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Does different orientation of the methoxy groups of ubiquinone-10 in the reaction centre of *Rhodobacter sphaeroides* cause different binding at Q_A and Q_B?

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The different roles of ubiquinone-10 (UQ_{10}) at the primary and secondary quinone (Q_A and Q_B) binding sites of *Rhodobacter sphaeroides* R26 reaction centres are governed by the protein microenvironment. The 4C=O carbonyl group of Q_A is unusually strongly hydrogen-bonded, in contrast to Q_B. This asymmetric binding seems to determine their different functions. The asymmetric hydrogen-bonding at Q_A can be caused intrinsically by distortion of the methoxy groups or extrinsically by binding to specific amino-acid side groups. Different X-ray-based structural models show contradictory orientations of the methoxy groups and do not provide a clear picture. To elucidate if distortion of the methoxy groups induces this hydrogen-bonding, their (ring-)C-O vibrations were assigned by use of site-specifically labelled [5-^{13}C]UQ_{10} and [6-^{13}C]UQ_{10} reconstituted at either the Q_A or the Q_B binding site. Two infrared bands at 1288 cm^{-1} and 1264 cm^{-1} were assigned to the methoxy vibrations. They did not shift in frequency at either the Q_A or Q_B binding sites, as compared with unbound UQ_{10}. As the frequencies of these vibrations and their coupling are sensitive to the conformations of the methoxy groups, different conformations of the C(5) and C(6) methoxy groups at the Q_A and Q_B binding sites can now be excluded. Both methoxy groups are oriented out of plane at Q_A and Q_B. Therefore, hydrogen-bonding to His M219 combined with electrostatic interactions with the Fe^{2+} ion seems to determine the strong asymmetric binding of Q_A.

Keywords: electron transfer; Fourier-transform infrared spectroscopy; isotopic labelling; photosynthetic reaction centre; ubiquinone.

The photosynthetic reaction centre (RC) of the purple nonsulphur bacterium *Rhodobacter sphaeroides* is a transmembrane pigment–protein complex, the structure of which has been determined with up to 2.2 Å resolution [1–4]. Upon light excitation, an electron is transferred from the primary donor P (bacteriochlorophyll *a* dimer) via a monomeric bacteriochlorophyll *a* and a bacteriopheophytin *a* molecule to the primary quinone Q_A and finally to the secondary quinone Q_B. Although ubiquinone-10 (UQ_{10}) is found at Q_A and Q_B, the two molecules differ in function: Q_A is tightly bound to the RC. By accepting one electron, a semiquinone anion radical $Q_A^{-\bullet}$ is created which quickly transfers the electron to Q_B. Q_B is less tightly bound. After the formation of a nonprotonated semiquinone anion radical $Q_B^{-\bullet}$, a second electron and two protons are accepted here to form a hydroquinone (Q_BH_2), which is finally released from the RC; for a recent review see [5].

To elucidate the protein–cofactor interactions that determine the different functions of UQ_{10} at Q_A and Q_B, Fourier-transform infrared (FTIR) difference spectroscopy has been applied [6–9]. By the use of UQ_{10} specifically ^{13}C-labelled at the ring positions 1, 2, 3, and 4, the 1C=O and 4C=O and 2/3C=C stretching vibrations of UQ_{10} in the RC have been assigned in the $Q_A^- - Q_A$ and $Q_B^- - Q_B$ difference spectra [10–13]. At the Q_A site, the mode dominated by the 4C=O vibration is dramatically downshifted compared with unbound UQ_{10}, indicating unusually strong hydrogen-bonding to the protein environment [10,11]. In contrast, the 1C=O group is only weakly bound to the protein. This asymmetric binding is conserved in the charge-separated state [10,11]. At the Q_B site, two fractions of UQ_{10} are found. The minor fraction is loosely bound and almost unaffected by the protein. In the major fraction, both C=O vibrations show symmetric hydrogen-bonding, but weaker than the hydrogen bond of 4C=O at the Q_A site [12,13]. These results for the charge-separated state are supported by EPR [14] and NMR spectroscopy [15].

It is proposed that this difference in binding governs the different roles of UQ_{10} at the Q_A and Q_B sites. However, the molecular origin of the strong binding of the 4C=O group is not clear. The conformation of the C(5) and C(6) methoxy substituents of UQ_{10} may differ at both binding sites as

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Abbreviations: FTIR, Fourier-transform infrared; IR, infrared; LDAO, lauryldimethylamine N-oxide; Q_A, primary acceptor quinone; Q_B, secondary acceptor quinone; *Rb.*, *Rhodobacter*; RC, reaction centre; UQ_{10}, ubiquinone-10.

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