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A switch in the electron transfer from heme a to binuclear centre of cytochrome c oxidase*

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New experimental evidence that a switch controls the reduction of the heme a_3–Cu_B binuclear centre has been observed in the N_2-dried thin film of purified cytochrome oxidase. When immersing the enzyme film into the acid phosphate buffer with extremely low concentration of dithionite, a spectrum was given to show a reduction of heme a with no electrons resting on Cu_A. By increasing dithionite, electrons could be accumulated gradually on Cu_A, but the binuclear centre still remains in the oxidized state. When the accumulation of electrons on Cu_A and/or heme a exceeded a threshold, a turnover of reduction of the binuclear centre and oxidation of heme a occurred abruptly. This switch-like action is pH-dependent.

Keywords: switch, electron transfer, cytochrome c oxidase, film
PACC: 7290, 8720

We are interested in the property of the internal electron transfer of cytochrome c oxidase in the dried film, because it not only offers the information of electron transfer inside the enzyme, but also a possible application in molecular electronics.[1,2] The oxidase (cytochrome aa_3, complex IV, EC 1.9.3.1) is the terminal complex of the mitochondrial respiratory chain, responsible for about 90% of oxygen consumption in mammals, and essential for virtually all energy production in cells. It is located in the mitochondrial inner membrane, and the enzyme catalyses the reduction of oxygen to water by ferrocytochrome c. The electron transfer from cytochrome c to oxygen through the cytochrome c oxidase is coupled with proton translocation across the membrane and adenosine triphosphate (ATP) synthesis.[1,3] Sodium dithionite can be used as an artificial electron donor instead of ferrocytochrome c in vitro.[4] The electron transfer inside the oxidase is via a way of electron donor→Cu_A–heme a→heme a_3–Cu_B binuclear centre→oxygen.[1,3,5] The reduction of the binuclear centre is a key step to reducing oxygen. The distance between heme a and heme a_3 is less than 1.3nm in the three-dimensional structure of cytochrome c oxidase. Such a short distance supports the view of fast electron transferring between heme a and a_3.[6] However, the rate constant of electron transfer is not well determined. It ranges from 1×10^4 to $3 \times 10^5 s^{-1}$ by laser photolysis, but from 0.1 to $30 s^{-1}$ by stopped-flow experiments at 445nm.[7] Little information is known about the detailed mechanism of electron transfer from heme a to the binuclear centre a_3-Cu_B, although research has given very important results about the pathway and the rate constant.

In order to gain more information about the electron transfer between the active centres of the enzyme, a N_2-dried thin film of purified cytochrome c oxidase has been used. The results show that a switch-like action can be observed in the electron transfer from heme a to the binuclear centre of heme a_3–Cu_B in cytochrome c oxidase.

Cytochrome c oxidase was purified from pig heart muscle according to the method mentioned in Refs.[3], [8] and [9]. The last precipitate of oxidase preparation was stored at 4°C in a sealed tube filled with N_2. The thin film was formed on a plastic sheet by centrifugation or on the inner wall of the quartz cell by spreading the oxidase suspension[10] and then dried under N_2 gas flow. Following this, it was washed with the buffer to remove the trace of detergent and then dried

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again under N_2 gas flow for two or three times. Finally, the film was kept in N_2 atmosphere and stored at room temperature. The absorption measurement was carried out with a 1601 spectrophotometer.

Immersing the N_2-dried film of cytochrome c oxidase in a cuvette containing 50mmol/L sodium phosphate buffer, pH 5.80, a spectrum with a peak at 418nm in the Soret region and a peak at 598nm in α band region was observed, as shown in Fig.1*a*. This peak shifted to 411nm when adding dithionite until 286mmol/L (Fig.1*c*). More than 286nmol/L of dithionite changed the peak 418nm in the Soret region into a double peak of 423 and 436nm, and shifted the peak at 598nm in α band region to 596.5nm (Fig.1*d*). Washing the enzyme film to remove the dithionite resulted in the decrease of the peaks at 423 and 436nm (Fig.1*e*), and the peak 436nm became a shoulder.

