Pure & Appl. Chem., Vol. 55, No. 7, pp. 1049-1058, 1983.
Printed in Great Britain.

0033-4545/83 $3.00+0.00
Pergamon Press Ltd.

REGULATION OF ELECTRON TRANSFER IN METALLOPROTEINS

Maurizio Brunori, Alfredo Colosimo and Maria Chiara Silvestrini(*)

Department of Biochemistry, Faculty of Medicine, II University of Rome, Tor Vergata and
(*) C.N.R. Center of Molecular Biology c/o Institute of Chemistry and Biochemistry, Faculty of Medicine, I University of Rome, 00185 Rome - Italy

Abstract - The close functional interplay between the prosthetic group and the polypeptide chain in redox enzymes is the theme of this paper, and represents a natural extension of the well established regulation exerted by the protein mojety on the ligand binding properties of hemoglobins and other oxygen carriers.
Two relevant examples are discussed: in the first one (azurin) the molecular mechanism controlling the electron transfer reactions is restricted to the immediate chemical environment of the metal center, while in the second one (mitochondrial cytochrome oxidase) it involves a conformational transition of the whole quaternary structure of the enzyme. The power of the kinetic approach in detecting significant intermediates is emphasized.

INTRODUCTION

The multiplicity of functions carried out by metalloproteins in the economy of the living systems is well established. Studies along this line of research provide continuously new and unexpected information, whose interpretation in detailed structural terms is a standing challenge. Investigation of the relationships between structure and function in metalloproteins is a "melting-pot" of diverse interests, and the development and application of new technologies has been stimulated by the curiosity to solve the problems to higher and higher level of resolution. Along this stream of ideas and experiments, there has been one well established and outstanding theme of research represented by the understanding of the role played by the protein moiety in controlling the reactivity of metal centers in biochemical systems.

The oxygen carrying hemeproteins, such as hemoglobin and myoglobin, represent a prototype of such a trend, and their behaviour has been investigated in depth. It has been established that the globin exerts a strict control on the physical state, stability and reactivity of the heme, and to achieve control of rates and binding energies in the formation of the oxygen complex stereochemical and electronic effects are involved (Ref. 1÷3). The modulation of reactivity by the immediate environment to the prosthetic group has been explored by correlating structural and functional properties of different monomeric hemeproteins, and by extensive and successful synthesis of sophisticated model compounds (Ref. 4 & 5). In the multi-site proteins, regulation of oxygen binding is achieved by the interplay of tertiary and quaternary conformational changes of the protein. The allosteric behaviour of hemoglobin has been investigated in great detail, and has proven to constitute a model of great generality (Ref. 6).

Electron transfer metalloproteins play fundamental and diversified roles in biological systems. "Oxygen activation" by cytochrome c oxidase, which allows to make use of all the oxidizing equivalents of dioxygen without release of radical species in the bulk (Ref. 7 & 8), is but one example of complex functions carried out by complex metalloproteins. In view of the crucial significance of intermediate states in enzymatic catalysis, kinetic investigation in the transient time regime represents the best approach to test a reaction mechanism (Ref. 9 & 10). Since the relevant intermediates on the enzyme are generally short lived (especially at higher protein concentrations) rapid reactions techniques, near equilibrium or far from it, have been used successfully.

Structural interpretation of equilibrium, steady-state and transient kinetics, however, demands information on the structure of the metal site and of the protein as a whole. The obvious relevant questions with reference to a structural interpretation are (i) the formal charge on the metal(s), (ii) the atoms directly bound to the metal and thus the geometry of the metal-ligand(s) complex, (iii) the interactions with the proteins backbone and the possible pathways for the transfer of electrons and/or binding of small molecules. The tridimensional structure obtained by crystallography, which begins to be available for some electron transfer metalloproteins (Ref. 11-13), in conjunction with spectroscopic data, is essential for a detailed molecular interpretation of mechanism.

In this paper we shall present experimental information and mechanistic interpretation concerning the reactions of azurin and cytochrome-c-oxidase which, at different levels of complexity, illustrate the role of the protein in controlling the reactivity of the metallic sites. These cases may serve as prototypes to exemplify the importance of correlating, whenever possible, functional kinetic data with biochemical, spectroscopic and crystallographic results.

