FEBS Letters 472 (2000) 114–116 FEBS 23582

Structure of the photosynthetic reaction centre from *Rhodobacter sphaeroides* reconstituted with anthraquinone as primary quinone Q_A

Andreas Kuglstatter[a], Jaroslava Miksovska[b], Pierre Sebban[b], Günter Fritzsch[a,*]

[a] *Max-Planck-Institut für Biophysik, Frankfurt a.M., Germany*
[b] *Centre de Génétique Moléculaire, CNRS, Gif-sur-Yvette, France*

Received 1 November 1999; received in revised form 16 December 1999

Edited by Richard Cogdell

Abstract In the photosynthetic reaction centre (RC) from the purple bacterium *Rhodobacter sphaeroides*, the primary quinone, a ubiquinone-10 (Q_A), has been substituted by anthraquinone. Three-dimensional crystals have been grown from the modified RC and its structure has been determined by X-ray crystallography to 2.4 Å resolution. The bindings of the head-group from ubiquinone-10 and of the anthraquinone ring are very similar. In particular, both rings are parallel to each other and the hydrogen bonds connecting the native ubiquinone-10 molecule to AlaM260 and HisM219 are conserved in the anthraquinone containing RC. The space of the phytyl tail missing in the anthraquinone exchanged RC is occupied by the alkyl chain of a detergent molecule. Other structural changes of the Q_A-binding site are within the limit of resolution. Our structural data bring strong credit to the very large amount of spectroscopic data previously achieved in anthraquinone-replaced RCs and which have participated in the determination of the energetics of the quinone system in bacterial RCs.

Key words: Photosynthetic reaction center; X-ray structure; Primary quinone; Cofactor exchange

1. Introduction

In purple bacteria, the primary processes of photosynthesis occur in membrane-bound reaction centres (RCs) which catalyse light driven electron transfer across the membrane. Different structures of the RC from the photosynthetic bacterium *Rhodobacter sphaeroides* have been determined by X-ray crystallography [1–5]. This protein complex consists of three subunits called L, M and H, and which molecular weights range between 30 and 35 kDa. L and M which contain five transmembrane helices form the core of the RC and carry all pigments and cofactors: four bacteriochlorophylls, two bacteriopheophytins, two quinone, one non-heme iron atom and one carotenoid molecule are present. These molecules are arranged along the complex through two symmetrical branches ('A' and 'B'). Both branches span the membrane and 'start' at the level of the primary electron donor, a non-covalently linked bacteriochlorophyll-*a* dimer (D). Two monomeric bacteriochlorophyll-*a* (B_A and B_B), two bacteriopheophytin-*a* (Φ_A and Φ_B) and two ubiquinone-10 (Q_A and Q_B) molecules are symmetrically arranged.

The initial excitation energy is transferred from the light harvesting antenna to the primary electron donor D. This results into the creation of D*, the lowest singlet electronic excited state. D* is a highly reducing species and rapidly transfers an electron to Φ_A via B_A and then to the primary quinone Q_A in about 200 ps. The electron is transferred further to the secondary quinone Q_B in about 1–100 μs [6,7]. In contrast to Q_A, Q_B can accept two electrons in series. The negative charges are compensated by the uptake of two protons from the cytoplasm. Q_B is finally converted into ubiquinol (Q_BH_2), which dissociates from the RC and diffuses to the cytochrome bc_1 complex.

