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Characterization and Discovery of a Selective Small Molecule Modulator of Mitochondrial Complex I Targeting a Unique Binding Site

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MJC Presentation

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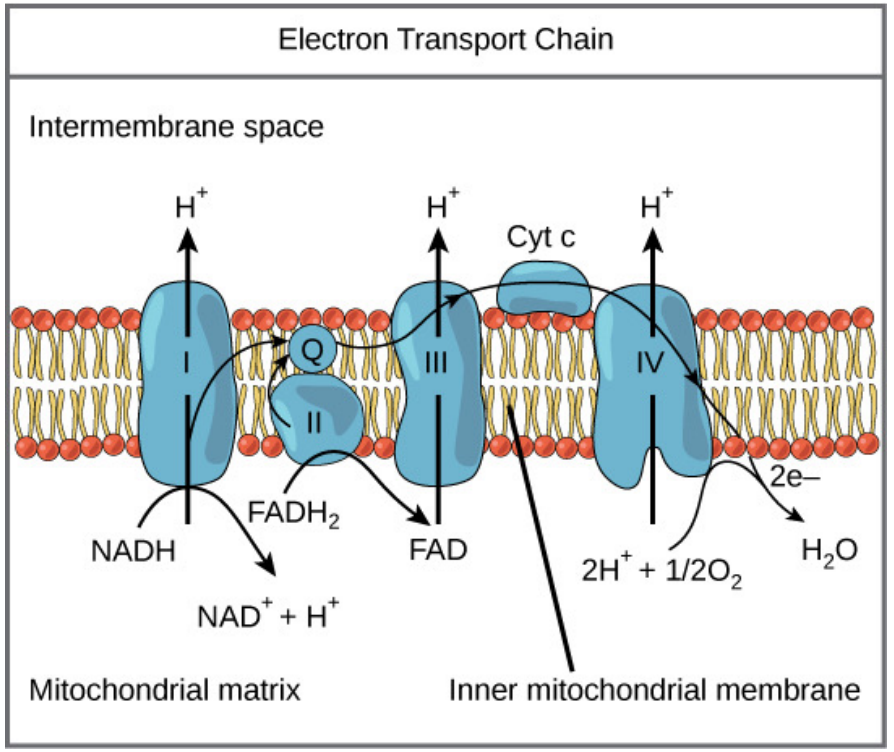
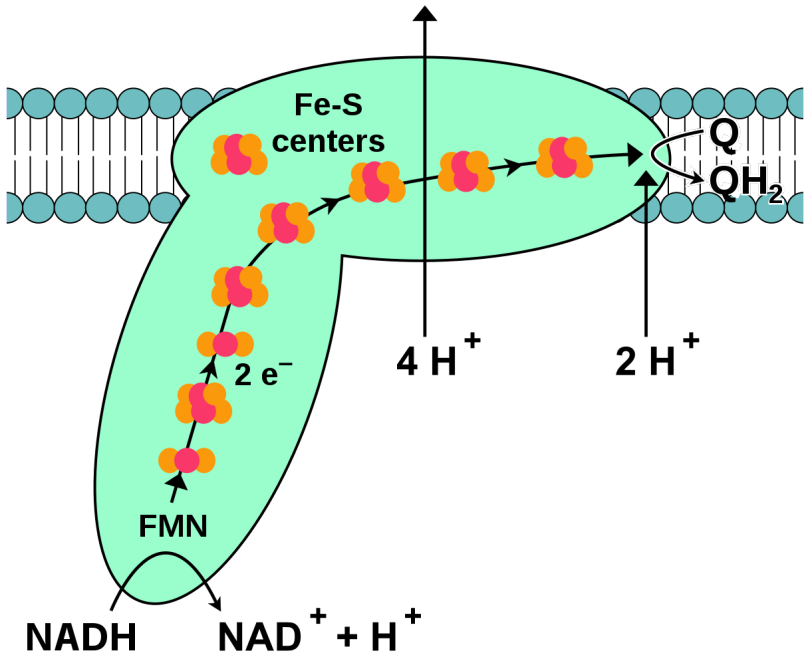
Electron Transport Chain

