Experiment 10

Building Better Blood: Co(Salen) Synthesis and Kinetics

Naturally occurring oxygen carriers and storage proteins contain a transition metal ion to which O_2 can reversibly bind, typically iron (in the form of ferrous heme in proteins such as myoglobin and hemoglobin) or copper (hemocyanin). In this experiment a simple cobalt complex will be prepared which also reversibly binds dioxygen. Many complexes of this type have been used as models to aid in the understanding of how the proteins function. The complex Co(salen) [where salen = N,N-bis(salicylaldehyde)ethylenediimine] reversibly binds O_2, thereby acting as a functional model for myoglobin. As one might imagine, most spectroscopic and measurement techniques are easier to perform and interpret on a molecule which has two orders of magnitude fewer atoms than a protein.

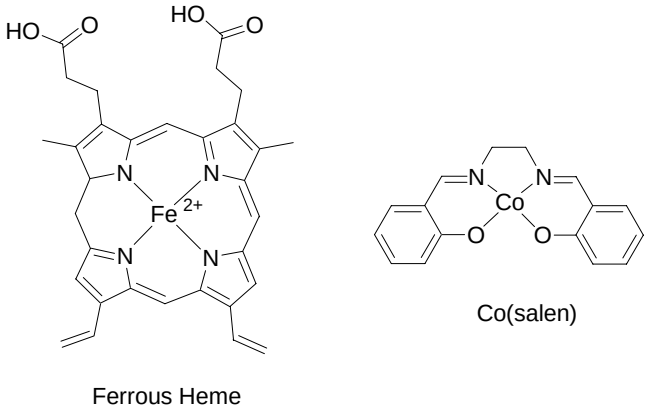


Figure 1. Ferrous heme is the cofactor which binds O_2 reversibly in hemoglobin and myoglobin. Co(salen) is a coordination compound that can also bind O_2 reversibly.

When Co(salen) was first prepared, it was observed that the red-brown crystals darkened on exposure to air. However, it was not until five years later that it was established that the color change was due to reversible uptake of O_2. SalenH_2 (figure 2) is a Schiff-base ligand formed by the condensation of two molecules of salicylaldehyde (sal) with ethylenediamine (en).

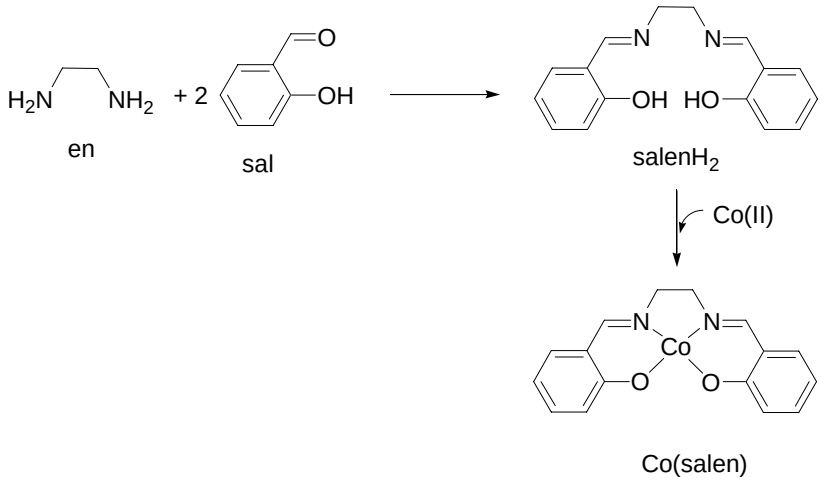


Figure 2. Synthesis of Co(salen).

Later, it was found that different crystalline forms existed depending on the solvent used in the preparation, and that these crystalline forms had varying capacity for oxygenation in the solid state. This variation in oxygenation has been related to the presence of voids in the crystal lattice, sufficient to allow the passage of molecular oxygen. This suggestion is supported by the x-ray crystal structure determination of the so-called "inactive" form which shows that the structure consists of dimeric units $[Co(salen)]_2$. The active forms of Co(salen) are presumed to contain dimeric units with a more-open lattice packing relative to the inactive form.

