Unexpected outcome of surface-engineering an integral membrane protein: Improved crystallization of cytochrome *ba*$_3$ from *Thermus thermophilus*

Bin Liu, V. Mitch Luna, Ying Chen, C. David Stout, James A. Fee
Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA

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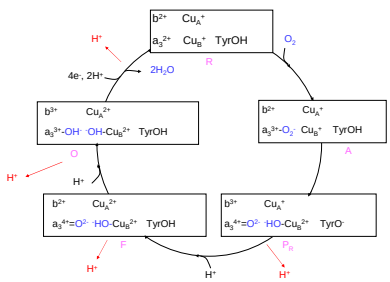
Abstract

Crystallization and the resolution of X-ray diffraction are general limiting factors in membrane protein crystallography. Past works have shown that it is feasible to mutate surface residues of soluble proteins in order to improve their crystallization behavior. Here we demonstrate a successful application of this approach to an integral membrane protein. Two mutant forms of *T. thermophilus* cytochrome *ba*$_3$ oxidase (I-K258R and I-K258R/II-E4Q) were created in which observed symmetrical crystal contacts were modified. These proteins had greatly shortened crystallization times, decreasing from ~30 days for wild-type to 1-3 days for the mutants, and crystallization was highly reproducible. The wild-type and His-tagged recombinant proteins crystallize in space group $P4_32_12$ whereas the mutant proteins crystallize in space group $P4_12_12$ with a new packing arrangement. The $P4_32_12$ crystal form diffracts occasionally to 2.4-2.3 Å resolution, following controlled dehydration, while the $P4_12_12$ crystal form diffracts routinely between 3.0 and 2.6 Å for crystals that are cryoprotected but not dehydrated.

Background and proposed mechanism

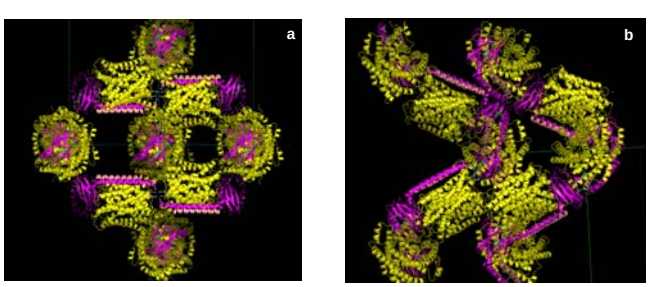
- Complex IV of the respiratory chain
- Site of O_2 reduction to water
- Proton pump
- Adds to electrochemical gradient used to drive ATP synthesis



Experimental procedure

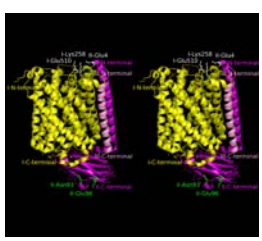
- Performed site-directed mutagenesis of recombinant cytochrome *ba*$_3$ oxidase (II-E4Q and I-K258R).
- Purified mutant recombinant cytochrome *ba*$_3$ oxidase from Thermus thermophilus as previously discribed[1, 2].
- Crystallized oxidized mutant recombinant cytochrome *ba*$_3$ oxidase under the previously used conditions[3, 4].
- Checked to show fully active in a standard cytochrome *c* oxidase assay.
- Collected datasets at SSRL.
- Determined 3-dimensional structure of cytochrome *ba*$_3$ oxidase with single or double mutation using model 1XME (PDB code).
- Analyzed intermolecular contacts (symmetry generated) using CCP4 program CONTACT with a limit of 0 to 3.1 Å in contact distance.
- We are currently screening for new conditions that retain new crystallization properties but have enhanced diffraction and trying dehydration.
- New surface residues modifications are being developed.

New lattice packing



(**a**) Packing of fourteen molecules of recombinant, wild type (1xme) cytochrome *ba*$_3$ in the left-handed space group $P4_32_12$; five cylinders facing the reader are dimers while two dimers lie horizontally in the plane. (**b**) Packing of ten molecules of either single- or double-mutant cytochrome *ba*$_3$ in the right-handed space group $P4_12_12$; sets of five molecules exhibit the 4_1-screw axis symmetry. The cyan or green box in **a** or **b** represents the unit cell. In both **a** and **b**, the view is along the (110) direction (unit cell diagonal in the a×b plane) and the c-axis is vertical.

