Understanding the Molecular and Structural Selectivity of Oxidant-induced Nitration and its Reversal in Sarcoplasmic Reticulum Ca^{2+}-ATPase SERCA2a vs. SERCA1a

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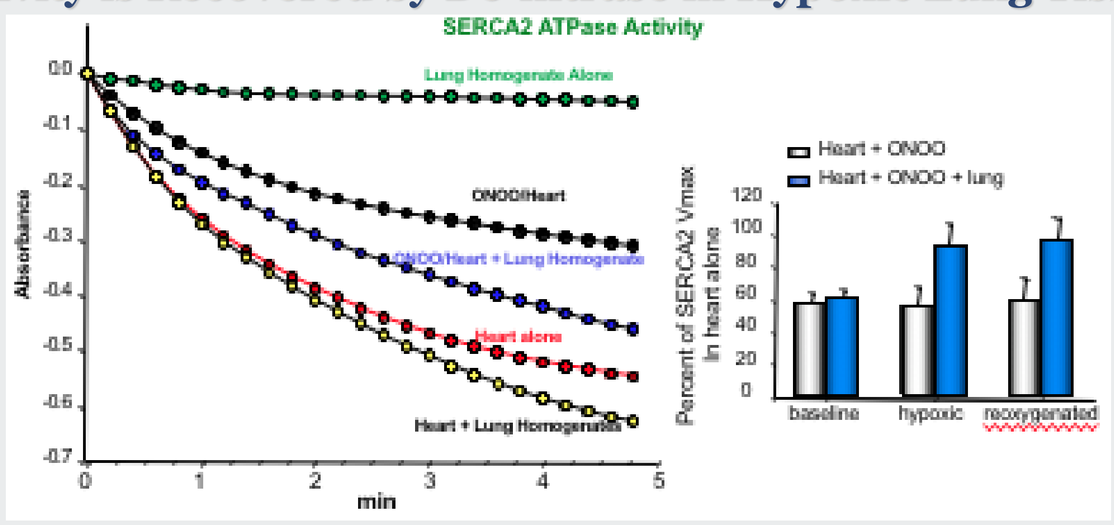
De-nitrase Activity: A Potential Repair Mechanism in Lung Tissue

- Beneficial nitric oxide (NO) participates in numerous physiological processes, but can rapidly become toxic in pathological settings where superoxide (O_2^-) levels are elevated[1]
- NO is transformed by O_2^- into RNS that exert multiple bioactivities *via* selective modifications of biomolecules
- RNS-induced tyrosine nitration of proteins is marked by the incorporation of a NO_2 group and is mostly established as an irreversible in-activator of protein function
- Recent studies show that protein-3-NT can be reversed by a biologically-regulated de-nitrase activity that is enriched in lung tissue[2]

Proof of Concept: RNS-Attenuated Cardiac SERCA2a Activity is Recovered by De-nitrase in Hypoxic Lung Tissue[3]



Hypoxia induced de-nitrase activity in an atelectatic lung. This restored the functionality of $ONOO^-$-inactivated SERCA2a in cardiac tissue

Background:

- SERCA2a, a critical controller of cardiomyocyte contraction and relaxation, is a target for RNS-induced nitration, inactivation and calcium cycling abnormalities in association with oxidative stress[4]
- RNS-induced SERCA2a Tyr-nitration is selective and specific and occurs via molecular processes that are not yet known
- Skeletal SERCA1a is not subject to Tyr-nitration although it displays similar enzymatic function, a great degree of sequence homology, and identical Tyr content and sequence location to SERCA2a[5]
- A crystal structure for SERCA1a is available but not for SERCA2a[5]

Study Design:

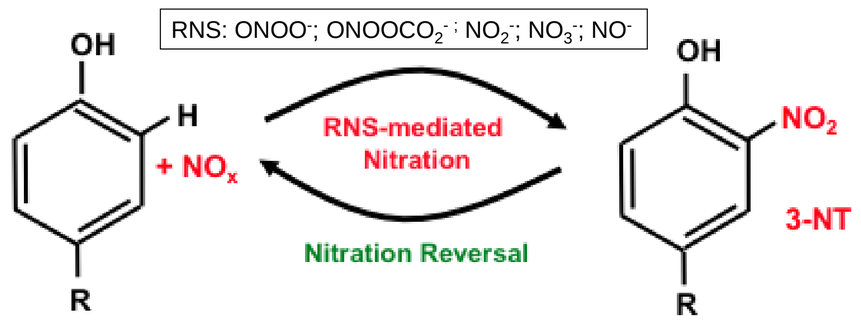
- A PDB (Protein Data Bank file) file is created using I-Tasser (Iterative Threading ASSEmbly Refinement) for protein structure and function predictions[6]
- VMD (Visual Molecular Dynamics) is used for structural modeling of SERCA[7]
- NAMD (Nanoscale Molecular Dynamics) is used for the interactive simulations between SERCA and RNS and also for data analysis[8]
- CHARMM (Chemistry at Harvard Macromolecular Mechanics) Force field is utilized in the form of topology and parameter files for the molecular simulations and data analysis[9]

SERCA1a	SERCA2a
• Compact, linear helices • Y-754 is more closely flanked by other helices • In the conserved region surrounding Y-294/Y-295, there are 19 amino acid differences relative to SERCA2a – Resulting in secondary structure variations	• Y-294/Y-295 are more exposed to solvent compared to SERCA1a • Single nucleotide base shift in amino acid sequence at position 600. – Highly conserved regions from SERCA1a still exist in SERCA2a indicating the similarity between the two proteins.

