3-2016

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

Craig Goodman
Clemson University

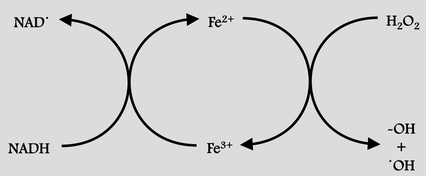
Julia L. Brumaghim
Clemson University

Understanding the Role of NADH in Cellular Fe^{2+} Generation of Hydroxyl Radical and the Effects of Polyphenol Antioxidants

Craig Goodman and Julia L. Brumaghim*
Department of Chemistry, Clemson University, Clemson, SC 29634-0973, USA

MOTIVATION

NADH as a catalyst for cellular damage. DNA damage caused by the hydroxyl radical (˙OH) is a primary cause of cell death.[1] This radical is generated via the reaction of Fe^{2+} and hydrogen peroxide:



In cells, iron-mediated hydroxyl radical production is cyclic - NADH can reduce the generated Fe^{3+}. This is the rate-limiting step *in vivo* and increases oxidative damage.[2]

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.[3,4]

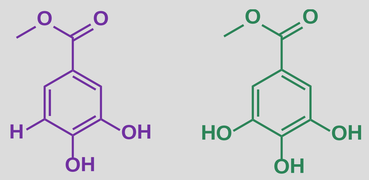


Figure 1. Structures of chosen polyphenols. Left: Methyl-3,4-dihydroxybenzoate (MEPCA). Right: Methyl-3,4,5-trihydroxybenzoate (MEGA).

***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

We explored the interactions among NADH, $Fe^{2+/3+}$, MEGA, and MEPCA in two ways.

UV-Vis kinetics. To assess polyphenol antioxidant affects on the NADH-Fe system, the rate of NADH reduction of Fe^{3+} was monitored in the presence of MEGA and MEPCA. NADH absorbs at 340 nM while NAD+ does not, allowing for straightforward analysis of the rate of NADH consumption.

***E. coli* antioxidant assays.** To explore polyphenol antioxidant effects in *E. coli*, strains AN387 (wild-type) and SLC22 (ndh mutant) were challenged with H_2O_2 (2.5 mM) following incubation with MEGA or MEPCA (30 min). 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.

RESULTS

What effect do polyphenol antioxidants have on the rate of Fe^{3+} 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 MEPCA inhibits the rate of Fe^{3+} reduction by NADH while the gallol MEGA slightly accelerates it (Figure 3).
- MEGA autoxidizes Fe^{2+} to Fe^{3+} more quickly in air than MEPCA,[4] potentially regenerating available Fe^{3+} for NADH to reduce.

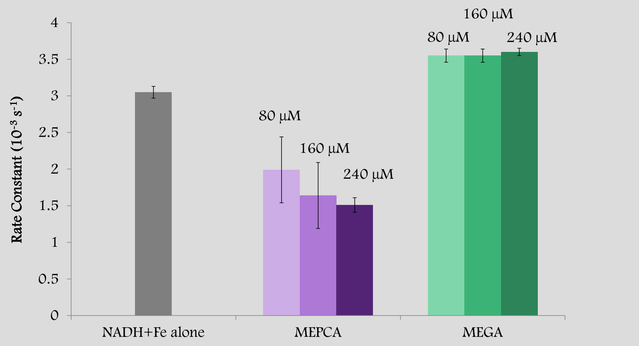
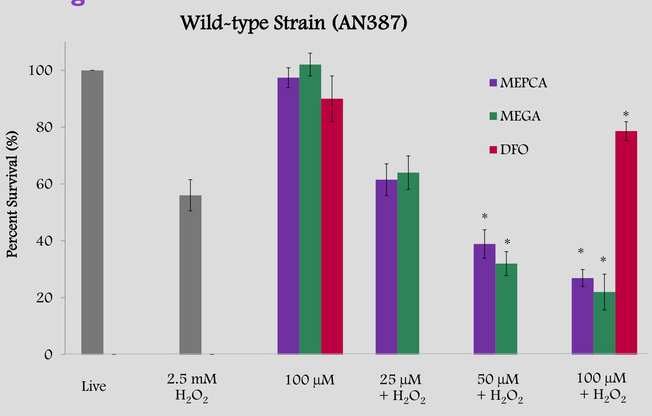


Figure 3. Comparison of first-order rate constants obtained from UV-vis experiments: pH = 6, $FeCl_3$ (80 µM), NADH (16 µM), ethanol (100 mM), and polyphenol (up to 3 molar equivalents relative to Fe^{3+}: 80, 160, and 240 µM) were all monitored at 340 nm and 25 °C. Error bars are the standard deviations of at least three trials.

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

Figures 4 & 5. Logarithmically growing *E. coli* (AN387) were challenged with H_2O_2 (2.5 mM) at 37 °C for 30 min following a 30 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. Asterisks denote data significantly different from appropriate controls ($p < 0.05$).



