Proton transfer in the photosynthetic reaction center of Blastochnorhis viridis

Maria A. Kozlova\textsuperscript{a,b}, Hanno D. Juhnke\textsuperscript{c}, Dmitry A. Cherepanov\textsuperscript{d}, C. Roy D. Lancaster\textsuperscript{c}, Armen Y. Mulkinidjan\textsuperscript{a,b,*}

\textsuperscript{a} A.N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, 119991 Moscow, Russia
\textsuperscript{b} School of Physics, University of Osnabrück, 49069 Osnabrück, Germany
\textsuperscript{c} Department of Molecular Membrane Biology, Max Planck Institute of Biophysics, 60438 Frankfurt am Main, Germany
\textsuperscript{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

Abstract Photosynthetic reaction centers of Blastochnorhis viridis require two quanta of light to catalyse a two-step reduction of their secondary ubiquinone Q\textsubscript{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\textsubscript{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 \(E_a\) ~ 60 kJ/mol.

© 2007 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.

Keywords: Photosynthetic reaction center; Ubiquinone; Proton transfer; Capacitive potentiometry; Blastochnorhis 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 \(\alpha\)-proteobacterium Blastochnorhis 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 \(Q_B^-\). The reduction of \(Q_B^-\) 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 proteoliposomes [9]. They have reported a \(Q_B\)-related voltage generation with an apparent time constant (\(\tau\)) 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 \(Q_B^-\) 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 \(Q_B^-\) after the second flash were found to be kinetically coupled in Rb. sphaeroides [11]. In Bl. viridis, however, the voltage generation with \(\tau\) of 400 μs [9] has seemed to be slower than the corresponding ET with \(\tau\) 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

0014-5793/$32.00 © 2007 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.febslet.2007.12.010
20 mM HEPES, 100 mM KCl, 2 mM potassium ascorbate, 25 μM \(N,N',N',N''\)-tetramethyl-p-phenylenediamine, and methylene blue, the concentration of which was optimized at each temperature and pH value to have OB 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 Q_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. 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 phytol 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].](image1)

![Fig. 2. Light-induced voltage generation in the RCs of Blastochloris viridis (pH 7.5, \(T = 20^\circ\)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. Insert: difference traces \(\pm\)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.]
the primary charge separation and attributable to ET from heme-3 of cytochrome c to P (see Fig. 1), as well as a component of ~2 μs making 10% of the primary charge separation and owing to ET from heme-4 to heme-3, in good correspondence with data of Dracheva and co-workers who had studied these kinetic components in more detail [9]. These three steps of voltage generation are the same after each flash, so that they are nullified upon the calculation of the ±terbutryn difference traces (see Section 2 for the procedure). The difference traces, as obtained for the first and second flash, respectively, are shown in the inset to Fig. 2A; they reflect the voltage generation at the Qb site upon Qb/Q0b and QbH2 formation [10,14]. In this work we focused on the kinetic analysis of these difference traces with the aim of understanding the proton transfer at the Qb site of Bl. viridis.

As shown in Fig. 3A, the relative amplitude of the terbutryn-sensitive voltage generation after the first flash, in response to the semiquinone Qb formation, depending on pH, made 2-8% of the charge separation A (see Section 2). Its kinetics could be approximated by two components that were well discernible at t < 20 °C (Figs. 2B and 3B). The faster component (~30 μs at 20 °C, pH 7.5) was virtually temperature-independent (see Table 1). The slower component (~200 μs at 25 °C) showed temperature dependence with apparent activation energy (Ea) of 50 ± 2 kJ/mol.

The relative amplitude of voltage generation after the second flash, in response to the QbH2 formation, made 12-18% of charge separation A (see Fig. 3C). The kinetics featured a very fast component with an apparent τ of ~5 μs (20 °C, pH 7.5) and slower component(s) with τ of ~440 μs if fitted by a single exponential function. The double exponential fit of the slower component allowed better approximation with τ values of 200 and 600 μs at neutral pH and 25 °C (Figs. 2C and 3D). The 5 and 200 μs components were virtually temperature-independent (see Table 1). The slowest 600 μs component showed temperature dependence with Ea of 63 ± 5 kJ/mol.