The role of protein conformation, both at the tertiary and quaternary levels, in modulating the biological requirements of the system under study is therefore the main theme of this paper.

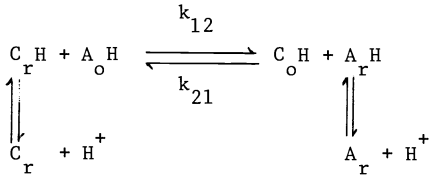
THE FUNCTIONAL ISOMERIZATION IN REDUCED AZURIN FROM PSEUDOMONAS AERUGINOSA

Azurins are small molecules (M.W. = 14000), containing a single blue or "type I" copper redox center, which have been purified from a number of nitrite reducing bacteria, where they are involved as electron carriers in the respiratory chain. They share with plastocyanins some characteristic features (Ref. 14), not yet reproduced by model compounds: a broad absorption band, centered around 620 nm with an extinction coefficient of $\simeq 4000\ M^{-1}\ cm^{-1}$, an EPR spectrum characterized by a small hyperfine constant, and a rather high redox potential.

The study of structural and functional properties of azurin from Pseudomonas aeruginosa has attracted a great deal of attention; the high level of resolution attained by spectroscopic and X-rays analyses allows to attempt a structural interpretation of its kinetic behaviour.

Azurin from Ps. a. is characterized by very rapid electron transfer with another small redox protein, cytochrome c_{551}, and acts as an efficient reducing substrate for the nitrite reductase from the same microorganism. The reaction with the reductase is complex, both in steady state (Ref. 15) and pre-steady state (Ref. 16) conditions. The reaction with cytochrome c_{551}, which is also a very good substrate for the nitrite reductase, has been investigated in detail by rapid reaction techniques.

In spite of the apparent simplicity of the system the kinetics is again complex (see scheme I), and very fast (Ref. 17) ($k_{12} = 6 \times 10^6\ M^{-1}\ sec^{-1}$ and $k_{21} = 3.5\ 10^6\ M^{-1}\ sec^{-1}$ at pH 7.0 and 20°C), suggesting some type of specific protein-protein interaction. Moreover Temperature-jump experiments gave clear evidences that reduced azurin exists in two different interconvertible forms (Ref. 17), one of which is inefficient in transferring electrons rapidly to ferricytochrome c.



SCHEME I: minimum reactional mechanism for the electron transfer between azurin and cytochrome c_{551} from Pseudomonas aeruginosa. C and A represent Ps. a. cytochrome c_{551} and azurin, H indicates the protonated "active" form of both proteins, the suffixes o and r denote the oxidation state. The need to include in the scheme a pH dependent isomerization also for cytochrome c_{551} arises from kinetic, equilibrium and spectroscopic results (see Ref. 18).

It has been estimated that the inactive form could reduce cytochrome c_{-551} with a second order rate constant two-to-three orders of magnitude lower than that of the active one. The reequilibration between the two species has a reciprocal relaxation time of 40-80 sec^{-1}, and has been demonstrated to be pH dependent, with a pK $\cong$ 7. Temperature jump experiments on reduced azurin in the presence of a pH indicator showed that an increase in temperature favours the inactive species, with proton release from the molecule: thus it was proposed (Ref. 18) that the protonated species is the active form.

May these kinetic features be understood in the light of the available structural and spectroscopic information on the same protein? The tertiary structure of Ps. a. azurin has been refined to 2.7 Å resolution (Ref. 13). The Cu atom is coordinated to four ligands (see fig. 1): two histidines (His 46 and His 117), a cysteine (Cys 112) and a methionine (Met 121). These four aminoacids are invariant in the nine azurines sequenced up to now (Ref. 14).