- At the end of the electron transport chain, energy (ATP) is generated by oxidative phosphorylation
- Through this process, high energy electrons are used in complexes I-IV to move protons into the inner mitochondrial membrane from the matrix
- The excess of protons in the inner membrane space is used to generate ATP at ATP Synthase (complex V) by shuttling them back into the matrix



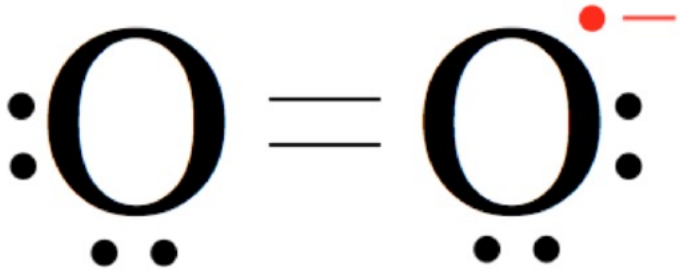
Complex I

- Complex 1, also known as NADH dehydrogenase, takes two electrons from NADH, a byproduct of the breakdown of glucose
- Inhibiting this complex using selective inhibitors is known to lead to decrease reactive oxygen species generation
- Reactive oxygen species (ROS) are oxygen compounds that have unpaired electrons, making them highly unstable
- In the present study, the researchers produced a simple complex I inhibitor to prevent ROS generation



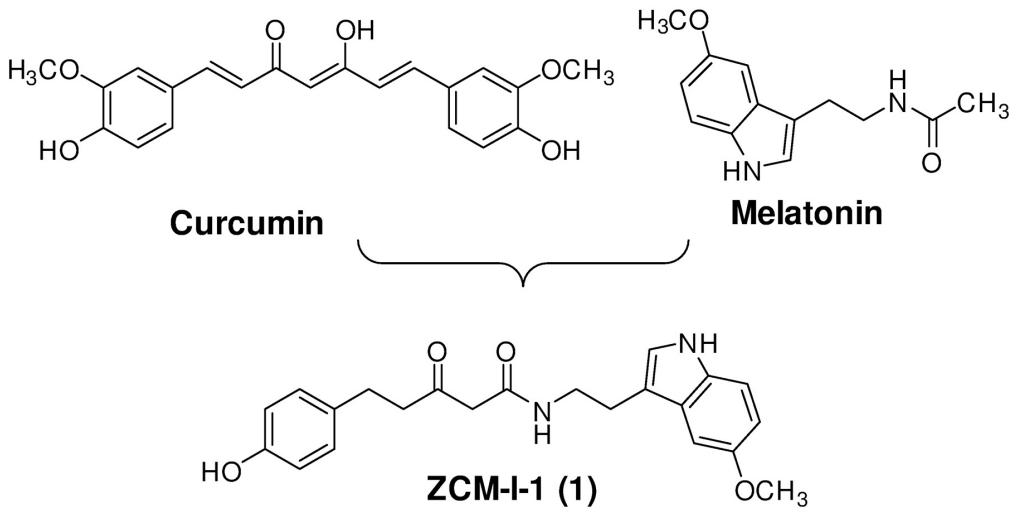
Reactive Oxygen Species (ROS)

- Reactive oxygen species are unstable due to the presence of an unpaired electron, making the orbitals in which they lie unstable
- Antioxidants, however, can donate electrons, making them stable
- If uncontrolled, reactive oxygen species can lead to apoptosis (programmed cell death) and neurodegenerative diseases like Alzheimer's diseases
- ROS are also one of the theories for aging
- Complex 1 is a primary generator of ROS, making it a target for inhibition