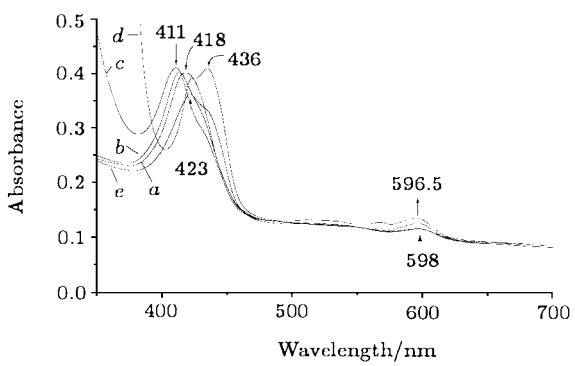


Fig.1. Spectra of cytochrome c oxidase film immersed in 50mmol/L sodium phosphate buffer, pH 5.80, (*a*), with 91 mmol/L (*b*), 286mmol/L (*c*), ~10mol/L (*d*) dithionite, and removal of dithionite (*e*).

Figure 2 illustrates the difference spectra of the reduced N_2-dried oxidase film obtained by increasing the amount of dithionite in the sodium phosphate buffer, pH 5.80. Both the negative peak at 426nm and the positive peak at 402nm are enhanced simultaneously with the increasing amount of dithionite. When the concentration of dithionite increases over a threshold value, an abrupt change occurs with the new peak-pair of the negative peak at ~412nm and the positive peak at ~445nm, instead of the original peak-pair of 426 and 402nm (Fig.3*d*). The increase of the peak-pair 426 and 402nm indicates that the population of the reduced heme a increases with the increasing amount of dithionite. The turnover of the peak-pair of 426 and 402nm and the peak-pair of 412 and 445nm implies that the transportation of electrons from the heme a and Cu_A centre to the heme a_3–Cu_B binuclear centre is in cluster, just like a switch action between the heme a and the binuclear centres.

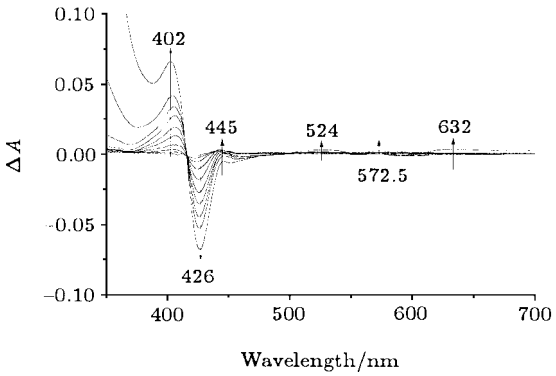


Fig.2. Dithionite-induced difference spectra of cytochrome c oxidase film immersed in 50mmol/L phosphate buffer, pH 5.8. From top to bottom at 426nm (or from bottom to top at 402nm) are for 1, 2, 4, 10, 23, 28, 91, 143, 211 and 286 mmol/L dithionite, respectively.

Dithionite has a strong absorption at 350nm. If using $\Delta A_{350\mathrm{nm}}$ to denote the change of free dithionite and $-\Delta A_{426\mathrm{nm}}$ the reduction of heme a, then the curves of $\Delta A_{350\mathrm{nm}}$ and $-\Delta A_{426\mathrm{nm}}$ versus the concentration of dithionite can be drawn as in Fig.4. The plot of $\Delta A_{350\mathrm{nm}}$ shows two phases. For less than 143mmol/L, the straight line with a lower slope represents the oxidative rate of dithionite, while for more than 143mmol/L, the $\Delta A_{350\mathrm{nm}}$ increases sharply; the increase of $\Delta A_{350\mathrm{nm}}$ means that the free dithionite begins to accumulate in the solution. The plot of $-\Delta A_{426\mathrm{nm}}$ at extremely low concentration (less than 25mmol/L) shows only the reduction of heme a. The Cu_A is not reduced until the dithionite concentration rises up to more than 25mmol/L. The reduction of the binuclear centre only occurs in the second phase of dithionite at more than 143mmol/L. And once the binuclear centre reduces, the heme a centre becomes oxidized (Fig.3*d*). This redox turnover implies that the electrons in the heme a centre are transported to the binuclear centre in batch. The action of redox turnover suggests an on–off switch action in the electron transfer between heme a and the binuclear centres.

