SUMMARY

Many reactions in the body do not proceed spontaneously, therefore cells constantly need an influx of energy from external sources. The blood transports sugar (glucose) and oxygen to all parts of the body and after uptake by the cells these two components are burnt down to water and carbon dioxide. The energy that is released can be partly stored in the form of the high-energy containing compound adenosine triphosphate, abbreviated ATP. ATP serves as an energy "currency" for energy-demanding processes, such as muscle contraction and for the synthesis of cellular building blocks such as proteins, enzymes and genes. The process is divided into many small steps. The last part of this chain of enzyme-catalyzed reactions takes place in a specialized organelle of the cell, the mitochondrion. Four major enzyme complexes take care of the transformation of energy from the high-energy intermediate NADH (reduced nicotinamide adenine dinucleotide) to ATP. This process is called oxidative phosphorylation and its enzymes form the so-called respiratory chain or electron-transport chain. The first enzyme of the chain is NADH: ubiquinone oxidoreductase, more commonly known as NADH dehydrogenase. It is the biggest enzyme, but with regard to its structure and function, also the most poorly understood part of the electron-transport chain. This enzyme catalyses the oxidation of NADH to NAD⁺, coupled to the reduction of ubiquinone, abbreviated Q. The energy generated in this reaction is used to pump protons across the inner mitochondrial membrane, thus generating a proton-gradient across the membrane. This proton-gradient supplies the energy for the ATP synthesis.

Many details about the structure and function of the respiratory chain have been worked out, but one crucial point has remained puzzling. It is not clear how the transfer of electrons through the series of enzyme complexes is coupled to the synthesis of ATP. A general way to obtain a better understanding about the function of enzymes is to determine their three-dimensional structure. This thesis describes an investigation into the structure of NADH dehydrogenase with electron microscopy in combination with image analysis techniques.
As a general introduction, chapter 1 summarizes the literature concerning NADH dehydrogenase and three of the other big enzyme complexes catalyzing oxidative phosphorylation. The structural aspects of the enzymes have been emphasized.

Chapter 2 describes the crystallisation of NADH dehydrogenase with the aid of a microdialysis-technique. The crystals are very suitable for electron microscopy because they measure about 1x1 micrometer and exist mostly as a monolayer of molecules. The unit cell that is the repeating unit of the crystal has dimensions of 153x153 Å and contains one enzyme molecule.

The removal of noise from the information of the two-dimensional crystals, as recorded in electron micrographs, is an important step in resolving the structure. This image processing is the scope of chapter 3. Noise-filtered images have been obtained by application of a correlation averaging method. The signal-to-noise ratio is enhanced by superposition of the images of small fragments into which the crystals were divided. The ultimate resolution is close to the optimum for the conditions used. By summing up the images of 4300 projections, the two-dimensional projection of the enzyme was determined to a resolution of 13 Å. A higher resolution has only been achieved in a few other cases by the application of different methods of embedding and supporting the material for microscopy. A promising technique to prepare and visualize biological material without strong deformation is cryo-electron microscopy. We have been able to record images of crystals with a resolution of 30 Å. After image processing the resolution of the filtered image was improved to 25 Å, but this is much lower than we ultimately expect to obtain.

Chapter 4 deals with the three-dimensional structure of the enzyme. One two-dimensional projection of the object is not enough for the calculation of the three-dimensional structure. Therefore, information about the missing third direction, perpendicular to the plane of the two-dimensional projection, was obtained by tilting the crystals in the electron beam using an eucentric goniometer stage. Three tilt series, each consisting of 10-16 projections, with tilt angles of up to 55 degrees, were computer processed. In the first step, each of the projections was noise-filtered with correlation averaging, as described in chapter 3 for the untitled projection.

After determination of the common origin in the projections of a tilt series, the aligned and filtered images of all three tilt series were combined after rotational reconstruction scheme (GS), which represents the density contour plots of protein molecules in the plane of the crystal. The NADH dehydrogenase molecule is very similar in shape, but the enzyme is in fact not composed of monomers a central pore running through the plane of the crystal. At the moment the function of this pore is not clear.

In chapter 5 some applications of the technique of correspondence analysis were examined. Two sets of images were selected, they were interrelated using the building block of the crystallographic reconstruction method, correspondence analysis, and averaged. In the averages the molecules, which may be comparable with possible hydrophilic parts of the molecule, were the unit cell of the two-dimensional crystal, with scanning transmission electron microscopy, 1,600,000 was obtained. Furthermore, the enzyme has a molecular weight of 1,600,000. On the basis of our results we have been able to establish the literature on NADH dehydrogenase.
izes the literature on another big enzyme.

The structural NADH dehydrogenase crystals are very useful because they contain about 1x1 molecules. The unit cell has dimensions of... series, the aligned and filtered projections were used for a three-dimensional reconstruction calculated with a real-space iterative reconstruction scheme (GSIRT algorithm). This procedure was followed for all three tilt series. The two better reconstructions could be combined after rotational alignment to a common origin. A model representing the density distribution of protein was made from contour plots of protein in the sections calculated parallel to the plane of the crystal. The model shows a height of 105 Å for the NADH dehydrogenase molecule. The four monomers forming the molecule are very similar in shape, but are only pairwise identical. Therefore the enzyme is in fact not a tetramer but only a dimer. In all four monomers a central pore runs through the structure, perpendicular to the plane of the crystal. They have a diameter of about 15 Å. At the moment the function of these pores is a mystery, but perhaps they have something to do with proton channeling through the enzyme.

In chapter 5 some additional experiments are described. Firstly, the projections of single molecules have been analyzed with the technique of correspondence analysis, a special form of multivariate statistical analysis. Two views of single particles have been selected, they were interpreted as the top and side view of the building block of the crystal. After computer image alignment and correspondence analysis, clusters of similar particles have been averaged. In the averages an uneven stain distribution is seen around the molecules, which may result from preferential staining of hydrophilic parts of the molecule. In another experiment the mass of the unit cell of the two-dimensional crystals has been determined with scanning transmission electron microscopy (STEM). A value of 1,600,000 was obtained. From the literature it is known that the enzyme has a molecular weight of 700,000 or two to three times that value. On the basis of our determination we conclude that the enzyme has been isolated as a dimer. This conclusion is in agreement with the literature on NADH dehydrogenase, and with the three-dimensional reconstruction of chapter 4.