termediate form was entirely unexpected until the X-ray structure became apparent.

Next, I wish to examine the proton pump, or the active transport of the proton. One of the most important aspects in understanding the mechanism of the proton pump, is identifying the changes that occur in X-ray structure coupled with the oxidation-reduction reaction.

Figure 8 shows the redox coupled conformational change of the enzyme detectable in the X-ray structure.[8] The oxidized state is shown in red, and the reduced state in green. Dots indicate the surface of the molecule on the outer surface of the inner mitochondrial membrane in the oxidized state. While many changes occur, we feel the most important is that which takes place in the amino acid called aspartic acid-51.

In the oxidized state, aspartic acid-51 is buried completely inside the molecule, so that it does not come into contact at all with the water in the outer part of the mitochondrion. When reduction occurs, however, the COOH group of the aspartic acid moves to the surface of the molecule and comes into contact with the water of the outer part of the mitochondrion. So, although this group is at first not accessible, it becomes accessible during the reduction phase of the oxidation-reduction reaction. Furthermore, in the oxidized state, the carboxyl group of aspartic acid-51 is bound by a hydrogen bond network to the inner side of the inner mitochondrial membrane as shown in Fig. 9.[8]

A hydrogen bond inside a protein is considered an excellent pathway for protons. Thus, because it is connected by a network of hydrogen bonds, aspartic acid-51 is able to take up protons from the inner side of the inner mitochondrial membrane and in that sense is accessible to the inner part of the mitochondrion. In the reduced state, aspartic acid-51 is accessible on the outer side. When this happens, however, the hydrogen bond connecting with the hydrogen bond network is broken, so it is accessible only on the outer side. In this way, the accessibility of aspartic acid-51 changes during the course of the oxidation-reduction reaction.

Even more importantly, the protonated carboxyl group, or COOH, at the end of the aspartic acid molecule is found to a considerable degree as COO^-, that is, in a dissociated state, in aqueous solution. In protein, however, in places where the dielectric constant is low, it is almost always found as COOH, not COO^-, showing high affinity for the proton. In the oxidized state of cytochrome oxidase, the COO^- is in the inside of the protein, so it shows high affinity for protons. In the reduced state, however, it becomes exposed on the surface of the molecule, and, since this is almost the same as being in aqueous solution, the affinity is reduced.

Table 1 summarizes the actions of the carboxyl group of aspartic acid-51. In the oxidized state, it is

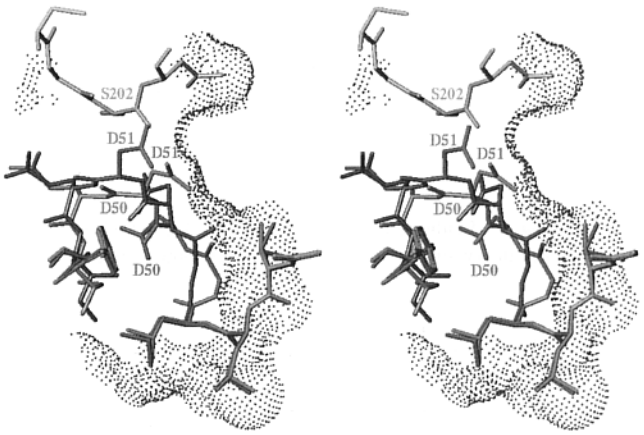

Fig. 8 Redox-coupled conformational change in the D51 segment. The accessible surface for the fully oxidized state is indicated by dots.

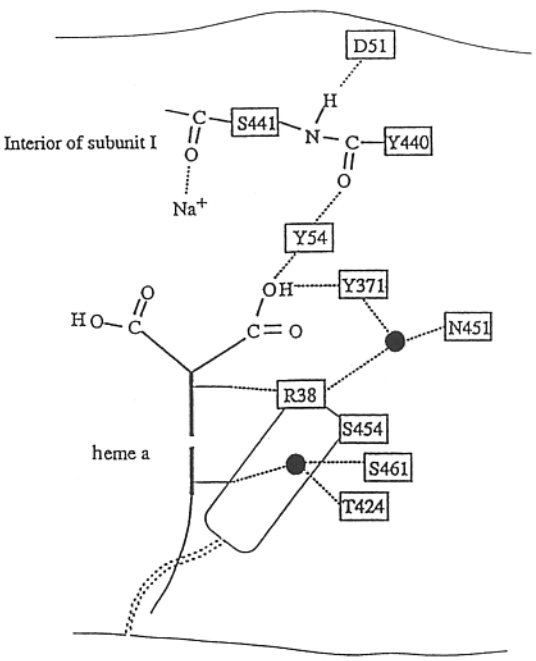

Fig. 9 The hydrogen bond network from Asp51 to the matrix surface. The small dark circles indicate fixed water. A thick stick denotes a side view of the porphyrin plane of heme a, and thin sticks from top to the bottom are side chains, propionates, formyl, and hydroxyl farnesylethyl.

accessible to the interior of the mitochondrion in the sense that it can take up protons from the inner part. In that state, it is located inside the protein, so its affinity must be extremely high. In the reduced state, however, it is accessible only to the outer part of the mitochondrion, and because it is surrounded by water, its affinity