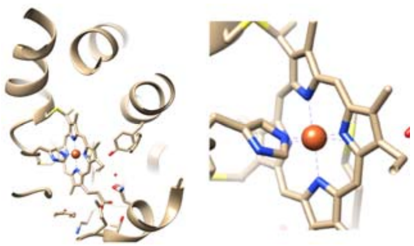
The Role of Cytochrome c in the Electron Transport Chain

Rebecca Rosamond, Ashleigh Keeler, Josh Diaz
Texas A&M University, College Station, TX 77843

A&M CHEMISTRY TEXAS A&M UNIVERSITY

Introduction

Iron is an important element for sustaining life. Iron appears in many different forms in the body, one of which is in a heme type protein, a cytochrome. These cytochromes bind heme as a cofactor and function as electron transfer agents, most commonly in the electron transport chain. The electron transport chain (ETC) is a series of complexes and molecules that transfer electrons from donors to acceptors via redox reactions coupled with the transport of protons across the inner mitochondrial membrane to create a concentration gradient.[1] This gradient is then used to supply the energy for ATP synthase to generate ATP, the principle molecule for providing energy to cells. The complexes and molecules the ETC consists of are Complex I, Ubiquinone, Complex II, Complex III, cytochrome c, and Complex IV (cytochrome c oxidase). Of these complexes and molecules Complex III, cytochrome c, and Complex IV contain heme type proteins. Cytochrome c is unique as it is not part of a larger complex, and freely diffuses through the inner membrane to react with Complex III and cytochrome c oxidase.[2] During the electron transport process, the coordinated iron of heme C is converted between Fe(II) and Fe(III) as the iron accepts and donates electrons.[1]

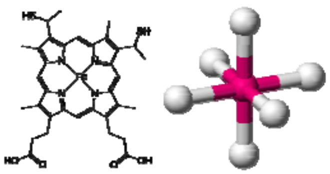


Structure of Cytochrome c [3]

In this presentation, the mechanism in which cytochrome c shuttles electrons between complexes III and IV of the ETC is explained by investigating the geometry and interconversion of the Fe center using Ligand Field Stabilization Energy (LFSE). Due to the fact that cytochrome c is an essential electron transport protein that facilitates the production of ATP, the energy molecule that fuels our cells, studying this electron transport heme-like protein is of utter importance. Therefore, the inorganic model compounds that are described in this presentation are used to study the transport of electrons between cytochrome c and cytochrome c oxidase. In this way, the important biological role of cytochrome c in the electron transport chain can be investigated using inorganic chemistry.

Cytochrome c

- Heme iron metal center
  - Octahedral geometry
  - Coordinated by 6 ligands
    - 4 nitrogen atoms of the porphyrin ring
      - Tetradentate chelating ligand
    - 1 sulfur atom of methionine residue
    - 1 nitrogen atom of histidine imidazole ring



- Role in the ETC
  - e^- transferred from Complex III to heme iron metal center
  - Iron is reduced from Fe^{3+} to Fe^{2+}
  - Cytochrome c transfers e^- to Complex IV
  - Iron oxidized back to Fe^{3+}
- Ligand Field Stabilization Energy
  - Iron metal center always adopts octahedral low spin geometry

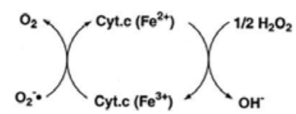
LFSE= $(-0.4x + 0.6y)\,\Delta_o$
x=5; y=0
LFSE= $(-0.4)(5)= -2\,\Delta_o$

LFSE= $(-0.4x + 0.6y)\,\Delta_o$
x=3; y=2
LFSE= $[-0.4\,(3) + 0.6\,(2)] = 0\,\Delta_o$

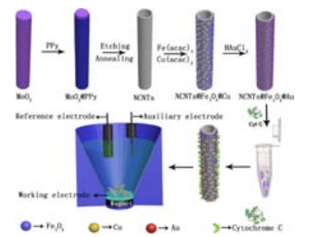
Electron Splitting Diagrams and LFSE Values for Low Spin (left) and High Spin (right) Fe^{3+}
x = Number of d-electrons in the Low Energy t_{2g} Orbitals
y = Number of d-electrons in the High Energy e_g Orbitals

Applications in Research

- Electrochemistry[12]
  - Cytochrome c encapsulated within a methyl-modified silica film to enhance electrochemical reduction rates
  - Advancements in this field help to create more efficient biotechnologies



Cyt c redox reaction to sense H_2O_2



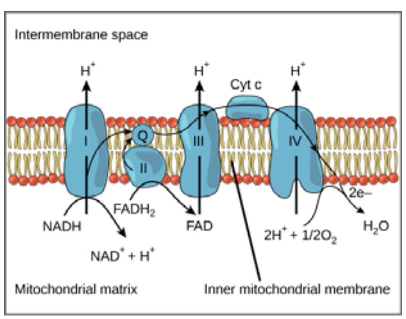
NCNT's coated with cyt c

- Biosensors[11]
  - Nitrogen doped carbon nanotubes coated with Fe_3O_4 and Au nanoparticles as a platform for cytochrome c molecules to act as biosensors of H_2O_2
  - Applications in industry (pharmaceutical, food, clinical) and environmental analyses

Conclusions

Cytochrome c is an essential part of the electron transport chain and without it the ATP required to fuel life would not be produced. Cytochrome c is only able to function in this capacity due to its heme iron metal center that undergoes redox reactions to transport electrons. Further research to aid understanding of how this complex performs this electron transfer will not only help to understand how cells function but also have applications in areas such as electrochemistry and biosensor technology.