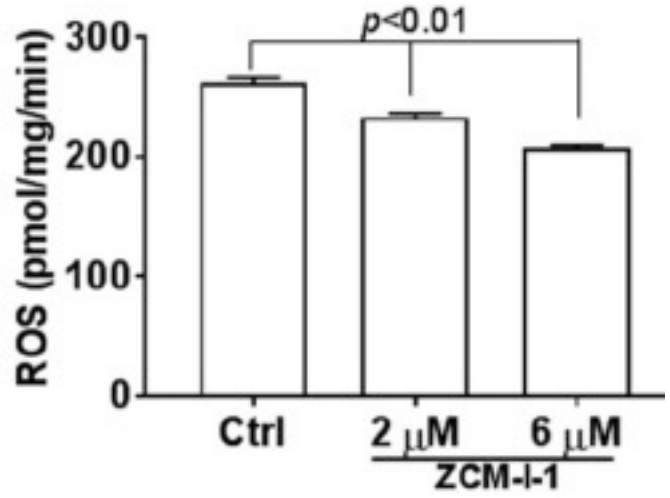


Superoxide

ZCM-I-1



- ZCM-I-1 is made up of two antioxidants



- These results show that ZCM-I-1 could itself serve lower ROS generation

Location of ZCM-I-1 Binding

- Before this study, the location of ZCM-I-1 binding was unknown
- It was known from previous studies that ZCM-I-1 suppressed total ROS
- The researchers then began to examine its role in the mitochondria
- Complexes I and III produce the greatest amounts of ROS, causing the researchers to focus on the binding (if present) of ZCM-I-1 to these complexes
- The researchers used cortical neurons for testing

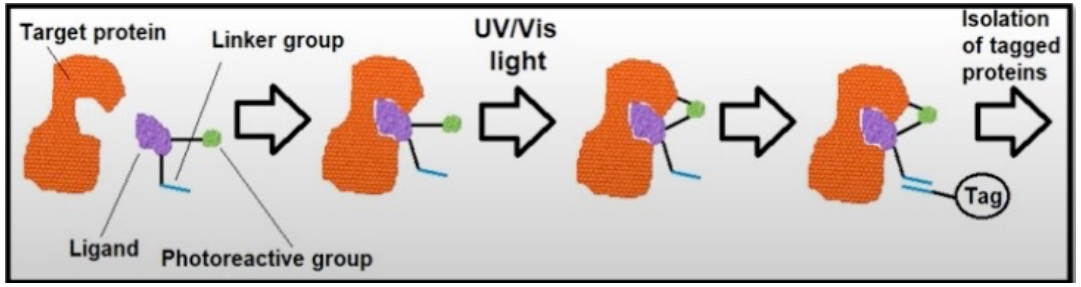
Location of ZCM-I-1 Binding

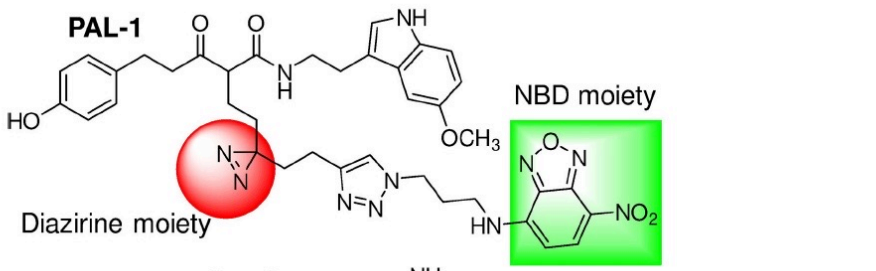
- Rotenone is known to increase ROS in the mitochondria (mitoROS) at complex 1
- With rotenone alone, the researchers observed greater mitoROS production
- However, ZCM-I-1 + rotenone produced lower mitoROS levels, leading the conclusion that ZCM-I-1 competes with rotenone has a selective inhibitor
- Antimycin was used to stimulate complex III by itself, and adding ZCM-I-1 did not lead to lower mitoROS, meaning ZCM-I-1 interacts with complex I alone

