Chemistry 146B, Spring 2009

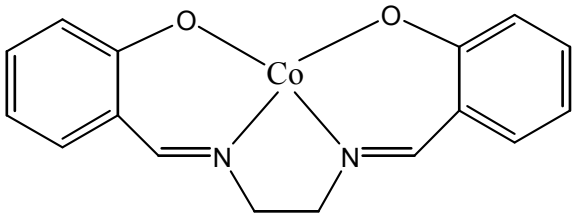
Experiment 1. Synthesis and Characterization of an Oxygen-Carrying Cobalt Complex which Mimics Hemoglobin: *An Introduction to Bioinorganic Chemistry*

Introduction

The discovery that many simple coordination compounds have properties similar to those of metal-containing proteins has caused an explosion of interest in the area of *bioinorganic chemistry*. Many enzymes that catalyze important biological reactions do so with the aid of metal ions. In fact, the metal site is often the critical portion of the enzyme or so-called "active site." It appears that Mother Nature is an excellent inorganic chemist, since much of the chemistry of these biological metal sites can be mimicked with small molecular weight coordination compounds. Examples of metaloenzymes include hemoglobin and myoglobin, which contain iron-porphyrin. Other metals found in nature include nickel in enzymes such as hydrogenase, cobalt in vitamin B12 and vanadium in bromoperoxidases.

The main goal of bioinorganic research is to better understand the roles that transition metal ions play in biological systems. An area of particular interest is understanding the mechanism by which metal centers in proteins bind or activate oxygen. Proteins like hemoglobin contain an Fe(II)-heme unit which reversibly binds oxygen. Cytochrome P450, however, contains a Fe(II)-heme unit similar to hemoglobin and activates oxygen for the hydroxylation of organic substrates. Mollusks bind oxygen with Cu(I) rather than Fe(II)-heme and some worms use a non-heme Fe(II) site for binding and activating oxygen. The earliest models of hemoglobin used ligands simpler than porphyrins as well as metals that were less reactive than Fe(II), such as Co(II). Remarkably, these simple metal complexes successfully mimicked the behavior of hemoglobin.

The purpose of this experiment will be to synthesize and utilize the Co(salen) complex, **1**:



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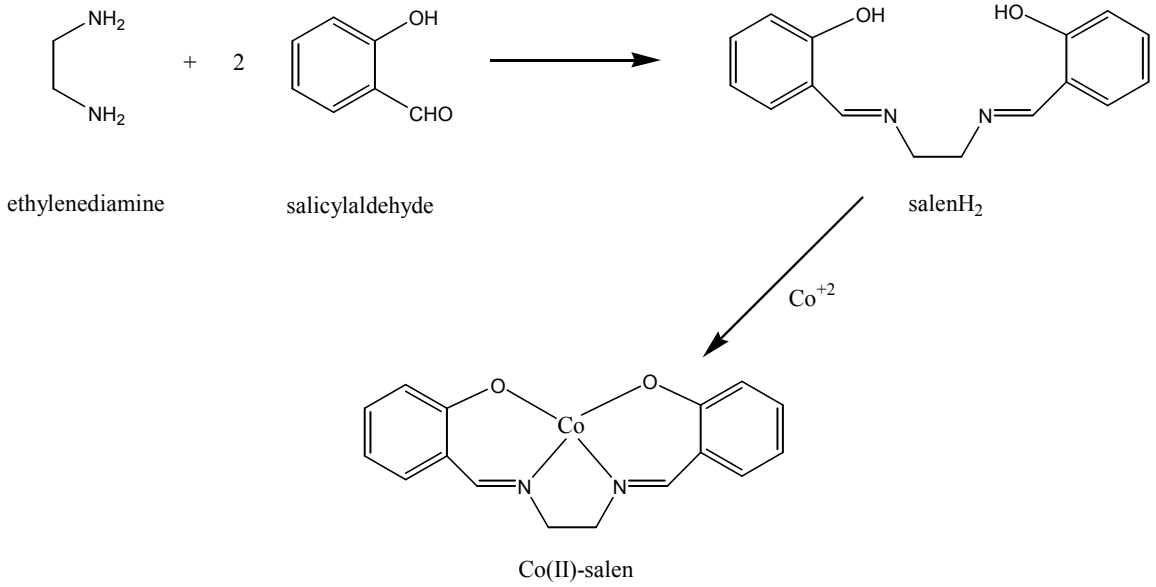
This complex is relatively easy to synthesize and uses a typical inorganic synthesis strategy involving condensation reactions to form Schiff base ligands (Scheme 1).