The different functional behaviour of Q_A and Q_B is likely to arise from the different structures of their respective binding protein pockets. Q_B is surrounded by many charged and polar residues whereas Q_A is situated in a relatively less hydrophilic environment. The head-group of Q_A is tightly bound to the M-subunit. This is in part due to the development of two hydrogen bonds between the two carbonyls of Q_A and the side chains of HisM219 and the N backbone of AlaM260. At variance, the mobile two-electron acceptor Q_B is loosely bound to the L-subunit. The possibility to deplete and replace both quinones of the RCs from *R. sphaeroides* [8,9] has led to biochemical, spectroscopic and theoretical analysis concerning the energetics of the two quinone electron acceptor system [9–19]. It has been shown that, in *R. sphaeroides* RCs, only quinone type molecules can substitute the native ubiquinone in the Q_A site and transfer electrons to Q_B [9]. At variance, non-quinolic compounds can occupy the Q_B site and may act as electron acceptors [18]. Special emphasis has been put on the replacement of the native Q_A by quinones with low redox midpoint potentials [11–14]. These experiments aimed to modify the free energy difference between Q_A^- and Q_B and/or Q_A^- and Φ_A, and therefore the driving force for the electron transfer process. In native RC from *R. sphaeroides*, when electron transfer from Q_A^- to Q_B is blocked (in Q_B-depleted RCs or in the presence of inhibitors of this electron transfer reaction), the $D^+Q_A^-$ state recombines directly to DQ_A, by an electron tunnelling process. Interestingly, when the redox potential of the substituted quinone is so low that the free energy gap between the $D^+Q_A^-$ state and the excited state D* becomes smaller than 0.8 eV, the $D^+Q_A^-$ recombination occurs by a thermally activated pathway repopulating the $P^+\Phi_A^-$ [12]. Consequently, the measure of the rate of charge recombination from the $P^+Q_A^-$ states in such modified RCs is very sen-

*Corresponding author. Fax: (49)-69-96769 423.
E-mail: fritzsch@mpibp-frankfurt.mpg.de

PII: S0014-5793(00)01447-2

sitive to the free energy gap between the $P^+Q_A^-$ and $P^+\Phi_A^-$ states. Many studies using this possibility to finely modulate the free energy level of $P^+Q_A^-$ have been reported [10,19]. However, it had never been verified if structural changes caused by the quinone analogues or the exchange procedure might not contribute to the observed effects in addition to the differences in redox potentials. It was therefore of high interest to verify at a structural level how a low potential quinone of very different size accommodates the Q_A-binding pocket and what could be the protein structural changes induced by this replacement. We present the results of an X-ray structure analysis of the RC from *R. sphaeroides* where the ubiquinone-10 in the Q_A-binding pocket has been substituted by anthraquinone.

2. Materials and methods

2.1. Preparation of RCs and reconstitution of Q_A

RCs of *R. sphaeroides* wild-type 2.4.1 (ATCC 17023) were prepared for crystallisation as described before (see e.g. [19,20]). Q_A-depleted RCs were prepared as described in [8] with slight modifications [22]. The reconstitution of the Q_A-binding site was achieved by adding anthraquinone (dissolved in dimethylsulfoxide) (10 mM Tris–HCl, 0.05% (v/v) LDAO, pH 8.0) to the RC suspension in a 10-fold excess. The yield of Q_A depletion was determined by measuring the absorbance change associated with the formation of $D^+Q_A^-$ after a flash. Typically, about 85–90% of the native quinone was replaced by AQ in 'modified RCs'.

2.2. Crystallisation, data collection and model refinement

Crystals were grown using vapour diffusion with potassium phosphate as precipitant [20,21]. After exchanging Q_A, the RCs were purified again on a DEAE Sepharose FF column. Only fractions with an OD_{280}/OD_{800} ratio ≤ 1.25 were used. The RCs were concentrated up to an $OD_{800} = 60$. Crystals were prepared under various conditions by vapour diffusion: mother liquor: 95 μM RC, 0.7 M potassium phosphate pH 7.0, 1% hexane-1,2,3-triol, 3% heptane-1,2,3-triol, 3% dioxan and 0.1% (v/v) LDAO. Reservoir: 1.6 M potassium phosphate. Trigonal crystals with a maximum size of $2.0\times1.0\times1.0$ mm^3 appeared after about 2 weeks. The space group is $P3_121$, and the unit cell dimension is $a = b = 141.7$ Å, $c = 187.2$ Å. The crystals diffracted X-rays to 2.4 Å resolution. One crystal has been used for data collection (5°C) on a 345 mm MAR-Research imaging plate detector at BW7B beamline of the DORIS storage ring at EMBL outstation, c/o DESY, Hamburg, Germany.

