SUMMARY.

The three-dimensional structure of the enzyme bovine liver rhodanese has been determined by means of X-ray diffraction. Rhodanese is a sulfurtransferase and in vitro it catalyzes the reaction:

$$S_2O_3^{2-} + CN^- \rightarrow SO_3^{2-} + SCN^-.$$

It is an ubiquitous enzyme in nature and quite possible it has an important role in sulfur metabolism. However, its real function in vivo is still unknown.

In chapter 1 the purpose of this investigation has been described and in appendix 1 my view with respect to the theory of evolution is presented.

In chapter 2 a summary of the most important properties of the enzyme is given, including a discussion of its molecular weight. Contrary to the earlier theory, favouring a dimeric nature of rhodanese, we were able to prove that the molecule consists of a single polypeptide chain of 293 residues and has a molecular weight of 32,800.

In chapter 3 the structure determination is described. The rhodanese we studied was in the form of its enzyme-sulfur intermediate, because rhodanese has been isolated and crystallized in the presence of thiosulfate. The following compounds have been used to prepare heavy atom derivatives: PCMS, $K_2Pt(CN)_4$, $UO_2(Ac)_2$, $NaReO_4$, $NaAu(CN)_2$ and a dimercury compound (di-Hg). For the PCMS and $NaReO_4$ derivatives data have been collected to 2.5 Å resolution, for the other derivatives this has been done in a more restricted part of the reciprocal space only. The intensities of the reflections have been measured

with a four-circle diffractometer. The phases of the protein reflections were determined by the isomorphous replacement method, including anomalous scattering information. Electron density maps with resolutions of 3.0 Å, 2.8 Å and finally 2.5 Å have been calculated. After building a model of the protein molecule with an optical model building device, the atomic coordinates have been measured. With Diamond's modelbuilding program ideal geometry has been imposed on the measured coordinates. The result of the procedure was a r.m.s. shift of 0.17 Å for 2325 atoms.

In chapter 4 at first our cooperation in the amino acid sequence determination with Dr. R.L. Heinrikson and co-workers is described. From the high resolution map we concluded that at two positions a discrepancy still exists with Heinrikson's final sequence.

The map revealed a very clear double domain structure of the molecule. The two domains are of equal size and have a very similar structure. They are related by a pseudo-dyad. In spite of this symmetry for the polypeptide chain folding hardly any similarity is found between corresponding side chains.

The central part of each domain is a five stranded twisted parallel β-structure. On each side of this β-structure a hydrophobic clustering of residues occurs. Of all residues 37% are in the helices, 15% in the β-structures and 24% in turns. In one domain a spectacular network of saltbridges exists.

The active site is in a hole between the two domains. One side of this hole contains mainly hydrophobic residues, while the other side contains mainly hydrophilic residues. The transferable sulfur atom is bound covalently to Cys-247, which is at the bottom of the cleft. The extra sulfur atom is stabilized by five hydrogen bonds.

In chapter 5 binding studies of inhibitors and substrates to rhodanese are described. These studies have been performed initially by means of two-dimensional difference Fourier maps. In addition the binding of adenosine, $K_2Ni(CN)_4$ and $NaReO_4$ has been studied in more detail by three-dimensional difference Fourier maps. Using the same method, the action of KCN on the rhodanese-S complex has been studied in three dimensions: besides the disappearance of the extra sulfur atom we clearly see the binding of either a sulfate or a thiocyanate ion in the active site. On the basis of the three-dimensional structure, the difference Fourier studies and kinetic results of other investigators a mechanism of action of rhodanese has been proposed.

In chapter 6 a structural comparison has been made of rhodanese with alcohol dehydrogenase and flavodoxin. The similarities in structure between these proteins are mainly caused by the parallel pleated sheets and the righthanded β-strand - helix - β-strand units. We conclude that these similarities are a result of the laws governing protein folding.