Here we describe several new kinetic components of flash-induced voltage generation at the Qb site of the Bl. viridis RCs. These novel components were apparently overlooked in the earlier pioneering study [9] because the difference between the voltage traces in response to the second and first flash was taken as a measure of Qb-related voltage generation – under the apparently incorrect assumption of its absence after the first flash (cf. with Figs. 2A, B, and 3A, B).

The rates, relative amplitudes, as well as the pH- and temperature dependence of the voltage generation after the first flash (see Figs. 2B and 3A, C) resemble those reported for Rb. sphaeroides [10,14,17]. In R. sphaeroides, two kinetic

Table 1
The activation energy (Ea) values calculated for the kinetics components of the voltage generation at the Qb site after the first flash (ubisemiquinone formation) and second flash (ubiquinol formation), respectively, as measured in the range from 7 °C to 28 °C

<table>
<thead>
<tr>
<th>Kinetics component</th>
<th>Very fast</th>
<th>Fast</th>
<th>Slow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ea (kJ/mol)</td>
<td>Qb → QbQ0b</td>
<td>QbQ0b → QbH2</td>
<td>QbH2 → QbH2b</td>
</tr>
<tr>
<td>(kJ/mol)</td>
<td>&lt;20 kJ/mol</td>
<td>&lt;10 kJ/mol</td>
<td>&lt;10 kJ/mol</td>
</tr>
<tr>
<td>(kJ/mol)</td>
<td>50 ± 2 kJ/mol</td>
<td>63 ± 5 kJ/mol</td>
<td>63 ± 5 kJ/mol</td>
</tr>
</tbody>
</table>

Fig. 3. Voltage generation at the Qb site as function of pH. First flash: relative amplitudes (A) and time constants (B). Open circles: the 30 μs component, open squares: 200 μs component. Second flash: relative amplitudes (C) and time constants (D). Open stars: 5 μs component, open circles: 200 μs component, open squares: 600 μs component. The total amplitudes of the respective voltage changes are shown as filled stars.

Please cite this article in press as: Kozlova, M.A. et al., Proton transfer in the photosynthetic reaction center ..., FEBS Lett. (2007), doi:10.1016/j.febslet.2007.12.010
components were discernable, namely a fast component with $\tau$ of $\approx 80 \mu s$ and $E_a$ of $\leq 10$ kJ/mol and a slow component with $\tau$ of $\approx 500 \mu s$ at $10^\circ C$ and $E_a$ of $\approx 60–70$ kJ/mol [14]. The $E_a$ of the slow component was too large for PT proper and indicated a kinetic limitation by conformational change in a fraction of RCs. It was hypothesized that in some RCs Qh may dwell in a remote non-functional site that is separated by a high activation barrier from the closer, catalytic position [14]. The low-temperature X-ray structures of the Rh. sphaeroides RC have indeed revealed two Qh states, namely a remote “distal” position and a “proximal” one with Qh being 5 Å closer to Qa [18]. It has been argued that in the RCs with Qh in a catalytic proximal position the proton redistribution towards Qh proceeds with $\tau$ of $\approx 100 \mu s$ and $E_a$ $< 10$ kJ/mol whereas in the RCs with Qh in a distal “stand-by” site, the formation of Qh and the coupled proton displacements are limited by slow relocation of Qh into the catalytic position [3,14,19]. Concurrently with the work on the Rh. sphaeroides RC, but using a different experimental strategy, distal and proximal positions (Fig. 1B) for the native Qh species were found in the Bl. viridis RC [20]. Taking into account that the transfer of the first electron to Qh takes $\approx 20 \mu s$ in Bl. viridis [7], i.e. is somewhat faster than the components of voltage generation revealed here (cf. with Figs. 2B and 3B), the latter can be attributed, by analogy with Rh. sphaeroides, to the unimpeded and conformationally constrained proton redistribution upon the Qh stabilization in Bl. viridis, respectively.

