Investigating RNase Modification by 1,4-Benzoinone & 1,4-Hydroquinone

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The main goal of the research is to understand the biochemical behavior and biological outcome of diverse quinones and hydroquinones which are known to be the cellular metabolites of benzene and substituted benzenes. Benzene and polycyclic aromatic hydrocarbons impose risks to human health, some identified and some uncertain. These compounds emerge as public hazard via continuous accumulation in living organisms and general environment. Several studies suggest that the quinones/hydroquinones are involved in causing toxic abnormal cell behavior through protein modifications, oxidative damage, lipid modifications, and/or nucleic acid modifications. However, many details regarding their activities and their fate in biological systems are not clearly understood. In this context, our lab is studying the biological effect(s) of the benzene and substituted benzene metabolite family focused on RNase modification induced by \( p \)-benzoquinone and \( p \)-hydroquinone as a model system. Our lab is trying to elucidate if the target protein modification occurs via alkylation or oxidative damage caused by \( p \)-benzoquinone and/or \( p \)-hydroquinone. For the study, various analytical techniques such as SDS-PAGE, NMR, and UV/VIS spectroscopy are utilized in order to understand protein modification mechanisms.
The Binding of IMP to Ribonuclease A

Febs J. v272 pp. 3988-4001, 2005 (1Z6D )
Ribonuclease P and RNA

“Crystal Structure of a Bacterial Ribonuclease P and RNA (2A64)”
PNAS v102 pp.13392-13397, 2005
Ribonuclease A Sequence Map


KETAAA KFERQH MDMA SSTSAASSS NYCNQM MK
SRNLT KDRCKPVNTFVHESLADVQAVCSQKN
VACKNG QNCYQSYSTMSITDCRETGSSKYP
NCA YK TQQANKHII VACEGNPY VPVHFDASV

From Protein Data Bank at http://www.rcsb.org/pdb/explore/biologyAndChemistry.do
Model Study Methodology

- $p$-benzoquinone ($p$BQ) was reacted with benzylamine in an attempt to gain insight on the reaction mechanism that $p$BQ may modify lysine residues of RNase.

- Thin layer chromatography, UV/Vis spectroscopy, and $^1$H NMR spectroscopy were used.
Proposed Mechanism

\[
\begin{align*}
R &= \text{phenyl} \\
\text{H}_2\text{N} - \text{R} &\xrightarrow{H_2O} \text{OH} \\
\text{NH}_2 &+ \text{R}^- + \text{R}^+ \quad + \\
\text{OH} &\xrightarrow{H_2N - \text{R}} \text{OH} \\
\text{NH}_2 &+ \text{R}^- + \text{R}^+ \quad + \\
\text{OH} &\xrightarrow{H_2N - \text{R}} \text{OH} \\
\text{NH}_2 &+ \text{R}^- + \text{R}^+ \quad + \\
\end{align*}
\]
Model Reaction of pBQ and Benzylamine

Time Dependent $^1$H NMR Scale Reaction  (Benzylamine: pBQ = 4:1)
Time Dependent UV/Vis Spectra of Control RNase

0.5 mg RNase in pH 7 phosphate buffer, 3 mL
Representative Spectra of RNase Modification by \( pBQ \)

1.0 mM \( pBQ \) and 0.5 mg RNase in pH 7 phosphate buffer
Time Dependent Progress of RNase Modification by \( pBQ \)

1.0 mM \( pBQ \) and 0.5 mg RNase in pH 7 phosphate buffer
UV/Vis Reactions of $pBQ$ and RNase at Various [$pBQ$]

- 0.5 mM $pBQ$ + 0.5 mg RNase
- 2 mM $pBQ$ + 0.5 mg RNase
- 3 mM $pBQ$ + 0.5 mg RNase
- 5 mM $pBQ$ + 0.5 mg RNase
Kinetic Analysis

- Kinetic model: Pseudo first order condition
  \[ [\rho \text{BQ}] \text{ is excess relatively to RNase A.} \]
  \[
  \begin{array}{c}
  \text{A} \\
  \text{P}
  \end{array}
  \]
  A: Unmodified RNase
  P: Modified RNase

- Kinetic equation used:
  \[
  \ln \left( \frac{(A_\infty - A_t)}{(A_\infty - A_0)} \right) = -k_1 t
  \]
  \( A_\infty \): Absorbance of P at the time infinity
  \( A_t \): Absorbance of P at the time t
  \( A_0 \): Initial absorbance of P
  \( k_1 \): Pseudo first order rate constant
  \( t \): Reaction time
Rate Constants at Each Concentration of $[^p]BQ$ at 27 °C

All data points represent triplicated experimental data points ($n = 3$)
Temperature Dependent Reactions of pBQ and RNase

1 mM pBQ at 27° C

1 mM pBQ at 37° C

Time Progress of $A_{350}$ at 350 nm at 27° C

Time Progress of $A_{350}$ at 350 nm at 37° C
UV/Vis Reactions of $p$HQ+$p$BQ and RNase

1.0 mM $p$HQ

0.5 mM $p$HQ/0.5 mM $p$BQ

0.75 mM $p$HQ & 0.25 mM $p$BQ

0.25 mM $p$HQ/0.75 mM $p$BQ
Conclusion

- RNase A appeared to be modified upon incubation with \( p \)-benzoquinone at 0.5 mM to 5 mM.

- Based on the rate constants, the modification of RNase A shows a linear relationship toward \( p \)-benzoquinone concentration except the reaction carried out with 5 mM of \( p \)-benzoquinone.

- The modification of RNase A monitored at different temperature revealed that increasing the reaction temperature increases the rate of the reaction representing the RNase modification.

- The reactions containing a mixture of \( p \)-benzoquinone and \( p \)-hydroquinone at different concentrations were carried out, and the spectral features indicated that a complex redox cycle might affect the RNase modification.


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