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ORIGINAL PAPER

Ethylenediammine is not detrimental to the photoactivity of the bacterial photosynthetic reaction center

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Abstract

The effect of the exposure of the photosynthetic reaction center from the purple bacterium *Rhodobacter sphaeroides* to ethylenediamine (EDA) was investigated by transient absorption spectroscopy and UV–Visible-Near Infrared absorption spectroscopy. We show that EDA is not detrimental to the photoactivity of the protein even at pH close to 12. EDA instead appears to inhibit the secondary quinone binding site with an apparent binding constant of 19.05 mM^{-1}.

Introduction

The interest towards the integration between the components of the photosynthetic process and the organic electronics for biocompatible, eco-friendly and efficient energy conversion hybrid devices is swiftly growing [1, 2]. Unfortunately, even in the simplest device configuration, the final assembly encounters several drawbacks associated with the low efficiency of the interfaces between the (photo)biological component with the abiotic one [3].

The bacterial enzyme reaction center (RC) obtained from the bacterium *Rhodobacter* (*R.*) *sphaeroides* represents a model system for the photosynthetic enzymes [4]. Under illumination, it absorbs light and converts it in a hole-electron couple amenable for several applications. The RC is a membrane protein formed by three subunits forming the scaffolding for nine cofactors organized [5–7] as illustrated in Fig. 1. Upon absorption of one photon, isolated RC forms a charge–separated state with the positive charge sitting on a dimeric couple of bacteriochlorophylls (D) and a negative charge sitting on the quinone complex (Q_AQ_B) located on the opposite side of the protein, roughly 35 Å away from the dimer.

The fate of the photoinduced hole-electron couple in isolated proteins depends upon several factors that include the lifetime of the charge-separated state [8–13] and the kind of interface to which the protein is exposed. With the goal of using the RC in electronic devices, the protein has been immobilized in films [12, 14] or encapsulated in nanoaggregates [15] for the assembly of photocathods. The nanoaggregates of polydopamine (PDA), formed by oxidative polymerization of dopamine [16], represent an interesting solution for encapsulating the active form of the protein, ensuring a reliable light driven electron transfer between the enzyme and ITO electrode [17].

The PDA has been largely investigated as surface coating materials for entrapping active enzymes [18]. Unfortunately, even as nanoaggregates PDA retains its blackish color that represents a drawback for application involving light driven enzymatic reactions. Further engineering [19] of the nanoaggregates are hence in need for producing smaller and optically transparent PDA particles. Small nanoaggregates would also improve the electrode coverage. The small, highly water-soluble molecule ethylenediamine (EDA) is a simple and efficient agent for engineering PDA that lead to the production of final PDA aggregates with good water dispersity, good transparency, and fluorescent properties [20]. The EDA acts after dopamine polymerization reducing the internal π-stacking interaction of PDA. Moreover, EDA can also intercalate into preformed PDA structures, and chemically functionalize the polymer via Schiff-base formation or Michael addition reaction [21].

