The crystal structure of fully oxidized cytochrome c oxidase from bovine heart at 2.0 Å resolution.

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Cytochrome c oxidase, an integral membrane protein, is the terminal enzyme in the respiratory chains of most of the aerobic organisms. Bovine heart cytochrome c oxidase is the biggest terminal oxidase composed of 13 different protein subunits with the molecular mass of 210 KDa. Because of the physiological importance at the intriguing catalytic reaction, the enzyme has been studied as one of the most important subjects in bioenergetics since its discovery. We have proposed a novel structure of peroxide for the bridging ligand between Fe_{a3} and Cu_B from the crystal structure of the fully oxidized bovine heart cytochrome c oxidase at 2.3 Å resolution. The high resolution crystal structural analysis of the enzyme was initiated to clarify the structure of dioxygen reduction site.

X-ray diffraction experiment was carried out at BL41XU of SPring-8. Intensity was recorded by R-axis IV of Rigaku. Other experimental conditions were as follow; wave length, 0.708 Å; crystal-to-detector distance, 350 mm; oscillation range, 0.65 degree; and temperature at the crystal, 100 K. One-hundred forty-one and 71 diffraction images were obtained by exposure times of 78 seconds and 117 seconds, respectively. Although the space group was the same $P2_12_12_1$ as that of the crystal at room temperature, the unit cell with dimensions of a=184.7 Å, b=207.5 Å and c=178.4 Å, is significantly smaller than the room temperature crystal. Total of 3,685,319 reflections were measured at 2.0 Å resolution. Independent reflections with average $I/\sigma(I)$ of 14.3 were amount to 429,322. Completeness was 94.9 %, R-merge 7.5 %, average redundancy 8.6.

Initial structure was determined by the molecular replacement method using a monomeric structure of the enzyme determined at 2.3 Å resolution. R_{free} and conventional R factors were reduced to 0.26 and 0.22 by X-plor refinement. σ_{bond} and σ_{angle} obtained were 0.014 Å and 1.73 degree, respectively. Difference Fourier map was calculated at the final stage of the refinement with coefficients of $(F_o-F_c)\exp(i\alpha_c)$, where suffices o and c represent observed and calculated values, respectively. The difference map exhibitted a bimodal electron density distribution between Fea3 and CuB. Consequently the bridging structure of the peroxide proposed at 2.3 Å analysis was confirmed.