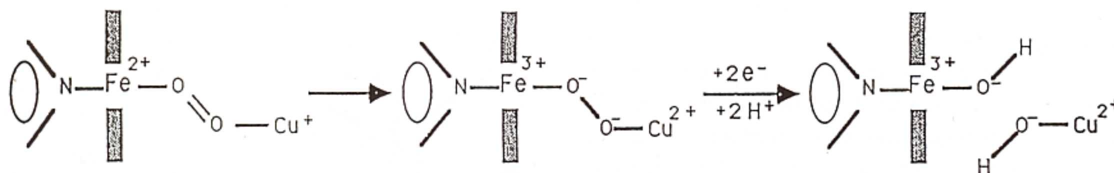

Fig. 5 Bridging peroxide mechanism for cytochrome *c* oxidase reaction. Fe and Cu denote Fe_{a3} and Cu_B, respectively, in the dioxygen reduction site of cytochrome *c* oxidase.

the reaction would be limited by the distance over which the electron is transferred, and, with the electron moving over a distance of only 2 Å, the reaction can be estimated to take only about a pico second, if all goes well. In other words, it should be almost impossible to detect this oxygen-bound form experimentally.

However, astonishingly enough, the oxygen-bound form was discovered by resonance Raman studies in collaboration with Dr. Kitagawa and Dr. Ogura.[7] I had never been so surprised in my life as when I learned that they had detected this form. Furthermore, they showed that the half-life of the form is 0.4 msec. While we had thought that the speed would be about a pico second, it was in fact about 0.4 msec. Although this is still rather fast, it is extremely slow if we assume that the bridging peroxide mechanism is correct. Why is this oxygen-bound form so stable? The crystal structure gives us a very important indication of the answer.

Figure 6 shows the coordination structure of Cu_B, the copper atom close to the oxygen-binding site. This is the reduced state, in which the Cu_B is in a state to which the oxygen will bind.[8] In this state, only three histidine ligands are present at the site, and, moreover, they are in trigonal, or triangle planar form. According to inorganic chemistry, a Cu^{1+} complex with a triangle planar structure is extremely stable. In the stable state, it is difficult to transfer electrons there or to accept a new ligand. We think that this is the reason for the abnormal stability of the oxygen-bound form.

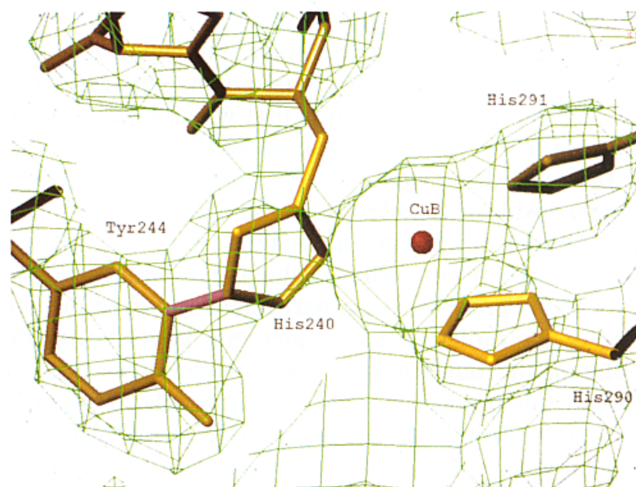

Fig. 6 Coordination structure of Cu_B. Cu_B is placed on the triangle formed by the three nitrogen atoms of these histidine imidazoles.

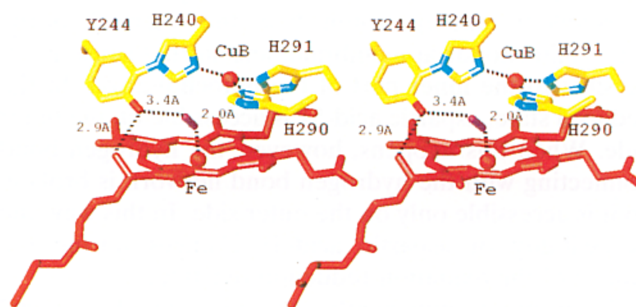

Fig. 7 X-ray structure of the O_2 reduction site of bovine heart cytochrome *c* oxidase in the fully reduced state with a model of O_2 placed at Fe_{a3}^{2+}, shown stereoscopically.

Looking again at the X-ray crystal structure, we found that the tyrosine near the oxygen binding site is bound by a covalent bond to one of the histidine ligands of Cu_B. As a result, the OH group of the tyrosine is in close proximity to the oxygen-binding site, as shown in Fig. 7. Using a model, we see that oxygen can bind in a way that hydrogen bonds can form quite adequately. Thus, although we had thought for over thirty years that when oxygen binds to cytochrome oxidase it forms a bridge between iron and copper, these X-ray structures showed that the bridge is in fact formed between the iron and the tyrosine. Electrons are unlikely to be transferred from Cu_B to the bound oxygen. Whereas Cu_B is thought to become involved at a subsequent stage, there does not seem to be any electron transfer at least in this initial stage. So, the crystal structure strongly indicates that, at least in the initial stage of the mechanism of oxygen reduction by cytochrome oxidase, a hydrogen bond is formed with the OH group of tyrosine. Moreover, the electron in fact does not come from Cu_B but from another iron near this iron to form a hydroperoxo form, Fe^{3+}-O-O-H. I believe such an in-