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Low potential manganese ions as efficient electron donors in native anoxygenic bacteria

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ABSTRACT

Systematic control over molecular driving forces is essential for understanding the natural electron transfer processes as well as for improving the efficiency of the artificial mimics of energy converting enzymes. Oxygen producing photosynthesis uniquely employs manganese ions as rapid electron donors. Introducing this attribute to anoxygenic photosynthesis may identify evolutionary intermediates and provide insights to the energetics of biological water oxidation. This work presents effective environmental methods that substantially and simultaneously tune the redox potentials of manganese ions and the cofactors of a photosynthetic enzyme from native anoxygenic bacteria without the necessity of genetic modification or synthesis. A spontaneous coordination with bis-tris propane lowered the redox potential of the manganese (II) to manganese (III) transition to an unusually low value (~400 mV) at pH 9.4 and allowed its binding to the bacterial reaction center. Binding to a novel buried binding site elevated the redox potential of the primary electron donor, a dimer of bacteriochlorophylls, by up to 92 mV also at pH 9.4 and facilitated the electron transfer that is able to compete with the wasteful charge recombination. These events impaired the function of the natural electron donor and made BTP-coordinated manganese a viable model for an evolutionary alternative.

1. Introduction

One of the critical milestones of the evolution of life on Earth is linked to the transition from anoxygenic to oxygen producing photosynthesis about 2.8–2.4 billion years ago [1–4]. Models have been proposed to account for the numerous molecular requirements that had to be satisfied over the transition period [5,6]. One of the key elements required for oxygen evolution is the ability to use manganese ions as rapid secondary electron donors [7]. Efficient utilization of manganese in the native bacterial reaction center (BRC) from purple anoxygenic photosynthetic bacteria has been considered energetically unfavorable (Fig. 1 left) as the primary electron donor (P) has lower potential (0.5 V) than that of the manganese sources readily available [5,8].

Contrarily, the primary electron donor (P_{680}) in Photosystem II can deploy high potential electron donors (Fig. 1 right), including manganese ions from the oxygen evolving complex (OEC) [7]. Photosystem II and BRC are evolutionary related as plants and cyanobacteria share a common ancestor with purple photosynthetic bacteria [9]. Despite the differences in the energetics surrounding the primary and secondary donors, the structural details and the mechanism of the transmembrane electron transfer are analogous in these two enzymes [5,8,10,11]. Generation of the proton gradient across the membrane, a key feature for the survival of the organism, requires subsequent electron transfer steps that lead to the double reduction and double protonation of the terminal electron acceptors (secondary quinones; Q_B) in both systems. This process is only possible if the oxidized primary electron donors, P^+ and $P_{680}{}^+$, are rapidly reduced by secondary electron donors in BRC and PSII, respectively. Electron transfer reactions from donors unique to oxygenic photosynthesis, such as light-driven tyrosine and manganese oxidation have been introduced to BRCs, however, thus far exclusively via multiple genetic modifications [12–14]. In these modified BRCs the redox potential of P was elevated by up to ~0.3 V by design and introduction of up to four mutations. Two-to-five mutations were necessary to observe manganese oxidation and at least five mutations were required to witness tyrosine oxidation. The electron transfer in these modified BRCs from the secondary electron donors to P^+ was effective as it successfully competed with the charge recombination reactions. A slow photo-oxidation of manganese(II) ions in native BRCs was reported in the presence of bicarbonate (BCT), however, the electron transfer from Mn^{2+} to P^+ there was found at least two orders of

Abbreviations: BRC, Bacterial Reaction Center; P, Primary electron donor of bacterial photosynthesis; P_{680}, Primary electron donor of oxygenic photosynthesis; OEC, Oxygen Evolving Complex; Q_B, terminal electron acceptor; WT, Wild type; R-26, carotenoid-less strain; *Rba. Rhodobacter*; LDAO, *N*-lauryl-*N*-*N*-dimethylamine-*N*-oxide; EDTA, Ethylenediaminetetraacetic acid; ubiquinone, UQ, 2,3-dimethoxy-5-methyl-6-polyprenyl-1,4-benzoquinone; BTP, bis-tris propane; BChl, Bacteriochlorophyll; BPheo, Bacteriopheophytin

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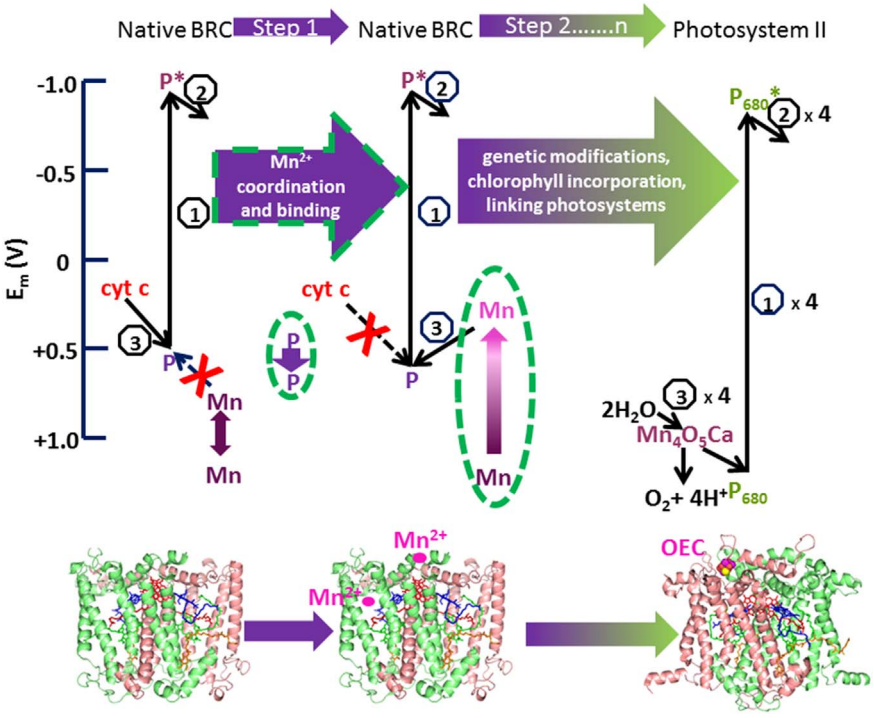