In Bl. viridis, the voltage generation in response to the second flash could be described by three kinetic components (see Figs. 2C and 3C, D). In the case of Rh. sphaeroides, the respective kinetics could be fitted by two kinetic components, namely a faster, almost temperature-independent one with $\tau$ of $\approx 100 \mu s$ and a slower component with $\tau$ of $\approx 500 \mu s$ at $20^\circ C$ and $E_a$ of about 60 kJ/mol; these components were attributed to the first and second protonation of Qh, upon the Qh$^-$ to Qh$^+$ transitions, respectively [19,21,22]. The two faster components that were observed in Bl. viridis (with $\tau$ values of 5 and 200 $\mu s$) were weakly temperature dependent, whereas the slowest one displayed an $E_a$ of about 50 kJ/mol (see Table 1). From the relative amplitudes of the components (see Fig. 3C) and by analogy with Rh. sphaeroides, the two faster components could be attributed to the first protonation of Qh, while the slower one apparently reflects the second protonation.

Unlike the neutral Qh, which can attain several positions in the Qh site [20], the Qh$^+$ anion radical is attracted by the positively charged non-heme iron [23] and is expected, by analogy with the binding of a semiquinone-mimetic stigmatellin [20,24] and also by analogy with Rh. sphaeroides [14,18], to occupy a defined position in the pocket [20]. Therefore the heterogeneity of the voltage generation found upon the Qh$^+$ to Qh$^+$ transition in Bl. viridis RCs is rather unexpected. It is noteworthy that the 5 $\mu s$ component has never been observed with Rh. sphaeroides chromatophores either in the earlier works [10,21,22] or in the same high-resolution set-up [3] that was here used to investigate the RC of Bl. viridis. Another striking finding is that the 5 $\mu s$ component of voltage generation is apparently faster than the reported time constant of ET from Qh to Qh$^+$ after the second flash ($\approx 50 \mu s$ [7,8]). One possibility is that the 5 $\mu s$ component reflects a terbutryn-sensitive charge displacement at the Qh-site in response to Qh$^+$ formation. It has been shown that the formation of Qh$^+$ after the first flash triggers proton binding [25] and conformational changes [26] at the Qh site of Rh. sphaeroides. Electrostatic calculations do not rule out that in Bl. viridis already the formation of Qh$^+$ can cause a prompt, electrogenic proton redistribution at the Qh site [27]. Alternatively, the 5 $\mu s$ component might reflect the heterogeneity in the kinetics of the second electron transfer in Bl. viridis. Unlike Rh. sphaeroides, the RCs of Bl. viridis have shown heterogeneity of their Qh$^+$ states, as inferred from the Qh$^{+}\rightarrow$Qh$^+$ backreaction kinetics [28,29]. The heterogeneity was explained by the existence of two fractions of RCs becoming differently protonated in response to Qh formation, and, accordingly, having different redox potentials of their Qh/Qh$^+$ redox pairs [29]. According to Marcus theory [30], the ET from Qh to Qh$^+$ has to be faster in the RCs with lower redox potential of the Qh/Qh$^+$ pair. This low-potential fraction made about 20% in Bl. viridis RC preparation at neutral pH and increased upon alkalisation [28,29]. Hence, one can expect two kinetic components for the Qh$^+$ to Qh$^++$ transition, with a faster component contributing 20% at neutral pH and increasing at higher pH values. These expectations, in principle, corroborate the pH-dependence of the relative amplitudes of the 5 $\mu s$ and 200 $\mu s$ components (see Fig. 3C).

In conclusion, the voltage generation at the Qh site shows fundamental similarity in Bl. viridis and Rh. sphaeroides. At the same time, the kinetics of the first Qh$^+$ protonation after the second flash contain a novel minor 5 $\mu s$ component that has no counterpart in Rh. sphaeroides. Further studies of site-specific mutants are needed to clarify the nature of this fast component.

Acknowledgements: Very useful discussions with Dr. W. Leibl are greatly appreciated. The research was funded by the Volkswagen Foundation, Max Planck Society, and by Grants from the Deutsche Forschungsgemeinschaft (Mu-1285/1, SFB 431-P15, SFB 472-P19, 436-RUS-113/210).

References


Please cite this article in press as: Kozlova, M.A. et al., Proton transfer in the photosynthetic reaction center ..., FEBS Lett. (2007), doi:10.1016/j.febslet.2007.12.010