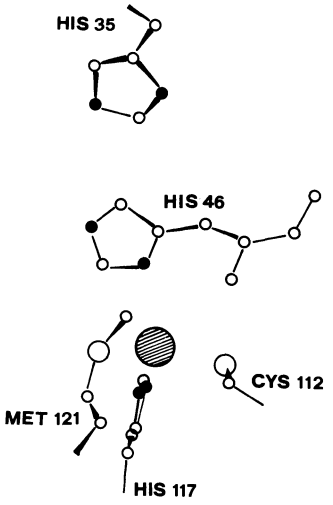


Fig. 1. The copper site in Ps. a. oxidized azurin and the relative position of histidine 35 according to crystallographic data in Ref. 22 (modified).

The site has a distorted tetrahedral array, very similar to plastocyanin, in which again two sulfur atoms and two nitrogen atoms of imidazole groups are the ligands of the metal (Ref.12). As pointed out by Freeman and coworkers for plastocyanin (12) the nature of the ligands and the geometry of the site enhance the redox potential and are advantageous for an outer-sphere mechanism of electron transfer. Indeed azurin was shown to react via an outer sphere mechanism with inorganic complexes (Ref. 19) and with small redox proteins (Ref. 20). The environment of the copper center is hydrophobic, the metal being shielded from the solvent and surrounded by apolar aminoacids. Among the aminoacidic residues present in the immediate surroundings of the copper, histidine 35 is also invariant (Ref. 14); it is this residue which is considered to play a crucial role in the switch mechanism from the active to the inactive form of reduced azurin. In fact, it has been shown by H-NMR spectroscopy that His 35 participates in a slow proton exchange process (Ref. 21) having a rate constant consistent with the reciprocal relaxation time observed by temperature jump; the ΔH and the pK associated to this process are also compatible with the values expected for an histidine (Ref. 18). To reconcile the almost normal pK value (pK = 7) with the slow acid-base exchange rate, a model assuming full hydrogen

bonding with groups of the protein of both the protonated and the deprotonated forms of this His has been proposed (Ref. 18), the conformational transition between the two states being the rate limiting step. An alternative model proposes a switch of the imidazole ring from one position, where it is accessible to water, to another inaccessible to water but suitable for hydrogen bonding (Ref. 22).

How is it that the protonation state of a histidine not directly bound to copper, although in its immediate vicinity, affects so drastically the efficiency of electron transfer to cytochrome c_{551}. A recent H-NMR investigation (Ref. 22) has shown quite clearly that increase in pH and in temperature, the two conditions favouring the inactive form of reduced azurin, enhance the movement of Met 121, with subsequent lenghtening and perhaps breaking of this coordination bond. This would lead to a different geometry of the active site, presumably stabilizing the cupric state, with a decrease of redox potential. It is noteworthy that in plastocyanin (Ref. 12) the pH dependence of the redox potential was also explained with the dissociation of one of the imidazole-copper ligands, leading, in this case, to a stabilization of the reduced species. Although it is clear that the deprotonation of His 35 and the movements of Met 121 are related, it is not possible at this stage to establish if this linkage occurs through the copper atom or through a conformational change of the peptide backbone. It must be recalled that H-NMR studies carried out on reduced and oxidized azurin indicate that the overall conformation of the protein is only slightly affected by the redox state of the metal. This suggests that also the isomerization between the two forms of reduced azurin does not involve major changes in the whole structure of the protein, and hence that the switch mechanism is achieved through fine adjustments of the polypeptide moiety.

The crucial role that the aminoacids around the copper, and particularly His 35, exert in modulating its reactivity finds additional support in affinity-labeling studies carried out with Cr(III). In Ps. a. azurin, the site of attack of the reactant Cr(II) was found to be very specific and identified with Lys 85 and/or Glu 91 (Ref. 23). The peptide between Lys 85 and Glu 91 is essentially conserved and constitutes an opening into the metal site, through His 35 and His 46, a copper ligand. Although it is not established if this represents the actual pathway the electrons follow when the transfer occurs with the physiological partners, a mechanism has been proposed in which the Cr(II) transfers its electrons through a coordinated water molecule hydrogen bonded to His 35, and from here through the imidazole of His 46 to copper II. Thus, the electron would find its way to the metal delocalizing through the conjugated pathway of the almost parallel histidine rings. Again an analogous mechanism has been envisaged for poplar plastocyanin (Ref. 12).

