Investigating G-Quadruplex Mimicked Enzyme Kinetics for Molecular Sensing

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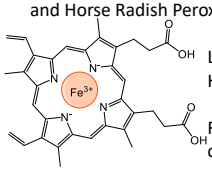
Introduction

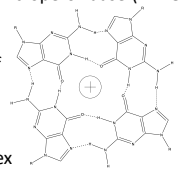
- Molecular assays that utilize hemin are abundant in biochemical research and diagnostics.
- The Hemin cofactor can catalyze oxidation-reduction reactions of a multitude of substrates, including ABTS.
- A G-Quadruplex, (G4 or G-quad) is a 4-guanine complex usually formed by a single G-rich single strand of DNA.
- When Hemin is bound to G-Quadruplex, its ability to catalyze redox reactions is enhanced. (Abbv. GQH for G-Quad+Hemin)
- While G-Quadruplex/Hemin has a propensity to catalyze reactions for many assays, we document the impact of buffer type and pH conditions on the kinetics of G-Quadruplex/Hemin compared to two other peroxidases. (Microperoxidase (m-POD) and Horse Radish Peroxidase (HRP))



hemin

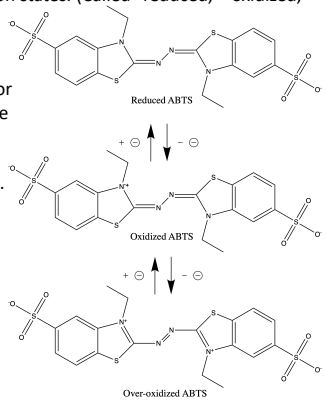
Left: Structure of Hemin

Right: Structure of a G-Quadruplex


Interactions of Guanine Bases in a G-Quadruplex

Background

- Previous research shows substrate toxicity of hydrogen peroxide on hemin activity.
- H2O2 can destroy the hemin cofactor, leading to inactivation.
- ABTS has three oxidation states. (Called "reduced," "oxidized," and "over-oxidized")
- "Reduced" and "Over-oxidized" states of ABTS are pale blue or near transparent, while the "oxidized" state is dark blue with strong absorbance at 420 nm.



References

By G-quadruplex.jpg: Julian Huppert Jlh29derivative work: Iridos (talk) - G-quadruplex.jpg, CC BY-SA 2.5, https://commons.wikimedia.org/w/index.php?curid=15670781

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Methodology and Analysis

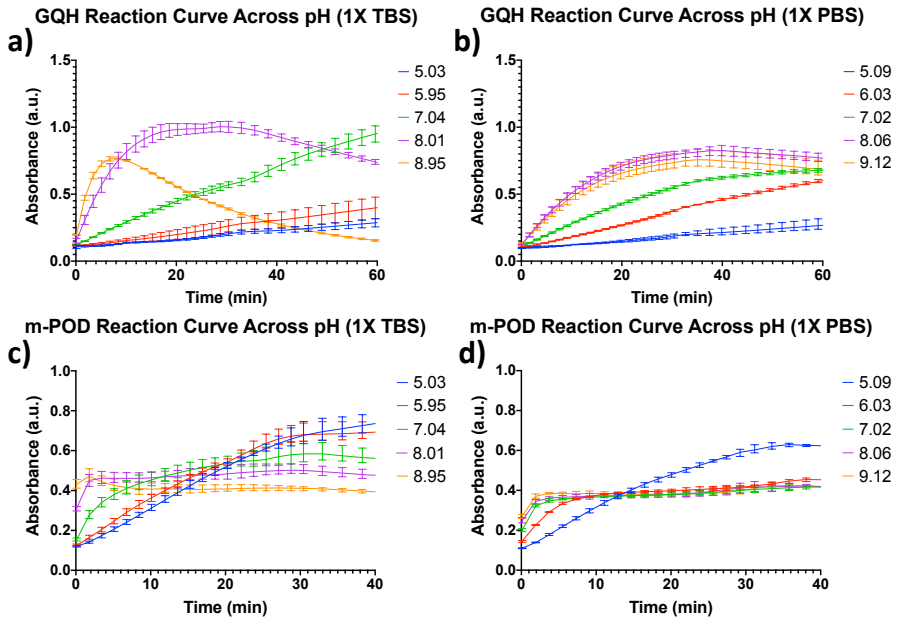

Figure 1) Raw reaction curve of **a)** G-Quad/Hemin in 1X TBS buffer **b)** G-Quad/Hemin in 1X PBS buffer **c)** m-POD in 1X TBS buffer **d)** m-POD in 1X PBS buffer. Error bars indicate data range. Legend shows measured pH of buffer.

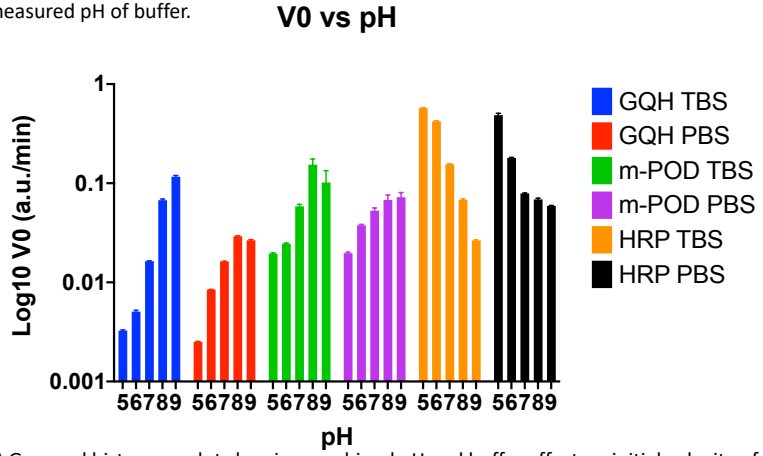

Figure 2) Grouped histogram plot showing combined pH and buffer effect on initial velocity of oxidation of ABTS.

Discussion

- GQH shows reduced kinetic activity in PBS buffer vs TBS buffer.
- In basic conditions, signal is degraded faster in TBS buffer vs PBS buffer.

These two combined trends suggest that there is a critical role that the buffer system and pH level contribute to the reduction in activity and the loss of signal from ABTS. Additionally, m-POD shows significant loss of activity under basic conditions, suggesting that enzyme destruction may also contribute to signal decay.

Conclusions and Directions for Future Research

In the ABTS/H_2O_2 reaction system of GQH, we demonstrate a buffer-dependent and pH dependent effect on it's enzymatic activity.

Future research will investigate this effect using other indicator species in place of ABTS, (e.g. Amplex Red and TMB) and determine the impact that enzyme destruction could contribute to the loss of signal.

From these aims, we hope to document peroxidase behavior under different conditions in order to ensure the accuracy and reproducibility of hemin-based assays in future applications.

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