Bonding Patterns of Hydroxynapthoquinones

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Biophysical Chemistry

Introduction

•The bc complex is important for a number of reasons including:

A. its presence in both mitochondrial and phostosynthetic electron transport chains (ETC)

B. it being the rate limiting step in both ETC's

C. its possession of a unique forked electron path

•The natural substrates of the bc complex are quinols, the specific one used in these studies was ubiquinol (UQH_2) (Figure 1).

•In the quinol oxidase (Q_o) site of the bc complex, $UQH_2$is converted to ubiquinone (UQ) (Figure 2)

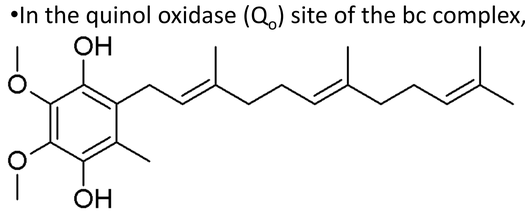


Figure 1. Ubiquinol

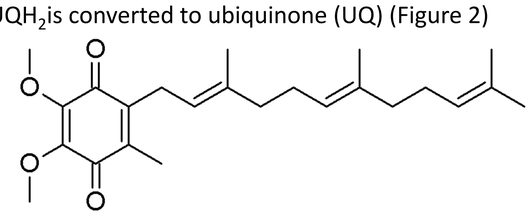


Figure 2. Ubiquinone

•The specifics of how natural substrates are bound in the Q_o site are unknown because a crystal structure of the bc complex with a bound substrate has not been achieved.

•Because of this we are dependent on looking at how inhibitors bind to the Q_o site using crystal structures, and how the inhibitors affect the activity of the bc complex in order to gain knowledge of the binding of substrates to the Q_o site.

•Crystal structures of the bc complex has revealed that the Q_o site appears to be occupied by only two hydrophillic amino acids, a glutamate and histidine, both of which believed to be involved in the binding of both substrates and inhibitors.

•The class of inhibitor studied here, hydroxynapthoquinones (OH-NQ), includes the anti-malarial drug atovaquone (Figure 3) which is believed to be bound as shown in Figure 6. [1]

•The inhibitors used in these studies were 2-(3,7-dimethyloctyl)-3-OH-NQ (Figure 4) and its reduced counterpart (Figure 5)

•Kinetic tests monitored the activity of the bc complexes by measuring the reduction of cytochrome c using change in UV absorbance.

•Half maximal inhibitory concentrations (IC_{50}) values were calculated using the program found at http://www.changbioscience.com/stat/ec50.html