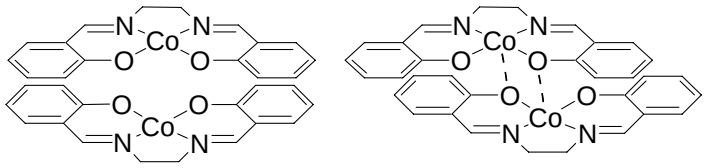


Figure 3. Structures of active and inactive Co(salen) in the solid state.

In anaerobic solution, it has been found that, the cobalt(II) may be four, five or six coordinate, depending on the solvent. For example, in a strongly coordinating solvent such as pyridine (py, C_5H_5N) both [Co(salen)(py)] and $[Co(salen)(py)_2]$ exist, while in chloroform, the only species is Co(salen). When 5- or 6-coordinate species can exist, O_2 will form a complex with Co(salen). The oxygenated product may be a 1:1 ($Co:O_2$) or a 2:1 ($2Co:O_2$) complex. It is thought that O_2 adds as the sixth ligand to a 5-coordinate complex, or replaces one of the coordinating solvent molecules. The final structure may be either a 6-coordinate 1:1 complex, or a 6-coordinate 2:1 complex. It is worth noting that the conventional way to view the formation of an O_2-adduct is as a redox process, in which O_2 accepts one electron from Co^{2+}, thereby forming superoxide, O_2^- bound to Co^{3+}.

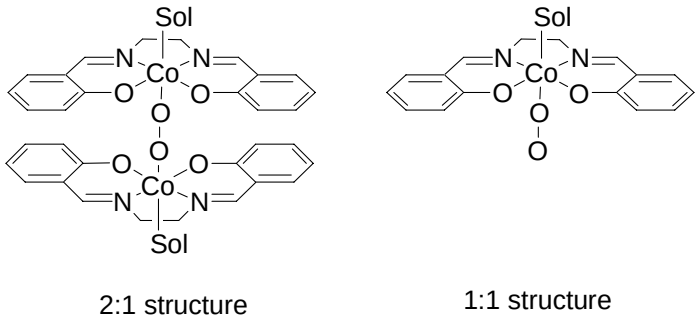


Figure 4. Two possible structures for the O_2 adduct of Co(salen), in which "Sol" represents a coordinating solvent molecule.

In this experiment, the inactive form of Co(salen) is prepared. The uptake of dioxygen is then investigated for the complex in DMSO solution to establish whether a 1:1 or a 2:1 complex is formed under these conditions.

Week 1: Synthesis

H_2salen

Synthesize the salen ligand by placing 3 mL of 95% ethanol in a test tube that also has a small magnetic stirring bar. Heat the ethanol to boiling in an oil bath on a magnetic stirrer/hotplate. Immediately, with continued heating and stirring, add 0.30 mL (2.8 mmol) of salicylaldehyde and then 0.10 mL (1.4 mmol) of ethylenediamine (98%). Stir the solution for 3 to 4 min and then cool in an ice-water bath to precipitate the yellow salen. Filter and wash your crystals and after air-drying, weigh your product and determine your percent yield. Measure the melting point of your ligand as an additional measure of ligand purity.

Co(salen)

NOTE: This preparation is sensitive to air, so it MUST be performed under a N_2-filled balloon.

Add H_2salen (0.23 g, 0.86 mmol) to a round-bottom flask containing a stir-bar, and fitted with a reflux condenser; seal the top of the condenser with a septum. To a separate flask, add cobalt(II) acetate tetrahydrate (0.2 g) to 1.5 ml of water while swirling, and affix a septum. Flush both flasks with N_2 to create an anaerobic atmosphere; approximately 5-minutes is sufficient.