- Both iron-binding polyphenols *increase* cell killing upon oxidative challenge.
- Conversely, the iron chelator DFO prevents ~75% of H_2O_2-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[3], the iron-binding abilities of these two polyphenols do not prevent iron-mediated cell death.

SUMMARY & OUTLOOK

Structurally similar polyphenols have opposite effects on rates of Fe^{3+} 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 Fe^{3+}.
- **What occurs in the presence of H_2O_2 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.

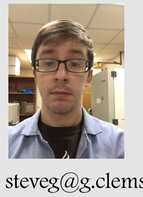
Iron-binding polyphenols display an increased lethality in conjunction with H_2O_2 challenge for both wild-type *E. coli* and the *ndh* mutant. However, iron chelation by DFO was able to rescue a significant amount of H_2O_2-challenged cells. To better understand polyphenol antioxidant activity in *E. 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.

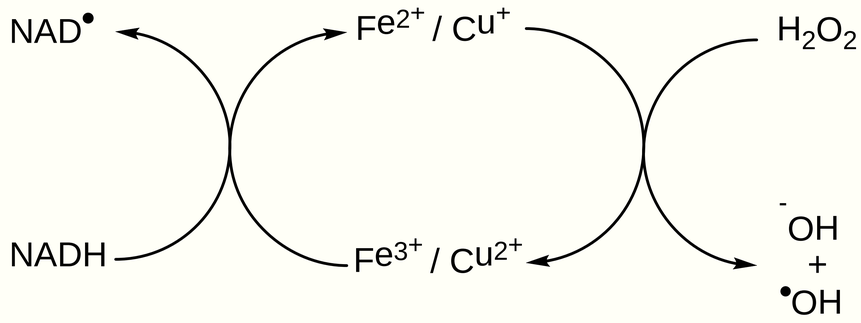
REFERENCES & ACKNOWLEDGEMENTS

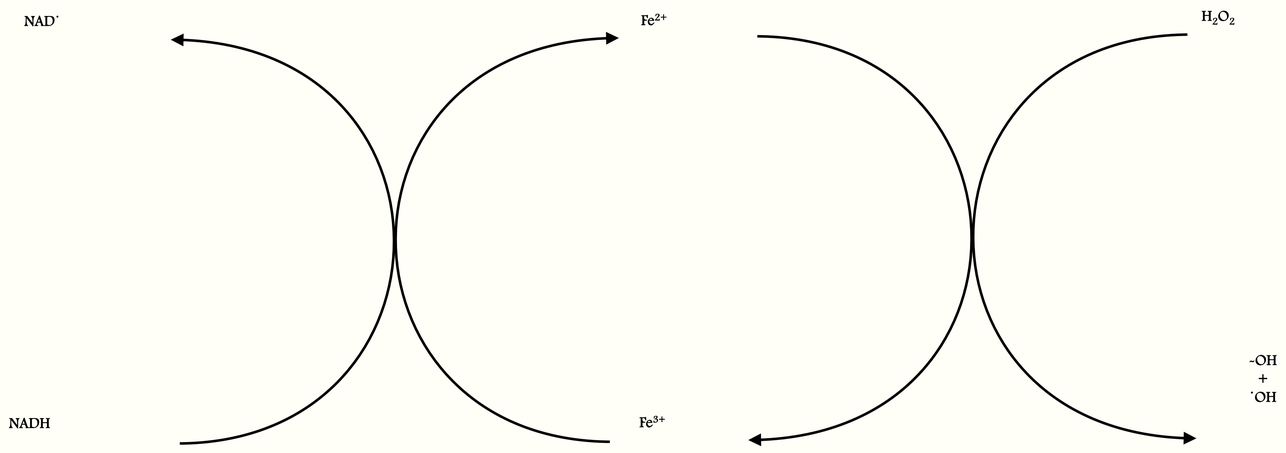
1. Imlay, J. A.; Linn, S. *Science.* **1988**, *240*, 1302-1309.
2. Brumaghim, J.L.; Li, Y.; Henle, E.; Linn, S. *J. Biol. Chem.* **2003**, *278*, 42495-42504.
3. Perron, N.R.; Hodges, J.N.; Jenkins, M.; Brumaghim, J.L. **2008**, *47*, 6153-6161.
4. Angelé-Martínez, C.; Goodman, C.; Brumaghim, J.L. **2014**, *6*, 1358-1381.

The authors thank NASA, the South Carolina Space Grant, and Clemson University for funding this work.



Contact: steveg@g.clemson.edu


NAD•
NADH
Fe2+ / Cu+
Fe3+ / Cu2+
H2O2
-OH
+
•OH


NAD·
NADH
Fe2+
Fe3+
H2O2
-OH
+
·OH