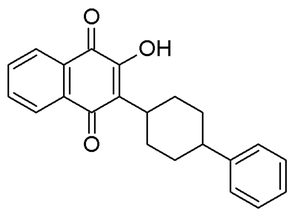


Figure 3. Atovaquone

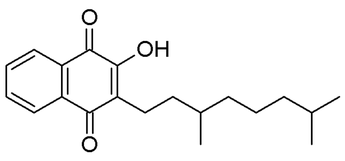


Figure 4. 2-(3,7-dimethyloctyl)-3-OH-NQ

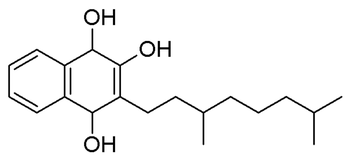


Figure 5. 2-(3,7-dimethyloctyl)-3-OH-NQH_2

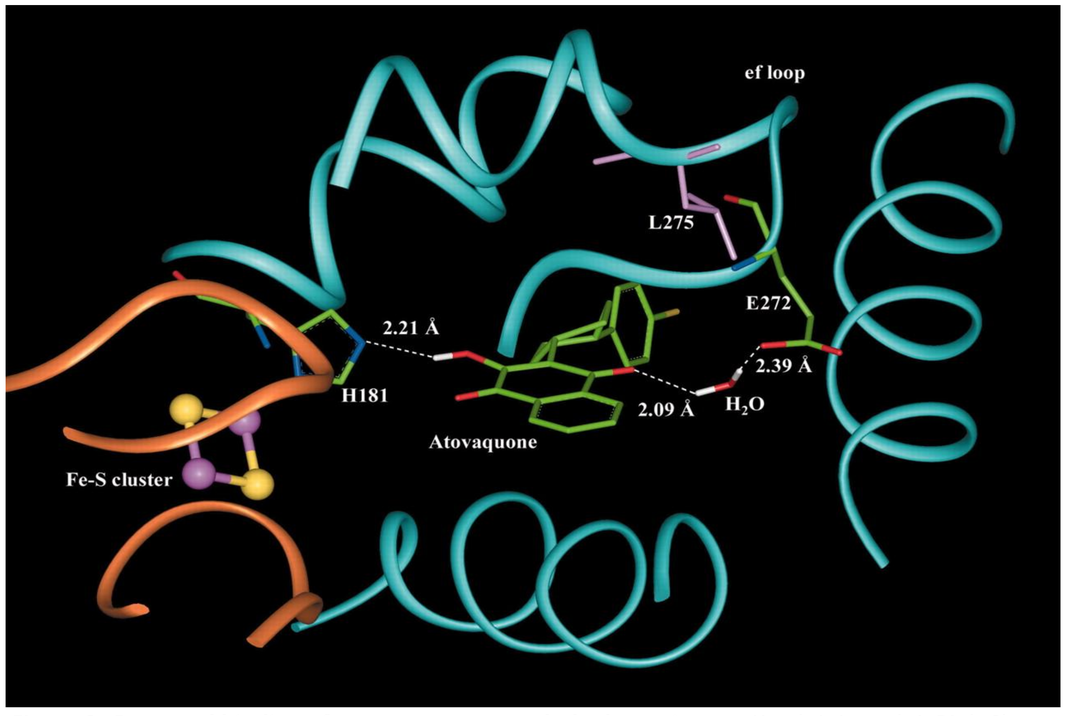
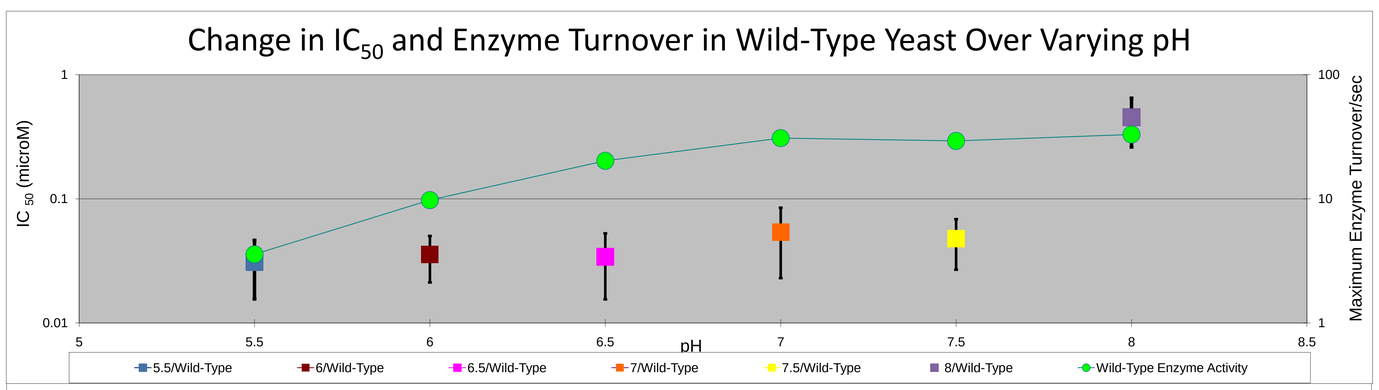


Figure 6. **Proposed binding of atovaquone, a napthohydrxyquinone.** Kessl, J. J. et al. J. Biol. Chem. 2003;278:31312-31318.