Finally, recent work on Alcaligenes faecalis azurin must be recalled. Temperature jump experiments (Ref. 24) on the reaction between this azurin and cyt c_{551}, have indicated that no unimolecular transition in the reduced state of azurin is observed. In agreement with this finding H-NMR (Ref. 25) has revealed that His 35 is not protonated down to pH 4.0 and thus electrons are efficiently transferred by the unprotonated species. Although this conclusion may appear in contrast with the mechanism discussed above, some of the differences in primary structure between the two azurins have to be taken into consideration. Thus Ser 34 and Pro 36 (the nearest neighbours to His 35) are substituted in Alcaligenes azurin by Threo and Ser, respectively. These substitutions may deeply alter the structure of that polypeptide stretch and the solvent accessibility, thus affecting the protonation behaviour of His 35. Therefore, it is not surprising that Alcaligenes azurin displays a kinetic behaviour different from that of Ps. aeruginosa.

The kinetics of electron transfer involving azurin, and the structural interpretation of the unimolecular events in this small copper protein, represent a type-case of the role exerted by small conformational fluctuations of the protein matrix in the functional control of reactivity. It should be pointed out, however, that the exact physiological significance and the possible role of the proposed mechanism in the regulation of the in vivo electron transfer are not yet defined.

QUATERNARY STATES AND CATALYTIC PROPERTIES OF CYTOCHROME c OXIDASE

Cytochrome c oxidase, the terminal component of the respiratory chain in all aerobic cells,

is a multisubunit enzyme of M.W. 120,000 per functional unit containing 4 metal centers and localized as an integral component on the internal mitochondrial membrane. A number of excellent reviews (Ref. 8 & 26) summarize the considerable bulk of knowledge presently available on this important and complex system; thus, in what follows, only the basic information necessary to the present discussion will be outlined.

Control and regulation of the catalytic activity of cytochrome c oxidase by the protein moiety is especially evident in the following typical properties of the enzyme: i) the very different and highly specialized functions of the otherwise identical prosthetic groups, ii) the proposed coupling between the primary function of shuttling electrons from cytochrome c to O_2 and that of transporting protons through the internal mitochondrial membrane.

To describe the kinetic data on cytochrome c oxidase, and the significance of conformational states of the enzyme we have proposed a 2-state model focussing on long-range rearrangements in the protein moiety, probably involving more than one polypeptide chain. This model accounts for the kinetic behaviour of the enzyme under pre steady-state and steady-state conditions (Ref. 27) and can be related to the stereochemical and electronic rearrangements of the binuclear oxygen binding complex during the catalytic cycle.

The binuclear oxygen binding center

The great interest in studying the detailed structure of the oxygen binding center of cytochrome oxidase lies in its outstanding efficiency in overcoming the kinetic inertness of oxygen, without release in the bulk of undesirable oxygen radicals such as superoxide or hydrogen peroxide (Ref. 7). Unfortunately, cytochrome oxidase has not been crystallized, which excluded up to now the use of X-rays diffraction. Moreover, EPR characterization of the metal center directly involved in the binding of oxygen, has been impaired in the oxidized native form of the enzyme, by the antiferromagnetic coupling between Fe a_3 and Cu_B (see note a), the latter being called EPR undetectable under these conditions. Such a phenomenon is a clear evidence for the close vicinity of Fe a_3 and Cu_B which provided a firm basis for the assumption, present in all the recently proposed structural models, of their functional cooperation during oxygen reduction.

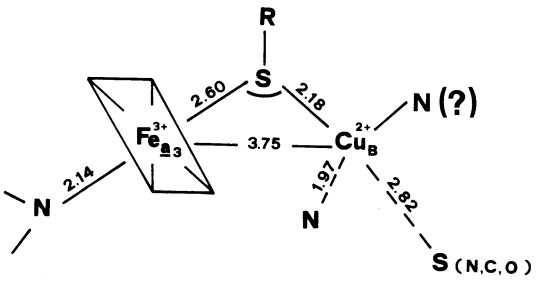


Fig. 2. Graphical representation (taken from Ref. 28) of the binuclear oxygen binding site for the oxidized resting state of cytochrome oxidase. The numbers indicate the internuclear distances (in Å) as obtained from EXAFS measurements. In parenthesis other possible ligands are indicated, and (?) refers to a ligand not observed but postulated from crystallographic data on similar systems. The errors are omitted for the sake of clarity, but are given and discussed at length in the original paper (Ref. 28).

