Interconversion between states in cytochrome oxidase: Interpretation of kinetic data on mixed-valence oxidase

Maurizio Brunori, Alfred Colosimo, Michael T. Wilson*, Paolo Sarti and Eraldo Antonini

Institutes of Chemistry and Biochemistry and C.N.R. Centre for Molecular Biology, Faculty of Medicine, 1st University of Rome, and Department of Biochemistry, Faculty of Medicine, 2nd University of Rome, Italy, and **Department of Chemistry, University of Essex, Colchester, England*

Received 19 November 1982

Pulsed cytochrome c oxidase *Respiratory chain* *Oxygen activation*

1. INTRODUCTION

We have proposed [1] a two-state model to account for the functional properties of cytochrome *c* oxidase. The basis of the model was the demonstration that the enzyme may exist either in a 'resting' (R) or in a 'pulsed' (P) state, the two states being interconvertible and characterized by different kinetic and spectral properties. Pulsed oxidase as an activated state of the enzyme was originally discovered in [2] on the basis of rapid mixing experiments in which the kinetic properties of the enzyme were tested immediately (milliseconds) after exposure of reduced oxidase to oxygen. Its properties have since been investigated [3–6], and a pulsed state has been identified for oxidases from different organisms. Pulsed oxidase may also be obtained by oxidation of the reduced enzyme by ferricyanide [7] and thus its formation is not absolutely dependent on the presence of dioxygen.

Our proposed two-state model (a simplified version of which is given in fig.1A) implies that the transition in the fully reduced derivative, $R_r \longrightarrow P_r$ (the equilibrium position of which favours the P_r state) is in kinetic competition with the reaction of R_r with O_2. This latter is a very fast process [8] with an apparent second order rate constant $k_2 \simeq 10^8\ M^{-1}s^{-1}$, which leads to the formation of R_o (the fully oxidized resting state) (fig.1A). This kinetic competition can account for the rather slow (seconds) self-activation of the enzyme, which during turnover shifts from resting to pulsed form [4].

Simulation of the time course of activation using a complete reaction scheme [1], has led to an estimate of the rate constant for the transition $R_r \longrightarrow P_r$ ($k_1 = 10^2 - 10^3\ s^{-1}$). Here, we report the interpretation of flow-flash experiments carried out some years ago [9] starting from the mixed-valence CO derivative of cytochrome *c* oxidase isolated from beef heart, and showing that the observations are quantitatively consistent with the predictions of the two-state model. Moreover, it appears that interconversions between oxidised

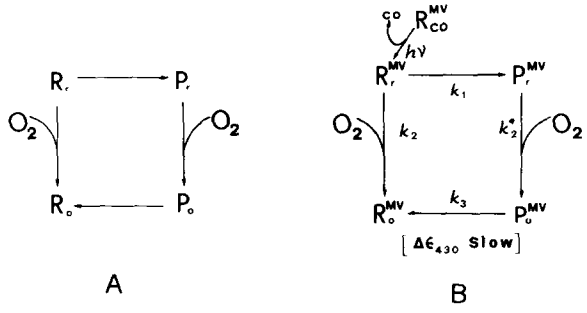

Fig.1. Two-state model for the reactions of cytochrome *c* oxidase in the native (A) or in the mixed valence (B) forms. In each scheme two states of the enzyme (called R for resting and P for pulsed) are shown. For the sake of simplicity only the overall reaction with molecular O_2 ($k_2 = k_2^* = 10^8\ M^{-1}\ s^{-1}$) and the interconversion rate constants (k_1 and k_3) are indicated. For complete kinetic scheme of the native enzyme see [1].

Published by Elsevier Biomedical Press
00145793/83/0000–0000/$3.00

species derived from mixed-valence oxidase may be more rapid than those in the fully oxidised enzyme, and that there exist ligand-linked transitions between states of the enzyme.

RESULTS AND DISCUSSION

In the mixed-valence CO derivative of oxidase, cytochrome *a* and Cu_A are both oxidized [10], while cytochrome a_3 and Cu_B are both kept reduced by carbon monoxide, the system being, therefore, schematically indicated by $[a^{3+}\ Cu_A^{2+}\ a_3^{2+}\ Cu_B^{+}\ CO]$. When this derivative of the enzyme is rapidly mixed with a solution of buffer containing O_2 and an intense flash of light is fired immediately after the flow stops, photochemical removal of CO yields a species which is reactive towards O_2. The time course of this reaction was expected to be a relatively simple process in the case of the mixed-valence CO derivative of oxidase since cytochrome *a* and Cu_A (the other two chromophores) are already oxidised. The main phase of the process, occurring in the μsecond range and relating to the reaction of cytochrome a_3 with O_2, was indeed very similar to that in [8] starting from the fully reduced CO derivative of oxidase. However, starting with the mixed-valence CO derivative, an additional kinetic component has been observed [9], in the second time range, characterized by the following properties:

(i) Its amplitude was O_2 concentration dependent, being smallest at high oxygen concentrations;

(ii) Its spectral distribution in the Soret region showed a peak at ~414 nm, a trough at ~433 nm, and an isosbestic at 422 nm (see insert in fig.2).