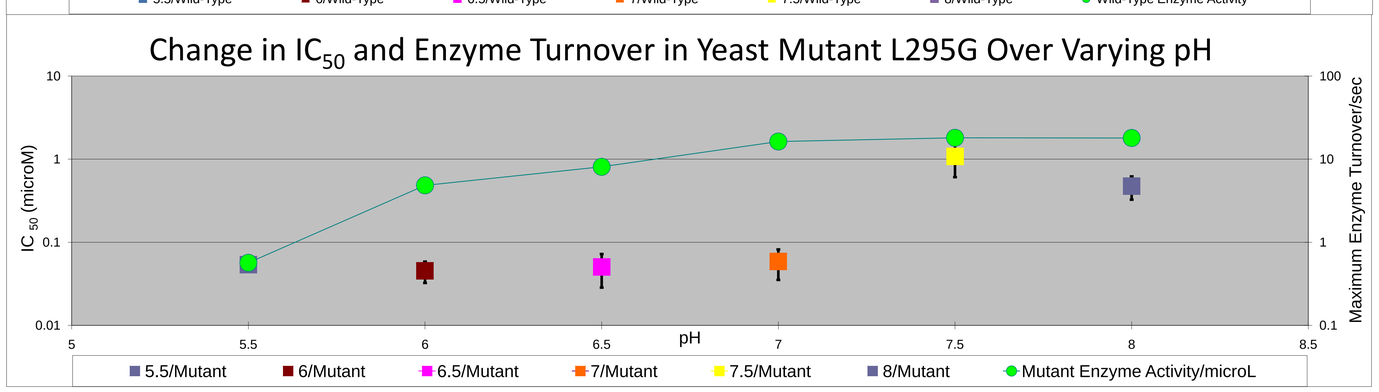


Figure 7 (Top) and 8 (Bottom). **Effects of changes in pH on both the maximum enzyme turnover and IC_{50} values of wild type yeast and a L295G yeast mutant.** Squares correspond to the primary y-axis (left) and green circles correspond to the secondary y-axis(right). IC_{50} values were obtained using a program from http://www.changbioscience.com/stat/ec50.html which also provided the standard deviations.

Results/Discussion

•Initially 2-(3,7-dimethyloctyl)-3-OH-NQ and 2-(3,7-dimethyloctyl)-3-OH-NQH_2 were tested as inhibitors in yeast bc complex. These tests resulted in no clear difference between the reduced and oxidized forms of the inhibitor. (Data not shown) .

•Kinetic assays were then carried out on the yeast wild type bc complex at different pH levels. Also, inhibitory assays were conducted at identical pH levels in order to find the corresponding IC_{50} values. Results are shown in Figure 7.

•These assays were then also performed on a yeast mutant that contained a leucine residue, whose R-group is a isobutyl, instead of a glutamate, whose R-group contains a carboxylic acid. This was done to disrupt the hydrogen bonding network that is theorized to aid in the binding of the inhibitor and UQ.[1,2] Results are shown in Figures 8.

•Comparing the trends of the maximum enzyme turnover in the wild type and mutant show no significant difference. This suggests that the major contributor to the bonding of UQH_2 is the histidine residue and that its deprotonation is integral to the effectiveness of the enzyme. Also this data suggest that the pKa of the histidine is approximately 6.5.

•The change of the IC_{50} value in the wild type and mutant bc complex are at approximately 7.75 and 7.25 respectively, indicating that the histidine is not a major contributor to the bonding of OH-NQ to the Q_o site.

•Two possible implications of this data is that the presence of the glutamate is significant to the bonding of OH-NQ.

•Also, the increase of the IC_{50} values an entire log unit in only half a pH unit indicates two protonations are taking place that each contribute to the bonding of the OH-NQ. The only possible groups that would be available for protonation in the mutant would be the hydroxyl group of the NQ-OH and the histidine residue.

•Having already accounted for the pKa of the histidine this leaves two possible explanations:

A) There is a unknown residue that is currently unaccounted for affecting the binding of the OH-NQ

B) Technical error in experimental technique.

•Further testing would be required to identify which of these is in fact the correct hypothesis. It should be noted though that if there is in fact an unaccounted for group it calls into question the identity of the histidine as the group affecting binding of UQ at pH 6.5.

•Future work would include

A) Titrations to identify the pKa of 2-(3,7-dimethyloctyl)-3-OH-NQ,

B) Further trials of the experiments shown to confirm results more rigorously.

References

1. Kessl, Jacques J., et. al. Molecular Basis for Atovaquone Binding to the Cytochrome bc_1 complex. *J. Biol. Chem*. **2003**, 278, 31312-31318.
2. Palsdottir, Hildur, et al. Structure of the Yeast Cytochrome bc_1 complex with a Hydroxyquinone Anion Q_o Site Inhibitor Bound. *J. Biol. Chem*. **2003,** 278, 31303-31311.

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