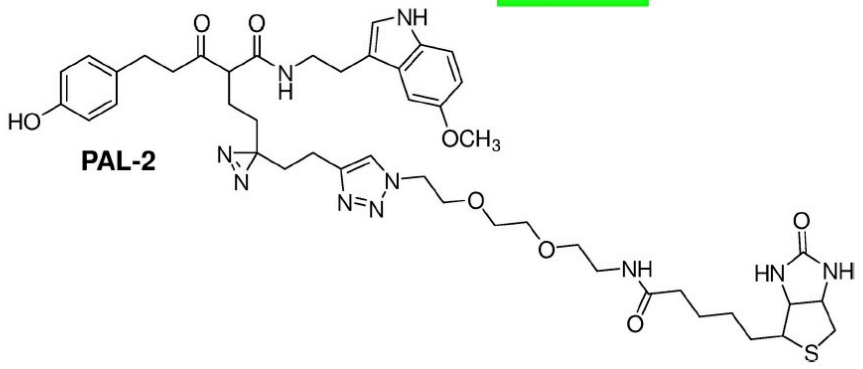
Specificity of Location

- Now that the researchers know that ZCM-I-1 selectively binds to complex I as an inhibitor, the researchers wanted to know where specifically it binds
- Knowing where it binds specifically would help scientists uncover the “the exact pathological role of mitochondrial dysfunction, especially for mitochondrial reactive oxygen species associated oxidative stress”
- Thus, the researchers marked ZCM-I-1 using photoaffinity probes to trace it

