Biochimica et Biophysica Acta xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

Reaction of wild-type and Glu243Asp variant yeast cytochrome *c* oxidase with O_2 ☆,☆☆

Linda Näsvik Öjemyr [a], Amandine Maréchal [b], Henrik Vestin [a], Brigitte Meunier [c], Peter R. Rich [b], Peter Brzezinski [a,*]

[a] Department of Biochemistry and Biophysics, The Arrhenius Laboratories for Natural Sciences, Stockholm University, SE-106 91 Stockholm, Sweden
[b] Glynn Laboratory of Bioenergetics, Institute of Structural and Molecular Biology, University College London, Gower Street, London WC1E 6BT, UK
[c] Centre de Génétique Moléculaire du CNRS, UPR 3404, avenue de la Terrasse, 91198 Gif-sur-Yvette Cedex, France

ARTICLE INFO

Article history:
Received 5 December 2013
Received in revised form 7 March 2014
Accepted 21 March 2014
Available online xxxx

Keywords:
Electron transfer
Membrane protein
Respiration
Redox reaction
Metalloprotein
Cytochrome aa_3

ABSTRACT

We have studied internal electron transfer during the reaction of *Saccharomyces cerevisiae* mitochondrial cytochrome *c* oxidase with dioxygen. Similar absorbance changes were observed with this yeast oxidase as with the previously studied *Rhodobacter sphaeroides* and bovine mitochondrial oxidases, which suggests that the reaction proceeds along the same trajectory. However, notable differences were observed in rates and electron-transfer equilibrium constants of specific reaction steps, for example the ferryl (**F**) to oxidized (**O**) reaction was faster with the yeast (0.4 ms) than with the bovine oxidase (~1 ms) and a larger fraction Cu_A was oxidized with the yeast than with the bovine oxidase in the peroxy ($\mathbf{P_R}$) to **F** reaction. Furthermore, upon replacement of Glu243, located at the end of the so-called D proton pathway, by Asp the $\mathbf{P_R} \rightarrow \mathbf{F}$ and $\mathbf{F} \rightarrow \mathbf{O}$ reactions were slowed by factors of ~3 and ~10, respectively, and electron transfer from Cu_A to heme *a* during the $\mathbf{P_R} \rightarrow \mathbf{F}$ reaction was not observed. These data indicate that during reduction of dioxygen protons are transferred through the D pathway, via Glu243, to the catalytic site in the yeast mitochondrial oxidase. This article is part of a Special Issue entitled: 18th European Bioenergetic Conference.

1. Introduction

In aerobic organisms electrons that are extracted from nutrients are transferred via a series of membrane bound respiratory chain proteins to dioxygen. The free energy is conserved by translocation or pumping of protons (or in some cases sodium ions) across the membrane and stored in the electrochemical proton gradient. This is used, for example, for transmembrane transport or synthesis of ATP. In mammalian and *Saccharomyces* (*S.*) *cerevisiae* yeast mitochondria, O_2 reduction is catalyzed by cytochrome *c* oxidases (CytcOs) with electrons delivered by cytochrome *c*. The first electron acceptor from cytochrome *c* is Cu_A, found in SU II, and electrons are then transferred within SU I via heme *a* to the bimetallic catalytic oxygen-binding site, consisting of heme a_3 and Cu_B. These heme-copper oxidases are members of a diverse range of homologous oxidoreductases [1,2]. The mammalian and *S. cerevisiae* CytcOs belong to the large A-class of oxidases, which also includes aa_3-type CytcOs from many bacteria such as *Rhodobacter sphaeroides* and *Paracoccus denitrificans* [1,2]. Their O_2 reduction reaction is energetically linked to proton pumping across the membrane, from the negative (*n*) side to the positive (*p*) side, with a typical stoichiometry of one pumped proton per electron transferred to O_2.

In the bacterial A-class oxidases, two proton pathways, denoted by letters D (after a conserved Asp residue at the orifice) and K (after a conserved Lys residue within the pathway), have been identified based on functional and structural studies. The K pathway transfers 1–2 protons upon reduction of the catalytic site, while the D pathway transfers the remaining substrate protons to the catalytic site as well as all pumped protons. The structure and function of CytcO have been reviewed, for example, in [3–12].