These observations may be interpreted quantitatively in the framework of the two-state model and may represent additional support for the validity of the model and allow some general conclusions to be drawn regarding the interpretation of recent spectroscopic data on cytochrome oxidase [6,12].

The scheme given in fig.1B was used to describe the system. The spectral distribution of the slow process ($t_{1/2}$ ~1 s at 20°C) observed in the flow-flash experiments starting with the mixed-valence CO derivative corresponds quite closely (insert to fig.2) with the difference spectrum described in going from pulsed oxidized (P_o) to resting oxidized (R_o) oxidase, the former having a spectrum similar to the so called 'oxygenated' oxidase [13,14]. Thus the slow process is assigned on spectral grounds to the transition $P_o^{MV} \longrightarrow R_o^{MV}$, with k_3 ~0.5 s^{-1}. The amount of P_o^{MV} formed at the end of the reaction between O_2 and the photochemically stripped mixed-valence oxidase (R_r^{MV}) depends on the fraction of oxidase molecules converted to the pulsed state during the time of the O_2 reaction (and therefore on the competition between the two possible pathways for R_r^{MV}, as indicated by k_1 and k_2). Thus, at very high O_2 concentrations (e.g., $\gtrsim 100\ \mu$M) the amplitude of the slow phase becomes vanishingly small, and increases as the O_2

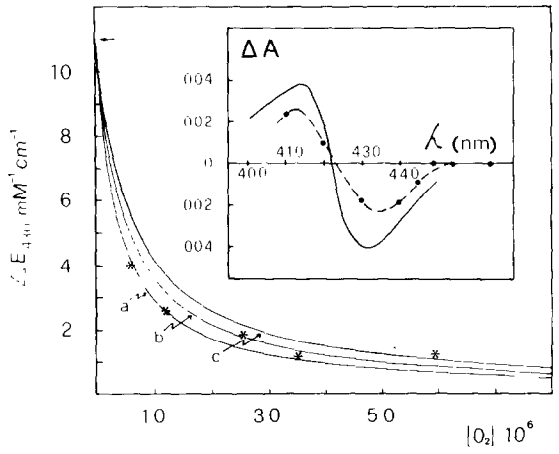

Fig.2. Fit of the amplitude of the slow phase observed in the reaction of the mixed-valence oxidase after photolytic removal of CO. The experimental points (*) are the results obtained in [9] and the solid lines have been calculated on the basis of the scheme in fig.1B, taking: $k_2 = k_{-2} = 10^8\ M^{-1}\ s^{-1}$; $k_1 = 300$ (a), 400 (b) and 500 (c) s^{-1}, and $k_3 = 0.5\ s^{-1}$. The arrow indicates the difference in extinction (ΔE_{430} $mM^{-1}\ cm^{-1}$) between pulsed oxidized and resting oxidized oxidase computed from independent data (see text). The inset shows the difference spectra relative to: (i) the slow phase observed in the O_2 reaction of the mixed-valence oxidase as obtained at 2 μM heme *a* and 6.5 μM O_2 in a 5-cm light path cell used for the flow-flash experiments (dashed line, data from [9]; (ii) the spectroscopic decay $P_o \longrightarrow R_o$ observed, starting from the completely reduced enzyme after anaerobic oxidation by ferricyanide (solid line, data from [7]). These experiments were performed at 3.6 μM oxidase in a 1-cm cell.

concentration is decreased (see fig.2), in agreement with the experimental observations.

Quantitative analysis according to the scheme of fig.1B allows one to estimate the value of k_1 (300–500 s^{-1}) from the O_2 concentration dependence of the amplitude data. The value of the difference extinction coefficient at 430 nm found by extrapolation to zero oxygen concentration is 11.0 mM^{-1} cm^{-1} [9], consistent with the independent estimate of the difference between P_o and R_o under the same experimental conditions (unpublished).

