

tronic 20 does not have to be set to 0.00 absorbance upon initial mixing. The cuvet can be thoroughly mixed and settled before setting the absorbance and time to 0.00. If proper technique is used then a relative standard deviation of $\pm 3\%$ can be obtained for repetitive runs. The rate varied from about 0.01 abs/min at a pH of 5 to 0.04 abs/min at a pH of 7.0 with a 0.0015 M GPNA.

The optimum pH was about 7.5 which agrees well with literature values (3). The denaturation temperature was around 45°C. The K_m , V_{max} and k_{cat} values were found to be about $3 \times 10^{-3} M$, $1 \times 10^{-5} M/min$, and 2 mole GPNA/mole CT/min, respectively. These values also agree well with the literature values (1-3). We are presently using the assay system to determine whether or not various inhibitors are competitive or noncompetitive and to determine the K_i values. We hope to present these results shortly.

Procedures A, B, C, and D were used in several senior level biochemistry classes. Good results were obtained by students working alone or in small groups. The experiments were also performed as demonstrations and, again, the results were good. If the solutions are available and if Eppendorf type pi-

pets are used, then the whole experiment can easily be completed in two 3 hr laboratory periods.

Other good enzyme experiments have been published in *this Journal* (1, 2, 4-7) but none of these have the versatility and ease of performance as this series of experiments has. The equipment is common and inexpensive, the chemicals are all readily available and inexpensive, the effects of temperature, pH, and inhibitors are demonstrated, the values of K_m , V_{max} , and k_{cat} are determined, the experiments can easily be done as a demonstration or as individual experiments, and the time element is short.

Literature Cited

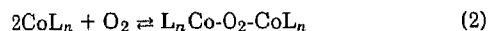
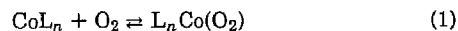
- (1) Hurlbut, J. A., Ball, T. N., Pound, H. C., and Graves, J. L., *J. CHEM. EDUC.*, **50**, 149 (1973).
- (2) Hurlbut, J. A., Bishop, C. V., Brittain, P. C., and Preheim, C. W., *J. CHEM. EDUC.*, **52**, 100 (1975).
- (3) Erlanger, B. F., Edel, F., and Cooper, A. G., *Arch. Biochem. Biophys.*, **115**, 206 (1966).
- (4) Bender, M. L., Kézdy, F. J., and Wedler, F. C., *J. CHEM. EDUC.*, **44**, 84 (1967).
- (5) Devine, J. E., and Toom, P. M., *J. CHEM. EDUC.*, **52**, 816 (1975).
- (6) Splitzgerber, A. G., Mitchell, K., Dahle, G., Puffer, M., and Blomquist, K., *J. CHEM. EDUC.*, **52**, 680 (1975).
- (7) Reigh, D. L., *J. CHEM. EDUC.*, **53**, 386 (1976).

Trevor G. Appleton
University of Queensland
Brisbane 4067
Australia

Oxygen Uptake by a Cobalt(II) Complex

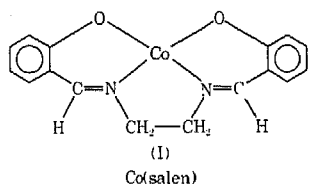
An undergraduate experiment

Natural oxygen-carrier and storage proteins contain a transition metal to which the oxygen reversibly coordinates—e.g., iron (myoglobin, hemoglobin), copper (hemocyanin), vanadium (hemovanadin). Various simpler coordination compounds will also reversibly coordinate oxygen, and have been extensively studied as “model compounds” (1, 2). Cobalt(II) complexes are among the most studied. Two types of bonding of dioxygen to cobalt(II) have been distinguished

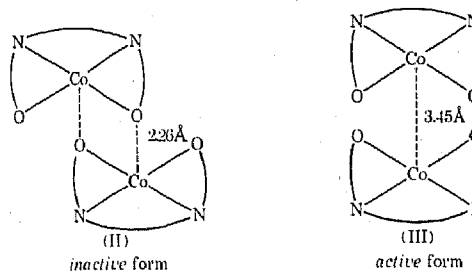
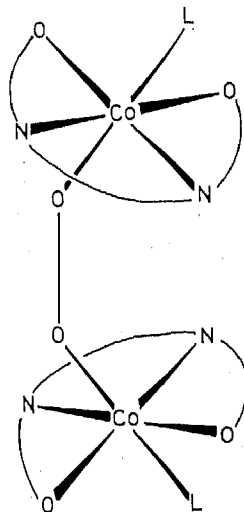


That is, the Co:O₂ ratio may be 1:1 or 2:1, depending on the nature of the ligands, temperature, solvent, etc. (2-7).

One of the best known cobalt(II) “oxygen carriers” is Co(salen) (I), where salenH₂ represents N,N'-bis(salicylaldehyde)ethylenediimine



Co(salen) exists in two different solid forms, depending on method of preparation: a brown form *active* toward absorption of oxygen, and a dark red *inactive* form. The *inactive* form consists of dimeric units (II) in which the Co atom of one Co(salen) molecule interacts with an O-atom of the second (8). The *active* form also has dimeric units, but with one Co atom directly above the other (III) (9). Channels in the structure allow oxygen to enter easily. The *active* form absorbs oxygen at room temperature, and releases it at higher temperatures. This cycle may be repeated many times, although the activity of the compound toward oxygen uptake slowly decreases on continued cycling, owing to decomposition (10).