To date most investigations of the mitochondrial CytcOs have been performed with bovine heart CytcO (*Bos taurus*, *Bt*CytcO). However, in recent years *S. cerevisiae* yeast CytcO (*Sc*CytcO) has emerged as a model mitochondrial CytcO in which effects of mutations on function can be studied [13,14] (Fig. 1A). Both D and K pathways are conserved between these two CytcOs, apart from minor differences that are unlikely to be functionally relevant [13]. In *Sc*CytcO the D pathway starts near Asp92 (corresponding to Asp132 in the *R. sphaeroides* CytcO (*Rs*CytcO)

Abbreviations: CytcO, cytochrome *c* oxidase; *n* side, negative side of the membrane; *p* side, positive side of the membrane; **R**, the four-electron reduced CytcO; **A**, reduced CytcO with O_2 bound to heme a_3; $\mathbf{P_R}$, the "peroxy" state formed after transfer of a third electron to the catalytic site; **F**, the ferryl state formed at the catalytic site after protonation of $\mathbf{P_R}$; **O**, the oxidized CytcO; SU, subunit; WT, wild-type

☆ This article is part of a Special Issue entitled: 18th European Bioenergetic Conference.

☆☆ Funding: These studies were supported by grants from the Swedish Research Council (grant number 2010-4987), the Knut and Alice Wallenberg Foundation, the Biotechnology and Biological Sciences Research Council (grant number BB/K001094/1) and EU COST Action 0902.

* Corresponding author. Tel.: +46 70 609 2642; fax: +46 8 153679.
E-mail address: peterb@dbb.su.se (P. Brzezinski).

