NANOSECOND RECOMBINATION LUMINESCENCE STIMULATED BY *o*-PHENANTHROLINE

Sensitivity to the light-induced membrane potential in *Rhodospirillum rubrum* chromatophores

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1. Introduction

In bacterial photosynthesis initial light-driven charge separation occurs between the pigment molecules of the reaction center complex prior to transfer of an electron to the quinone (so-called 'primary') acceptor [1,2]. For example, reduction of this quinone gives rise to nanosecond bacteriochlorophyll afterglow due to the radiative recombination of photooxidized primary electron donor $P870^+$ and photoreduced intermediary acceptor I^- [3–5]. In [6] the lifetime and intensity of this short-lived afterglow strongly depended on the transmembrane electric potential difference ($\Delta\Psi$). The electric field across the chromatophore membrane was created by the hydrolysis of ATP or PP_i, whereas membrane potential generation coupled with the photosynthetic electron flow was completely suppressed by reduction of the primary quinone under anaerobiosis. Here, we show that the effect of the light-induced membrane potential on the recombination luminescence can be observed under appropriate conditions. The energy-linked luminescence variations are shown to correlate with carotenoid electrochromic absorbance changes.

2. Materials and methods

Rhodospirillum rubrum cells (wild-type strain no. 1 MGU) were grown and chromatophores were prepared as in [7].

Abbreviations: CCCP, carbonylcyanide *m*-chlorophenylhydrazone; ΔA, absorbance change; $\Delta\Psi$, transmembrane electric potential difference; τ and φ, lifetime and relative quantum yield of chromatophore emission

The measurements of the luminescence lifetimes and relative quantum yields were performed with a phase-type fluorometer [4]. Luminescence was excited by 404 and 436 nm mercury lines and detected through a combination of glass filters transmitting the $\lambda >$ 760 nm range.

Light-induced absorbance changes (ΔA) in the 400–600 nm spectral range were determined as in [8] with a home-made dual-beam spectrophotometer. The actinic light of saturating intensity was provided by a tungsten lamp and passed through a combination of KS-19 glass (transmitting $\lambda > 680$ nm range) and IF-894 interference (VEB Carl Zeiss Jena) filters.

All experiments were carried out at room temperature.

The incubation mixture contained 220 mM sucrose (or 75–100 mM KCl), 50 mM Tris–HCl buffer (pH 7.6), 2 mM $MgSO_4$, chromatophores ($A^{1\,cm}_{880\,nm}$ amounted to 0.75–1 for luminescence measurements and 1–1.6 for ΔA measurements).

3. Results and discussion

Nanosecond bacteriochlorophyll luminescence occurs in chromatophores of purple bacteria upon either chemical [4], or photochemical reduction of the primary quinone [6,8,9]. The latter is attained by a light-induced electron transfer from an exogenous donor to the quinone, if its reoxidation by oxygen via the secondary quinone [7] is prevented, e.g., under anaerobic conditions [6,10]. The light-induced accumulation of the reaction centers with the reduced