Note a. Fe a_3 indicates the haem iron in cytochrome a_3 and Cu_B the copper ion which is spin coupled to it in the oxidized form; Fe a and Cu_A indicate the other two metal centers in the functional unit of the enzyme.

Among the various techniques employed in recent years to obtain structural data on the oxygen binding site, the EXAFS (Extended X Rays Absorption Fine Structure) spectroscopy and the extensive use of spin probes inducing a high-spin cytochrome $\underline{a}_3$ EPR signal from the oxidized enzyme have provided essential information.

Fig. 2 reports the proposed structure of the oxidized binuclear complex as deduced by Power et al. (Ref. 28) from a comparison of the EXAFS patterns of the enzyme with those of a number of other proteins and model compounds of known structure.

In the oxidized resting form, where Fe $\underline{a}_3$ and Cu_B are spin-coupled, the atom providing the physical bridging between the two is identified (but only with 35% occupancy) with a sulphur from a cysteinyl residue. The Fe $\underline{a}_3$ first shell, besides the heme nitrogen at 2.01 $\pm$ 0.03 Å, has another nitrogen as a proximal ligand and the bridging sulphur in the sixth position. The Cu_B first shell has a distorted tetrahedral arrangement and shows 2 nitrogens besides the bridging sulphur, while the nature of the fourth ligand is still uncertain. Upon reduction, the sulphur bridge is broken and the first shell of Fe $\underline{a}_3$ becomes identical to that of oxyhemoglobin, with CO (used as a stabilizer for the reduced form) instead of oxygen, and the sulphur atom remains bonded to Cu_B. Further information by this technique has also been reported on the Pulsed state of the enzyme (see below), in which the internal bridge provided by the protein seems to be broken making the iron of cytochrome a_3 available to external ligands (Ref. 29).

Structural information on the binuclear complex has been obtained, in the last 2-3 years, from EPR spectroscopy using as a ligand of oxidized Cu_B nitric oxide, which is known to be an excellent spin label (Ref. 30). The most interesting results of this investigation indicate: i) the existence of a mixture of forms (at least three) for the oxidized enzyme, forms which differ in the kinetics of reactions with reductants and oxygen; ii) a tetragonal arrangement of ligands around Cu_B; iii) a number of possible candidates for the ligand bridging Fe $\underline{a}_3$ and Cu_B in the oxidized form, such as water, hydroxyl, carboxylate and tyrosinate.

Nothwitstanding the discrepancies between the latter two conclusions and the picture shown in Fig. 2, the essential point emerging from these investigations is that a multiplicity of forms, characterized by different electronic and structural arrangement at the oxygen binding site, exists for the oxidized enzyme.

A two-state allosteric model

We have shown (Ref. 31 & 32) that freshly oxidized oxidase is catalytically more efficient than the enzyme which has been exposed to O_2 for a long time. Such a difference in the catalytic properties has been ascribed to the existence of two different molecular states of the enzyme, named Pulsed and Resting, the former being the more active one.

A typical result of rapid-mixing experiments, upon which the two-state model was originally developed, is reported in fig. 3. The minimum kinetic scheme initially proposed (Ref. 31) to describe the kinetics of oxidase has been included in a more general model with the following basic features: a) the existence of two interconvertible states of the enzyme, which differ in their catalytic properties; b) the effect exerted on the population of the states by external factors, such as the relative abundance of reducing and oxidizing equivalents. The experimental basis of the model has been subsequently extended to cover a number of different conditions including more complex and integrated systems, like Keilin-Hartree particles (Ref. 33), artificial proteoliposomes (Ref. 34) and, very recently, even crude heart tissue homogenates (Antonini et al., submitted). Results accumulated over the last five years indicate that both the Resting and the Pulsed state undergo a complete catalytic cycle (Fig. 4). Such a model, although simplified, allows a very satisfactory simulation of the time course observed under steady-state and transient regimes (Fig. 5).