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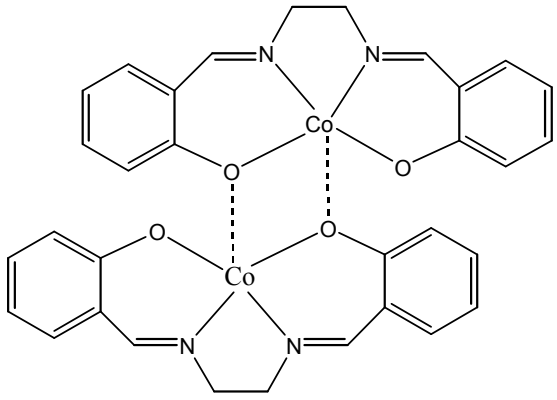
Scheme 1. Synthesis of Co(II)-salen.

Co(II)-salen is a four-coordinate complex that readily adds ligands to become five or six-coordinate. In the solid state, Co(II)-salen can exist in two different forms: **2** is dark red, while **3** is brown. Only one of these forms is capable of binding oxygen.



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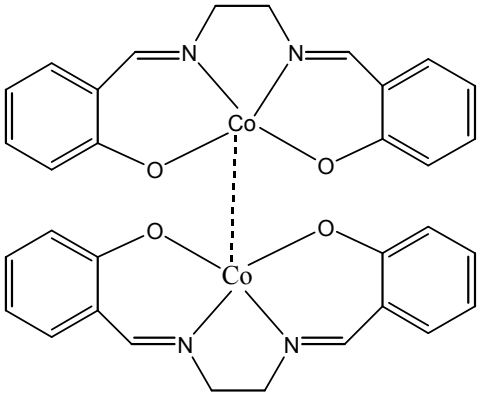

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The propensity of Co(II)-salen to bind oxygen can be rationalized by remembering some of the principles of Chem 151. A Co(II) ion is d^7 and can exist as high spin or low spin configurations. A relatively strong-field ligand can promote oxidation of Co(II) to Co(III) (why do you think this is?). Consider also the electronic configuration of oxygen. Oxygen is a paramagnetic complex with two unpaired electrons and readily reacts with Co(II), where Co(II) is oxidized to Co(III) and the Co-O bond is formed. If the Co(III) ion can become octahedral this makes the reaction energetically feasible. Based on this discussion, it appears that neither of the above forms of Co(II)-salen is poised for reaction with oxygen. In this experiment, you will study the oxygen

binding to Co(II)-salen and determine the active form which is capable of reversible binding.

Experimental Procedures

Safety precautions:

1) You will be using DMSO, which is potentially hazardous since it can rapidly transport impurities into the bloodstream. Thus, DMSO solutions of toxic substances are particularly dangerous. Exercise special care when using DMSO and be sure to clean all spills thoroughly with soap and water.
2) Ethylenediamine is strongly alkaline, caustic, burns on contact and targets the liver and kidneys. The odor is quite strong and it should be used only in the fumehood. Always wear gloves.

Synthesis of N,N-bis(salicylaldehyde)ethylenediimine acid, salenH_2. Add 1.7 g of salicylaldehyde to 20 ml of 95% ethanol in a 50 ml Erlenmeyer flask. While stirring, bring this solution to a boil on a stirring hot plate. Using a small syringe, add 0.5 ml of ethylenediamine in small portions. Stir the solution for approximately 5 min. Remove the flask from the hot plate and allow it to cool to room temperature. Cool the flask in an ice/water bath then collect the bright yellow flaky crystals on a Buchner funnel using the water aspirator. Wash the crystals with a small amount of cold ethanol, then air-dry the product thoroughly. Determine the yield and melting point of your product.

Synthesis of [N,N-bis(salicylaldehyde)ethylenediimino]cobalt(II), Co(salen), the inactive form. Add approximately 1.25 to 1.50 g of salenH_2 into a 100 ml 3-neck flask fitted with a magnetic stir bar and a condenser capped with a nitrogen inlet. Record the actual amount of salenH_2 used in your notebook. Add approximately 40 ml of 95% ethanol and flush the apparatus with nitrogen while stirring for ~ 30 min. Connect the addition funnel, adjust the nitrogen flow to a steady rate and immerse the flask in a 70-80 °C water bath while providing a slow, steady flow of cool water through the condenser. *After* all of the salenH_2 is dissolved, weigh out 0.93 g of cobalt(II) acetate, $Co(CH_3CO_2)(H_2O)_4$, for each gram of salenH_2 weighed out. Dissolve the cobalt(II) acetate in approximately 10 ml of hot water and place into the addition funnel. Add this solution drop wise over a period of 10 min. Additions taking longer than 15 min. run the risk of oxidizing the cobalt. After addition of cobalt is complete, reflux for ~ 5 min and then allow the solution to cool under N_2. Discontinue the nitrogen flow and then collect the product on a Buchner funnel in air. Rinse the product three times with 5 ml of diethyl ether and allow to air dry. If further drying is necessary, use a vacuum dessicator. Determine the yield of the Co(salen) product.

