FACILITATION OF ELECTRON TRANSFER AS NOVEL THERAPEUTICAPPROACH

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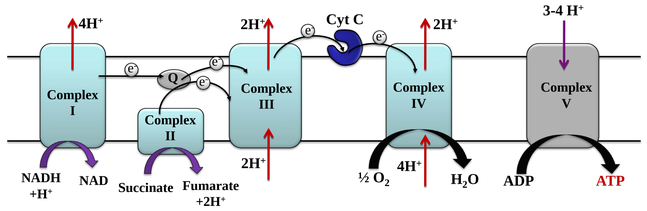
This research aims to characterize the electron transfer kinetics along the electron transport chain (ETC) in mitochondria for pharmacological development. Electron transfer is responsible for facilitating a number of biological processes in various organisms. One of the most vital of these processes is adenosine tri-phosphate (ATP) production via mitochondrial oxidative phosphorylation. The inability to transfer electrons efficiently in an organism, specifically along the ETC, can compromise ATP production which results in age associated diseases, including but not limited to: diabetes, cancer, and neurodegenerative diseases. Presently, there are no treatments that seek to promote cellular energy production by targeting mitochondria and enhancing electron transfer reactions. We used time-dependent absorption and photoluminescence spectroscopy to study electron transfer kinetics involving cytochrome c, a specific component of the ETC. Absorption and luminescence spectra were taken to demonstrate the dose dependent effect of mitochondrion-targeting molecule SS31 on cytochrome c reduction. Spectral weight shifting as a function of time show that SS31 can facilitate electron transfer to oxidized cytochrome c in the ETC to accelerate ATP production. The ability to understand and influence the mechanism for electron transfer provides a novel approach to managing cellular energy and could revolutionize remedies for mitochondrial dysfunction.

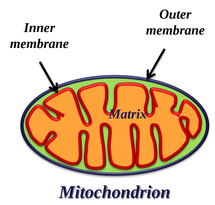
Background and Motivation

Electron Transport in Mitochondria

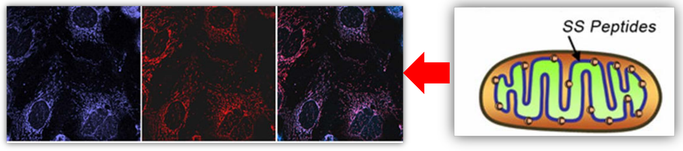


The electron transport chain: responsible for producing cellular energy via ATP synthase

Electron transfer reactions play crucial role for the production of cellular energy required for the life of an organism. The electron transport chain (ETC) on the inner mitochondrial membrane is the major source of intracellular adenosine triphosphate (ATP). The energy released by the flow of electrons through the ETC is used to pump protons out of the mitochondrial inner membrane through complexes I, III, and IV. This creates an electrochemical proton gradient across the mitochondrial inner membrane. The potential energy stored is coupled to ATP synthesis by complex V. Cytochrome c (cyt c), a mobile redox molecule shuttles electrons between complexes III and IV and therefore the enhancement of the electron transfer kinetics along cyt c leads to improved ATP synthesis at complex V.



Currently, there are no drug molecules that act to facilitate cyt c reduction. SS31 is a cell-permeable tetrapeptide that selectively targets the inner mitochondrial membrane. SS31 has been reported to promote ATP production during ischemia-reperfusion injury and reduce cell death. In this study, we have obtained evidence that SS31 can facilitate electron transfer to the heme group embedded in cyt c.



Motivation and Purpose

- Characterization of intracellular electron dynamics by utilizing time-dependent optical spectroscopy and electrochemical methods;
- Mechanism for the enhancement of cellular energy production by targeting and/or enhancing electron transfer reactions;
- Revolutionize pharmacological remedies for mitochondrial dysfunction.

References:

Szeto, H.H. *Mitochondria- targeted cytoprotective peptides for ischemia-reperfusion injury.* Antioxid Redox Signal 10, 601-619 (2008);

C. Zang, J. A. Stevens, J. J. Link, L. Guo, L. Wang and D. Zhong. *Ultrafast proteinquake dynamics in cytochrome c.* J. Am. Chem. Soc. 131, 2846 (2009);

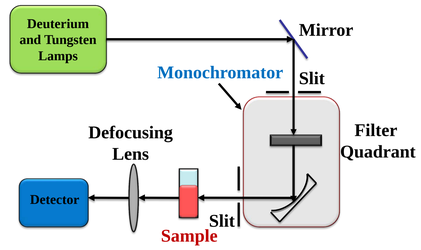
H. Park, *Electrochemical and in situ UV–visible spectroscopic behavior of cytochrome c at a cardiolipin-modified electrode, et al.* /Journal of Electroanalytical Chemistry 514 (2001) 67–74;

V. Fridman, *Electrochemical investigation of cellobiose oxidation by cellobiose dehydrogenase in the presence of cytochrome c as mediator,* Biochemical Society Transaction (2000), Volume 28, part 2, 63-70.