At least four major points must be stressed in the present context:

1. The increased catalytic efficiency of the Pulsed form is related, as clearly shown by the experiment reported in fig. 3, to a higher rate of the intramolecular electron transfer between the electron accepting sites (Fe_a and Cu_A) and the binuclear O_2 binding site. This

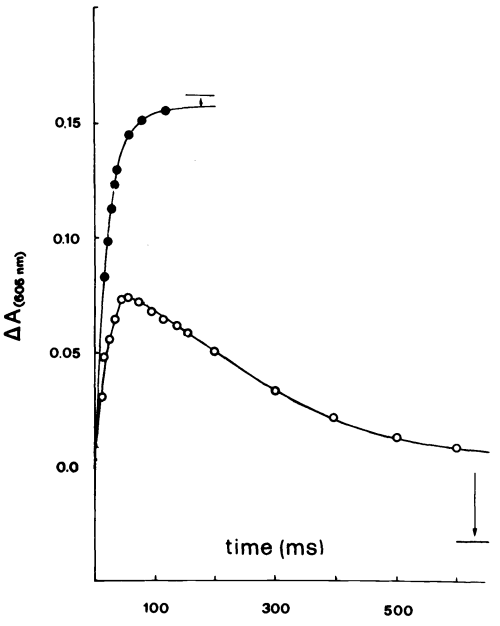


Fig. 3. Time courses for the reactions in the presence of CO between reduced cytochrome c and cytochrome c oxidase in the resting (●) and pulsed (⊙) states (modified from ref. 27). The reactions were followed in a stopped flow apparatus after mixing cytochrome oxidase (35 μM) with cytochrome c (100 μM) in the presence of Na Ascorbate (500 μM) and CO (900 μM). The buffer was K phosphate 0.1 M, pH 7.4 containing 1% Emasol 4130 and the temperature was 22°C. Binding with CO (monitored in the present case by a decrease in the O.D. at 605 nm) is only possible after reduction of cytochrome a_3, the bimolecular combination process being never rate limiting under the present conditions. Reduction of cyt a_3 has a $t_{\frac{1}{2}} \simeq 200$ ms for the Pulsed enzyme, while for the Resting one the process is not appreciable over the whole time range explored here (0 ÷ 1000 ms).

has been attributed to a different conformation of the protein moiety which modulates the electron transfer among different sites.

2. The R⟶P interconversion occurs during turnover, as demonstrated in Fig.**5** by the experiment starting with the Resting state in which the continuous sliding of the oxidation level of cytochrome c tends towards that of the Pulsed state. This result indicates a specific role for the P⟷R interconversion in the regulation of the overall process.

3. The difference between the two states of the enzyme is associated to spectroscopic changes which have been observed by absorption (Ref. 32), EPR (Ref. 30) and EXAFS (Ref. 28 & 29). Beinert and his associates (Ref. 35) reported the identification of a thus far unrecognized EPR signal with g=5, 1.78 and 1.69, obtained after mixing reduced oxidase with oxygen, in the absence of the typical g=6 signal of the oxidized and resting cyt a_3. The attribution of the new signal to the Pulsed state has been made since.

4. The different catalytic properties of the Resting and Pulsed state of the oxidase embedded into artificial proteoliposomes are observed from the rate of appearance of a pH gradient between the interior of the vesicles and the external medium. Whatever the intimate mechanism of the phenomenon and the exact value of the H^+/e^- ratio (Ref. 8), there is no doubt that the job of providing a physical linkage between the redox reactions at the metallic centers and the proton binding responsible for the formation of the pH gradient is carried out by the polypeptide backbone. Whether the P⟷R interconversion plays a role in this process remains, however, to be established.

