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M234Glu is a component of the proton sponge in the reaction center from photosynthetic bacteria

Hélène Cheap [a], Sophie Bernad [a], Valérie Derrien [a], László Gerencsér [a], Julia Tandori [a], Pedro de Oliveira [a], Deborah K. Hanson [b], Péter Maróti [c], Pierre Sebban [a,*]

[a] Laboratoire de Chimie Physique, UMR 8000, University of Paris-Sud 11/CNRS, 91405 cedex, France
[b] Biosciences Division, Argonne National Laboratory, 9700 South Cass Avenue, Argonne, IL 60439, USA
[c] Department of Biophysics, University of Szeged, H-6722, Szeged, Hungary

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ABSTRACT

Bacterial reaction centers use light energy to couple the uptake of protons to the successive semi-reduction of two quinones, namely Q_A and Q_B. These molecules are situated symmetrically in regard to a non-heme iron atom. Four histidines and one glutamic acid, M234Glu, constitute the five ligands of this atom. By flash-induced absorption spectroscopy and delayed fluorescence we have studied in the M234EH and M234EL variants the role played by this acidic residue on the energetic balance between the two quinones as well as in proton uptake. Delayed fluorescence from the $P^+Q_A^-$ state (P is the primary electron donor) and temperature dependence of the rate of $P^+Q_A^-$ charge recombination that are in good agreement show that in the two RC variants, both Q_A^- and Q_B^- are destabilized by about the same free energy amount: respectively ~100 ± 5 meV and 90 ± 5 meV for the M234EH and M234EL variants, as compared to the WT. Importantly, in the M234EH and M234EL variants we observe a collapse of the high pH band (present in the wild-type reaction center) of the proton uptake amplitudes associated with formation of Q_A^- and Q_B^-. This band has recently been shown to be a signature of a collective behaviour of an extended, multi-entry, proton uptake network. M234Glu seems to play a central role in the proton sponge-like system formed by the RC protein.

1. Introduction

Reaction centers (RC) convert light energy into chemical free energy. This membrane protein is composed of three subunits (L, M and H) with a total molecular weight of about 100 kDa. The 3D structure of this model complex is known at 1.87 Å resolution [1]. In photosynthetic purple bacteria the energy conversion is achieved through the excitation of a dimer of bacteriochlorophyll (P) (situated on the periplasmic side of the protein) to its singlet excited state of lowest energy, P*, therefore triggering an electron transfer (ET) chain. An electron is transferred to a bacteriochlorophyll monomer and then in few picoseconds to a bacteriopheophytin, H_A ("A" relates to one of the two symmetrical branches formed by cofactors in the protein). H_A^- is then reoxidized in about 200 ps by electron transfer to the primary quinone, Q_A. The electron is then transferred to a secondary quinone molecule, Q_B situated in a symmetrical position to Q_A in regard to a non-heme iron atom, Fe^{2+}. This ET process takes about 150 μs in WT RCs from *Rhodobacter (R.) sphaeroides*. Indeed, this process is much slower than the Marcus theory [2] would predict it to be on the basis of the edge-to-edge distance between Q_A and Q_B (~15.4 Å, edge-to-edge distance). As all ET steps from P to Q_A occur at a maximal rate, following the Marcus theory (*i.e.* in an activation-less process), the electron transfer process from Q_A to Q_B is rate-limited by a "gating" process [3,4] which is likely to be associated with proton uptake by the protein following Q_A^- formation [5,6]. Water molecules associated in hydrogen-bond clusters might be involved in these processes [7–12]. It has very recently been demonstrated by a combined analysis of the surface amino acid conservation and of hydrogen-bond network that the reaction center functions as a *proton sponge*-like system [12].

The protein medium joining Q_A to Q_B can be viewed in part as a molecular motif containing the two quinones, the Fe atom, and their ligands. The latter consist of four histidines – L190His, L230His, M219His, M266His – and one glutamic acid, M234Glu, which develops two bonds with the Fe atom (Fig. 1). These five residues are conserved in all photosynthetic bacteria. The four His are also conserved in photosystem 2 of oxygen-evolving systems. M234Glu is absent in photosystem 2 RCs [13]. L190His and M219His are symmetrically bound to the Fe atom and also to Q_A and Q_B, with which they develop H-bonds. The Q_A-M219His-Fe-L230His-Q_B "wire"

Abbreviations: B, Blastochloris; M234EL, GluM234→Leu variant; M234EH, GluM234→His variant; LDAO, *N*,N'-dimethyldodecylamine N-oxide; P, primary electron donor, a non-covalently linked bacteriochlorophyll dimer; Q_A and Q_B, primary and secondary quinones; *R, Rhodobacter*; RC, reaction center; Triton X-100, octylphenol polyethylene glycol ether; UQ, ubiquinone, 2,3-dimethoxy-5-methyl-6-hexaisoprenyl-1-4-benzoquinone; WT, wild type

* Corresponding author.
E-mail address: pierre.sebban@u-psud.fr (P. Sebban).