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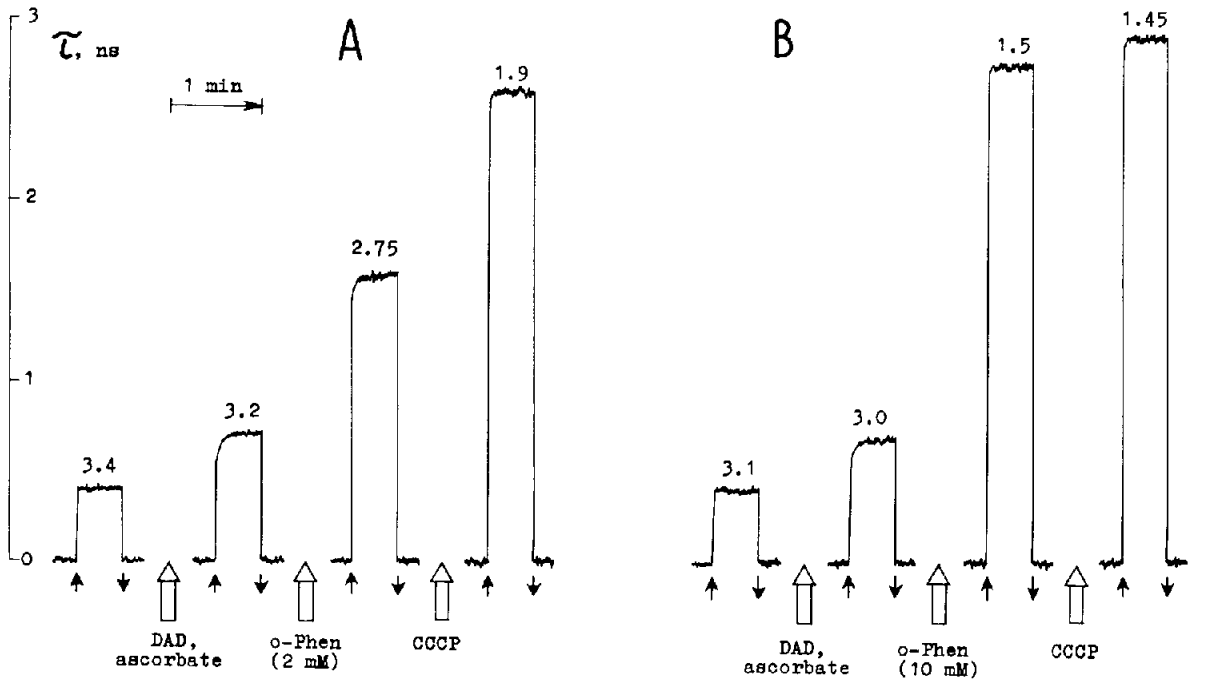

Fig.1. Effect of *o*-phenanthroline and CCCP on the lifetime (τ) and relative quantum yield (numbers above the columns) of the total emission of *R. rubrum* chromatophores incubated with electron donors under aerobic conditions. Measurements of τ were made as follows. First, the zero level of τ was determined by placing a latex suspension into an exciting beam and recording a phase-shift of the scattered light as compared to the reference signal. Then, the latex was replaced by a luminescent sample and the phase-shift of the luminescence in relation to the zero level was recorded. The value of this shift was used to calculate τ of the luminescence, according to the formula in [4]. (↑) and (↓) designate the moments when the sample was placed into the exciting light and removed. Additions: 5 mM Tris-ascorbate, 0.1 mM diaminodurene (DAD), 2 mM (in A) or 10 mM (in B) *o*-phenanthroline (*o*-Phen), 1×10^{-6} M CCCP. Chromatophores were preincubated with *o*-phenanthroline for 10 min before the measurement. $A^{1\text{ cm}}_{880\text{ nm}} = 0.8$ (in A) and 0.75 (in B).

primary acceptor also takes place in chromatophores incubated aerobically, provided that the electron transfer between the primary and secondary quinones is impeded by an inhibitor [7–9]. Indeed, the addition of 2 mM *o*-phenanthroline to aerobic *R. rubrum* chromatophores, supplemented with diaminodurene (2,3,5,6-tetramethyl-*p*-phenylenediamine) or TMPD (*N,N,N',N'*-tetramethyl-*p*-phenylenediamine) and ascorbate, leads to a marked increase in the lifetime (τ) of chromatophore emission (fig.1A). This increase is indicative of the appearance of a nanosecond component in luminescence [6,9]. However, the value of τ in this case is considerably lower than that in the presence of sodium dithionite [6]. The subsequent addition of protonophorous uncoupler CCCP to *o*-phenanthroline-treated chromatophores causes increase in τ from 1.6–2.6 ns, accompanied by a drop in the luminescence yield (φ).

The pattern of the CCCP-induced τ and φ changes, shown in fig.1A, is opposite to that observed upon pyrophosphate-dependent energization of the chromatophore membrane under reducing conditions (cf. fig.1A in [6]). Therefore, the CCCP effect on τ and φ may be associated with dissipation of the transmembrane difference of H^+ electrochemical potentials. This suggestion was tested by using valinomycin, a compound known to abolish $\Delta\Psi$. As seen in fig.2, this ionophorous antibiotic added after *o*-phenanthroline produces the same effect on the luminescence parameters as CCCP. Thus, in both cases, we dealt with the response of nanosecond luminescence to transmembrane electric field collapse, which is in line with the data in [6].

