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Review

Gating and regulation of the cytochrome c oxidase proton pump ☆

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ABSTRACT

As a consumer of 95% of the oxygen we breathe, cytochrome c oxidase plays a major role in the energy balance of the cell. Regulation of its oxygen reduction and proton pumping activity is therefore critical to physiological function in health and disease. The location and structure of pathways for protons that are required to support cytochrome c oxidase activity are still under debate, with respect to their requirements for key residues and fixed waters, and how they are gated to prevent (or allow) proton backflow. Recent high resolution structures of bacterial and mammalian forms reveal conserved lipid and steroid binding sites as well as redox-linked conformational changes that provide new insights into potential regulatory ligands and gating modes. Mechanistic interpretation of these findings and their significance for understanding energy regulation is discussed. This article is part of a Special Issue entitled: Respiratory Oxidases.

1. Introduction

The proton pumping function of cytochrome c oxidase (CcO) has been the subject of intense study and controversy for many years, beginning with the question of whether CcO pumps protons at all [1]. While the pumping question is no longer in dispute and much progress has been made in defining the underlying mechanism of oxygen reduction, it remains unclear how this exergonic process drives proton translocation across a membrane. Part of the difficulty in addressing the issue relates to the apparently critical role of water in facilitating and gating the movement of protons [2–5]. Mutagenesis and crystallography are powerful tools for determining the structural features important for protein function, but they are less incisive when it comes to detecting the positions and roles of water molecules. Residues can be mutated but water cannot, and very high resolution crystal structures are necessary to reliably track the whereabouts of water. Obtaining the necessary resolution (usually better than 2.5 Å) with a membrane protein is a challenge. In this regard, some success has been achieved with CcO isolated from bovine and bacterial sources, clarifying some aspects of the pumping process but leading to conflicting conclusions regarding others.

A major physiological question that drives the quest to understand the pumping mechanism is: how is the efficiency of the process controlled? As the consumer of 95% of the oxygen we breathe and the terminal member of the mitochondrial electron transfer chain, cytochrome c oxidase is a major player in the energy equilibrium of organisms, contributing to the balance between ATP and heat production, exerting control over the level of aerobic metabolism, and affecting upstream production of reactive oxygen species [6,7]. The intrinsic efficiency of the CcO proton pump has been proposed to be regulated by the rate of proton backflow through the protein [8–12] possibly via reversal of the normal exit pathway for pumped protons. However, neither the exit nor the backflow pathway has been located, although various routes are postulated [5,13,14]. It is clear that defining the proton pathways and understanding what structural features determine the rates of flow, during uptake, exit and backflow, are of fundamental importance to understanding energy balance and metabolic control in health and disease.

This review will summarize the insights gained from studies on the *Rhodobacter* enzyme (RsCcO) focusing on evidence for the importance of conserved lipid and steroid binding sites in structure and regulation, and for a role of conformational change and water positioning in the gating of proton pathways.

2. Conserved lipid binding sites

The original RsCcO crystal structure ([15]; PDB ID: 1M56) revealed six phospholipids embedded in the structure, two buried in a cleft in subunit III and four surrounding the single transmembrane helix of subunit IV, in an arrangement that almost completely separated the latter subunit from interaction with the rest of the protein (Fig. 1). The structure was unprecedented in its demonstration of major involvement of lipid in a membrane protein's structural integrity.

Also striking were the images of lipids associated with the bovine enzyme: when structures were obtained at 1.8 Å resolution, a total of 13 different lipids per 13-subunit monomer were resolved and each binding site was specific for a particular lipid [16] (PDB ID: 2DYR) (Fig. 2).

The importance of specific lipid associations was further emphasized when a second structure of the *Rhodobacter* enzyme [17] (PDB

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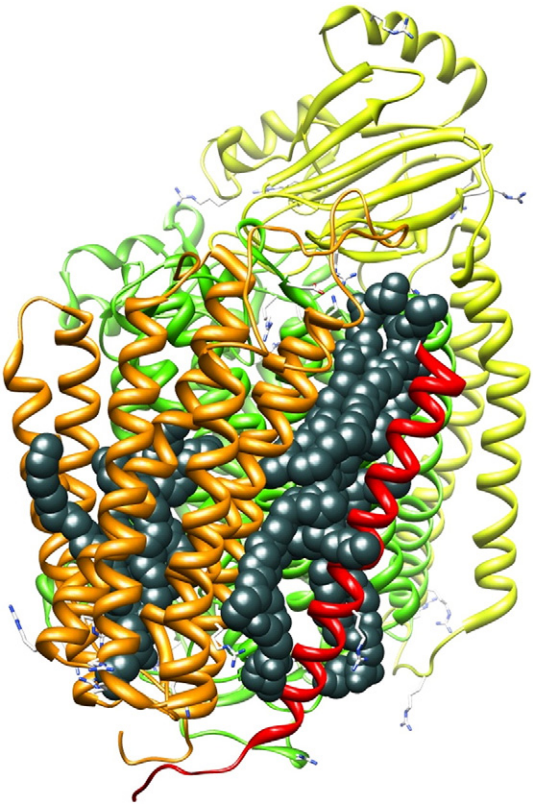

Fig. 1. *Rhodobacter sphaeroides* cytochrome c oxidase (RsCcO) showing associated lipid. Four subunits: I, green; II, yellow; III, orange; IV, red. Lipid, phosphatidylethanolamine (PE), indicated as dark gray spheres. Drawing created in Chimera (UCSF) from PDB ID: 1M56.

