Studies of lysozyme modifications by substituted benzoquinones

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Abstract

Amyloidosis, which involves the precipitation of mis-folded protein aggregates, is a prominent process that occurs in many neurodegenerative conditions such as Alzheimer’s disease and Parkinson’s disease. Our laboratory has focused on lysozyme modifications induced by a metastable form of benzoquinones known as polycyclic aromatic hydrocarbons (PAHs). PAHs are found in substances ranging from grilled meats to cigarette smoke to cosmetics, and they can be metabolized into a family of biological toxins known as benzoquinones. The molecules studied for this research project were 1,4-benzoquinone (BQ), 2-chloro-1,4-benzoquinone (CBQ), and 2-methyl-1,4-benzoquinone (MBQ). The effect these benzoquinones had on lysozyme was studied by first creating samples through time- and concentration-dependent incubations in physiologic conditions. These samples were then examined through SDS-PAGE analysis, fluorescence assays, and UV-Vis spectroscopy to determine the products created through this modification. Our findings revealed the effective oligomerization and aggregation of lysozyme modified by benzoquinones.

Introduction

Aggregation of mis-folded proteins in the form of amyloid plaques is a relevant process to many neurodegenerative diseases. In Alzheimer’s disease, there is the β-amyloid, while in Parkinson’s disease, α-synuclein is the suspect 1. The process by which these polymeric aggregates self-assemble into fibril plaques of cross β skeletons is not completely understood 2. Our laboratory focused on this process of aggregation as induced by benzoquinones. Benzoquinones are some of the common metabolites of PAHs 3. Quinones resulting from the reduction of PAHs (Figure I), such as the three in this study, have been suspected to cause their toxicity by reacting with proteins through redox cycling and adduct formation 3,4.

Methods

Time- and concentration-dependent incubations were performed in physiologic conditions of pH 7.0 and a 50 mM phosphate buffer at 37 °C. Aliquots were taken at times from 10 minutes to 24 hours and compared against controls. Four concentrations (3.0 mM, 1.0 mM, 0.5 mM, and 0.1 mM) of each quinone were tested against 0.1 mM lysozyme. These samples were run through sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) for analysis. Each gel compares the time variance at each concentration of quinone.

Results

Figures II-IV represent the results of the SDS-PAGE experiments for BQ, CBQ, and MBQ, respectively. From these experiments, it was determined that CBQ caused oligomerization and aggregation both faster and more intensely than BQ or MBQ, with MBQ having the smallest effect on lysozyme.

Conclusions

The results of this experiment showed the effective oligomerization and aggregation of lysozyme leading to amyloidosis as induced by BQ, CBQ, and MBQ. During the experiments, it was also noted that a fibrous plaque formed in the SDS-PAGE wells at higher concentrations of all quinones, further suggesting this mechanism of toxicity. This project is being continued through fluorescence assays and UV-Vis spectroscopy, however, optimizing a methodology is proving difficult due to the plaque that forms on cuvettes walls. This further work will optimally lead to a more definite conclusion for the mechanism that occurs during these reactions.

Acknowledgments

- UTC Undergraduate Research Program funded by the Grote Chemistry Fund
- UTC Department of Chemistry
- Provost Student Research Award

References

7. Albert, R. V., Biological implications of 2-chlorobenzoquinone-2,5-dione-1,4-dione toward ribonucleoside A. Advances in Biscience and Biotechnology 2013, 54.