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Isolation of yeast complex IV in native lipid nanodiscs

Irina A. Smirnova ^{a, d} ... Peter Brzezinski ^a  

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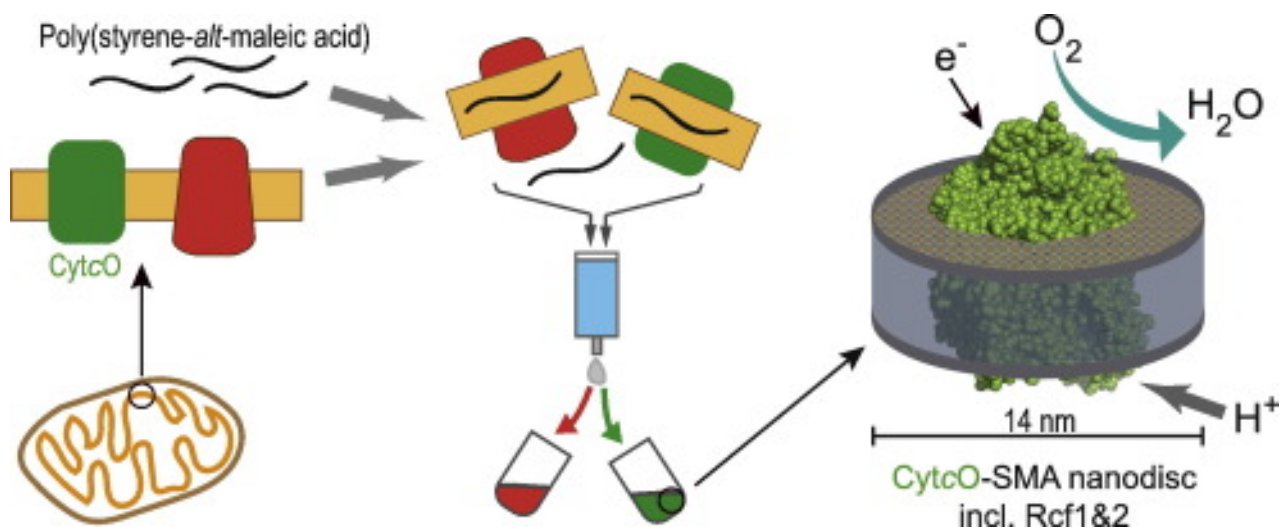
Highlights

- Styrene maleic acid (SMA) was used to purify cytochrome *c* oxidase without detergent.
- The native nanodiscs contained one cytochrome *c* oxidase and ~100 lipids per disc.
- The same major phospholipids as those found in the native membrane were isolated.
- The discs contained Rcf1 and Rcf2, but no cytochrome *bc*₁.
- Cytochrome *c* oxidase in the lipid discs was fully active.

Abstract

We used the amphipathic styrene maleic acid (SMA) co-polymer to extract cytochrome *c* oxidase (Cyt *c*O) in its native lipid environment from *S. cerevisiae* mitochondria. Native nanodiscs containing one Cyt *c*O per disc were purified using affinity chromatography. The longest cross-sections of the native nanodiscs were 11–14 nm. Based on this size we estimated that each Cyt *c*O was surrounded by ~100 phospholipids. The native nanodiscs contained the same major phospholipids as those found in the mitochondrial inner membrane. Even though Cyt *c*O forms a supercomplex with cytochrome *bc*₁ in the mitochondrial membrane, cyt. *bc*₁ was not found in the native nanodiscs. Yet, the loosely-bound Respiratory SuperComplex factors were found to associate with the isolated Cyt *c*O. The native nanodiscs displayed an O₂-reduction activity of ~130 electrons per Cyt *c*O and the kinetics of the reaction of the fully reduced Cyt *c*O with O₂ was essentially the same as that observed with Cyt *c*O in mitochondrial membranes. The kinetics of CO-ligand binding to the Cyt *c*O catalytic site was similar in the native nanodiscs and the mitochondrial membranes. We also found that excess SMA reversibly inhibited the catalytic activity of the mitochondrial Cyt *c*O, presumably by interfering with cyt. *c* binding. These data point to the importance of removing excess SMA after extraction of the membrane protein. Taken together, our data shows the high potential of using SMA-extracted Cyt *c*O for functional and structural studies.

Graphical abstract





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Abbreviations

Bis-Tris, 2,2-Bis(hydroxymethyl)-2,2,2-tris(nitrotriethanol); cyt. *c*, cytochrome *c*; Cyt *c*O, cytochrome *c* oxidase; DDM, *n*-dodecyl β -D-maltoside; EDTA, ethylenediaminetetraacetic acid; HEPES, 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid; MES, 2-(N-Morpholino)ethanesulfonic acid; PMS, phenazine methosulfate; SDS, sodium dodecyl sulfate; SMA, poly(styrene-*alt*-maleic acid); TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine; Tris, Tris(hydroxymethyl)aminomethane

Keywords

Bioenergetics; Proton transfer; Membrane protein; Energy conservation

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- ¹ Current address: Department of Neurology, UCSF School of Medicine, San Francisco, CA 94143, USA; Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94158, USA.

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