Results



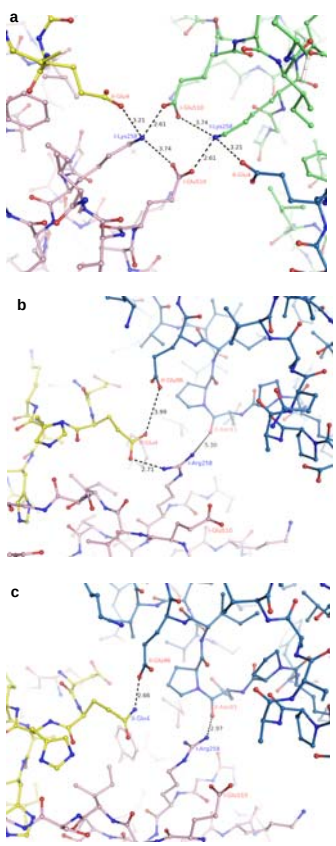
Stereo-view of wild type cytochrome *ba*$_3$ oxidase structure (wall-eye stereo): Three subunits are labeled using colors: yellow for subunit I, magenta for subunit II, and pink for subunit IIa. N and C termini of the three subunits are labeled. I-K258, I-E510, and II–E4 are shown with white sticks. II-E96 and II-N93 are demonstrated with green sticks.

Crystals of wild-type *ba*$_3$ oxidase (**a**) with rhomb-like shape and crystals of I-K258R (**b**) and I-K258R/II-E4Q (**c**) with rhomb and prism-like shapes are shown. The crystals are ~0.2 mm along their longest axis.

Table 1

	Wild-type	I-K258R	I-K258R/II-E4Q
PDB code	(1xme)	(2qpd)	(2qpe)
Space group	$P4_12_12$	$P4_12_12$	$P4_12_12$
Cell dimensions (Å , *a, b, c*)	114.90, 114.90, 177.06	115.19, 115.19, 149.14	114.64, 114.64, 148.57
Unit cell volume (1x10^6 Å^3)	2.338	1.979	1.953
Matthews coefficient (Å ^{3}/dalton)	3.44	2.91	2.87
Solvent content (%)	64.24	57.76	57.19
Protein concentration (mM) †	5.68	6.71	6.80
Crystal morphology	Tetragonal bipyramidal	Rhomb shaped	Prismatic

Effect of the mutations on the crystallization of *ba*$_3$ oxidase, † Protein concentration in a unit cell calculated from the cell parameter and a MW of about 85 kDa.



Lattice contacts of cytochrome *ba*3 oxidase are shown. In wild-type structure (**a**), two salt bridges (2.61Å in distance) between I-K258/I-E510 and I-E510/I-K258 in the symmetric mate were observed. In addition, hydrogen bonds are observed involving I-K258, I-E510 and II-E4 within each molecule. For I-K258R mutant (**b**), a salt bridge (2.71 Å) between I-R258 and II-E4 is formed while these residues are now proximal to subunit II of *ba*$_3$. In the I-K258R/II-E4QR mutant structure (**c**), two new hydrogen bonds were demonstrated: between II-Q4 and symmetric II-E96 (2.66 Å) and between I-R258 and symmetric II-N93 (2.97 Å). The latter interaction with the main chain of the Cu_A domain (subunit II) may contribute to the improved diffraction resolution of the double mutant. Subunit I and II in the reference molecule are labeled with pink and yellow respectively. For the symmetric mate, they are labeled with green and blue respectively.

Selected references

- 1. Soulimane, *et. al.* (2000) *EMBO J.*, **19**, 1766-1776.
- 2. Chen, *et al.* (2005) *Protein Expression and Purification,* **40,** 299-318.
- 3. Hunsicker-Wang, *et al.* (2005), *Acta. Cryst.*, **D61**, 340-343.
- 4. Liu, *et al*. (2007) *Acta. Cryst*. **F**, In press.