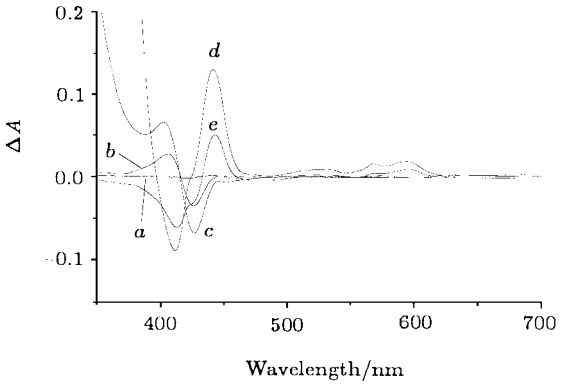


Fig.3. Dependence of reduction of cytochrome c oxidase thin solid film immersed in 50mmol/L sodium phosphate buffer, pH 5.80, on the concentration of dithionite as an electron donor. The concentrations of dithionite are 1mmol/L (*a*), 91mmol/L (*b*), 286mmol/L (*c*) and ∼10mol/L (*d*), and *e* is for dithionite removed.

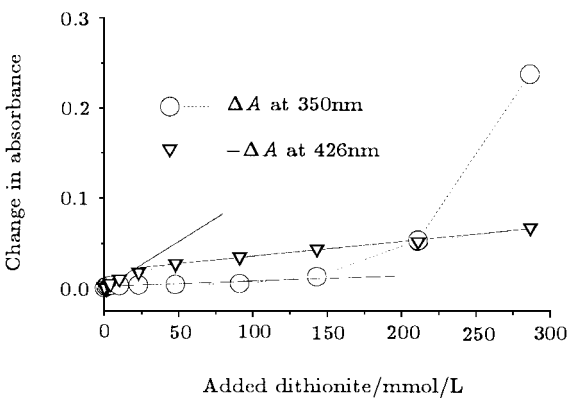


Fig.4. Plots of ΔA at 350nm ($\cdots\bigcirc\cdots$, ΔA at 350nm) and 426nm ($\cdots\triangledown\cdots$, $-\Delta A$ at 426nm) against the concentration of sodium dithionite.

The switch action mentioned above is pH-dependent. Under alkaline conditions, the reduction by dithionite differs from that under acid conditions (Figs.5 and 6). A weak negative peak at ∼426nm can be found at 50mmol/L dithionite added at pH 7.40 (Fig.5), but it is undetectable at pH 10.0 (Fig.6). This implies that the retaining-ability of electrons in heme a and/or Cu_A decreases in higher pH solution.

Lower pH is favourable to the electron–donor-controlling switch property. This suggests that the proton plays an important role in the formation of the switch. A study of the detailed mechanism is underway.

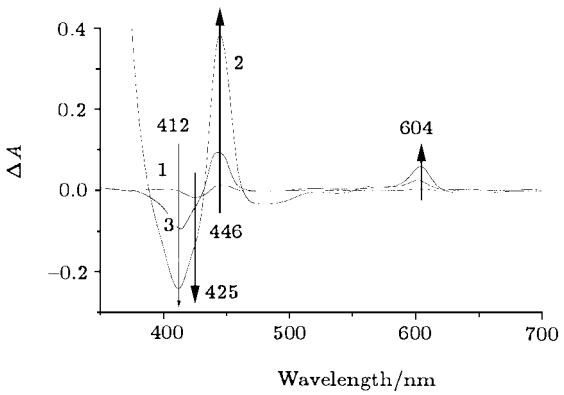


Fig.5. Difference spectra of cytochrome c oxidase film in 50mmol/L sodium phosphate buffer, pH 7.40, induced by 50mmol/L (curve 1), 100mmol/L (curve 2) of dithionite. Curve 3 is for the removal of dithionite.

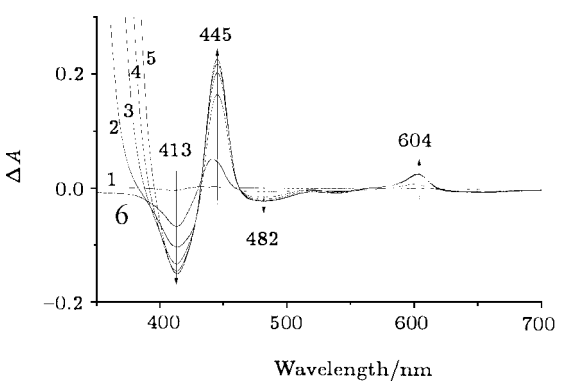


Fig.6. Difference spectra of cytochrome c oxidase film in 50mmol/L sodium phosphate buffer, pH 10.0, with and without dithionite. Curves 1–5 are for 1, 5, 20, 50 and 100mmol/L dithionite, respectively, and curve 6 is for the removal of dithionite. The numbers of the curves are 6, 1, 2, 3, 4 and 5 from top to bottom at 412nm or from bottom to top at 445nm.

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