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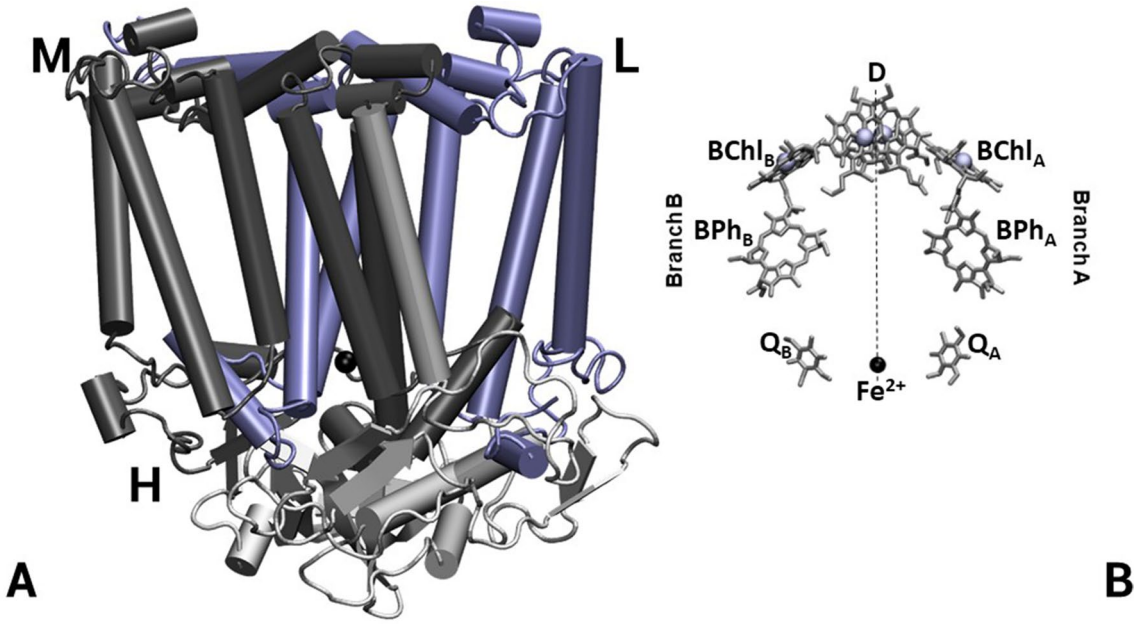

Fig. 1 **a** The reaction center from *R. sphaeroides* strain R26 is a membrane-spanning enzyme composed by three protein subunits named M (dark grey), L (cyan), and H (light grey). **b** The enzyme contains four bacteriochlorophylls *a* (BChl), two of which organized as dimer (*D*), two bacteriopheophytins *a* (BPh), two ubiquinones-10 (*Q*), and one bivalent iron ion (Fe^{2+}), arranged in two almost symmetrically branches A and B. The cofactors, excluded *D* and Fe^{2+}, are represented with the subscript A or B according to their branch. The binary symmetry axle is shown as broken line. For sake of simplicity, the hydrophobic chains of the cofactors are not shown

To apply EDA as versatile and simple engineering strategy for PDA nanoaggregates containing the RC, it is compulsory to show that the diamine is not detrimental for the enzymatic activity. This task was tackled by studying the bare RC under the worst possible conditions, i.e. exposing it to increasing and massive amount of EDA in poorly buffered solutions, allowing the pH to reach values well above the physiological range.

Experimental section

Microorganisms and culture conditions

The strain R26 of the purple bacterium *Rhodobacter sphaeroides* was grown under continuous illumination in anaerobic conditions [22] using the medium 27 of the German Collection of Microorganisms and Cell Cultures (DSMZ).

Extraction of reaction center from *Rhodobacter sphaeroides* strain R26

The biomass from the anaerobic cultures was used for the extraction of the photosynthetic reaction center according to a previously published procedure [23]. Briefly, harvested cells were disrupted using a French Press cell and centrifuged to eliminate cellular debris. The photosynthetic RC was extracted from the bacterial membranes in the supernatant with the detergent LDAO (*N*,*N*-Dimethyldodecylamine N-oxide), separated by ultracentrifugation, and eventually purified ion-exchange chromatography. Specific peaks of the absorption spectrum of the reaction center were used to assess purity and integrity of the protein [24, 25]. Stock solutions of the RCs were stored at − 20 °C in Tris (tris-hydroxymethil-aminomethane) 20 mM, EDTA (Ethylenediaminetetraacetic acid) 1 mM, and LDAO 0.025% $T_{20}E_1L_{0.025}$ at pH 8.0.

Absorption and transient absorption spectroscopies

The RC has a rich absorption spectrum with peaks spanning from ultraviolet to near infrared (see text) [24]. The absorbance ratio A_{280}/A_{802} is used for assessing RC purity and should range from 1.2 to 1.4; the absorbance ratios A_{760}/A_{802} and A_{802}/A_{865} are diagnostic of protein integrity and must be equal to 1 and 2 respectively.

