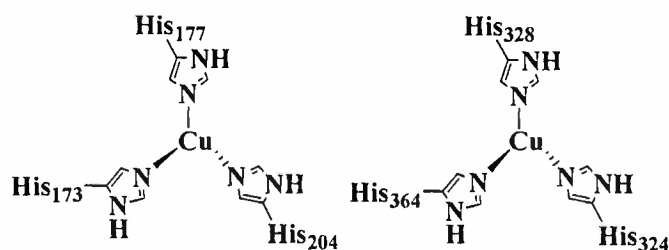


# Mid Term Examination

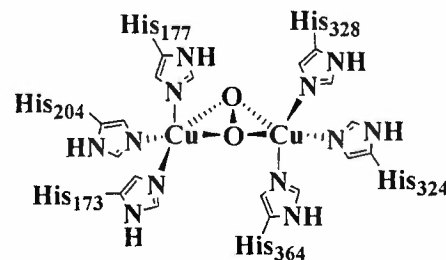
CH4201

Total Marks:20; Time 90 min; Answer all Questions

1. Hemocyanins, non-heme bimetallic copper proteins, are oxygen carriers in anthropods such as spiny lobster. Below are core structures of deoxy- and oxyhemocyanin. Deoxyhemocyanin has both copper ions in the I oxidation state. Oxyhemocyanin is diamagnetic.



Deoxyhemocyanin

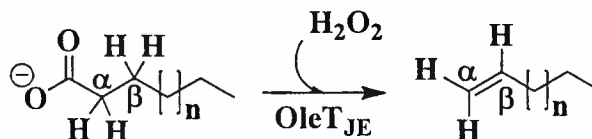


Oxyhemocyanin

- Indicate the oxidation state of the copper ions and of the  $O_2$  ligand in oxyhemocyanin.
- What single method: mass spec, UV-vis, or IR/Raman spectroscopy would be best suited to verify the oxidation state of the  $O_2$  ligand in oxyhemocyanin? Briefly explain your answer-for example; would oxygen isotopes such as  $^{18}O_2$  be useful?
- Model studies indicate that the  $\mu-\eta^2: \eta^2 Cu_2^{II}$  peroxide intermediate is in equilibrium with the  $\mu$ -bis-oxo dimer. Write the structure of the  $\mu$ -bis-oxo  $Cu_2$  dimer and indicate the oxidation state(s) of the Cu centers.
- Using molecular orbital interaction diagrams explain why the O-O stretching frequency of  $\mu-\eta^2: \eta^2 Cu_2^{II}$  peroxide is lower than that of the  $\mu$ -1,2-peroxo  $Cu_2^{II}$  peroxide complex.

(6 marks)

2. Cytochrome P450s are known to catalyse several reactions including the oxidative decarbonylation reaction as has been taught in class. Recently a new member of the Cytochrome P450 family from *Jeotgalicoccus sp.* has been discovered which metabolizes  $n$  chain length fatty acids to produce  $(n - 1)$  alkenes, utilizing hydrogen peroxide ( $H_2O_2$ ) as an oxidant in the reaction (see below).



During the mechanistic investigation, the researchers were able to trap one heme-based reactive intermediate which had the following characteristics: (i) EPR spectra characteristic of  $S=1/2$  species (ii) EXAFS indicating a short Fe-O bond distance of 1.6 Å characteristic of "ferryl" complex (iii) Mossbauer parameters ( $\delta_{Fe} = 0.06$  mm/sec) that was similar to well-known  $Fe^{IV}$ -porphyrin complexes. When the reaction was carried out in  $D_2O$ , no

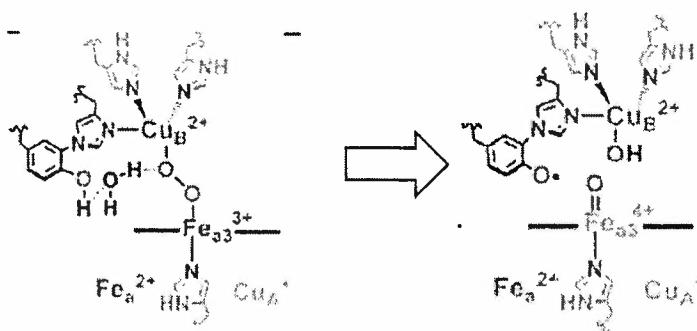
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incorporation of D was observed in the product. In addition, when the deuterated fatty acid was used as substrate, a KIE of 3.0 was observed from kinetic studies

Based on these observations, propose a reaction mechanism for this decarboxylation reaction which is consistent with the experimental observations. As in all Cyt P450, the resting state of the enzyme is  $\text{Fe}^{\text{III}}$ .

(6 marks)

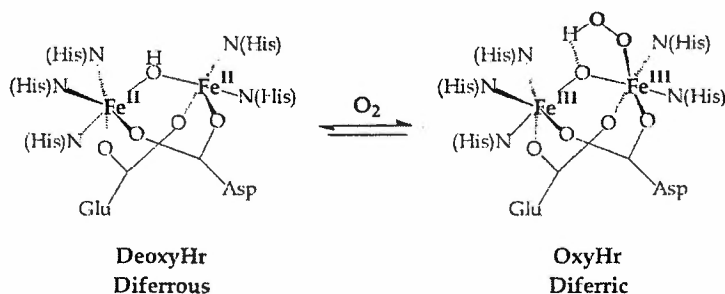
3. In the four-electron reduction of  $\text{O}_2$  by cytochrome c oxidase, the following step shows the O-O bond cleavage of the peroxy species leading to the formation of the ferryl intermediate.



- How is the ferryl (iron oxo) intermediate formed in this case different from the one formed for Horseradish Peroxidase
- The Cu is co-ordinated to one histidine which is covalently linked to the tyrosine. Hypothesise why this unusual tyrosine-histidine dimer is present in this enzyme.

(4 marks)

4. Hemerythrin is an iron-based protein which carries dioxygen. The equilibrium between the deoxy and oxy form is given below:



In OxyHr, the peroxo intermediate is unsymmetrical with the hydroperoxo group bound to only one iron centre. In the absence of crystal structure of OxyHr, the other two possibilities could have been the corresponding  $\mu$ -1,2-peroxo or the  $\mu$ - $\eta^2$ : $\eta^2$  peroxide (both dimeric  $\text{Fe}^{\text{III}}$ ). How would you design Resonance Raman experiments to distinguish between these three binding modes discussed above?

(4 marks)

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