Kinetic Studies of Amino Acid Modification Induced by PAH Quinones

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Over the decades, there has been increasing air pollution induced by polycyclic aromatic hydrocarbons (PAHs) generated through various industry activities and incomplete fuel combustion. Quinone is one of the PAH metabolites suspected to modify macromolecules such as proteins, lipids, and nucleic acids in cells, which might lead to cancer. In order to understand how quinones induce protein modification at the molecular level, we studied the kinetics of selected amino acid modification induced by quinones of interest. The choices of acids are lysine, serine, cysteine, threonine and aspartate which can behave as a nucleophile. This study was carried out by using Ultraviolet-Visible (UV-Vis) spectroscopic approach to investigate how different experimental conditions can influence the speed of a chemical reaction and offer insight on the reaction mechanism. Once the time-dependent UV-Vis spectral data is optimized, the reaction rate constant can be calculated.
Polycyclic Aromatic Hydrocarbons (PAH)

- Widespread environmental toxins
- Exposure occurs
  - Cigarette smoke
  - Grilled meat
  - Paint & furniture polish
  - Food preservatives
  - Petroleum products
    - Medicine
    - Cosmetics

http://blog.newsok.com/watchdog/2009/04/24/smoking-or-non/
http://www.nanoid.co.uk/nano_cosmetics.html
Mechanism

Oxidative damage

Pathway I

O2

O2-

[O]

Pathway II

Protein

Nu

Adduct formation

Pathway III

Protein crosslink

H2N-Lys

O=Lys + H2N-Lys
Structures of Quinones

1,4-Benzoquinone ($p$BQ)

2-Chloro 1,4-benzoquinone ($Clp$BQ)

2-Methyl 1,4-benzoquinone ($Mep$BQ)
Sequence Map of Ribonuclease A

KETAAA KFERQHQMSSTSAASSSNSYCNQMMKSRNLTKDRCKPVNTFVHE SLADVQAVCSQKNVACKNGQTNCYQSYSTMSITDCRETGSSKYPNCAYKTTQANKHIVACEGNPYVPVHFDASV

Structures of Selected Amino Acids

Lysine (K)

Aspartate (D)

Serine (S)

Threonine (T)

Cysteine (C)
Reactivity Conditions of Kinetics Analysis

- Temperature: 37°C
- Buffer: Phosphate buffer (50mM, pH7.0)
- Pseudo-first order condition ([A.A.] is in excess)

\[ \text{A.A.} + \text{Q} \xrightleftharpoons{k} \text{P} \]

Rate = \( k \text{[A.A.]}\text{[Q]} = k_{\text{obs}}\text{[Q]} \)

- Instrument: Shimadzu UV-Vis Spectrometer
**Optimization:** $[\rho BQ]=0.05\text{mM}, [\text{Lys}]=2.0\text{mM}$

- The spectrum was scanned every 10 minutes for 5 hours. The spectral data demonstrates that lysine is modified by $\rho BQ$. Arrows represent absorbance changes in a time dependent manner.

* Scanned every 10min for 5hrs*
Optimization

[pBQ]=0.05 mM, [Lys]=2.0 mM

[pBQ]=0.05 mM, [Lys]=20 mM

Each $\lambda_{\text{max}}$ represents reactants, intermediates and products.
Time Dependent UV/Vis Spectral Change

- [pBQ]=0.01mM, [Lys]=20mM
- [pBQ]=0.025mM, [Lys]=20mM
- [pBQ]=0.05mM, [Lys]=20mM
- [pBQ]=0.075mM, [Lys]=20mM
Representative Standard Curve: \( pBQ \)

* Data triplicated.
Representative Standard Curve: \( \rho \text{BQ} \)

![Graph showing a linear relationship between absorbance at 246nm and [\( \rho \text{BQ} \)] (mM). The equation of the line is given as \( y = 18.22x + 0.2665 \) with a \( R^2 = 0.993 \).]

