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O_2 activation in cytochrome oxidase and in other heme proteins

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In applying room-temperature, time-resolved optical and resonance-Raman spectroscopies to the cytochrome oxidase/dioxygen reaction, we have been fortunate in being able to detect intermediates in this process both before and after the O-O bond scission reaction occurs (e.g., Refs. 1–9). This behavior contrasts markedly with the situation in the cytochromes *P*-450 (P450), for example, where the structure of key intermediates, subsequent to the initial oxy complex [10], in the bond cleavage and substrate hydroxylating reactions have been inferred from model studies, chemical reasoning, and genetic modification, but have not been detected directly [11,12].

Recently, Babcock and Wikström have combined the kinetic work on cytochrome oxidase with results from reversed electron-transfer experiments [13,14] and low-temperature trapping measurements [15–17] to propose a detailed mechanism for the dioxygen reduction reaction [18]. An adaptation of this mechanism is shown in Fig. 1. A preliminary simulation of the time evolution of several intermediates in this process, beginning with the ferrous-oxy species (compound A), is shown in Fig. 2; the rate constants used in this simulation are available in the literature or from our own work and are discussed in detail elsewhere [19]. This analysis describes flow/flash results for the fully reduced enzyme, where the transition from the oxy complex (A) to the initial three-electron reduced peroxy species (B) is likely to be artifactually fast [18]. Nonetheless, the simulation shows that bound, partially reduced intermediates in the catalytic cycle build up to substantial concentrations during the reaction. It is this facet of oxidase catalysis that accounts for the relative ease with which the process can be monitored kinetically and spectroscopically.

A consideration of the P450 catalytic cycle in relation to that of cytochrome oxidase provides insight into the basis for the contrasting behavior of the two en-

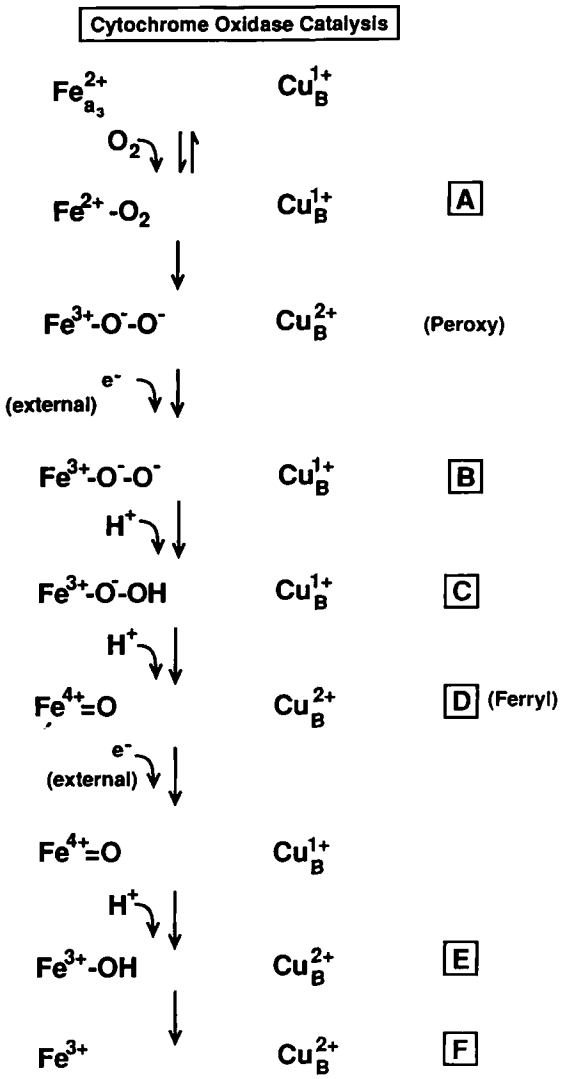

Fig. 1. Postulated dioxygen reduction mechanism in cytochrome oxidase (adapted from Ref. 18).

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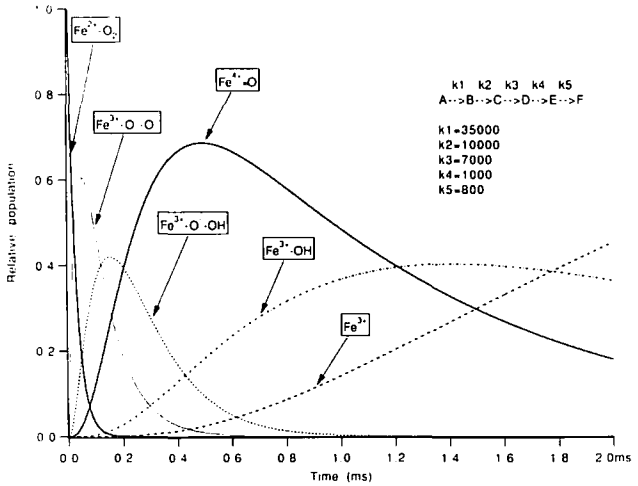

Fig. 2. Kinetic simulation of the reaction sequence shown in Fig. 1. The intermediates A–F are used as defined in Fig. 1. Their chemical structures are used to label their respective progress curves. The rate constants used are given below the reaction sequence (adapted from Ref. 19).