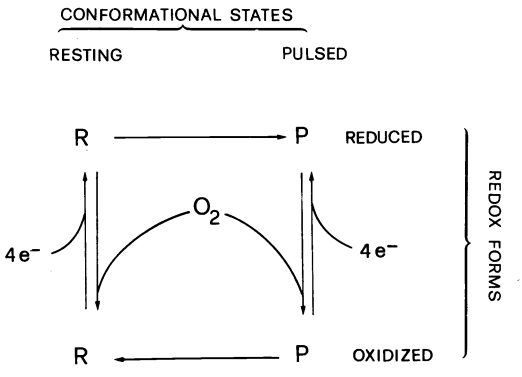


Fig.**4** . Schematic diagram of a two-state allosteric model for cytochrome oxidase (modified from Ref. 27). The Pulsed or the Resting states can both undergo reduction and oxidation as well as reciprocal interconversion. The latter are represented as irreversible to indicate that the Resting state is the more populated one in the oxidized form and the Pulsed state in the reduced one.

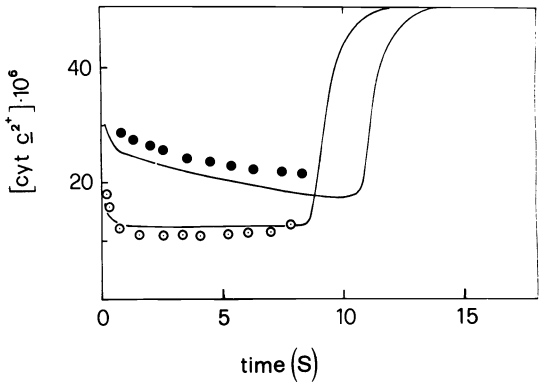


Fig.**5** . Comparison between experimental and simulated time courses for the oxidation of cytochrome c catalyzed by resting and pulsed cytochrome c oxidase in the presence of oxygen.
The reactions have been followed in a stopped-flow apparatus after mixing 50 μM cytochrome c in the presence of Na Ascorbate (5 mM) and TMPD with Resting (●) and Pulsed (○) cytochrome oxidase(10 μM).Buffer and temperature conditions as in fig. 3. The continuous lines are the computer solutions for the kinetic scheme used to describe the data (see Ref. 27) on the basis of a two-state model.

As a conclusion, it should be mentioned that the differences between resting and pulsed oxidase cannot be explained on the basis of oxygen or oxygen byproducts being still bound to the enzyme in the Pulsed state, but is of conformational origin, as suggested by:

i) the results of isotope labeling experiments (Ref. 35) in which no correlation was found between the presence of the EPR signals at g=5, 1.78, 1.69 and the presence of oxygen in the ligand binding site;

ii) a recent experiment (Ref. 37) in which the Pulsed state could be produced using Ferricyanide instead of O_2 as an electron acceptor.

This is in full agreement with the idea that changes in functional properties occurring during the interconversion from Resting to Pulsed oxidase, and vice-versa, must be explained in terms of a conformational rearrangement of the protein moiety and shows its fundamental role as a controller of the chemical activity of metal centers.

Acknowledgement - A Research Grant from Consiglio Nazionale delle Ricerche (Progetto "Chimica Fine e Secondaria") is gratefully acknowledged.

