltage generation in *Bl. viridis* by employing a specific inhibitor terbutryn to discriminate the reactions at the Q_B site.

2. Materials and methods

The *Bl. viridis* cells were grown and their RCs were purified as described elsewhere [12]. Proteoliposomes were prepared according to Ref. [13]. The flash-induced voltage generation was traced by capacitive potentiometry, as described in detail elsewhere [10,14]. Proteoliposomes were fused to a nitrocellulose film impregnated with the solution of 150 mg/ml soybean phosphatidylcholine (type II, Sigma) and 20 mg/ml ubiquinone-10 in *n*-decane. The voltage changes were monitored by a home-made electrometer-amplifier (constructed by N. Spreckelmeier) and digitalised on a Nicolet Pro-90 oscilloscope (point resolution 1 μs). The samples were equilibrated in the dark for 20 min before being illuminated by series of two consecutive saturating flashes of light with 1 s interval. A Surelite Nd:Yag laser (532 nm, FWHM 6 ns, Continuum, USA) was used as an excitation source. The dark adaptation time between the series was 5–10 min. The sign of the flash-induced voltage changes indicated that RCs incorporated into liposomes with their cytochromes pointing outside. In order to separate the Q_B related voltage signal from the total response, the traces obtained in presence of the Q_B site inhibitor terbutryn (10 μM) were point-by-point subtracted from those measured after the first and the second flash, respectively, without the inhibitor. All traces were normalized to the amplitude of the terbutryn trace at 2 μs after the flash, and this amplitude, *A*, was taken as 100% on estimating the relative amplitudes of difference traces. The kinetic traces were fitted with exponentials; all points were considered with equal weight. The Pluk software (kindly provided by Dr. Y. Kalaidzidis) and Microcal Origin 6.0 package (OriginLab, USA) were used. The incubation medium contained routinely

*Corresponding author. Address: School of Physics, University of Osnabrück, 49069 Osnabrück, Germany. Fax: +49 541 9692656.
E-mail address: amulkid@uos.de (A.Y. Mulkidjanian).

0014-5793/$32.00
doi:10.1016/j.febslet.2007.12.010

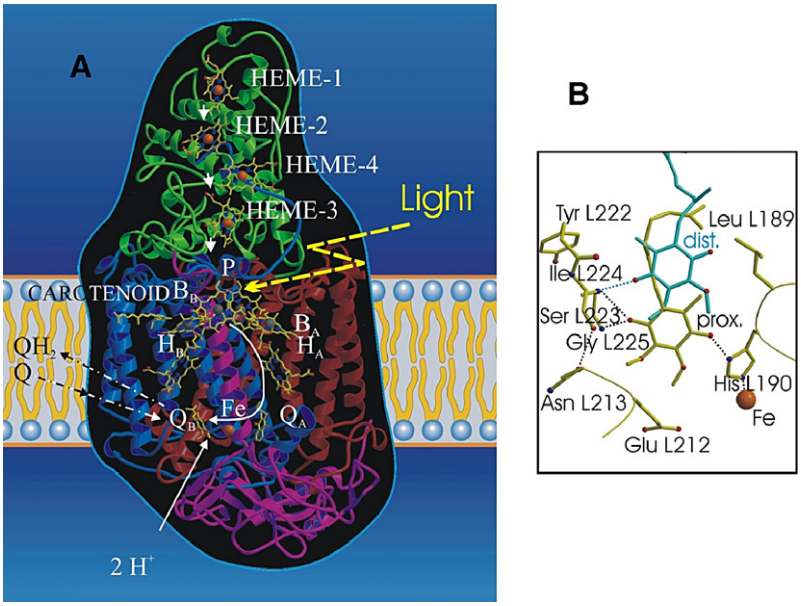

Fig. 1. Structure of the photosynthetic RC from *Blastochloris viridis*. (A) the structure of the *Bl. viridis* RC is represented schematically showing the heterotetramer of C, L, M, and H subunits as Cα traces in green, brown, blue, and purple, respectively, plus the 14 cofactors, which have been projected on to the molecule for better visibility. Also for the sake of clarity, the quinone tails are truncated after the first isoprenoid unit and the phytyl side chains of the bacteriochlorophyll and bacteriopheophytin molecules have been omitted, as have those atoms of the carotenoid molecule which were not observed in the electron density and assigned zero occupancy in the PDB entry 2PRC [20]. (B) Comparison of distal ($1PRC_{new}$, cyan) and proximal (2PRC, yellow) ubiquinone-binding sites [20].