The data were processed with DENZO and reduced with the SCALEPACK package [23]. The structure was phased by rigid body refinement of the wild-type model [24] against the anthraquinone data. Initial coordinates of the anthraquinone molecule were taken from the Cambridge Structural Database [25]. Crystallographic refinement was completed using iterative cycles of isotropic overall B-factor refinement, bulk solvent correction, simulated annealing, conventional positional refinement, and individual B-factor refinement with the program package CNS [26] and manual rebuilding with the graphics program O [27]. Geometric parameters for the polypeptide chain of Engh and Huber [28] and for the cofactors of Treutlein et al. [29], Lancaster and Michel [30], and Ermler (unpublished) have been used for model refinement. The figures have been produced with the graphics program SETOR [31].

3. Results and discussion

Although the protein was subjected to a drastic chemical treatment, the crystal quality was identical to that of native RC. The crystal structure of the anthraquinone-modified RC at 2.4 Å resolution was determined by difference Fourier techniques and Simulated Annealing Omit Maps using coordinates of the trigonal crystal from native *R. sphaeroides* RC [32]. The structure has been refined to an R_{cryst} of 20.9% and an R_{free} of 23.1%. These values are considerably lower than those obtained from tetragonal crystals [5] which amounted to 22.0% and 27.0%, respectively. The data collection and refinement statistics are displayed in Table 1. Due to the residual occupancy of the Q_A-binding pocket with ubiquinone-10, the electron density from the anthraquinone molecule is less meaningful than for other parts of the modified RC. Nevertheless, the electron density map shows the position of the reconstituted anthraquinone unambiguously. Although the head-group of this quinone possesses two additional benzene rings compared to the native ubiquinone, it is easily accommodated by the Q_A-binding pocket.

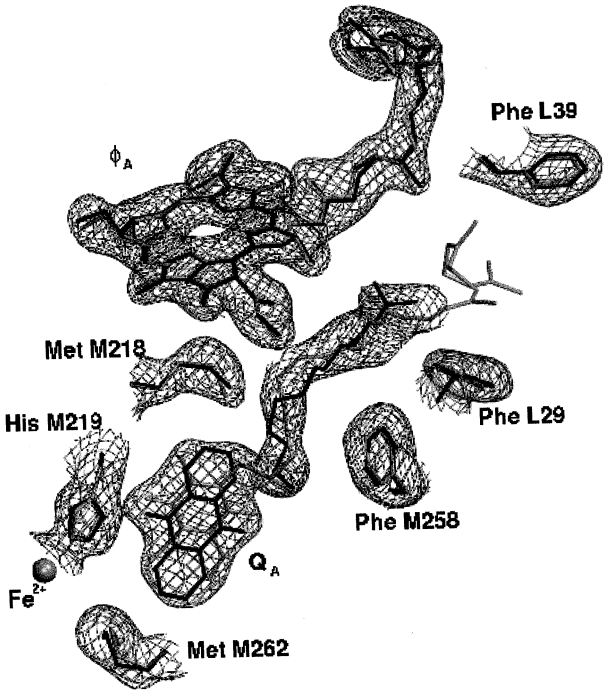

Fig. 1. The $2|F_{obs}|-|F_{calc}|$ electron density map of the RC–anthraquinone complex contoured at 1 σ. Shown are Q_A, Φ_A, Fe^{2+} and surrounding amino acid residues. Grey: the RC–anthraquinone complex including the additional LDAO molecule. Black: the native ubiquinone RC.

Fig. 1 shows details of the refined structure. The $(2|F_{obs}|-|F_{calc}|)$ map, contoured at 1 $\sigma(\rho)$, indicates a 1 Å

Table 1
Data collection and refinement statistics

Data processing	
Resolution range (Å)	50.0–2.4
Unique reflections	82 966
Completeness (%)	97.0
R_{sym} (%)	4.8
Mosaicity (°)	0.07
Model refinement	
Resolution (Å)	50.0–2.4
R_{free} (%)	23.1
R_{cryst} (%)	20.9
$\langle\|\Delta r\|\rangle_{cv}$ (Å)	
Luzzati	0.39
σ_A	0.30
$\langle B\rangle$ (Å^2)	52.6
rms	
bond distance (Å)	0.008
bond angle (°)	1.05