Electron Transport Chain



Electron Transport Chain [4]

- **ETC** consists of several proteins and complexes that exchange electrons and pump protons from inside the mitochondrial matrix to the intermembrane space to power ATP synthase
- **Complex I** [5]
  - Accepts e^- from NADH reduction
  - Transports 4 protons across membrane [6]
- **Complex II** [7]
  - Accepts e^- from $FADH_2$ reduction
- **Ubiquinone (Q)** [2]
  - Organic molecule that accepts e^- from Complexes I & II and transports them to Complex III
- **Complex III** [8]
  - Accepts e- from Q
  - Transports 4 protons across membrane [6]
- **Cytochrome c** [2]
  - Accepts e^- from Complex III and transports them to Complex IV
  - Freely moves through inner mitochondrial membrane
  - Small heme group containing protein
- **Complex IV** [2]
  - Final e- acceptor
  - Accepts e- from cytochrome c
  - Transports 2 H^+ across membrane [6]
  - Uses e^- to perform reaction:
    $2H^+ + ½\,O_2 + 2e^- \rightarrow H_2O$
- **ATP Synthase** [2]
  - Uses the proton gradient generated by ETC complexes to form ATP
  - Mechanical pump

References

1) Weller, M.; Overton, T.; Rourke, J.; Armstrong, F. A. *Inorganic chemistry*, 6th ed.; Oxford University Press: Oxford, 2014.
2) Electron Transfers in Oxidative Phosphorylation. http://www.chemistry.wustl.edu/~edudev/LabTutorials/Cytochromes/moviedescription.html (accessed Mar 7, 2019).
3) Cytochrome C. https://chem.libretexts.org/Courses/Saint_Mary's_College,_Notre_Dame,_IN/CHEM_342:_Bio-inorganic_Chemistry/Chapters/Metals_in_Biological_Systems_(Saint_Mary's_College)/Cytochrome_C (accessed Mar 23, 2019).
4) Biology for Majors I. https://courses.lumenlearning.com/wm-biology1/chapter/reading-electron-transport-chain/ (accessed Mar 23, 2019).
5) Sazanov, L. A. *Nature Reviews Molecular Cell Biology* **2015**, 16 (6), 375–388.
6) Wikström, M. *FEBS Letters* **1984**, 169 (2), 300–304.
7) Voet, D.; Voet, J. G.; Pratt, C. W. *Fundamentals of biochemistry: life at the molecular level*, 4th ed.; Wiley: Hoboken, NJ, **2013**.
8) Cytochrome b. https://en.wikipedia.org/wiki/Cytochrome_b (accessed Mar 7, 2019).
9) Roat-Malone, R.M. *Bioinorganic Chemistry- a short course*; Wiley & Sons: Hoboken, NJ, 2007.
10) Xinshan, K. & Carey, J. (1999). *Role of Heme in Structural Organization of Cytochrome c Probed by Semisynthesis.* Biochemistry, 38 (48), 15944-15951.
11) Zhang, M.; Zheng, J.; Wang, J.; Xu, J.; Hayat, T.; Alharbi, N. S. *Sensors and Actuators B: Chemical* 2019, 282, 85–95.
12) López-Bernabeu, S.; Gamero-Quijano, A.; Huerta, F.; Morallón, E.; Montilla, F. *Journal of Electroanalytical Chemistry* 2017, 793, 34–40.

Cytochrome c is a water soluble electron transport protein that is loosely associated with the mitochondrial inner membrane. It contains a heme iron metal center that is essential to it's function in the ETC.[9] The iron metal center in cytochrome c has an octahedral geometry and is coordinated by six ligands - 4 nitrogen atoms of the porphyrin ring, a sulfur atom of a methionine residue, and a nitrogen atom of a histidine imidazole ring. Each of the 6 electron-rich ligands stabilize the positively charged iron ion. The rigid, square planar porphyrin ring in cytochrome c is considered a tetradentate chelating ligand because the four nitrogen atoms of the porphyrin ring bind to the central iron and form a stable organometallic complex.[10] In the ETC an electron is transferred from Complex III to the heme Fe^{3+} of oxidized cytochrome c which reduces it to Fe^{2+}. Cytochrome c then releases the electron to Complex IV. The iron center then returns to the Fe^{3+} oxidized state. Even though the heme iron metal center changes oxidation state during the electron transport, cytochrome c always adopts the octahedral, low spin geometry regardless of the oxidation state on the iron since this geometry is preferred based on Ligand Field Stabilization Energy (LFSE). LFSE is the total energy of the d-electrons of a metal complex relative to the theoretical midpoint energy and a more negative LFSE value is indicative of a more stable complex. When comparing the electron splitting diagrams and LFSE values shown below for both low spin Fe^{3+} and high spin Fe^{3+}, the more negative LFSE values for low spin iron compared to high spin iron indicate that this low spin conformation is more energetically favorable. This is also the case when comparing low ad high spin Fe^{2+}. Therefore, the heme iron metal center of cytochrome c will always adopt the low spin octahedral geometry, regardless of the oxidation state on the iron.