Transient absorption spectroscopy (TSA) experiments were performed using an instrumental set-up of local design [9]. Experiments were performed at room temperature using single flashes as excitation source; the decay of the excited state generated by the flash was recorded at 865 nm and the traces were fitted to biexponential decay function.

A solution containing the RCs in $T_{20}E_1L_{0.025}$ in presence of decylubiquinone (DQ) 100 µM was used as control. An identical sample was added stepwise with increasing amount of EDA to obtain the concentrations 1, 10, 50, 100, 500, and 1000 mM. After each EDA addition the pH, the absorption spectrum, and the kinetic decay were recorded.

Results and discussion

PDA nanoaggregates encapsulating proteins or enzymes were recently used with the photosynthetic bacterial reaction center to investigate the possibility of generating efficient, cheap, and biocompatible photocathodes for electrochemical cells [17]. Since ethylene diamine is often used to chemically modify PDA nanoaggregates, we explored the biocompatibility of EDA with proteins by testing its effect on the RCs dissolved in buffer solution. As mentioned, the RC converts the photons absorbed into a hole-electron couple with the positive charge sitting on the bacteriochlorophyll dimer *D* and the negative charge sitting on the electron acceptor system formed by two quinones; one sitting in the Q_A-binding site is tightly bound, and the second loosely bound to the Q_B-binding site. The hole-electron couple has a lifetime that depends on the occupancy of the Q_B-site. A fully occupied Q_B-site decays with a kinetics of 1 s^{-1}; when the Q_B-site is empty or inhibited, the recombination kinetics is ten times faster. The kinetics of the charge-separated state can hence be used to monitor the occupancy of the Q_B-site. The amount of charge-separated state generated by saturating amount of light, instead, does not depend on the occupancy of the Q_B-site and relates to the functional integrity the of the protein [23].

The RCs exposed to increasing amount of EDA were investigated by TSA obtaining two complementary information, namely the occupancy of the final electron acceptor Q_B obtained by the changes in the recombination kinetics of the charge-separated state (see Fig. 2a) and the integrity of the protein from the initial value of the absorbance change associated to the photogenerated signal (Table 1). The charge-recombination reaction of $D^+(Q_AQ_B)^-$ can be fitted to the equation

$$A_{tot}(t) = A_f e_f^{(-kt)} + A_s e_s^{(-kt)} \quad (1)$$

where A_{tot} is the total amplitude of the signal generated by the flash of light, proportional to the initial concentration of the charge-separated state, while A_f and A_s are the amplitude of the contributions from the $D^+Q_A^-$ and $D^+Q_B^-$ populations that decay with the kinetic constants k_f and k_s. The relevant fit parameters to Fig. 2 are reported in Table 1.

The EDA inhibits the Q_B-site and, although no inhibition mechanism can be inferred, an apparent binding constant $K_D = 19.05$ mM can be retrieved by the curve in Fig. 2b. The

