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Cryo-EM structure of the four-subunit *Rhodobacter sphaeroides* cytochrome *bc*$_1$ complex in styrene maleic acid nanodiscs

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Cytochrome *bc*$_1$ complexes are ubiquinol:cytochrome *c* oxidoreductases, and as such, they are centrally important components of respiratory and photosynthetic electron transfer chains in many species of bacteria and in mitochondria. The minimal complex has three catalytic components, which are cytochrome *b*, cytochrome *c*$_1$, and the Rieske iron–sulfur subunit, but the function of mitochondrial cytochrome *bc*$_1$ complexes is modified by up to eight supernumerary subunits. The cytochrome *bc*$_1$ complex from the purple phototrophic bacterium *Rhodobacter sphaeroides* has a single supernumerary subunit called subunit IV, which is absent from current structures of the complex. In this work we use the styrene–maleic acid copolymer to purify the *R. sphaeroides* cytochrome *bc*$_1$ complex in native lipid nanodiscs, which retains the labile subunit IV, annular lipids, and natively bound quinones. The catalytic activity of the four-subunit cytochrome *bc*$_1$ complex is threefold higher than that of the complex lacking subunit IV. To understand the role of subunit IV, we determined the structure of the four-subunit complex at 2.9 Å using single particle cryogenic electron microscopy. The structure shows the position of the transmembrane domain of subunit IV, which lies across the transmembrane helices of the Rieske and cytochrome *c*$_1$ subunits. We observe a quinone at the Q$_o$ quinone-binding site and show that occupancy of this site is linked to conformational changes in the Rieske head domain during catalysis. Twelve lipids were structurally resolved, making contacts with the Rieske and cytochrome *b* subunits, with some spanning both of the two monomers that make up the dimeric complex.

cytochrome *bc*$_1$ | quinone | *Rhodobacter sphaeroides* | evolution | photosynthesis

Significance

Cytochrome *bc*$_1$ complexes are found in mitochondria and many species of bacteria and are related to the cytochrome *b*$_6$*f* complexes of plants, algae, and cyanobacteria. The simplest cytochrome *bc*$_1$ complexes are comprised of three core proteins, but many have acquired supernumerary subunits during evolution. The cytochrome *bc*$_1$ complex of *Rhodobacter sphaeroides* has a unique supernumerary subunit, named subunit IV, which enhances its activity. We have determined the structure of the four-subunit cytochrome *bc*$_1$ complex from this model phototroph, which provides insight into how and why cytochrome *bc*$_1$ complexes evolved supernumerary subunits. Our structure also reveals the position of bound lipids and rationale for how the binding of quinones to the catalytic sites may coordinate the conformational changes that underpin the Q-cycle.

Cytochrome (cyt) *bc*$_1$ complexes are quinol:cytochrome *c* oxidoreductases (E.C. 1.10.2.2) and are central components of respiratory and photosynthetic electron transport chains in bacteria and mitochondria. These multisubunit complexes translocate protons across bioenergetic membranes, generating a proton-motive force (pmf) that can be used to directly power cellular functions (e.g., flagella rotation and substrate transport) or stored in a chemical form as adenosine triphosphate (ATP). Similar to the related cytochrome *b*$_6$*f* complex (cyt *b*$_6$*f*) found in chloroplasts and cyanobacteria, cyt *bc*$_1$ complexes operate via a modified Q-cycle, in which oxidation of two quinols at the Q$_o$ site (also known as Q$_P$) leads to the release of four protons on the positive side of the membrane, the reduction of soluble electron carriers in the lumen/intermembrane space, and the regeneration of a quinol at the Q$_i$ site (also known as Q$_n$) coupled to the uptake of two protons from the cytoplasm/matrix (1, 2). This bifurcated electron transfer mechanism increases the number of protons translocated per quinol oxidized and explains the high coupling efficiency of cyt *bc*$_1$ and cyt *b*$_6$*f* complexes. Quinol oxidation by these complexes is the rate-limiting step in photosynthetic electron transport, further underlying the central importance of cyt *bc*$_1$ and cyt *b*$_6$*f* in electron transfer chains (3–11).

The simplest cyt *bc*$_1$ complexes, such as the bacterial complexes from *Rhodobacter capsulatus* or *Paracoccus denitrificans,* comprise three subunits: cyt *b*, which binds two *b*-type hemes (*b*$_L$ and *b*$_H$) one quinol and one quinone; cyt *c*$_1$ that binds one *c*-type heme; and the Rieske subunit that binds a two-iron two-sulfur cluster (FeS). The two heme-binding domains are static during the Q-cycle, whereas the membrane-extrinsic head of the Rieske subunit is mobile and undergoes a conformational change during turnover (12, 13). These three catalytic subunits associate to form a dimer with C2 symmetry, and are common to all Rieske/cyt *b* complexes, but the mitochondrial cyt *bc*$_1$ complexes (known as complex III) have a varying number of supernumerary subunits, of which there are seven in yeast and plants and eight in mammals (14). In thermophilic bacteria such as *Aquifex aeolicus,* the cyt *c*$_1$ subunit has an additional N-terminal transmembrane helix (TMH) that provides a fused supernumerary component of the

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The authors declare no competing interest.

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