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STRUCTURE AND MECHANISM OF QUINOL:FUMARATE REDUCTASE, A RESPIRATORY MEMBRANE PROTEIN COMPLEX FROM *WOLINELLA SUCCINOGENES*

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The structure of the respiratory membrane protein complex quinol:fumarate reductase (QFR) from *Wolinella succinogenes* has been determined by X-ray crystallography at 2.2 A resolution [Lancaster et al., Nature 402, 377-385 (1999)]. Based on the structure of the three protein subunits A, B, and C and the arrangement of the six prosthetic groups (a covalently-bound FAD, three iron-sulfur clusters, and two haem b groups) a pathway of electron transfer from the quinol-oxidising dihaem cytochrome b in the membrane to the site of fumarate reduction in the hydrophilic subunit A has been proposed. Based on crystallographic analysis of three different crystal forms of the enzyme, indicating interdomain movement at the site of fumarate reduction, and the results from site-directed mutagenesis, we have derived a mechanism of fumarate reduction and succinate oxidation [Lancaster et al., Eur. J. Biochem. 268, 1820-1827 (2001], which should be generally relevant throughout the superfamily of succinate:quinone oxidoreductases. The structure of the membrane-integral dihaem cytochrome b reveals that, firstly, all transmembrane helical segments are tilted with respect to the membrane normal, secondly, the dihaem binding motif is very different from those of the cytochrome bc1 complex and of the formate dehydrogenase/nitrate reductase/hydrogenase group, and thirdly, the g-hydroxyl group has an important role in stabilizing a kink in transmembrane helix IV. By combining the results from site-directed mutagenesis, functional and electrochemical characterization, and X-ray crystallography, a residue was identified which was found to be essential for menaquinol oxidation. [Lancaster et al., Proc. Natl. Acad. Sci. U.S.A. 97, 13051-13056 (2000)]. The distal location of this residue in the structure indicates that the coupling of the oxidation of menaquinol to the reduction of fumarate in dihaem-containing succinate:quinone oxidoreductases could in principle be associated with the generation of a transmembrane electrochemical potential. However, it is suggested [Lancaster, Biochim. Biophys. Acta Special Issue on Membrane Protein Structure, submitted] that in W. succinogenes QFR, this electrogenic effect is counterbalanced by the transfer of two protons via a proton transfer pathway (the 'E-pathway') in concert with the transfer of two electrons via the membrane-bound haem groups. According to this 'E-pathway hypothesis', the net reaction catalysed by W. succinogenes QFR does not contribute directly to the generation of a transmembrane electrochemical potential.

Keywords: FUMARATE REDUCTASE SUCCINATE DEHYDROGENASE MEMBRANE PROTEIN

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TOWARD ACTIVATION MECHANISM OF GPCRs

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The first structure of seven transmembrane (7TM) receptor, bovine rhodopsin bound 11-cis-retinal revealed several interesting features with the common 7TM bundle in an inactive state. Additional amphiphilic short helix VIII locates immediate after 7th TM helix along to putative membrane surface. These helices VII and VIII are almost right angle supported by the aromatic ring stacking of Tyr306 and Phe313. N-Terminal domain folds compact in two-layered structure. The two layer composes of N-terminal region and the long loop between helices IV and V with the S-S bridge of Cys187 and Cys110 at the end of helix III, while all cytoplasmic loops are almost opening as the putative interface with the G-protein, transducin. Most of highly conserved amino-acid residues among rhodopsin family members are forming non-covalent bonding to support the inactive form. The GPCR-motif of Glu134-Arg135-Tyr136 interacts with Glu247 and Thr251 of helix VI at the cytoplasmic end of helix-III, and the hydrogen-bond network consists of Asn55, Asp83, Asn78 and Trp161. Many parts of seven transmembrane helices are irregular due to many Gly and Pro, residues in the 7TM-helices. In facts, α-helices II, VI and VII are kinked, furthermore, in helix VII several residues around Lys296 bound 11-cis-retinal by Schiff base is rather 310-helix. These bent-helices form spacious cavity more than the accommodation of the bound retinal, while the cytoplasmic side of the helices is tightly bundled. The reverse agonist, 11-cis-retinal configuration is bow shaped in 6s-cis, 12s-trans, and anti-C=N of Schiff base with a salt bridge of Asp113 directly.

Keywords: RHODOPSIN G-PROTEIN COUPLED RECEPTOR MEMBRANE PROTEIN

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STRUCTURE AND FUNCTIONAL MECHANISMS OF AQP1 WATER CHANNEL

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Water channels facilitate the rapid transport of water across cell membranes in response to osmotic gradients. These channels are believed to be involved in many physiological processes that include renal water conservation, neuro-homeostasis, digestion, regulation of body temperature and reproduction. Defects of these proteins have been associated with a number of medical disorders such as *Nephrogenic Diabetes Insipidus*.

The structure of AQP1 from bovine red blood cells at 2.2 Å resolution, as determined by x-ray crystallography, shows that the channel consists of an extracellular and a cytoplasmic vestibule connected by an extended narrow pore. The narrow pore contains a constriction filter that establishes the steric upper limit of the channel and three hydrophilic nodes that punctuate an otherwise extremely hydrophobic narrow pore. This channel design has revealed the basis for water transport specificity that utilizes both the size of the constriction and an appropriate combination of long hydrophobic pore and minimal number of water binding sites to facilitate water transport through the channel.

Keywords: WATER CHANNEL AQUAPORIN MEMBRANE PROTEIN

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EARLY STRUCTURAL REARRANGEMENTS IN THE PHOTOCYCLES OF BACTERIORHODOPSIN & SENSORY RHODOPSIN II

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Archeal rhodopsins are a family of heptahelical transmembrane proteins. All contain a buried retinal chromophore covalently bound to a conserved lysine in helix G. Upon light-activation retinal is isomerized from an all-trans to a 13-cis configuration, which initiate a sequence of specific spectroscopic and structural rearrangements. For bacteriorhodopsin this results in the vectorial transport of a proton across the cell membrane. For sensory rhodopsins II light initiates a signal, which is propagated to a tightly bound transducer molecule. This, in turn, triggers a phosphorylation cascade inducing a negative phototaxis response in the host archae. Comparisons are drawn between the early structural rearrangements in the photocycles of bacteriorhodopsin and sensory rhodopsin II that illustrate how slight modifications of their respective retinal binding pockets lead to subtle differences in the early relaxation of photo-isomerized retinal.

Keywords: MEMBRANE PROTEIN BACTERIORHODOPSIN SENSORY RHODOPSIN II