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 b₅₆₇ 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 P₄₂₂₂ whereas the mutant proteins crystallize in space group P₄₂₂₂ with a new packing arrangement. The P₄₂₂₂ crystal form diffracts occasionally to 2.4-2.3 Å resolution, following controlled dehydration, while the P₄₂₂₂ crystal form diffracts routinely between 3.0 and 2.6 Å for crystals that are cryoprotected but not dehydrated.

Experimental procedure
- Performed site-directed mutagenesis of recombinant cytochrome b₅₆₇ oxidase (II-E4Q and I-K258R).
- Purified mutant recombinant cytochrome b₅₆₇ oxidase from Thermus thermophilus as previously described1, 2.
- Crystalized oxidized mutant recombinant cytochrome b₅₆₇ oxidase under the previously used conditions3, 4.
- Checked to show fully active in a standard cytochrome c oxidase assay.
- Analyzed intermolecular contacts (symmetry generated) with CCP4 program CONTACT with a limit of 0 to 3.1 Å in contact distance.
- Collected datasets at SSRL.
- Determined 3-dimensional structure of cytochrome b₅₆₇ oxidase with single or double mutation using model 1XM1 (PDB code).
- Analyzed intermolecular contacts (symmetry generated) with 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 b₅₆₇ in the left-handed space group P₄₂₂₂; 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 b₅₆₇ in the right-handed space group P₄₂₂₂; sets of five molecules exhibit the 4₃-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-axes is vertical.

Table 1

<table>
<thead>
<tr>
<th>Protein</th>
<th>Wild-type</th>
<th>I-K258R</th>
<th>I-K258R/II-E4Q</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein concentration (mM)</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Solvent content (%)</td>
<td>64.24</td>
<td>57.76</td>
<td>57.19</td>
</tr>
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<td>Unit cell volume (Å³)</td>
<td>1.979</td>
<td>1.953</td>
<td>1.923</td>
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<tr>
<td>Crystal morphology</td>
<td>Tetragonal</td>
<td>Rhomb</td>
<td>Prismatic</td>
</tr>
<tr>
<td>Space group</td>
<td>P₄₁₂₂₂</td>
<td>P₄₁₂₂₂</td>
<td>P₄₁₂₂₂</td>
</tr>
</tbody>
</table>

Results

The site of O₂ reduction to water is the CuB site and is involved in the reduction chain.

Selected references

Background and proposed mechanism
- Complex IV of the respiratory chain
- Site of O₂ reduction to water
- Proton pump
- Adds to electrochemical gradient used to drive ATP synthesis

Effect of the mutations on the crystallization of b₅₆₇ oxidase. Protein concentration in a unit cell calculated from the cell parameter and a MW of about 85 kDa.

Crystals of wild-type b₅₆₇ oxidase (a) with ribbon-like shapes and crystals of I-K258R (b) and I-K258R/II-E4Q (c) with dendritic and prismatic shapes are shown. The crystals are ~0.2 mm along their longest axis.

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