It should be noted that the maintenance of an appreciable membrane potential in the presence of *o*-phenanthroline is not evident, since this agent is

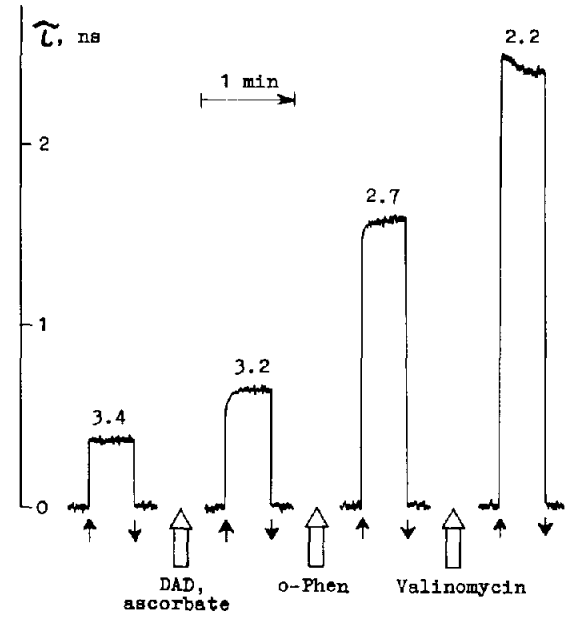

Fig.2. Effect of valinomycin on the lifetime (τ) and relative quantum yield (numbers above the columns) of the total emission of *R. rubrum* chromatophores incubated with *o*-phenanthroline and electron donors under aerobic conditions. Additions: 5 mM Tris–ascorbate, 0.1 mM diaminodurene (DAD), 2 mM *o*-phenanthroline (*o*-Phen), 5×10^{-7} M valinomycin. Chromatophores were preincubated in 50 mM Tris–HCl buffer (pH 7.6) containing 75 mM KCl and 2 mM $MgSO_4$ for 10 h at $A^{1\,cm}_{880\,nm} = 100$ and then diluted with the same buffer to $A^{1\,cm}_{880\,nm} = 0.8$.