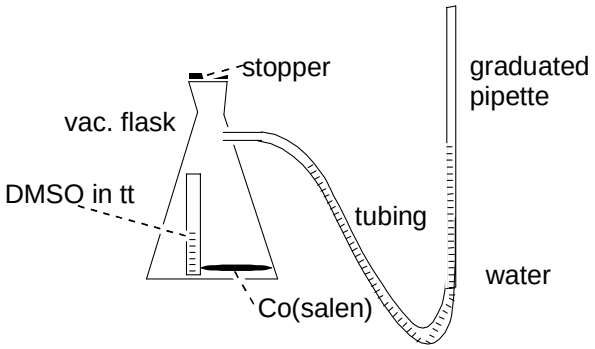
Once both flasks are flushed with N_2, add 12 ml of previously degassed ethanol to the H_2salen via a needle. Swirl to dissolve the H_2salen. Next, add the cobalt acetate solution to the ethanol solution of H_2salen via a syringe, and swirl. A brown, gelatinous precipitate should form.

Replace the septum with an N_2-filled balloon (why is this necessary?). Set the reaction on a sandbath, and heat at a reflux for at least 1 hour. During this period, the initially formed brown "active" complex slowly changes to the brick-red "inactive" complex. Once this has occurred, cool the solution to room temperature, filter to collect the crystals (in air) and wash with 1 ml of ice-cold ethanol. Dry the Co(salen) in a dessicator, then record the yield the following week.

Week 2: Kinetics of O_2 Absorption by Co(salen) in DMSO

Accurately weigh out 50-100 mg of a powder sample of Co(salen) and place it in a side-arm test tube. Larger amounts of Co(salen) make for more accurate results (why is this?) – a good strategy would be to use 1/3 of your material for the first run, and the remaining 2/3 for the second run (where you are more aware of experimental challenges, and are more likely to get good data). Transfer about 5 ml of dimethylsulfoxide (DMSO) to a small test tube that can fit inside the side-arm test tube and lower the tube carefully inside without spillage. (CAUTION: although DMSO is not itself poisonous, it is readily absorbed by the skin and can easily carry other compounds through the skin with it).

Connect up the apparatus as shown in the diagram, so that the movable arm reservoir can be adjusted to bring the water level of the graduated tube to a good starting point (based on how the water level will change, what is a good starting level for the water?), adding or removing water if necessary.



Flush the side-arm tube with a gentle stream of oxygen. Insert a tightly fitting rubber stopper in the mouth of the vacuum flask. Adjust the movable arm to make the water levels equal in both sides (this ensures that the pressure within the apparatus is atmospheric). Record the water level in the graduated tube.

Carefully tilt the filter flask to initiate the reaction, and record the time. The DMSO should be allowed to dissolve the Co(salen), but do not allow any DMSO to spill into the side-arm. As O_2 is absorbed, the water level in the graduated tube begins to drop. Note the changes occurring in the filter flask. Continue monitoring until no further change in water level occurs (taking a reading every one or two minutes for 20 minutes is usually sufficient). You must perform this experiment a second time to verify the reproducibility of your results, given the high sensitivity of your results to leaks in the experimental setup.

Draw a graph of volume changes versus time and extrapolate to estimate the overall total oxygen uptake. From this volume change at room temperature and atmospheric pressure, the number of moles of dioxygen absorbed per mole of Co(salen) should be calculated. In addition to plotting volume versus time, you should try alternate plot axes such as log(volume) or 1/time to more accurately determine the extrapolated volume limit.

Questions.

1. Draw the organic mechanism for Schiff-base formation, using salicylaldehyde and ethylenediamine as reactants. It is a dehydration reaction.

2. What chemical differences between chloroform and pyridine result in chloroform being unable to coordinate to Co(salen) while as pyridine can readily be coordinated? Draw Lewis dot structures of these solvents to support your explanation.

3. Why do you prepare Co(salen) in its *inactive* form prior to determining your yield for its formation?

4. Why does oxygen uptake begin only after DMSO is added? Draw on your knowledge of the chemistry of Co(salen) and DMSO to give a detailed explanation of the changes that occur and the molecular processes that are important. Make sure you include a Lewis dot structure of DMSO.

5. How many moles of dioxygen were actually absorbed per mole of Co(salen)? Give a qualitative estimate (*ie* your best guess) of your error in this stoichiometric ratio. [Your work in obtaining the answers for this question should be discussed in detail in the *Data Analysis* section]