Understanding the Role of NADH in Cellular Fe2+ Generation of Hydroxyl Radical and the Effects of Polyphenol Antioxidants

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Understanding the Role of NADH in Cellular Fe²⁺ Generation of Hydroxyl Radical and the Effects of Polyphenol Antioxidants

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MOTIVATION

What is the role of autoxidation?

Oxidative stress due to exogenous 2,4-dinitrophenylhydrazine (DNPH) is a primary cause of cell death. The radical is generated via the reaction of Fe²⁺ and hydrogen peroxide:

\[ \text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^+ + \text{OH}^- \]


What occurs in the presence of H₂O₂?

Perron, N.R.; Hodges, J.N.; Jenkins, M.; Brumaghim, J.L.

Antioxidants as a protective measure.

Polyphenol antioxidants in teas, fruits, and vegetables can ameliorate this damage. In vitro studies by our group have shown that these antioxidants protect plasmid DNA from this damage.1,2

E. coli as a cellular model.

A significant body of work exists exploring Fe-mediated oxidative damage in E. coli. We chose a mutant strain with excess NADH (SLC22; wild-type parent strain AN387) to explore the effects of the polyphenol antioxidants MEGA and MEPCA (Figure 1).

APPROACH

What effect do polyphenol antioxidants have on the rate of Fe³⁺ reduction by NADH?

UV-vis kinetics experiments explore the role of NADH in an iron- and antioxidant-containing system—research in this area does not often account for the presence of NADH.

Catechol MPCA inhibits the rate of Fe³⁺ reduction by NADH while the gallate MEGA slightly accelerates it (Figure 3). MEGA autoxidizes Fe²⁺ to Fe³⁺ more quickly in air than MPCA, potentially regenerating available Fe³⁺ for NADH to reduce.

What occurs in E. coli with excess levels of NADH when polyphenol antioxidants are supplemented prior to oxidative challenge?

Figure 4 & 5. Logarithmically growing E. coli (AN387) were challenged with H₂O₂ (2.5 mM) at 37 °C for 30 min following a 50 min incubation with the selected polyphenol. SLC22 was challenged for 5 min due to its extreme sensitivity to oxidative challenge. Cellular viability was assessed via plating. Error bars are the standard deviations of at least three trials.

Wild-type Strain (AN387)

- Both iron-binding polyphenols increase cell killing upon oxidative challenge.
- Conversely, the iron chelator DFO prevents ~75% of H₂O₂-mediated cell death in both strains.
- No significant dose-response was observed for the two polyphenols.
- In contrast to in vitro iron-mediated DNA damage results, the iron-binding abilities of these two polyphenols do not prevent iron-mediated cell death.

Figure 3. Comparison of first-order rate constants obtained from UV-vis experiments: pH = 6, FeCl₃ (60 µM), NADH (16 µM), ethanol (110 mM), and polyphenol (up to 3 molar equivalents relative to Fe³⁺, 80, 160, and 240 µM) were all monitored at 540 nm and 25 °C. Error bars are the standard deviations of at least three trials.

SLC22 (ndh mutant) were challenged with H₂O₂ (2.5 mM) at 37 °C for 30 min following a 50 min incubation with MEGA or MEPCA (30 µM) and hydrogen peroxide:

- MEGA and MEPCA increase cell killing upon oxidative challenge.
- The iron-chelating desferrioxamine B (DFO) was employed as a positive control, since iron chelation prevents cell death upon oxidative challenge. Cellular viability was determined by plating.

SUMMARY & OUTLOOK

Structurally similar polyphenols have opposite effects on rates of Fe³⁺ reduction by NADH. In future work, we anticipate exploring several avenues:

- What is the role of autoxidation? UV-vis kinetic experiments in an air-free atmosphere should reveal if MEGA is increasing the pool of reducible Fe²⁺.
- What occurs in the presence of H₂O₂ and/or increased levels of NADH? Mimicking the environment of a stressed cell should yield interesting information about antioxidant activity if the rates change significantly.

Iron-binding polyphenols display an increased lethality in conjunction with H₂O₂ challenge for both wild-type E. coli and the ndh mutant. However, iron chelation by DFO was able to rescue a significant amount of H₂O₂-challenged cells. To better understand polyphenol antioxidant activity in E. coli, several questions must be answered:

- Is the increased cell death upon polyphenol treatment due to DNA damage? These polyphenols may cause cell death via a pathway separate from iron binding and prevention of DNA damage.
- What is the relative importance of NADH and iron levels in cells with respect to antioxidant function? If iron-binding abilities of polyphenols cannot rescue these E. coli strains, it would be interesting to discover their effect in E. coli mutants that are deficient in ferric uptake regulator protein (fur) with high labile iron pools.

REFERENCES & ACKNOWLEDGEMENTS


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NADH + Fe^{2+} / Cu^{+} \rightarrow \text{Fe}^{3+} / \text{Cu}^{2+} + \text{H}_2\text{O}_2

NAD^{+} \rightarrow \text{Fe}^{2+} / \text{Cu}^{+} \rightarrow \text{H}_2\text{O}_2

\text{NADH} \rightarrow \text{Fe}^{3+} / \text{Cu}^{2+} \rightarrow \cdot\text{OH} + \cdot\text{OH}