Fig. 1. Energetics of light-induced electron transfer steps at the donor side of photosystems. The redox potentials of the primary and secondary electron donors are indicated in the BRC (left), in Photosystem II (right), and in native BRC, where manganese oxidation is enabled by coordination and binding (middle). The electron transfer sequence is labeled by numbers in octagons for the electronic excitation of P or P_{680}, for the charge separation, and for the electron donation from the secondary electron donors, respectively. Only reactions marked by thin downhill arrows are spontaneous. Vertical block arrows indicate tuning of redox potentials. To demonstrate the structural similarities, the L and M subunits of BRC (PDB: 1PCR) and the D1 and D2 proteins of Photosystem II (PDB: 3WU2) are shown in salmon and green colors, respectively. The location of the OEC (Mn_4O_5Ca) and the proposed binding of Mn^{2+} are also indicated.

magnitude slower than the charge recombination and the yield was only ~5% [15]. Here we describe how spontaneous coordination of manganese with hydroxyl and amine groups and its binding to native BRCs can tune the redox potentials of both manganese and P substantially. The electron transfer between Mn^{2+} and P^+ not only can compete with the charge recombination but also approaches 100% yield. As solely environmental factors were required to gain this attribute our system may serve as a proof of principle for the earliest step in the development of oxygenic photosynthesis (Fig. 1 middle) that does not require genetic modification.

2. Materials and methods

2.1. Bacterial growth and BRC isolation

Cells from wild type (WT) and the carotenoid-less R-26 strains of *Rhodobacter* (*Rba.*) *sphaeroides* were grown anaerobically under light. The RCs were isolated and purified using *N*-lauryl-*N*-*N*-dimethylamine-*N*-oxide (LDAO) according to methods described earlier [16,17]. Ethylenediaminetetraacetic acid (EDTA), a potent chelator, was removed from the samples by extensive dialysis. For some experiments the LDAO detergent was replaced with Triton X-100 (TX-100) by ion exchange chromatography. In some experiments 100 μM terbutryn was used to block the electron transfer between the quinones while in others the secondary quinone activity was reconstituted with either UQ_{10} or UQ_0 (2,3-dimethoxy-5-methyl-6-polyprenyl-1,4-benzoquinone).

2.2. Preparation of the manganese complex

Manganous acetate or chloride was dispersed in 0.03% LDAO containing 80 mM bis-tris propane (BTP) at pH 9.4. A 1 M stock was prepared and this stock was diluted to the desired final concentration. A fresh stock was used for each measurement. The manganese complex was added to BRCs that were previously pre-illuminated and allowed to recover from their corresponding charge-separated states. The measurements were performed after 30 min incubation time.

2.3. Optical spectroscopy

Optical spectra and some kinetics of the absorbance changes induced by continuous illumination or light pulse were measured using a Varian (Agilent) Cary 5000 spectrophotometer (Mulgrave, Victoria, Australia) according to methods described earlier [16–18]. The charge-separated states were induced either by continuous or pulsed illumination using high throughput fiber optics (Newport Corp., Irvine, CA, USA.). The continuous light source was either an Oriel 2129 tungsten lamp or an Oriel 6140 Arc lamp. The light intensity was varied between 0.13 and 1 W/cm^2. Laser flash-induced electron transfer reactions in the ms time scale were recorded with a miniaturized laser flash photolysis unit (LFP-112 from Luzchem Research Co., Ottawa, Ontario, Canada) as reported elsewhere [17]. Kinetic traces were analyzed by decomposition into exponentials using Marquardt-Levenberg nonlinear least-squares method.

2.4. Oxidation-reduction potential measurements

The oxidation-reduction midpoint potential of the P/P^+ and Mn^{2+}/Mn^{3+} couples were determined by spectroelectrochemical oxidation-reduction titrations as we described earlier [18]. The redox potential dependent light-induced absorption changes were determined using the same spectroelectrochemical redox cell. For these measurements the cell was tilted at ~45° angle with respect to the propagation of the monitoring beam and the actinic illumination was delivered perpendicular to the window of the cell to avoid stray light entering the detector chamber [18]. At each selected potential value a short (~10 s) illumination was applied until the light-induced optical changes reached their equilibrium values. The absorption changes recorded at different potentials were fit with a single or a two component Nernst-equation.

2.5. Dual polarization interferometry (DPI)

Surface depositions of the BRCs and cytochrome were done using AnaLight Bio200 interferometer (Farfield Ltd., Manchester, U.K.) as described earlier [19,20]. The samples were delivered to the sensor