Experimental Methods

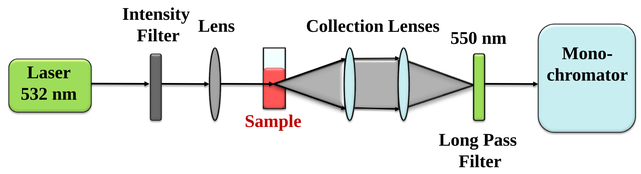
1. Time-Dependent Absorption Spectroscopy

Ultrospec 3300 pro (220-1100 nm)



A wide range of probing wavelengths from 220 nm to 1100 nm was recorded for revealing the intensity changes of absorption peaks in reduced cyt c, which is proportional to the rate of electron transfer to cyt c.

2. Photoluminescence spectroscopy

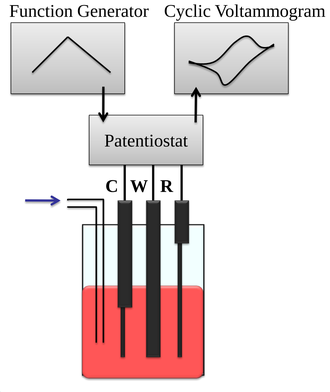


A Nd:YDO4 laser (532.8 nm) is used to photoexcite electrons in sample. Analyzed spectral data reveals peaks that represent a direct measure of the energy levels in cyt c.

3. Cyclic Voltammetry

BASi C3 Cell Stand

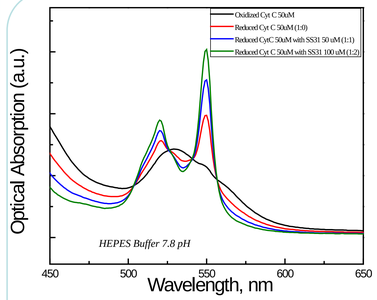
- Golden working electrodes
- Ag/AgCl reference electrode
- Platinum auxiliary electrode



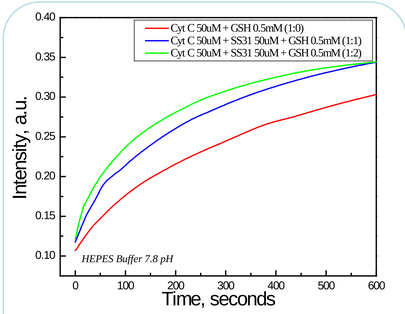
By changing the potential (V) as a linear function of time, we record change of the current (I) and thereby calculate the relative electrochemical potential change during reduction and oxidation processes.

We acknowledge the support from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH).

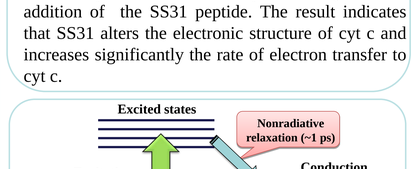
Results and Discussion



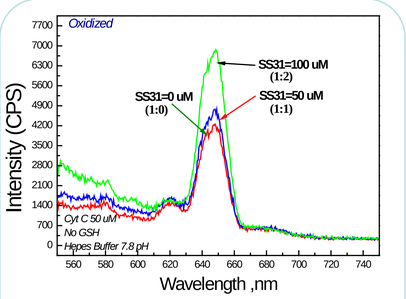
Absorption spectra in the oxidized and reduced states of cyt c in Q-band. Absorption maxima in the reduced cyt c band are clearly shifted up with an addition of the SS31 peptide. The result indicates that SS31 alters the electronic structure of cyt c and increases significantly the rate of electron transfer to cyt c.



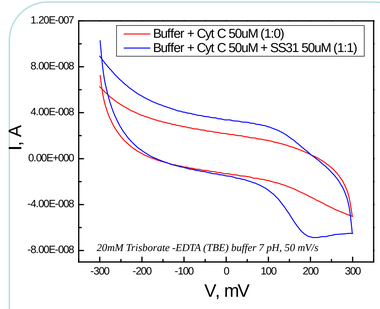
Reduction kinetics of the cyt c in presence of SS31 at 550 nm. SS31 increases rate of cyt c reduction dose-dependently.



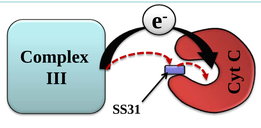
Schematic representation of the photo-luminescence (PL) emission experiment

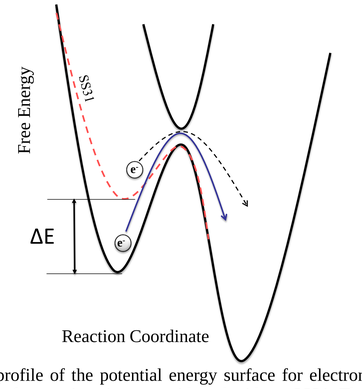


Strong PL emission for the reduced cyt c state can be clearly identified at 650 nm (the electron band is moved from 550 nm to 650 nm due to stock shift). The PL intensity increases significantly with addition of SS31.



Cyclic voltammogram for reduction and oxidation process of cyt c shows that SS31 increases electron diffusion rate (current) for both reduction and oxidation processes of cyt c .These results suggest that SS31 does not act by altering reduction potential of cyt c, but rather increases electron flow through cyt c.





A profile of the potential energy surface for electron transfer reactions in cyt c with and without SS31. The addition of SS31 decreases the energy barrier allowing the electron transfer to proceed faster. The left diagram illustrates SS31 accelerating electron flow through cyt c