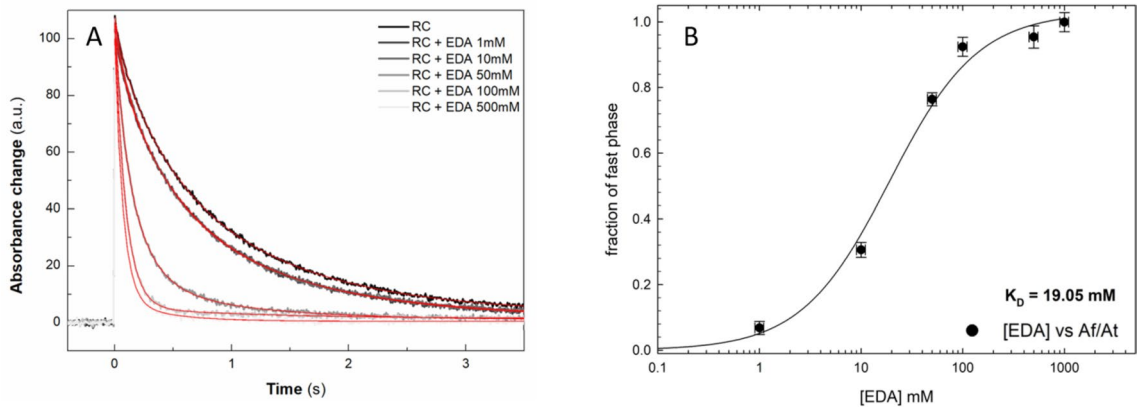

Fig. 2 **a** Transient absorption changes recorded at 865 nm in solution 1 µM of RC in $T_{20}L_{0.025}E_1$ at increasing EDA concentrations. Red line represents the results of the fitting procedures. **b** The ratio between the fast phase intensity A_f and the total intensity A_{tot} of the data in panel A are interpolated with an apparent binding constant for EDA to the RC of 19.05 mM

Table 1 Fit parameters obtained by fitting data in Fig. 2a to Eq. 1

Parameter	no EDA	1 mM EDA	10 mM EDA	50 mM EDA	100 mM EDA	500 mM EDA	1000 mM EDA
A_{tot}	103 ± 2	100 ± 1	102 ± 2	102.5 ± 0.3	104.1 ± 0.1	100.5 ± 0.4	101 ± 2
A_f/A_{tot}	0.01 ± 0.01	0.07 ± 0.02	0.31 ± 0.02	0.76 ± 0.02	0.92 ± 0.03	0.96 ± 0.03	0.98 ± 0.02
k_s	1.03 ± 0.01	1.10 ± 0.01	1.13 ± 0.01	1.58 ± 0.02	1.1 ± 0.2	0.9 ± 0.9	-

TSA experiments also show that the state $D^{+}(Q_AQ_B)^{-}$ decays faster with the increase of the EDA concentration. This effect is due to the known dependence of the recombination rate on the pH that in our solution increases [26], passing from 8.8 in absence of EDA to 11.6 at EDA 1000 mM.

The integrity of the photoenzyme was independently tested via absorption spectroscopy in the NIR region, where the main absorption peaks of the protein are located at 760 nm, 802 nm, and 865 nm (Fig. 3a). Peak positions are not influenced by EDA, confirming the results of the transient absorption spectroscopy. At wavelengths below 500 nm the large shoulder associated to the EDA absorption becomes dominant (Fig. 3b). Interestingly, a small absorption peak due to EDA appears at 1046 nm (Fig. 3c). The molar extinction coefficient is small, 0.057 mM^{-1} cm^{-1}, but this peak can be used as internal signal to assess the concentration of EDA in the solution containing the photosynthetic RC.

Conclusions

Polydopamine nanoaggregates encapsulating the photosynthetic reaction center are a versatile tool in the production of film that can be used in several applications to exploit the photoactivity of the bacterial enzyme. One recent successful application was the assembly of photocathode used for the production of photocurrents. Ethylenediamine is a molecule known to interact with PDA modifying the structure of the polymer in several aspects. With the purpose of further engineer the RC-PDA nanoaggregates for other electrochemical of photochemical applications, the effect of EDA on the photosynthetic RC was investigated. The activity of the RC is maintained in presence of 1 M EDA and a pH close to 12. Although EDA does have an effect on the occupancy of the final electron acceptor Q_B, the capability of the RC to form the