* Starndard curve of Cl\( \rho \text{BQ} \) and Me\( \rho \text{BQ} \) are not shown.
Control Spectra of Quinones

- $\lambda_{\text{max}}$
  - $p\text{BQ}$ : 246nm
  - Cl$p\text{BQ}$ : 255nm
  - Me$p\text{BQ}$: 250nm

* Data triplicated.
Absorbance Change at $\lambda_{\text{max}}$

- $[\text{pBQ}] = 0.05\text{mM}$, $[\text{Lys}] = 20\text{mM}$
- $[\text{ClpBQ}] = 0.05\text{mM}$, $[\text{Lys}] = 20\text{mM}$
- $[\text{MepBQ}] = 0.05\text{mM}$, $[\text{Lys}] = 20\text{mM}$

*Estimated $t_{1/2}$:
  - $\text{pBQ}$ : 10min
  - $\text{ClpBQ}$ : 5min
  - $\text{MepBQ}$ : 40min

* Data triplicated.
## Rate Law

<table>
<thead>
<tr>
<th>Order</th>
<th>Reaction</th>
<th>Rate law</th>
<th>Rate eq.</th>
<th>Reaction rate (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0th</td>
<td>A\rightarrow B</td>
<td>( r = k )</td>
<td>([A]=[A_0]-kt) (k unit: M( \cdot )time(^{-1} ))</td>
<td>Constant as the reaction progresses.</td>
</tr>
<tr>
<td>1st</td>
<td>A\rightarrow B</td>
<td>( r = k[A] )</td>
<td>( \ln([A]/[A_0]) = -kt ) (k unit: time(^{-1} ))</td>
<td>Directly proportional to the concentration. As the reactant is consumed during the reaction, the concentration and the rate of the reaction drops.</td>
</tr>
<tr>
<td>2nd</td>
<td>A+A\rightarrow B</td>
<td>( r = k[A]^2 )</td>
<td>( \frac{1}{[A]} - \frac{1}{[A_0]} = kt ) (k unit: M(^{-1} )time(^{-1} ))</td>
<td>Increases with the square of the concentration. r decreases rapidly as the reactant concentration decreases.</td>
</tr>
</tbody>
</table>

A+B \rightarrow C+D | \( r = k[A][B] \) | | | |
Kinetic Analysis

- Kinetic Equation Used:

\[
\ln\left(\frac{A_\infty - A_t}{A_\infty - A_0}\right) = -k(1)_{\text{obs}} t
\]

- $A_\infty$: Absorbance of Q at the time infinity
- $A_t$: Absorbance of Q at time $t$
- $A_0$: Initial absorbance of Q
- $k(1)_{\text{obs}}$: Pseudo-first order rate constant
- $t$: Reaction time
Calculation of Rate Constant

$[\text{Me}\rho\text{BQ}] = 0.05\text{mM}, [\text{Lys}] = 20\text{mM}$

\[
\ln\left(\frac{A_\infty - A_t}{A_\infty - A_0}\right) = -k(1)_{\text{obs}} t
\]
Rate Constants ( \([\text{Lys}] = 20\text{mM}\))

* Phosphate buffer (50mM, pH 7.0) at 37°C

\[
\begin{array}{cccccc}
\text{[Quinone]} (\text{mM}) & 0 & 0.05 & 0.1 & 0.15 & 0.2 & 0.25 \\
\text{k(1) obs (min}^{-1} \text{)} & & & & & & \\
\end{array}
\]

\(\text{pBQ} = 0.1\text{min}^{-1}\)
\(\text{ClpBQ} = 0.23\text{min}^{-1}\)
\(\text{MepBQ} = 0.023\text{min}^{-1}\)

* Data triplicated.
* Rate constant= \(k(1)_{\text{obs}} = k[A.A]\)
Rate Constants ( [Quinones]= 0.01mM)

* Phosphate buffer (50mM, pH 7.0) at 37°C

* Data triplicated.

* Rate constant= $k(1)_{obs} = k[A.A]$
Optimization Using Other Amino Acids

- [p BQ]=0.01mM, [Ser]=20mM
  - \( t_{1/2} \): 23min

- [p BQ]=0.01mM, [Thr]=20mM
  - \( t_{1/2} \): 20min

- [p BQ]=0.01mM, [Asp]=20mM
  - \( t_{1/2} \): 310min

- [p BQ]=0.01mM, [Cys]=20mM
Conclusions & Future Plans

- Pseudo-first order rate constants are independent of [Q].
- We will investigate the dependence of the rate constants on [A.A]
- Rate of product formation will be analyzed.
- The reactions with other selected amino acids will be carried out.
References


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