When Co(salen) is dissolved in donor solvents (e.g., dimethylsulfoxide DMSO, dimethylformamide DMF, pyridine) in the presence of oxygen, adducts rapidly form (6, 7). In DMSO, a dark brown solid, [(DMSO)Co(salen)]₂O₂ precipitates, which probably has a structure similar to that of the DMF analog (Fig. 1, L - DMF) (11). The dia-

Figure 1. Structure of the oxygen adduct.

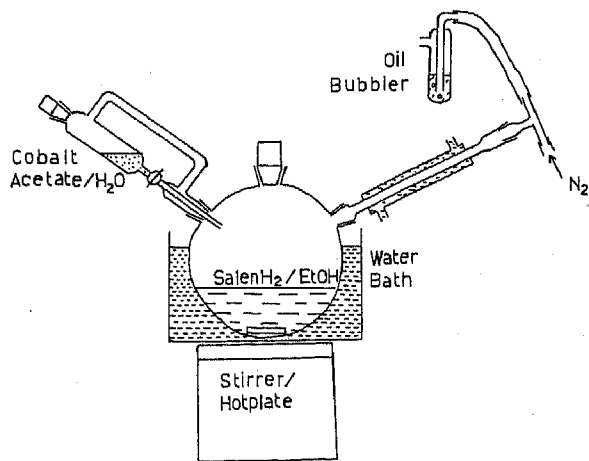


Figure 2. Preparation of Co(salen).

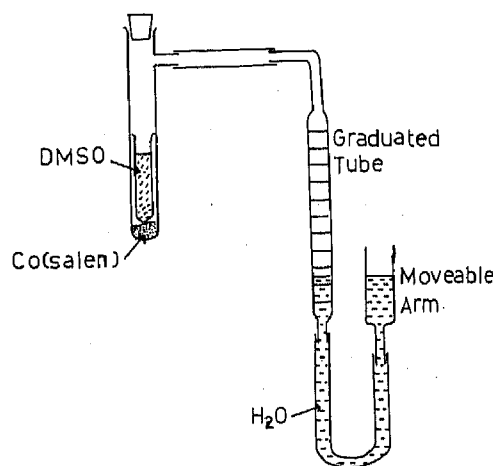


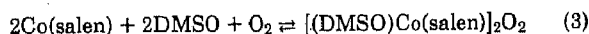
Figure 3. Oxygen uptake by Co(salen).

magnetic compounds may be formally regarded as containing two Co(III) atoms linked by a peroxo bridge.

In this experiment, the inactive form of Co(salen) is prepared by an adaptation of the method of Bailes and Calvin (5). An aqueous solution of cobalt(II) acetate is added to a hot ethanol solution of salenH₂ under nitrogen (Fig. 2). A mixture of *active* and *inactive* forms is initially obtained, which is entirely converted to the *inactive* form when heated in ethanol. Once *inactive* Co(salen) has been formed, it may be filtered off and handled in air.

Oxygen uptake is measured using the simple apparatus depicted in Figure 3. The side-arm test tube is flushed with oxygen to increase the rate of reaction, and to leave no doubt that the gas absorbed is, in fact, oxygen. It is not necessary to remove all traces of nitrogen. When the side-arm tube is inverted, allowing the DMSO to dissolve the Co(salen), oxygen is rapidly absorbed and a dark brown precipitate forms. Reaction is complete in 10–20 min, if the tube is continuously shaken. The small volume of the apparatus prevents small temperature changes from having a large effect on volume. In the two years that this experiment has been used in a third-year Bioinorganic Chemistry course at the University of Queensland, most student results for the O₂:Co ratio have been close to the theoretical value, 0.5, and all have been within the limits 0.44–0.60.

The oxygen adduct formed is a very fine precipitate, which cannot be easily filtered, but may be isolated by centrifuging. When chloroform is added to the adduct, it slowly dissolves, releasing fine streams of oxygen bubbles, to give a red solution of Co(salen). The student can be asked to discuss the factors which affect the equilibrium (3) in DMSO and chloroform solvents



Electron donation by a ligand, *L*, is required to stabilize the structure in Figure 1. Chloroform is a poor donor unlikely to coordinate to cobalt. In DMSO, the high concentration of DMSO provided by the solvent, and the low solubility of the adduct help to force the equilibrium to the right.

The total time required for experimental work, including characterization of compounds by ir and melting/decomposition behavior,

is about 6 hr, easily broken if necessary into two 3-hr sessions.

Experimental

Preparation of *N,N'*-Bis(salicylaldehyde)ethylenediamine, salenH₂

To a solution of 4.9 g (4.2 ml) salicylaldehyde in 50 ml boiling 95% ethanol add 1.2 g (1.35 ml) ethylenediamine. Stir the reaction mixture for 3½ min, and leave the solution to cool in an ice bath. Filter the bright yellow flaky crystals under suction, wash with a small volume of ethanol, and air-dry. Melting point 120°C. Yield 90–100%.