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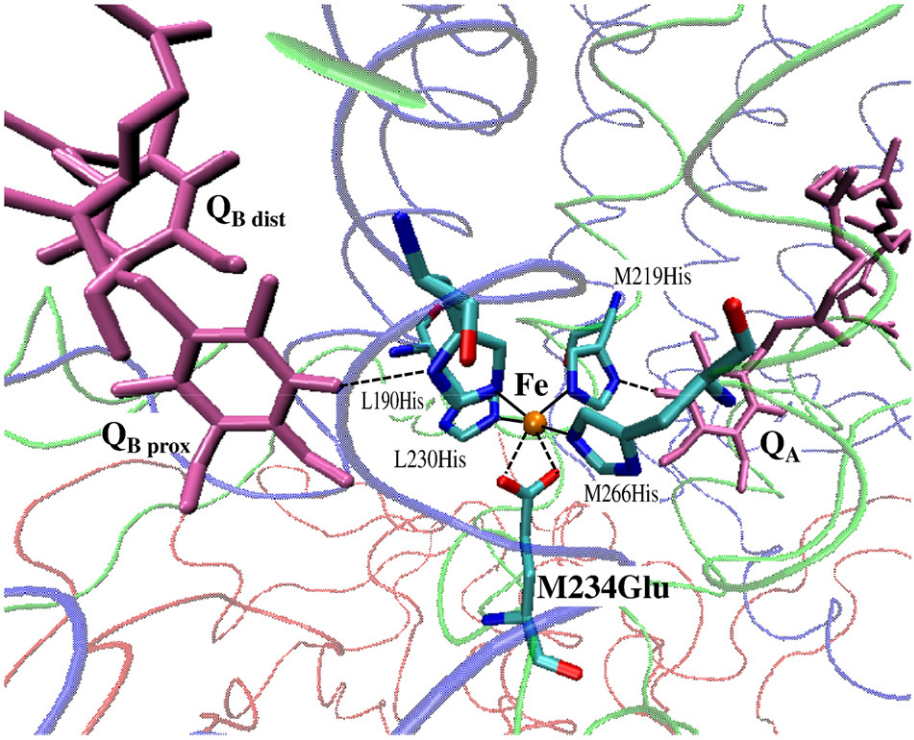

Fig. 1. Representation of the Fe-quinone region in the RC from *R. sphaeroides*. The 3D structure is taken from [1] (pdb 2J8C). The two quinones are represented in purple. The two positions of Q_B (distal and proximal) are represented. In the dark at pH 8, the distal and proximal occupancies respectively represent 0.35 and 0.65. The L, M and H subunits are respectively displayed in blue, green and red.

has been proposed as a structural and energetic connection of the two quinone pockets [14]. In addition to this "localized" connection, it has been suggested that broad, extended H-bond networks involving water molecules and protonatable and polar residues on the cytoplasmic side of the protein could also be involved in both the transient storage of protons and also in the proton uptake associated with the formation of both semiquinones, Q_A^- and Q_B^- [7,11]. All these structural motifs may be involved in the rate-limiting process (gating) associated with $Q_A^- \rightarrow Q_B$ ET. In particular, the role of M234Glu in the proton-coupled electron transfer reaction is still unknown [13].

In the WT RCs from *R. sphaeroides*, the charge recombination from the $P^+Q_A^-$ state occurs in about 100 ms through a tunneling ET process directly to the PQ_A ground state. When the native Q_A (ubiquinone$_{10}$) is replaced by a low potential quinone such as anthraquinone, or in the WT RCs from *Blastochloris viridis* (where Q_A is a menaquinone$_8$), $P^+Q_A^-$ decays *via* a thermal repopulation of a relaxed state of $P^+H_A^-$ [15–21].

The amplitudes of H^+/Q_A^- and H^+/Q_B^- proton uptake associated with the respective formations of Q_A^- and Q_B^- are due to shifts of the pK_as of proteic residues interacting by coulombic effects with the semiquinones [22,23]. Therefore, they are good probes of the internal electrostatic interactions within the protein. Moreover, since the dielectric constant in RCs is low [24–37], the electrostatic interactions extend to large distances resulting into a delocalized coupling of most of the involved protonatable residues. Recently, a signature of the presence of such delocalized interactions has been shown to be the high pH band (above ~9.0) of proton uptake [9]. Indeed, this band reveals the results of pairwise interactions displacing to higher pH the apparent highest pK_a of proton uptake. The absence of this band in variants lacking L212Glu, L209Pro, and M266His, and its recovery in variants modified near the Q_A site (M249Ala→Tyr) suggested the involvement of a broadly distributed network of interactions over the whole cytoplasmic side of the protein. This is in agreement with results obtained from Fourier Transform Infrared Spectroscopy [38–45].

Because of the central position of M234Glu, lying between the two quinones and binding the Fe atom, we have analyzed here the possible implication of this residue in the energetic balance between both quinone sites and therefore in the functioning of the coupled electron/proton transfer process in the RCs. We have targeted our study to two site-specific mutants, carrying respectively the M234Glu→His and M234Glu→Leu mutations. The former one was chosen in order to add a fifth His in the vicinity of the Fe. The latter was chosen as a bulky non-polar amino acid that is incapable of developing interactions with the Fe atom or allowing a cavity that could accommodate new water molecules.

2. Materials and methods

2.1. Bacterial strains and growth conditions

The design of the *R. sphaeroides* "wild type" was described previously [46]. Construction of mutant strains harboring the M234EL and M234EH mutations followed procedures discussed therein [47]. The cells were grown in Erlenmeyer flasks filled to 50% of the total volume with malate yeast medium [47] supplemented with kanamycin (20 μg/ml) and tetracycline (2 μg/ml). $ZnSO_4$ was present in the medium to supplement lower specificity iron in the mutant RCs as previously mentioned [48]. The cultures were grown in the darkness at 30 °C on a gyratory shaker (100 rpm).

2.2. Biochemical techniques

The WT, M234EL and M234EH RC variants were purified as previously described [9]. For the pH titration of kinetic parameters, buffers (10 mM) were used as follows: 2-(*N*-morpholino)-ethanesulfonic acid (MES; Sigma) between pH 5.5 and pH 6.5; 1,3-bis[tris(hydroxymethyl) methylamino]propane] (Bis–Tris propane; Sigma) between pH 6.3 and pH 9.5; Tris HCl (Sigma) between pH 7.5 and pH 9.0; 3-(cyclohexylamino) propanesulfonic acid (CAPS; Calbiochem) above pH 9.5.