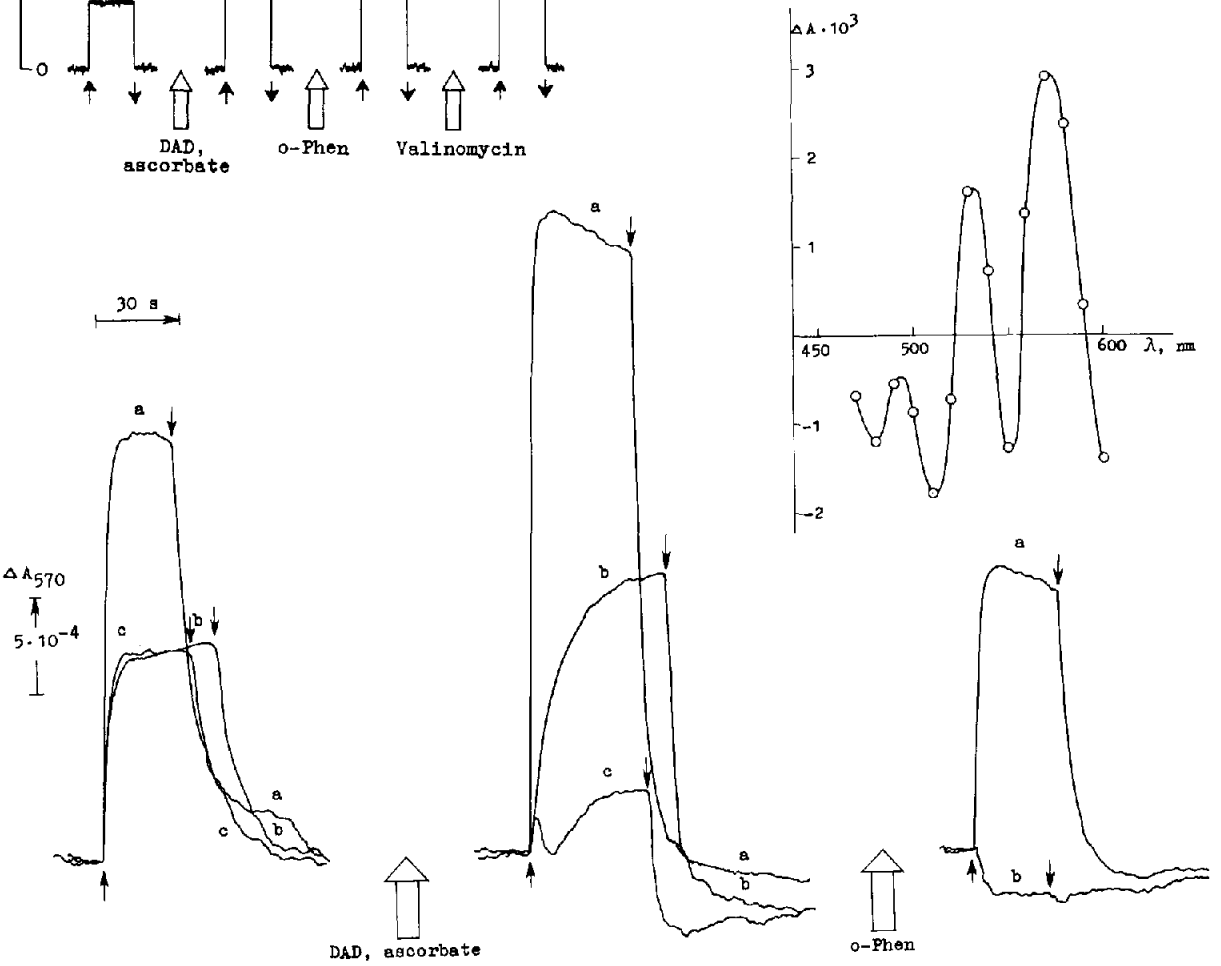

Fig.3. Light-induced absorbance changes of carotenoids at λ = 570 nm (ΔA_{570}) in *R. rubrum* chromatophores incubated aerobically. Successive additions: 5 mM Tris–ascorbate; 0.1 mM diaminodurene (DAD); 2 mM *o*-phenanthroline (*o*-Phen). Traces: (a) in the absence of valinomycin; (b,c) in the presence of 5×10^{-7} M and 2×10^{-6} M valinomycin, respectively. Chromatophores were preincubated with KCl as in fig.2. $A^{1\,cm}_{880\,nm} = 1.3$ (↑) and (↓), switching on and off the actinic light. Insert: Spectrum of the light-induced absorbance changes of aerobic *R. rubrum* chromatophores supplemented with 0.1 mM diaminodurene and 5 mM Tris–ascorbate, $A^{1\,cm}_{880\,nm} = 1.2$.

known to suppress light-induced $\Delta\Psi$ generation in chromatophores [7]. For this reason, a correlation between the luminescence changes obtained and some probes of membrane potential should be demonstrated.

It is generally accepted that the light-induced red shift in the absorbance spectrum of carotenoids of some purple bacteria results from the $\Delta\Psi$ generation across the chromatophore membrane [11,12]. This has also appeared to be true for *R. rubrum* [13–16]. Fig.3 shows traces of the absorbance changes (ΔA_{570}) at the maximum of the light-induced difference spectrum (see insert). Comparison of the traces recorded in the absence and presence of valinomycin reveals that in chromatophores incubated without electron donors only part (~50%) of ΔA_{570} represents a response to the transmembrane electric field. The other part is presumably due to the photochemistry of the reaction center [17,18], in particular to the oxidation of P870 and formation of a local electric field. The addition of diaminodurene in combination with ascorbate significantly stimulates the valinomycin-sensitive absorbance change and sharply decreases the insensitive one. Thus the light-induced ΔA_{570} in this case may be attributed almost exclusively to the transmembrane $\Delta\Psi$ generation. As seen in fig.3, a substantial part (~40%) of these $\Delta\Psi$-values is retained in the presence of 2 mM *o*-phenanthroline and is completely abolished by subsequent addition of valinomycin. Hence, at this concentration *o*-phenanthroline does not inhibit electron transfer in all reaction centers. The relatively high membrane potential observed under these conditions is possibly explained by the non-linear relationship between $\Delta\Psi$ and a portion of the photoactive reaction centers, as in [19]. In fact the rise in *o*-phenanthroline from 2–10 mM, which almost completely suppresses the light-induced $\Delta\Psi$ generation, increases τ of the uncoupled chromatophores only from 1.6–2.9 ns (fig.1A,B).

With intact cells of *R. rubrum*, the effect of the light-induced membrane potential on the nanosecond luminescence was also observed. CCCP caused increase in τ (from 2.0–2.6 ns) and ~15% decrease in φ of aerobic cells in the presence of dithionite.

These, data confirm the idea that nanosecond recombination luminescence is sensitive to the transmembrane electric field.

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