PAL-1 and PAL-2



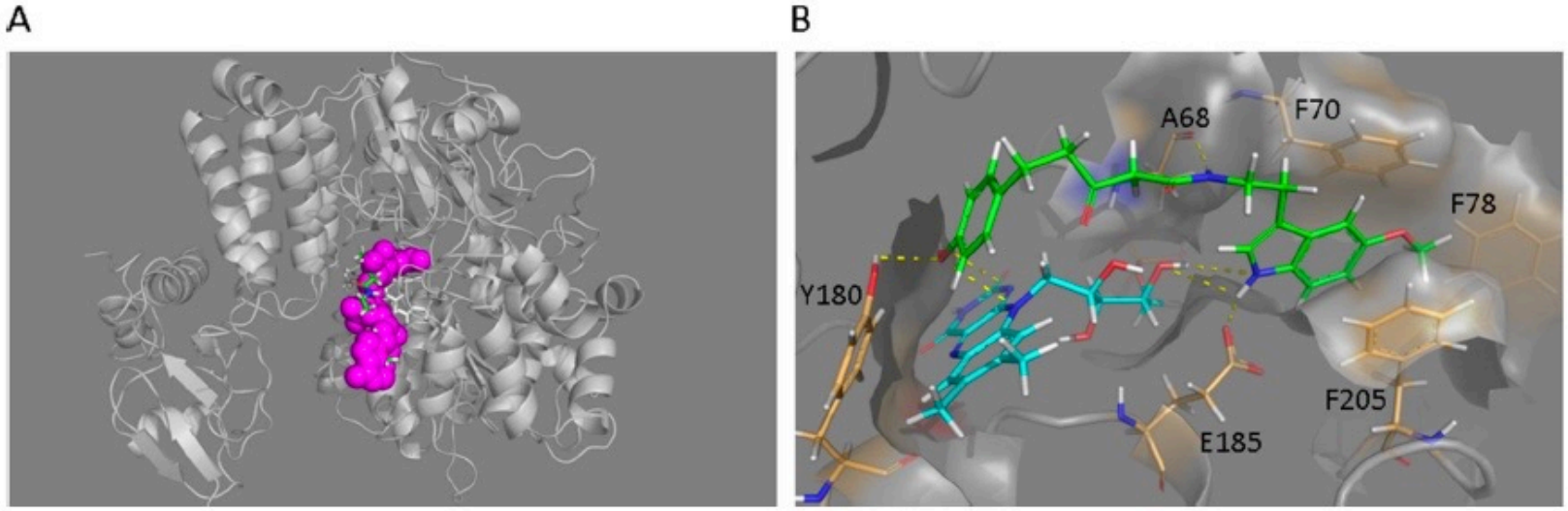




- PAL-1 were each individually bonded to ZCM-I-1, meaning that wherever ZCM-I-1 went, PAL-1 would go also
- By photoaffinity labeling, the researchers were able to find where ZCM-I-1 bound itself to
- PAL-2 was bonded to ZCM-I-1 in a separate experiment
- These photoaffinity labels covalently bind to enzyme, and fluorescence can reveal where the labels bonded to

PAL-1 and PAL-2 Binding

- It was found that PAL-1 bound to NDUFS1, NDUFV1, NDUFS2, NDUFV2, and NDUFS6 subunits, meaning that ZCM-I-1 bound to the I_F site on complex I
- This means that ZCM-I-1 specifically binds to the I_F site to inhibit complex 1 and lower ROS generation



ROS Decline: An Antioxidant Effect?

- So far, ZCM-I-1 has been shown to reduce ROS by binding to the I_F site of complex I
- However, could ZCM-I-1 also serve as an antioxidant?
- When trolox and NAC (known antioxidants) were added, the total ROS did not decrease when compared to ZCM-I-1 alone
- Thus, it can be concluded that ZCM-I-1 affects ROS generation by binding to the I_F site on complex I, not because it serves as an antioxidant

Potential Adverse Effects

- It has been established that ZCM-I-1 inhibits complex I at the IF site
- However, would this difference cause a decrease in membrane potential and less ATP generated?
- The researchers tested on mice nervous tissue and found that membrane potential and ATP levels were not affected by ZCM-I-1, suggesting ZCM-I-1 would be an effective drug to allow mitochondria to function while inhibiting the production of ROS

Conclusions So Far

- Previously it has been found that ZCM-I-1 lowered overall ROS
- This study showed that ZCM-I-1 lowered ROS by modulating the ETC within the mitochondria
- More specifically, the ZCM-I-1 inhibits complex I at the I_F site
- However, ZCM-I-1 did not lower membrane potential
- This makes ZCM-I-1 an exciting drug to administer to alleviate oxidative stress and prevent mitochondrial dysfunction

Connection to Neurodegenerative Diseases

- Until now, how ROS and mitochondrial dysfunction played a role in Alzheimer's disease was unknown
  - Was ROS a cause of AD or an effect?
- Theoretically, if ROS were a cause of Alzheimer's, lowering ROS using ZCM-I-1 would prevent the effects of Alzheimer's
- To test thus, the researchers used 3xTg mice
  - 1: AD – protein tangling
  - 2: AD – protein plaques
  - 3: Mitochondrial dysfunction/oxidative stress

Oxidative Stress and Alzheimer's

- By measuring the respiration of pyruvate in 3xTg mice, the researchers were able to determine that continuous treatment with ZCM-I-1 lowered mitochondrial dysfunction
- This means that if the oxidative stress model of Alzheimer's is true, ZCM-I-1 could serve as an effective tool in controlling Alzheimer's
- However, overdosing on ZCM-I-1 could mean the electron transport chain is unable to function, only further leading to more mitochondrial dysfunction

Discussion Questions

- This paper made use of many chemical techniques to determine the specific binding site of a single molecule within the certain type of cell. Do you think the specificity of research is too great?
- Do you think we should focus on more passive agents of disease (ROS) or more active agents of disease (excess sugar)?
- The complexity of our cells – even our mitochondria – are great. Why do you think it is that we have so much interest in characterizing things we cannot see with the naked eye?

Citation

- J Med Chem. 2020 October 22; 63(20): 11819–11830. doi:10.1021/acs.jmedchem.0c01021.