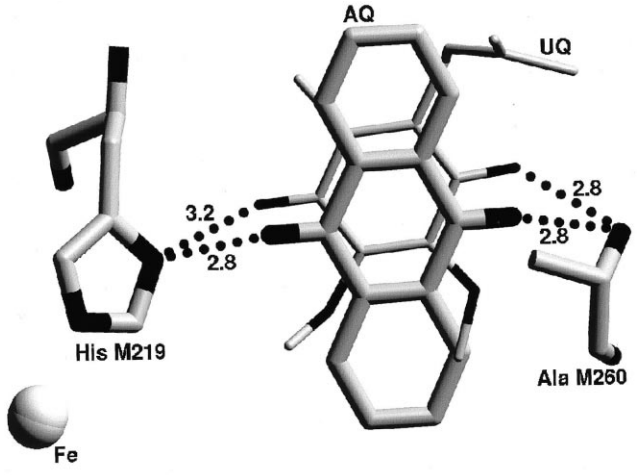

Fig. 2. The Q_A site of the anthraquinone-exchanged RC (thick) and of the native ubiquinone RC (thin). The hydrogen bond lengths are indicated in Å. The phytyl tails are omitted.

shift of the anthraquinone towards the cytoplasm in the Q_A-exchanged RC compared to the ubiquinone-10 of the native protein. The hydrogen bonds between the oxygens of ubiquinone-10 and the two amino acid residues HisM219 and AlaM260, respectively, are conserved. In the model, the length of the hydrogen bond between O^1 and AlaM260 is 2.8 Å as in the native RC, whereas the length between O^4 and HisM219 is reduced from 3.2 Å to 2.9 Å (Fig. 2). The positions and orientations of the amino acids forming the Q_A-binding pocket as well as the position of the iron ion are well conserved and the observed deviations are within the resolution limit. The alkyl chain of a bound detergent (LDAO) molecule is found at the position of the native ubiquinone–phytyl chain. The orientation of this LDAO molecule is not unique because the precise position of the LDAO head-group cannot be determined at the present resolution.

We have to mention that very similar results were obtained (data not shown) for the replacement of ubiquinone-10 in the Q_A-binding site by menaquinone-8 that functions as the primary quinone in the native *Rhodopseudomonas viridis* RC. The position and orientation of the menaquinone head-group are indistinguishable from those of the ubiquinone-10 molecule [22]. In these modified RCs, the hydrogen bond pattern of the native protein is conserved. These structural observations justify spectroscopic studies achieved with such modified RCs.

In this report, we show that well-diffracting crystals of the *R. sphaeroides* RC can be grown when the primary quinone Q_A has been replaced by an anthraquinone (and also menaquinone-8). The results indicate that substitution of ubiquinone-10 in the Q_A-binding pocket of the *R. sphaeroides* RC by other quinones does not cause any strong or long range structural changes. The differences between native and modified RC structures are restricted to the position of the quinone itself. However, the quinone rings in the native and the AQ-replaced RCs are parallel and the important H bonds conserved.

These results justify a posteriori the conclusions of spectroscopic observations that have been based on Q_A-replaced RCs. In particular, this especially concerns the studies of the Φ_A to Q_A electron transfer processes in relation to the 'Marcus' theory and works based on the analysis of the $P^+Q_A^-$ charge recombination state from which much of our understanding of the energetics of the Q_A–Q_B system has been derived.

Acknowledgements: We are grateful to Zlata Bojadzijev and Gregor Eichberger for technical assistance and Ulrich Ermler for help in data collection. We acknowledge help of Victor Lamzin in use of the DORIS storage ring facilities of the EMBL outstation, c/o DESY, Hamburg, Germany. The work was supported by PROCOPE Grant number 312/pro-gg.

