Synthetic models for the active site of alcohol dehydrogenase.
Kaptein, Bernardus
SUMMARY

In this thesis the synthesis of Zn\(^{2+}\) and Co\(^{2+}\) complexes with sulfur and nitrogen containing ligands as model compounds for the active site of Horse Liver Alcohol Dehydrogenase (HLADH) is described. HLADH is an enzyme which reversibly oxidizes alcohols to aldehydes or ketones, making use of the coenzyme NAD\(^+\)/NADH. The active site of HLADH contains a zinc ion, coordinated by two cysteines, a histidine and a water molecule (or hydroxide ion). This zinc ion is necessary for the catalytic activity of the enzyme (Figure 1).

\[
\ce{R^1-C-OH + NAD^+ <-> HLADH <-> R^1-C=O + NADH + H^+}
\]

Figure 1

Coordination of the zinc ion in the active site of HLADH.

A review of the properties of the enzyme HLADH and the coenzyme NAD\(^+\)/NADH is described in Chapter I. In addition, modifications of the active site of HLADH and model compounds for the action of this enzyme known so far in the literature are reviewed.

In Chapter II the synthesis of tridentate ligands is described, i.e. containing an imidazole group and two thiol (or sulfide) groups (Figure }
Complexation of Zn\(^{2+}\) or Co\(^{2+}\) ions by the dithiol containing ligand results in poorly soluble complexes. The thiol groups in these neutral complexes are deprotonated, which is analogous to the coordination site of the Zn\(^{2+}\) ion in HLADH. UV-VIS spectroscopy with the cobalt(II) complex also reveals a coordination identical to HLADH in which the zinc ion is substituted for a cobalt(II) ion. The central aromatic ring in the complexes serves as a cap, shielding one side of the complex.

In a model reaction for the enzyme-catalyzed process ethyl phenyl-glyoxylate is reduced to ethyl mandelate by N-benzyl-1,4-dihydronicotinamide in low yield upon addition of the complexes in catalytic amounts. For the cobalt(II) complex turnover numbers of 50-100 are reached. These imidazole-dithiolate complexes thus act as model compounds for HLADH. A model for their catalytic action is proposed. The sulfide containing ligands (Figure 2a) do not coordinate well with Zn\(^{2+}\) or Co\(^{2+}\) ions; only coordination by the imidazole group is observed. These complexes do not show catalytic activity in the model reaction.
In Chapter III pyridine-dithiol compounds (Figure 2b) are described as ligands for Zn$^{2+}$ ions. The tetramethyl- and tetraphenyl-substituted ligands form dinuclear complexes in which each Zn$^{2+}$ ion is coordinated by one pyridine-dithiolate dianionic ligand and by a sulfur bridge to the sulfur of a second ligand. This was established by $^1$H-NMR spectroscopy and by X-ray analysis. These complexes are inactive in the model reaction which tests the catalytic activity. The identical compound with bis-fluorene groups in the α-position of the thiol groups gave better shielding of these thiols, so dimerization via sulfur bridging is prevented. However, the mononuclear complex formed proves to be unstable, and rapidly decomposed.

In addition, in this chapter the corresponding pyridine-diols (Figure 2c) and their complexing properties are described. The X-ray structure of one of these zinc complexes was determined.

The synthesis of an identical ligand (Figure 2d) with an imidazole group instead of a pyridine group is described in Chapter IV. This imidazole-dithiol ligand is very sensitive to oxidation. Complexation with Zn$^{2+}$ ions results in an insoluble polymeric complex.

In Chapter V 1,4-dihydropyridines are described containing coordinating groups at the 4-position and in the ester groups in the 3- and 5-position. These compounds were prepared by the Hantzsch ester synthesis, as models for a binary complex of HLADH and NADH. The conformation of one of these compounds (Figure 3) was solved by X-ray analysis. The imidazole group at the 4-position of the boat-shaped 1,4-dihydropyridine is in a pseudo-axial position. Conformational analysis by Molecular Modelling for an analogous compound reveals that this is the most stable conformation. Complexation with Zn(NO$_3$)$_2$ only occurs with the nitrogen donors at the 4-position and not with the ether or sulfide substituents in the ester groups. Each zinc ion is coordinated by two dihydropyridine
ligands. These compounds do not reduce ethyl phenylglyoxylate, probably as a result of the pseudo-equatorial position of the hydrogen atom at the 4-position.