The above analysis allows the following general comments to be made:

(1) The results are quantitatively consistent with the predictions of the two-state model in [1] and may thus be taken as supporting evidence for the validity of this model and for the presence of two states of the macromolecular complex in the partially and totally reduced enzyme. The rate constant for the $R_r^{MV} \longrightarrow P_r^{MV}$ interconversion determined from the computations in fig.1 (k_1 = 300–500 s^{-1}) is consistent with the value estimated for the same interconversion in the fully reduced derivative $R_r \longrightarrow P_r$ (i.e., 100–1000 s^{-1}, as obtained from the rate of shift of the steady-state level during turnover [1].

(2) The results demand that the mixed-valence CO oxidase is in the resting conformation, and only after photodissociation of CO (R_r^{MV} in fig.1) does the pulsed state begin to be populated. Thus, while the fully reduced enzyme is largely in the pulsed conformation state, the fully reduced CO derivative may be in the resting state (R_r^{CO}). This interpretation seems consistent with the conclusion of the flow-flash experiments in [8] that, starting from the CO derivative of fully reduced oxidase, the product of the reaction with O_2 was the resting oxidised form of the enzyme. Moreover, this implies that upon binding carbon monoxide, a ligand-linked conformational transition may occur (in this case from P to R forms), reminiscent of the well known allosteric transition in hemoglobin [15].

(3) Given the consideration reported above, the postulated stabilization of the resting state by CO binding may clarify the interpretation of a number of low-temperature freeze trapping experiments carried out in the last few years on cytochrome *c* oxidase [11,16]. In particular, recent EXAFS measurements [6,12] have indicated that the resting state is the only one significantly populated at low temperatures when the O_2 reactions are initiated by starting from the CO derivative of fully reduced oxidase. This finding is in accord with our interpretation.

(4) The decay $P_o \longrightarrow R_o$ in the fully oxidized oxidase is a slow process, with half times in the range of 5–60 min at room temperature [7,17]. This may represent an obstacle in assigning a physiological role to pulsed oxidase, since the active state of the enzyme, once achieved during turnover, decays back to resting very slowly. However, according to present interpretation of the results in [9], it appears that state-reequilibration may occur much more rapidly at intermediate levels of oxidation, since the back decay $P_o^{MV} \longrightarrow R_o^{MV}$ is now occurring with $t_{1/2} \sim 1$ s and thus in a time scale compatible with a physiologically meaningful scheme.

ACKNOWLEDGEMENT

We gratefully acknowledge NATO grant no.1767.

REFERENCES

[1] Wilson, M.T., Peterson, J., Antonini, E., Brunori, M., Colosimo, A. and Wyman, J. (1981) Proc. Natl. Acad. Sci. USA 78, 7115–7118.
[2] Antonini, E., Brunori, M., Colosimo, A., Greenwood, C. and Wilson, M.T. (1977) Proc. Natl. Acad. Sci. USA 74, 3128–3132.
[3] Rosen, S., Bränden, R., Vänngard, T. and Mälmstrom, B.G. (1977) FEBS Lett. 74, 25–29.
[4] Brunori, M., Colosimo, A., Rainoni, G., Wilson, M.T. and Antonini, E. (1979) J. Biol. Chem. 254, 10769–10775.
[5] Shaw, R.W., Beinert, H. and Hansen, R.E. (1979) Biochim. Biophys. Acta 548, 386–396.
[6] Powers, L., Ching, Y., Chance, B. and Muhoberac, B. (1982) Biophys. J. 37, 403a.
[7] Brunori, M., Colosimo, A., Sarti, P., Antonini, E. and Wilson, M.T. (1981) FEBS Lett. 126, 195–198.
[8] Greenwood, C. and Gibson, Q.H. (1967) J. Biol. Chem. 242, 1782–1787.
[9] Greenwood, C., Wilson, M.T. and Brunori, M. (1974) Biochem. J. 137, 205–215.

[10] Leigh, J.S., Wilson, D.F. and Owen, C.S. (1974) Arch. Biochem. Biophys. 160, 476–486.
[11] Chance, B., Saronio, C. and Leigh, J.S. (1975) J. Biol. Chem. 250, 9226–9237.
[12] Powers, L., Chance, B., Ching, Y. and Angiolillo, P. (1981) Biophys. J. 34, 465–498.
[13] Okunuki, K. (1962) in: Oxygenases (Hayaishi, O. ed) p.409, Academic Press, New York.
[14] Lemberg, M.R. (1969) Physiol. Rev. 49, 48.
[15] Perutz, M.F. (1970) Nature 228, 726–739.
[16] Clore, G.M., Andreasson, L.E., Karlssen, B., Aasa, R. and Mälmstrom, B.G. (1980) Biochem. J. 185, 155–163.
[17] Tiesjema, R.N., Mujisers, A.O., Neuderson, R.W. and Van Gelder, B.F. (1972) Biochim. Biophys. Acta 267, 216–221.