Preparation of the Inactive Form of (*N,N'*-bis(salicylaldehyde)-ethylenediamine)cobalt(II), Co(salen)

Weigh 2.34 g salenH₂ into a 250-ml 3-necked flask. Add 120 ml 95% ethanol. Set up the apparatus as shown in Figure 2. Stir using a magnetic follower, and flush the apparatus with nitrogen. Adjust the nitrogen flow to a steady rate (~one bubble/5 s). Immerse the flask in a water bath maintained at 70–80°C. Dissolve 2.17 g cobalt(II) acetate, Co(CH₃CO₂)₂·4H₂O in 15 ml hot water. When the salenH₂ has all dissolved, add the cobalt acetate solution through the funnel. A brown gelatinous precipitate forms immediately. Continue heating and stirring for a further hour, during which time the precipitate turns dark red. Cool the flask by immersing in cold water. Discontinue the nitrogen flow and filter off the solid on a sintered glass funnel (in the air). Wash three times with 5 ml water, then with 5 ml 95% ethanol. Dry on the funnel, then in a vacuum desiccator. Decomp. ~300°C. Yield 70–90%.

Oxygen Uptake by Co(salen)

Weigh out between 0.05 and 0.1 g ground Co(salen) into a side-arm test tube (2 × 18 cm). Place approximately 5 ml DMSO in a beaker, and bubble oxygen through for a few seconds, to saturate it with oxygen (**caution—DMSO is absorbed through the skin**). Transfer the DMSO into a small test tube (1 × 7.5 cm) until it is filled to about 2 cm from the rim. Using forceps, carefully lower the small test tube into the side-arm tube without spillage.

Connect a length of glass tubing (graduated 0–10 ml—a pipet would suffice although the apparatus shown is easily made), to a second glass tube ("movable arm") using tubing. Arrange the tubes in a U-shape (Fig. 3) and fill with water. Connect the graduated tube to the side-arm tube with polyethylene tubing. Adjust the movable arm so that the water level is near the bottom of the graduated tube.

Flush the side-arm tube with a *gentle* stream of oxygen. Insert a tight-fitting rubber stopper in the mouth of the tube. Adjust the movable arm to make the water levels the same in both tubes (i.e. pressure within the apparatus is atmospheric). Note the water level in the graduated tube. Carefully invert the side-arm tube (holding near the stopper to minimize heating by the hand) so that the DMSO is introduced onto the Co(salen), but not spilled into the polyethylene tube. Gently shake the tube. As oxygen is absorbed the water level in the graduated tube begins to rise. Note the changes occurring in the tube. Continue shaking until no further change in water level occurs (10–20 min). Adjust the movable arm so that the water levels in the two tubes are again equal, and read off the new level in the graduated tube.

From the decrease in volume at room temperature and atmospheric pressure, calculate the number of moles of dioxygen absorbed per mole of Co(salen).

Reaction of the Oxygen Adduct in Chloroform

Remove the stopper from the side-arm tube, and remove as much as possible of the dark brown suspension into a centrifuge tube. Centrifuge until the precipitate has settled to the bottom of the tube (15–30 min). Carefully remove the supernatant DMSO. To the residue in the tube (drying is not necessary) add 5–10 ml chloroform without stirring. Observe the result, and attempt to explain your observations.

Literature Cited

- (1) Klevan, J., Peone, J., and Madan, S. K., *J. Chem. Educ.*, **50**, 670 (1973).
- (2) Basolo, F., Hoffmann, B. M., and Ibers, J. A., *Accounts Chem. Res.*, **3**, 384 (1975).
- (3) Pfeiffer, P., Breith, E., Lubbe, E., and Tsumaki, T., *Justus Liebig's Ann. Chem.*, **503**, 84 (1933).
- (4) Tsumaki, T., *Bull. Chem. Soc. Jap.*, **13**, 252 (1938).
- (5) Bailes, R. H., and Calvin, M., *J. Amer. Chem. Soc.*, **69**, 1886 (1947).
- (6) Floriani, C., and Calderazzo, F., *J. Chem. Soc. (A)*, 946 (1969).
- (7) Ochiai, E. I., *J. Inorg. Nucl. Chem.*, **35**, 1727 (1973).
- (8) Brückner, S., Calligaris, M., Nardin, G., and Randaccio, L., *Acta Crystallogr.*, **B25**, 1671 (1969); Delasi, R., Holt, S. L., and Post, B., *Inorg. Chem.*, **10**, 1498 (1971).
- (9) Schaefer, W. P., and Marsh, R. E., *Acta Crystallogr.*, **B25**, 1675 (1969).
- (10) Wilmarth, W. K., Aranoff, S., and Calvin, M., *J. Amer. Chem. Soc.*, **68**, 2263 (1946).
- (11) Calligaris, M., Nardin, G., Randaccio, L., and Ripamonti, A., *J. Chem. Soc. (A)*, 1069 (1970).