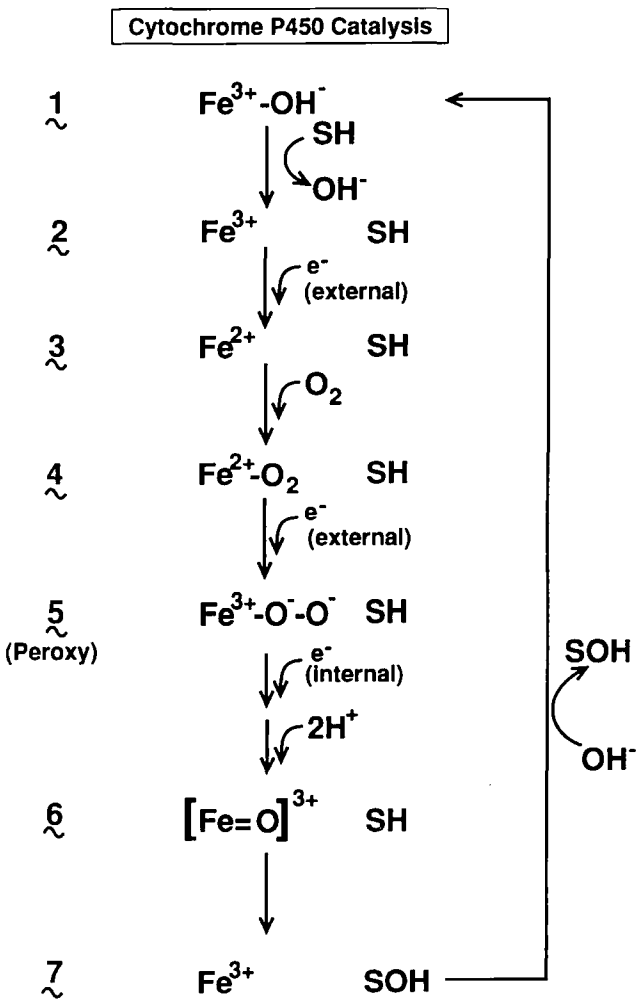

Fig. 3. Postulated dioxygen reduction and substrate hydroxylation mechanism in cytochrome *P*-450. SH corresponds to substrate and SOH is the hydroxylated product (adapted from Ref. 12).

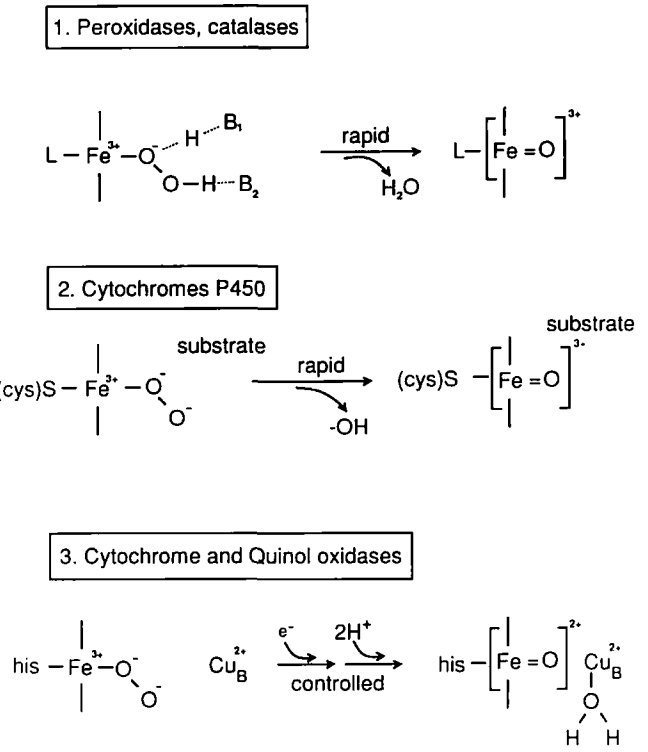

Fig. 4. Schematic representation of the different mechanisms used in peroxide bond cleavage in the peroxidases, catalases, cytochromes *P*-450, and cytochrome oxidase.

zymes. Fig. 3 shows the commonly accepted reaction sequence for P450 hydroxylation [12]. Substrate (SH) binds to the ferric enzyme, followed by reduction and dioxygen binding to form the ferrous-oxy species (**4**). Reduction of **4** produces the peroxy **5**; internal electron transfer, protonation and O-O bond cleavage generates the hydroxylating species (**6**), which is formally analogous to the compounds I that occur in peroxidase catalysis. Hydroxylation followed by product release regenerates the resting enzyme (**1**).

Comparison of Figs. 1 and 3 shows that peroxy species are key to the reactions catalyzed by both cytochrome oxidase and P450. In P450, the reaction is optimized to produce the hydroxylating species **6**; the active site is designed for the rapid internal reduction, protonation and cleavage of the peroxy O-O bond, and the energy released in this process is simply dissipated as heat. One aspect of this design, the electron donating thiolate, has been known for some time [11]; a second, regulation of the proton current, has been shown recently to be accessible by genetic/X-ray crystallographic approaches [12]. In oxidase, on the other hand, the free energy made available on reduction of the peroxy is critical to the proton-pumping efficiency of the enzyme. The design of the pocket is not predisposed to the rapid metabolism of this key intermediate; rather, the electron and proton transfers that are required to produce the ferryl species, D, are controlled

by factors external to the pocket (the negative redox cooperativity between *a* and the binuclear center [18] and protonation events that can be monitored in the external solution phase [20]). As a consequence, the rate constants for the reactions that lead from peroxy to ferryl are comparable to those for the other steps in the reaction sequence. This situation produces the kinetic behavior simulated in Fig. 2, i.e., appreciable buildup of several of the key intermediates in catalysis. Fig. 4 summarizes, schematically, the contrasting mechanisms for peroxide metabolism in the cytochromes P-450, the peroxidases (which function analogously to P450, in terms of peroxy O-O bond cleavage), and cytochrome oxidase.

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