ID: 2GSM) was obtained at higher resolution (2.0 Å). This structure contained the two core subunits I and II, but was missing subunits III and IV that were initially seen as having the major lipid interactions. In the new structure, alkyl chains are seen embedded in the membrane domain of the protein surface, some with defined head groups of the sugar-containing detergents used in the purification and others without a resolved head group. Interestingly, the sites occupied by lipids and detergents in the RsCcO structure were found to overlay precisely with positions occupied by lipid or detergent in both *Paracoccus* and bovine CcO. Analysis of the residues that created the grooves holding the alkyl chains also showed a high level of conservation in all three structures [18], emphasizing the significance of the lipid binding sites. Such conserved sites have also been noted in other membrane proteins [19–22]. What these specifically-bound lipids contribute to the structural and functional properties of intrinsic membrane proteins remains to be clarified. A possibility is that they could act as flexible caulking between subunits and helices, shielding internal water channels while allowing conformational change. In fact, both proton uptake pathways, D and K, when inhibited by removal of a carboxyl at their entrance, can be chemically rescued by addition of μmolar levels of lipidic molecules with a carboxyl group, indicating a lipid binding site in close proximity [8,23,24].

Another interesting facet of crystallographically-defined, specific lipid binding sites is the observation that certain detergents, particularly sugar-based detergents, are capable of substituting for lipid molecules in these sites. The positioning of the sugar head groups indicates that they are particularly good substitutes because they stack and hydrogen-bond effectively with the aromatic residues that are concentrated in the region of the protein at the membrane interface [17]. This stable interaction may account for their unusual success in purifying [25,26] and crystallizing membrane proteins [27].

3. A conserved steroid binding site

Of even greater significance with respect to the function and regulation of CcO is the discovery that one of the steroid/bile salt binding sites first recognized in the bovine crystal structure [28] and attributed to possible nucleotide binding, is also conserved in the bacterial enzyme [24]. In both cases, bile salts bound in this site appear to influence the activity of CcO by interacting with a carboxyl residue ($E101_{II}$ in Rs, $E62_{II}$ in bovine) at the entrance to a key proton uptake route, the K pathway (Fig. 3).

Initial clues about the existence of this site in RsCcO came from studies of a mutant in which the conserved carboxyl, $E101_{II}$, is replaced with an

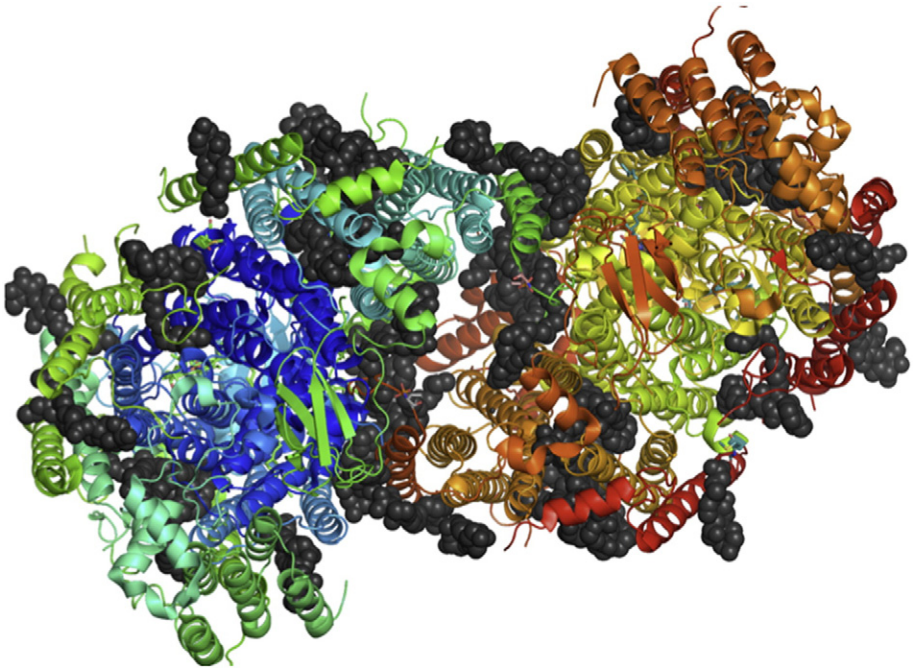

Fig. 2. Bovine cytochrome c oxidase dimer at 1.8 Å showing associated lipids (dark gray spheres). Drawing created in Pymol from PDB ID: 2DYR.