20 mM HEPES, 100 mM KCl, 2 mM potassium ascorbate, 25 μM *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylendiamine, and methylene blue, the concentration of which was optimized at each temperature and pH value to have O_B fully oxidized in 30 s [15]. To measure the pH-dependence, we used a pH–buffer mixture of Gly/GlyGly/potassium phosphate/potassium acetate (20 mM each).

3. Results and discussion

As shown in Fig. 2A, the flash-induced charge separation between P^+ and Q_A^- led to a kinetically unresolved voltage jump at <10 ns (see component A in Fig. 2A) followed by a slower rise. The rise could be due to electrogenic charge displacements both in the donor and in the acceptor parts of the RC [9]. However, only the voltage generation at the O_B site was expected to be sensitive to the Q_B antagonist terbutryn [16]. As shown in Fig. 2A, terbutryn partially suppressed the voltage rise both after the first and second flash. When the residual voltage rise in the presence of terbutryn (Fig. 2A) was resolved on a faster time scale (not documented), it was contributed by a component of <10 ns of the primary charge separation between P and Q_A, a component of ~200 ns making ~25% of

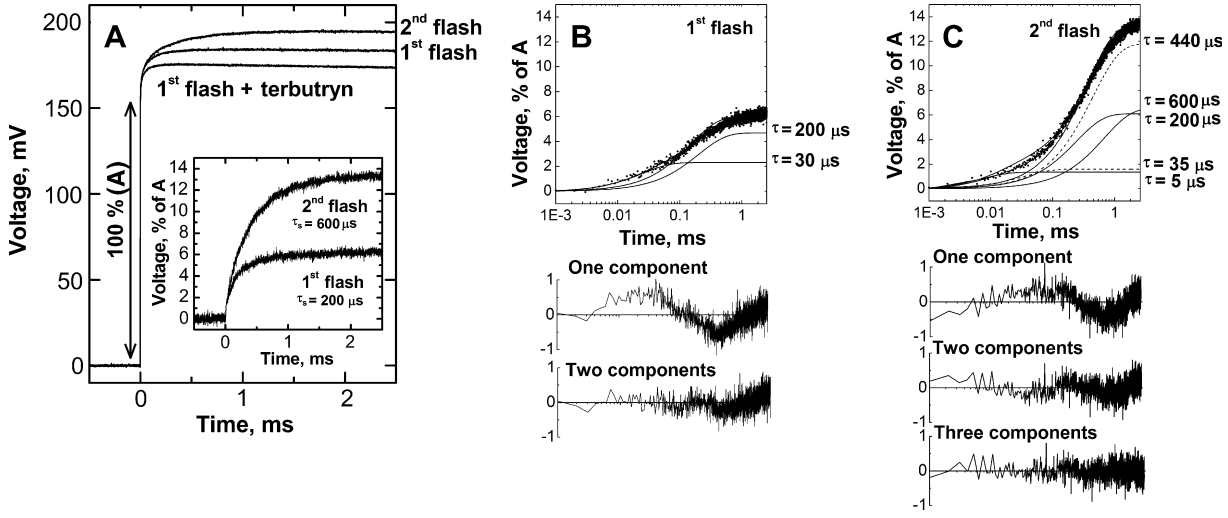

Fig. 2. Light-induced voltage generation in the RCs of *Blastochloris viridis* (pH 7.5, $T = 20$ °C). (A) Voltage traces as measured after the first and second flash, and after the first flash in the presence of Q_B-antagonist terbutryn. *Inset*: difference traces ±terbutryn reflecting the voltage changes at the Q_B site, as normalized to the charge separation component A. (B) Voltage generation after the first flash as approximated by one or two exponentials. The residuals of the fits are shown at the bottom of the plot. (C) voltage generation after the second flash as approximated by one, two or three exponentials. The components of the three exponential fit (5, 200 and 600 μs) are shown as solid lines. The components of the two exponential fit (440 and 35 μs) are shown as dashed lines.