Oxygen Binding Studies. The apparatus to be used for the oxygen binding studies is illustrated in Figure 1. Accurately weigh out ~ 0.050 g of finely pulverized, dry Co(salen) and place into the side-arm test tube portion of the apparatus. De-gas approximately 5 ml of DMSO with bubbling oxygen for 30 seconds. Transfer the DMSO

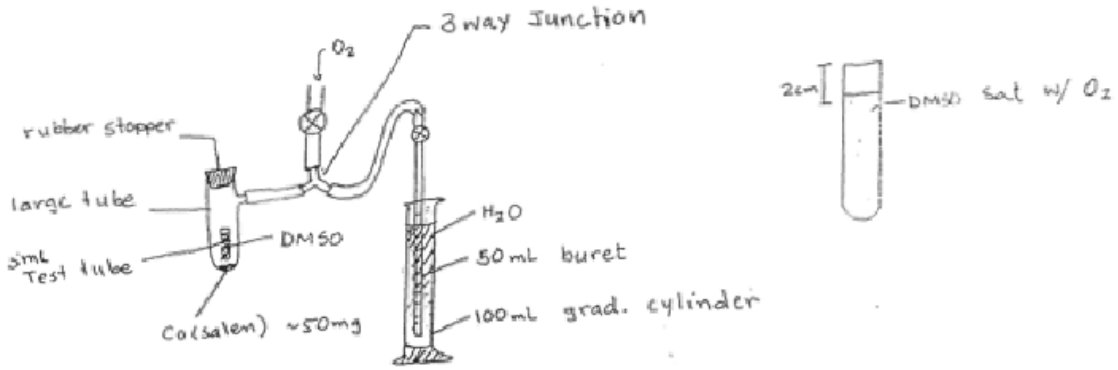

Figure 1. Apparatus to be used for oxygen binding studies.

to a small test tube until it is filled to within 2 cm of the top. Be sure that no solvent has spilled on the outside of the tube and if so, wipe dry with a kimwipe. Lower the small test tube into the side-arm test tube without spilling the DMSO. Flush the apparatus with a gentle stream of oxygen for a few seconds, then insert a tight-fitting rubber stopper into the side-arm test tube and continue flushing. Close the stopcock to the oxygen line, then squeeze the rubber tubing in order to force some oxygen out of the buret. Hold open until the water level is near the first line on the buret. Then open the stopcock until the water level is the same inside and out, i.e. there is no pressure difference, and record the buret reading.

Carefully invert the side-arm test tube so that the DMSO is introduced onto the Co(salen). Hold the apparatus near the stopper to minimize heating by your hand, *but do not push the stopper further onto the tube*. Gently shake the tube, being careful not to spill any of the mixture into the tygon connecting tube. As oxygen is absorbed, the water level inside the buret should rise. Continue shaking until no further changes occur (~ 5 to 10 min.). After the absorption is complete, record the new volume on the buret. Note any other changes that might have occurred. From the decrease in volume at room temperature and constant pressure, the number of moles of oxygen absorbed per mole of Co(salen) can be calculated. Repeat the above oxygen uptake experiment to test reproducibility. Save the precipitate for the next step.

Remove the stopper from the side-arm test tube and transfer as much of the dark-brown suspension to a centrifuge tube as possible. Centrifuge the mixture until the precipitate has settled to the bottom of the test tube. Remove the supernatant DMSO with a Pasteur pipette. To the wet residue, add 5 to 10 ml of chloroform without mixing. Observe and record any changes. Repeat the entire oxygen binding procedure with chloroform instead of DMSO in the small test tube in the side arm.

Report and Discussion: The following points are meant to provide some insight into the sort of issues you should be addressing in the results and discussion section of your report. Do not answer them as questions one by one, but rather use them as a starting basis for the discussion.

1) Report the yields and colors of the salenH_2 ligand and the various salen complexes. Describe any unusual observations and possible reasons for low yield. Explain all observations, precipitates, etc.

2) Report the amount (grams and moles) of Co(salen) used for the oxygen experiments and the total oxygen absorbed. Explain the stoichiometry of the adsorption reaction by drawing a structure for the oxygenated product.

3) What is the structure of the active form of Co(salen)? Explain why this form is active and the other is inactive.

4) What is the purpose of the DMSO? What role does it play in the oxygen adsorption process? Would other solvents work as well? Which ones and why?

6) Why was it necessary to perform the reaction of cobalt acetate with the salenH_2 under nitrogen? Why is the product not air sensitive once formed?

7) Which of the following molecules might also expect to form complexes with Co(salen) in DMSO: N_2, NO, NO_2, CO_2, CN, Cl, CH_3, CO, H_2, SO_3.

References

1) Haggin, J. "High-Capacity Oxygen Sorbents Show Promise In Air Separation Applications", *Chemical and Engineering News*, February 5, 1996, p. 35-36.

2) Calvin, M.; Bailes, R. H.; Wilmarth, W. K. *J. Am. Chem. Soc*. **1946**, *68*, 2254-2256.

3) Chen, D.; Martell, A. E.; Sun, Y. *Inorg. Chem*. **1989**, *28*, 2647-2652.

4) Diehl, H.; Hach, C. C. *Inorg. Syntheses*, **1950**, *3*, 196-201.