

Discussion questions Chem 111 (Oct 15, 2004)

Analysis of paper by Egawa et al: "Kinetic and Spectroscopic Characterization of Hydroperoxy Compound in the Reaction of Native Myoglobin with Hydrogen Peroxide"

- 1) What is the overall purpose of this research report?
- 2) What is the biological role of myoglobin?
- 3) What is the Soret absorption band?
- 4) Explain what does EPR measure
- 5) What are the common oxidation states of iron called?
- 6) What is metmyoglobin?
- 7) What are peroxidases?
- 8) Where is Compound I in Figure 1?
- 9) What approaches were used by researchers who were able to observe intermediates that form prior to Intermediate I in horseradish peroxidase?
- 10) What is ENDOR?
- 11) What evidence prior to this study suggested that the distal histidine is important in catalysis of peroxidases?
- 12) How was the instrument's dead time measured? How long was it?
- 13) How many absorbance values were recorded in each experiment?
- 14) Not all the collected data were used. Why?
- 15) Authors estimate the total accumulated amount of intermediate as $[I]_{\text{total}} / [R]_{\text{total}} = k_1 / k_2$. Is this relationship universally true at any given time?
- 16) What is Eq. 4 good for?
- 17) What coolant was used in the rapid-freezing experiment to obtain frozen flakes of myoglobin- H_2O_2 mixture
- 18) What method was used to characterize the composition of these frozen flakes
- 19) What does visual inspection of Fig 2a and 2b tell us about the presence of intermediates in the reaction.
- 20) Does spectrum 2b provide evidence for formation of Compound I
- 21) Why was SVD used to further analyze data on Figure 2B
- 22) What are matrixes U , S , and V^T
- 23) Explain plots A and D in Figure 3
- 24) Explain plots B and E in Figure 3
- 25) Explain plots C and F in Figure 3
- 26) How did the authors know the true spectrum of Compound I
- 27) What was so special about spectrum on plot F, Figure 3?
- 28) Why was analysis of kinetics from rows of matrix V^T problematic?
- 29) What is the standard statistical of diagonal elements in matrix S .
- 30) What chemical significance do elements of matrix S carry according to authors? Does this sound reasonable?
- 31) Show how Eq 5 arises from Eq 2.
- 32) So, how come they were able to observe this intermediate by SVD if it reached maximal value at 2 ms while their instruments dead time is 6 ms?
- 33) What was done to get further evidence for this short-lived intermediate?
- 34) How can one recognize $[\text{Fe(III)-O-O-H}]^-$ species based on EPR data?

Graduate student homework assignments:

Write short answers, using your own words, to the following questions:

- 1) What are the main characteristics of the Soret absorption band?
- 2) Explain what does EPR measure? How was this useful for Egawa and co-workers?
- 3) Explain the working principle of ENDOR?
- 4) Explain in detail how was the instrument's dead time measured? Make sure to show the chemical structures of reagents used and write the overall reaction mechanism. Give an appropriate literature reference for this method.
- 5) Derive Equation 3/
- 6) What are matrixes \mathbf{U} , \mathbf{S} , and \mathbf{V}^T
- 7) What was so special about spectrum on plot F, Figure 3?
- 8) Using *Mathematica*, show whether their deduction that $k_1/k_2 < 10^{-2}$ based on magnitudes of singular values, is reasonably accurate.
- 9) Discuss the importance of pH studies in understanding the mechanism of formation of Compound I.
- 10) Do you think that the species observed in freeze-quench experiments is the same as the one that was observed in the SVD analysis of UV-Vis spectra? Justify your reasoning?