Time-Resolved Resonance Raman Study on Oxygen Activation by Cytochrome *c* Oxidase in Intact Whole Mitochondria

Takashi Ogura, Toshinari Takahashi, Shigeki Kuroiwa and Shinya Yoshikawa

Graduate School of Life Science, University of Hyogo, Japan

Cytochrome *c* oxidase is the terminal component of the respiratory chain in mitochondrial inner membrane. It catalyzes dioxygen reduction to water and this electron-transfer reaction is coupled with proton translocation across the membrane. Thus produced electrochemical potential is utilized to synthesize ATP. The coordination structures of the Oxy, P, F and H intermediates have been established with resonance Raman spectroscopy for isolated and solubilized enzyme.

It has been estimated that three-quarters of the weight of the mitochondrial inner membrane is occupied by membrane proteins. Furthermore the phospholipid bilayer region consists of various sorts of lipids, and other hydrophobic molecules. Thus, all membrane proteins, which are contained within the mitochondrial inner membrane, interact strongly with various other membrane proteins and components of the phospholipid bilayer. On the other hand, such interactions are not expected to exist at all in the purified membrane protein systems. In order to examine difference of the environment of the enzyme in mitochondria and in the solubilized state, we have recorded time-resolved resonance Raman spectra of the enzyme in intact whole mitochondria.

In the present study, the process of dioxygen reduction by cytochrome *c* oxidase was investigated by examining intact porcine mitochondrial preparations using a novel time-resolved resonance Raman measurement system at experimental accuracy equivalent to those of the reaction system of the solubilized and purified enzyme. The resonance Raman bands assignable to the initial three intermediates were detected at 571/544, 804/764 and 780/750 cm^{-1} for $^{16}O_2/^{18}O_2$. These originate from Oxy, P and F intermediates, respectively. The frequencies are identical to those of the corresponding intermediates observed with purified enzyme preparations. However, the lifetime of the initial intermediate (the Oxy species) in the mitochondrial preparation was found to be significantly longer than that observed in purified preparations. Actually, the Raman band at 571 cm^{-1} of the Oxy species was detectable even at a delay time of 1.4 ms. This suggests that control of the stability of the oxygenated species is imposed by the mitochondrial membrane system.