References

[1] Allen, J.P., Feher, G., Yeates, T.O., Deisenhofer, J. and Huber, R. (1986) Proc. Natl. Acad. Sci. USA 83, 8589–8593.
[2] Chang, C.-H., Tiede, D., Tang, J., Smith, U., Norris, J. and Schiffer, M. (1986) FEBS Lett. 205, 82–86.
[3] Arnoux, B., Ducruix, A., Reiss-Husson, F., Lutz, M., Norris, J., Schiffer, M. and Chang, C.H. (1989) FEBS Lett. 258, 47–50.
[4] Ermler, O., Fritzsch, G., Buchanan, S.K. and Michel, H. (1994) Structure 2, 925–936.
[5] Stowell, M.H.B., McPhillips, T.M., Rees, D.C., Soltis, S.M., Abresch, E. and Feher, G. (1997) Science 276, 812–816.
[6] Tiede, D.M., Vázquez, J., Córdova, J. and Marone, P.A. (1996) Biochemistry 35, 10763–10775.
[7] Li, J., Gilroy, D., Tiede, D.M. and Gunner, M.R. (1998) Biochemistry 37, 2818–2829.
[8] Okamura, M.Y., Isaacson, R.A. and Feher, G. (1975) Proc. Natl. Acad. Sci. USA 72, 3492–3495.
[9] Gunner, M.R., Tiede, D.M., Prince, R.C. and Dutton, P.L. (1982) in: Function of Quinones in Energy Conserving Systems (Trumpower, B.L., Ed.), pp. 265–269, Acad. Press.
[10] Gunner, M.R. and Dutton, P.L. (1989) J. Am. Chem. Soc. 111, 3400–3412.
[11] Gunner, M.R. and Robertson, D.E. (1986) J. Phys. Chem. 90, 3183–3195.
[12] Woodbury, N.W., Parson, W.W., Gunner, M.R., Prince, R.C. and Dutton, P.L. (1986) Biochim. Biophys. Acta 851, 16–22.
[13] Shopes, R.J. (1987) Biochim. Biophys. Acta 893, 409–425.
[14] Sebban, P. (1988) FEBS Lett. 233, 331–334.
[15] Moser, C.C., Keske, J.M., Warncke, K., Farid, R.S. and Dutton, P.L. (1992) Nature 355, 796–802.
[16] Miksovska, J., Maroti, P., Tandori, J., Schiffer, M., Hanson, D.K. and Sebban, P. (1996) Biochemistry 35, 15411–15417.
[17] Kálmán, L. and Maróti, P. (1997) Biochemistry 36, 15269–15276.
[18] Giangiacomo, K.M. and Dutton, P.L. (1989) Proc. Natl. Acad. Sci. USA 86, 2658–2662.
[19] Graige, M.S., Paddock, M.L., Bruce, J.M., Feher, G. and Okamura, M.Y. (1996) J. Am. Chem. Soc. 118, 9005–9013.
[20] Buchanan, S.K., Fritzsch, G., Ermler, U. and Michel, H. (1993) J. Mol. Biol. 230, 1311–1314.
[21] Fritzsch, G. (1998) in: Photosynhesis: Molecular Biology of Energy Capture (McIntosh, L., Ed.), pp. 57–77, Acad. Press.
[22] Sebban, P. (1988) Biochim. Biophys. Acta 936, 124–132.
[23] Otwinowski, Z. and Minor, W. (1997) Methods Enzymol. 276, 307–326.
[24] Kapaun, G. (1997) Diploma thesis, J.W. Goethe-Universität, Frankfurt a.M.
[25] Allen, F.H. and Kennard, O. (1993) Chem. Des. Autom. News 8, 31–37.
[26] Brünger, A.T. et al. (1998) Acta Crystallogr. D 54, 905–921.
[27] Jones, T.A., Zou, J.-Y., Cowan, S.W. and Kjeldgaard, M. (1991) Acta Crystallogr. A 47, 110–119.
[28] Engh, R.A. and Huber, R. (1991) Acta Crystallogr. A 47, 392–400.
[29] Treutlein, H., Schulten, K., Brünger, A.T., Karplus, M., Deisenhofer, J. and Michel, H. (1992) Proc. Natl. Acad. Sci. USA 89, 75–79.
[30] Lancaster, C.R.D. and Michel, H. (1997) Structure 5, 1339–1359.
[31] Evans, S.V. (1993) J. Mol. Graph. 11, 134–138.
[32] Bundschuh, T. (1995) Diploma thesis, J.W. Goethe-Universität, Frankfurt a.M.