Bioinorganic Chemistry **Exam II** **Mar. 25, 2009**

Name ________________________________

(18) 1. Metal ions can contribute to phosphate ester hydrolysis in a number of different ways. Select a single specific enzyme from the examples presents in class and describe **in detail** at least two different modes of metal ion catalysis in the reaction catalyzed by that enzyme.

Possible modes of metal ion catalysis of phosphate ester hydrolysis:

Lewis acid activation
nucleophile activation
leaving group activation
general base catalysis
general acid catalysis

One example of an enzyme with multiple modes of metal ion catalysis is pyrophosphatase which catalyzes the cleavage of pyrophosphate into two phosphates.

Several metal ions are involved in binding the pyrophosphate substrate through the phosphate oxygens and polarizing the P-O bond to make phosphorus more electron deficient and more susceptible to nucleophilic attack (Lewis acid activation).

One metal ion promotes the ionization of a bound water molecule to generate the hydroxide nucleophile that attacks the phosphorus (nucleophile activation).

Finally, the coordination of the metal ions help to neutralize the charge on the leaving phosphate group (leaving group activation).

(20) 2. A large number of metalloproteins are found to require multiple metal ions for full biological activity. For **five and only five** of the examples given below list the function of each of the metal ions.

(a) Ni^{2+} and Fe^{3+} ions in hydrogenase

The Ni and Fe ions are bound in a binuclear center with a bridging cysteine and hydroxide. The hydrogen substrate coordinates to the axial position on the Fe.

(b) two Ca^{2+} ions in subtilisin

The weaker Ca site is 4-coordinate and binding at this site leads to higher enzyme thermostability. The strong Ca site is 7-coordinate and functions in catalysis.

(c) two Fe^{3+} and two Cu^{2+} ions in cytochrome oxidase

The two heme-Fe and two Cu ions each function to accept an electron from successive cytochrome c donors and then deliver the four electrons to reduce oxygen to water.

(d) two Zn^{2+} ions in carboxypeptidase

The physiological Zn has three protein ligands and a bound water that ionizes to generate the hydroxide nucleophile in the reaction. In the presence of excess zinc a second Zn ion binds and causes inhibition of enzyme activity.

(e) two Zn^{2+} ions in alcohol dehydrogenase

One Zn ion plays a structural role by stabilizing the enzyme through binding to four cysteines. The other Zn plays a catalytic role and has an open ligand position that binds the alcohol substrate.

(f) two Mg^{2+} and one K^{+} ions in pyruvate kinase

One Mg ion binds to ATP while the other Mg and K ions help to bind to the phosphorylated substrate.

(g) two Cu^{2+} ions in hemocyanin

The two Cu ions occupy a binuclear metal site that bind oxygen by bridging between the two metal ions.

(h) two Fe^{3+} ions in ribonucleotide reductase

The two Fe ions are in a binuclear site with bridging glutamate and oxygen ligands. These ions provide the binding site for the ribose substrate.

(16) 3. Carbonic anhydrase catalyzes a simple reaction, the hydration of carbon dioxide, with very high efficiency. Describe each of the contributions that the essential metal ion in carbonic anhydrase contributes to this high efficiency.

Carbonic anhydrase has an essential zinc ion that is required for catalytic activity. The Zn is coordinate by three histidines from the protein and has an open ligand position. The Zn contributes to the high efficiency of the enzyme by:

1. binding a water molecule and lowering its pK value to generate hydroxide which is the nucleophile in the reaction

2. positioning the Zn-coordinated hydroxide adjacent to the substrate binding site for attack on carbon dioxide

3. coordinates the bicarbonate product after its formation

4. facilitates proton transfer within bicarbonate to allow product release

(10) 4. The enzyme xylose isomerase requires bound metal ions both for stability and for catalytic activity. Describe the relative effectiveness of different metal ions on the stability of the enzyme and on the catalytic activity of xylose isomerase.

Xylose isomerase is less stable in the absence of bound metal ions, with Mn and Co leading to a significant increase in the thermostability of the enzyme, while Mg is much less effective in stabilizing the Bacillus enzyme form. The stability of xylose isomerases from thermophilic organisms is much greater and is further stabilized in the presence of bound metal ions.