REFERENCES

1. E. Antonini and M. Brunori, Hemoglobin and Myoglobin in their reactions with ligands, North-Holland Publishing Company - Amsterdam - London (1971).
2. M. Perutz, Ann. Rev. Biochem. 48, 327-349 (1979).
3. M. Brunori, B. Giardina and H.A. Kuiper in Inorganic Biochemistry 3, 126-182 (1982).
4. J.P. Collman, Acc. Chem. Res. 10, 265-273 (1977).
5. T.G. Traylor, D. Campbell and S. Tsuchiya, J. Am. Chem. Soc. 101, 4748-4752 (1979).
6. J. Monod, J. Wyman and J.C. Changeux, J. Mol. Biol. 12, 88-107 (1965).
7. B.G. Mälmstrom, Ann. Rev. Biochem. 51, 21-59 (1982).
8. M. Wikstrom, K. Krab and M. Saraste, Cytochrome oxidase: a synthesis, Ac. Press London-New York, (1981).
9. Q.H. Gibson, Ann. Rev. Biochem. 35, 435-456 (1966).
10. M. Eigen, Q. Rev. Biophys. 1, 3-26 (1968).
11. T. Takano and R.E. Dickerson, J. Mol. Biol. 153, 79-94 (1981).
12. P.M. Colman, H.C. Freeman, J.M. Guss, M. Murata, V.A. Norris, J.A.M. Ramshaw, and M.P. Venkatappa, Nature, 272, 319-324 (1978).
13. E.T. Adman and L.H. Jensen Isr. J. Chem. 21, 8-12 (1981).
14. L. Rydel and J.O. Lundgren, Nature 261, 344-346, (1976).
15. D. Barber, R.S. Parr and C. Greenwood Biochem. J. 157, 431-438 (1976).
16. S.R. Parr, D. Barber, C. Greenwood and M. Brunori, Biochem. J. 167, 447-455 (1977).
17. M.T. Wilson, C. Greenwood, M. Brunori and E. Antonini, Biochem. J. 145, 449-457 (1975).
18. M.C. Silvestrini, M. Brunori, M.T. Wilson and V. Darley-Usmar, J. Inorg. Biochem. 14, 327-338 (1981).
19. G.A. Lappin, M.G. Segal, D.C. Weatherburn, R.A. Henderson and A.G. Sykes, J. Am. Chem. Soc. 101, 2302-2306 (1979).
20. S. Wherland and I. Pecht Biochemistry 17, 2585-2591 (1978).
21. H.A.O. Hill, and B.E. Smith, J. Inorg. Biochem. 11, 79-93 (1979).
22. E.T. Adman, G.W. Canters, H.A.O. Hill and N.A. Kitchen, FEBS Letters 143, 287-292 (1982).
23. O. Farver and I. Pecht, Isr. J. Chem. 21, 13-17 (1981).
24. P. Rosen, M. Segal and I. Pecht, Eur. J. Biochem. 120, 339-344 (1981).
25. S. Mitra and R. Bersohn, Proc. Natl. Ac. Sci. U.S.A. 79, 6807-6811 (1982).
26. M. Brunori, E. Antonini and M.T. Wilson Metal ions in biological systems (H. Segel ed.), Vol. 13, 187-227, Marcel Dekker (N.Y. and Basel) (1981).
27. M.T. Wilson, J. Peterson, E. Antonini, M. Brunori, A. Colosimo and J. Wyman, Proc. Natl. Ac. Sci. U.S.A., 78, 7115-7118 (1981).
28. L. Powers, B. Chance, Y. Ching, and P. Angiolillo, Biophys. J., 34, 465-498 (1981).
29. L. Powers, Y. Ching, B. Chance, B. Muhoberac, Biophys. J., 37, 403A (1982).
30. G.W. Brudvig, T.N. Stevens, R.N. Morse and S.I. Chan, Biochemistry, 20, 3912-3921, (1981).
31. E. Antonini, M. Brunori, A. Colosimo, C. Greenwood and M.T. Wilson, Proc. Natl. Ac. Sci. U.S.A., 74, 3128-3132 (1977).
32. M. Brunori, A. Colosimo, G. Rainoni, M.T. Wilson and E. Antonini, J. Biol. Chem. 254,

10769-10775 (1979).
33. C. Bonaventura, J. Bonaventura, M. Brunori and M.T. Wilson, FEBS Letters, 85, 30-34 (1978).
34. P. Sarti, A. Colosimo, M. Brunori, M.T. Wilson and E. Antonini, Biochem. J., 209, 81-89, (1983).
35. R.W. Shaw, R.E. Hansen and H. Beinert, Biochim. Biophys. Acta, 548, 386-396, (1979).
36. M.T. Wilson, P. Jensen, R. Aasa, B.G. Malmstrom and T. Vänngard, Biochem. J., 203, 483-492 (1982).
37. M. Brunori, A. Colosimo, M.T. Wilson, P. Sarti and E. Antonini, FEBS Letters, 152, 75-78, (1983).