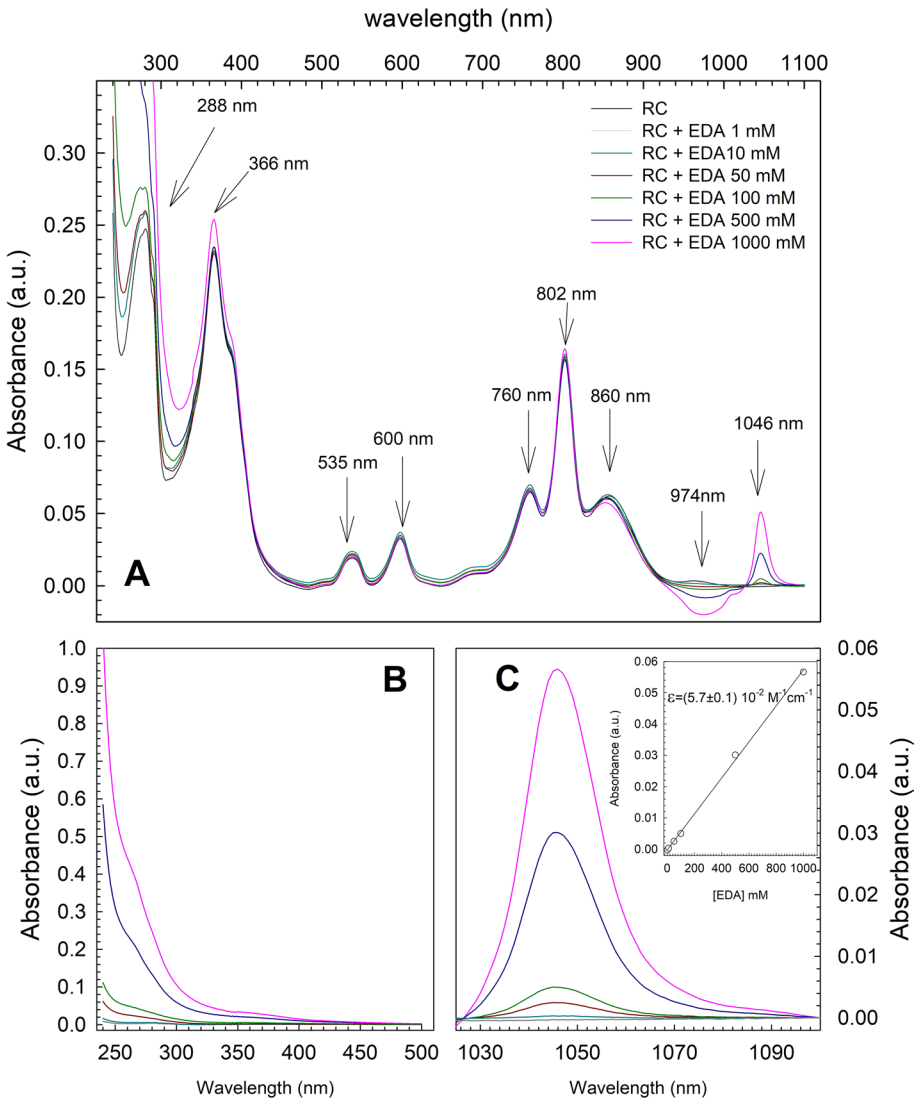

Fig. 3 Spectra of 1 μM RC solution recorded in the interval 240–1100 nm [24] with increasing amount of EDA. **a** UV–Vis-NIR spectrum showing the wavelengths of the most intense peaks of the RC, of EDA and of the water. **b** UV–Vis region of the spectra showing the increasing absorption due to EDA. **c** Specific absorption of EDA in the Near Infrared. In the inset is shown the linear correlation between [EDA] and absorption intensity from which the extinction coefficient is determined

charge-separated state is unchanged, showing that the use of ethylenediammine is not detrimental to the generation of the hole-electron couple even at very high pH. Transient and steady state absorption spectroscopy both indicated that the enzyme remains intact and fully active. Furthermore, it was shown that the EDA shows an apparent binding constant of roughly 20 mM.

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Data availability The authors declare that the data supporting the findings of this study are available within the article.

Compliance with ethical standards

Conflict of interest The authors have no financial or proprietary interests in any material discussed in this article.

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