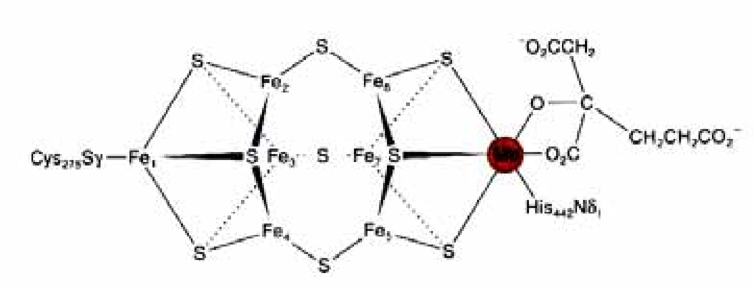
The Mn and Co forms of the Bacillus enzyme are not only more stable but are also more active than the Mg form. However, Mg is a much more effective activator of the Streptomyces enzyme forms at neutral pH, but becomes much less effective at lower pH.

(14) 5. Certain redox reactions require the transfer of more than one electron pair. For either nitrogen fixation catalyzed by nitrogenase or the conversion of sulfite to hydrogen sulfide catalyzed by sulfite reductase describe:

(a) the type of redox cofactors involved

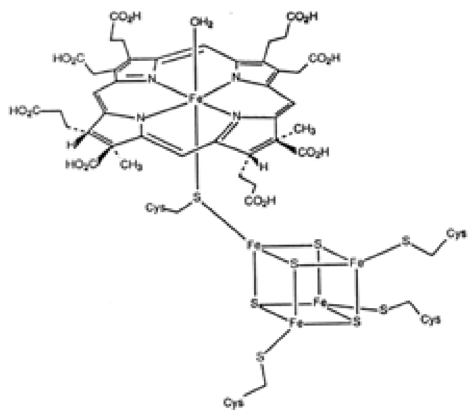
(b) the sequence of electrons transfers in the reaction

Nitrogenase consists of two subunits, an Fe protein containing a 4Fe/4S iron-sulfur cluster and a Mo-Fe protein composed of two 4Fe/4S clusters fused together with one of the Fe ions replaced by an Mo.



Nitrogen likely binds in the center of the fused Mo-Fe cluster and electrons are shuttled from the 4Fe/4S cluster in the Fe protein to the Mo-Fe protein to reduce nitrogen by six electrons to ammonia.

Sulfite reductase contains two different redox cofactors, a heme-Fe that has an open axial position to bind the sulfite substrate and a 4Fe/4S iron-sulfur cluster that is linked to the heme cofactor through a shared axial cysteine on the opposite face of the heme ring.



The electrons required to reduce sulfite to sulfide are channeled to the heme-Fe through the iron-sulfur cluster and serve to reduce sulfite by six electrons.

(10) 6. Describe how the anion binding sites of the sulfate and molybdate binding proteins achieve their exquisite specificity.

The sulfate and molybdate binding proteins each contain a tetrahedral binding site for their respective anions where the binding ligands consist of backbone amide nitrogens and side chain hydroxyl groups.

Despite the identity of the coordination geometry and the types of ligands each binding site is almost absolutely specific for its respective target anion. The molybdate binding protein (ModA) has over a 1000-fold preference for molybdate over sulfate, while the sulfate binding protein has over a 20,000-fold preference for sulfate over molybdate. Neither protein will bind phosphate with high affinity.

It appears that the anion binding sites in these proteins are fine-tuned to recognize the size differences between molybdate and sulfate, and can also recognize the charge differences on the oxygens of these anions to be selective for the appropriate anion.

(12) 7. Most dehydrogenases do not require metal ions to catalyze their oxidation-reduction reactions, but there are a few exceptions. Describe the role of the divalent metal ion in the unusual reaction catalyzed by isocitrate dehydrogenase.

Isocitrate dehydrogenase catalyzes the conversion of isocitrate to oxalosuccinate and then the subsequent decarboxylation to α-ketoglutarate. The enzyme can bind a variety of divalent metal ions, but only Mg and Mn are effective in supporting the reaction.

The role of the divalent metal ion is to:

1. aid in substrate binding through a metal bridged binding mode between isocitrate and the enzyme

2. facilitate decarboxylation by stabilizing the intermediate (oxalosuccinate) to allow carbon-carbon bond cleavage and the release of carbon dioxide