http://dx.doi.org/10.1016/j.bbabio.2014.03.012
0005-2728/

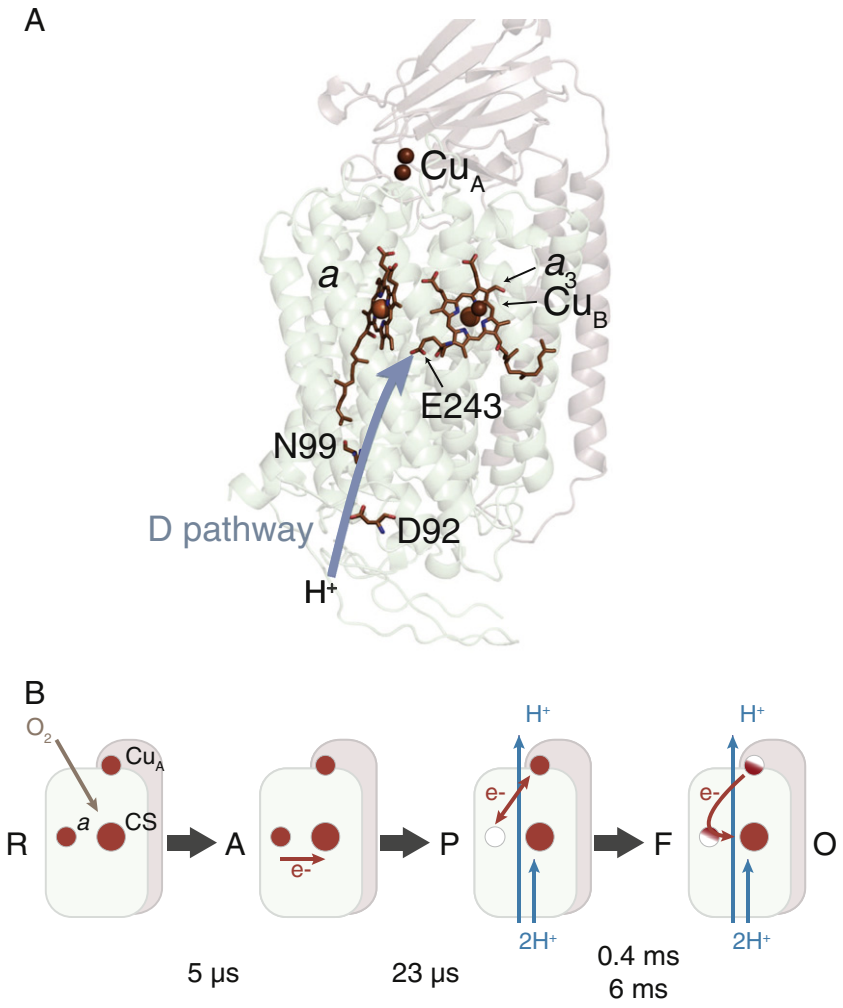

Fig. 1. (A) A structural model of subunits I and II of the *Sc*CytcO, based on the X-ray crystal structure of the equivalent subunits of the bovine mitochondrial enzyme. Shown is the D pathway used for proton uptake during O_2 reduction. (B) A schematic illustration of the reaction of the reduced oxidase with O_2, based on earlier results with e.g. the *Rs*CytcO and *Bt*CytcO. The filled and empty circles represent the reduced and oxidized redox centers, respectively. Flash photolysis of CO from the four-electron reduced CytcO yields the reduced enzyme, **R** (CS is the catalytic site, i.e. heme a_3 and Cu_B). Binding of O_2 to heme a_3 results in formation of **A**. Next, an electron is transferred from heme *a* to the catalytic site forming state $\mathbf{P_R}$. During the $\mathbf{P_R} \rightarrow \mathbf{F}$ reaction at the catalytic site there is a fractional electron transfer from Cu_A to heme *a*, proton uptake to the catalytic site and proton pumping across the membrane. Finally, in the last step of the reaction the electron from the Cu_A–heme *a* equilibrium is transferred to the catalytic site, linked to proton uptake and proton pumping across the membrane. The time constants are from the present study.

and Asp91 in *Bt*CytcO) at the *n* side of the membrane and spans a distance of ~25 Å towards the *p* side to Glu243 (Glu286 and Glu242 in the *R. sphaeroides* and bovine CytcOs, respectively). In *Bt*CytcO a third, H (His) pathway was suggested to be used for the transfer of the pumped protons [15–17]. Although there are structural differences between the *Sc*CytcO and *Bt*CytcO at the entry and exit regions of the H pathway, the membrane-spanning part appears to be similar [13,14]. This pathway is less obvious in the bacterial CytcOs and substitution of amino acids at positions equivalent to those of the H pathway in the *Bt*CytcO indicated that these residues are not involved in proton transfer [18,19].

The kinetics of electron transfer and associated proton uptake through the D pathway have been investigated, for example, using a "flow-flash" approach that involves pre-reduction of all metal sites in the presence of carbon monoxide (CO), which binds to heme a_3, followed by flash photolysis of the CO ligand after addition of O_2 (for review, see [7,8,10,20–22]) (Fig. 1B). O_2 binds with a time constant of ~10 µs at 1 mM O_2 to form ferrous-oxo compound **A**, followed in time by electron transfer from heme *a* to the catalytic site with a time constant of 30–70 µs (depending on the CytcO source). This results in O – O bond cleavage and formation of a ferryl intermediate that displays an absorption maximum at 607 nm, termed $\mathbf{P_R}$. In the next step, two protons are taken up from the *n*-side solution; one is transferred to the catalytic site to form the ferryl state (termed **F**) with an absorption maximum at 580 nm while the other is transferred through a "pump site" (presumably located "above" the hemes) and into to the *p*-side aqueous phase. In the bacterial A-type oxidases, both of these protons are thought to be transferred through the D pathway to Glu286 (*Rs*CytcO numbering, Glu243 in *Sc*CytcO), which is the branching point for connection to both the catalytic site and the proton exit pathways. Both proton uptake reactions and proton release to the *p*-side display the same time constant of ~100 µs at pH 7 [23,24]. Over the same timescale there is a fractional electron transfer from Cu_A to heme *a*. Finally, the remaining electron in the equilibrated Cu_A–heme *a* is transferred with a time constant of ~1 ms into the catalytic site, converting the **F** state into the oxidized (**O**) form. As for the $\mathbf{P_R} \rightarrow \mathbf{F}$ reaction, this $\mathbf{F} \rightarrow \mathbf{O}$ step is also linked in time to the uptake of two protons from the *n* side and release of one pumped proton to the *p* side.

With the bacterial A-type oxidases replacement of either Asp132 or Glu286 (*Rs*CytcO numbering; Asp92 or Glu243, in *Sc*CytcO) by non-protonatable analogs Asn or Gln, respectively, results in almost a complete loss of enzymatic activity and impaired proton uptake from

Please cite this article as: L. Näsvik Öjemyr, et al., Reaction of wild-type and Glu243Asp variant yeast cytochrome c oxidase with O_2, Biochim. Biophys. Acta (2014), http://dx.doi.org/10.1016/j.bbabio.2014.03.012