Common structural observations

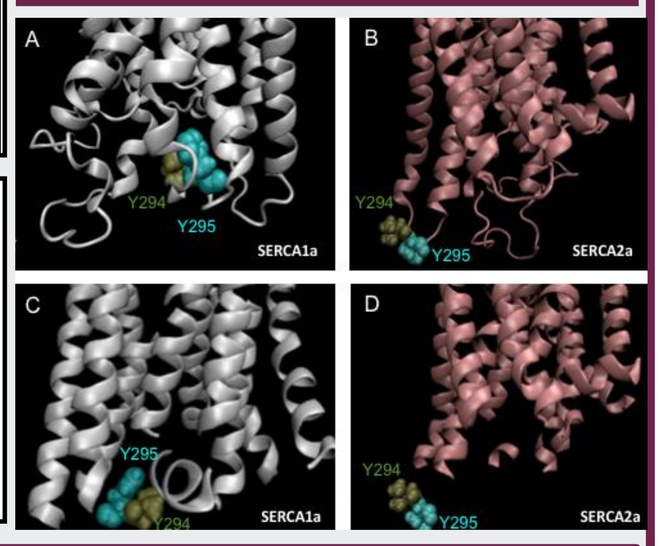
- Y-294/Y-295 are located on turns between alpha helices
- Y-754 is located on the longest alpha helix
  - In general, helices are more unstable than other secondary structures
  - Helices are the first to undergo conformational changes when interacting with other molecules
- Tyrs of interest are located in hydrophobic pockets
  - Y-294/Y-295 are flanked by Isoleucine and Phenylalanine
  - Y-754 is flanked by Isoleucine and Asparagine

The physiological RNS $ONOOCO_2^-$ rapidly homolyzes to $\bullet CO_3^-$ and $\bullet NO_2$. This causes a hydrogen atom to abstract from the 3-position of tyrosine to form 3-Nitrotyrosine[10]



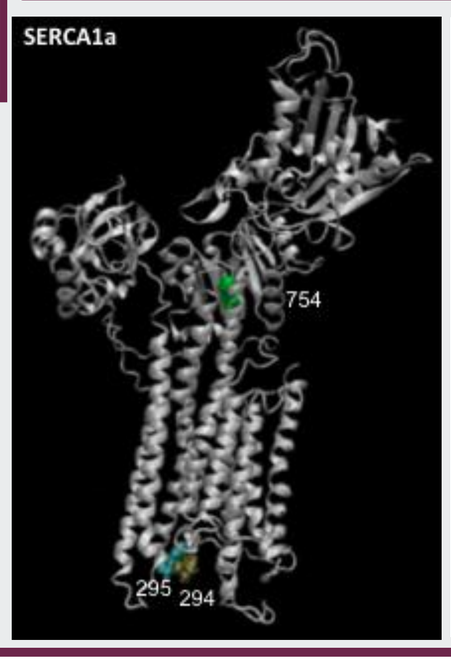
Investigative Questions:

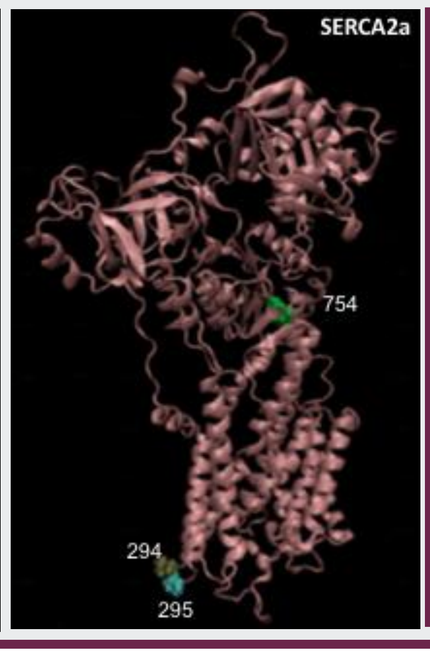
- The present study uses existing sequence (SERCA1a & SERCA2a) and structure (SERCA1a) information to extrapolate a tertiary structure construct for SERCA2a using computational modeling software
- A comparison of SERCA1a and SERCA2a models could reveal structural anomalies that explain the basis for selective and specific Tyr nitration in SERCA2a but not SERCA1a



Summary:

- SERCA1a and SERCA2a share 16 of 18 Tyr sites with sequence homology but SERCA1a is not sensitive to Tyr-nitration
- Despite conserved regions surrounding Tyrs, SERCA1a and SERCA2a are structurally different, specifically in the stalk region. These differences may 'protect' SERCA1a from $ONOO^-$ access and consequent nitration
- Tyr294/295 are surrounded by 3/10 helices that are the least stable and will likely undergo conformational changes as follows:
  1) A hypothesis is that SERCA1a undergoes conformational changes during interaction with $ONOO^-$ that stabilizes helices and shields Tyr residues
  2) Conversely, SERCA2a undergoes conformational changes during interaction with $ONOO^-$ that destabilize alpha helices resulting in Tyr nitration and consequent reversal
- In SERCA2a, Tyr294/295 are in direct contact with extracellular $ONOO^-$ whereas Tyr754 is not.
- A hypothesis is that there is a unobstructed hydrophilic path for $ONOO^-$ between Tyr294/295 to Tyr754
- Tyr754 is slightly more hydrophilic than its immediate neighbors, Phenylalanine and Isoleucine which can render it more sensitive to nitration.





Conclusions:

This study has identified:

- Key structural differences between SERCA1a and SERCA2a
- Possible mechanisms of Tyr-nitration in SERCA2a
- Potential nitration differences between Tyr754 and Tyr294/295 based on their location in the secondary structure on helices or turns.

Next Steps:

- Use NAMD simulation and data analysis tools to study the interactions of $ONOO^-$ and SERCA2a.
- Track secondary structure fluctuations and reconformations for increased $ONOO^-$ exposure
- Further determine what causes selective Tyr-nitration

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