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A Proposed Hydrogen Transfer Function for Cytochrome *c*

(electron transfer)

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ABSTRACT **Outlines of proposed mechanisms for cytochrome *c* oxidoreduction are presented which differ in detail from those previously published. The mechanisms, while accommodating the fact that cytochrome *c* is a net electron acceptor and donor in its catalytic cycle, dictate that the enzyme catalyses hydrogen transfer. It is shown that the mechanism of cytochrome *c* reduction fulfills a recent proposal that the transition state is an analogue of the structure of ferricytochrome c_2. The implications of a hydrogen transfer role of cytochrome *c* are discussed.**

Introduction

In recent reports from two laboratories, attempts were made to interpret the wealth of biochemical, x-ray, and other data on cytochrome *c* in terms of detailed and specific mechanisms of reduction and oxidation (1, 2). The mechanisms proposed for both reduction and oxidation differ markedly, but have in common the premise that cytochrome *c* catalyses the transfer of an electron between its reductase (cytochrome c_1) and oxidase. Oxidation–reduction reactions of the components of the respiratory chain are manifestations of the stepwise transfer of reducing equivalents, but the underlying processes involved are barely understood. The question of electron versus hydrogen transfer is fundamental to an overall understanding of electron transport and associated energy coupling reactions (3–5), and especially to the role of the proton in these processes (6, 7). The purpose of the present paper is to present the outlines of proposed mechanisms of cytochrome *c* oxidoreduction which involve hydrogen transfer to and from the enzyme with simultaneous proton loss and gain. The abandonment of the premise that cytochrome *c* must catalyse the mechanistic (as well as the overall) transfer of the electron allows a plausible mechanism of reduction to be written, which differs only in minor detail from the mechanism proposed by Salemme *et al.* (1). In this proposed reduction mechanism, cytochrome *c* attains a transition state completely analogous to the structure of cytochrome c_2. The latter proposal has been advanced by Salemme *et al.* (1) in a comprehensive review of the structure function relationship of the two related cytochromes.

Proposed Mechanism

The mechanism allows that cytochrome *c* interacts with its reductase (cytochrome c_1) essentially as described by Salemme *et al.* (1), binding being a function of lysine residues and the invariant 70–80 sequence (1, 2). The displacement of the ferric heme stabilizing anion (chloride) by phenylalanine 82 is followed, since this has been proposed to increase the electron affinity of the heme group in the transition state (1, 2). The present mechanism, like that most recently proposed, considers the role of the tyrosine 67-threonine 78 hydrogen bond in the physiological reduction mechanism. In contrast to the previous proposals, it is proposed that a basic residue (or the conjugate base of an acidic residue), located on the reductase, specifically deprotonates the hydroxyl of threonine 78; while the hydrogen bonded tyrosine 67 proton now bonds fully with the threonine 78 oxygen, the incipient phenoxide ion reduces the heme group as the ferric stabilizing anion is displaced. This first part of the mechanism, which can be briefly described as the base-induced oxidation of a phenol, is illustrated in Fig. 1. The threonine-tyrosine hydrogen bond relays the attack by base, while the methionine 90 sulfur (ligand) mediates the oxidation of tryrosine 67 by the heme group.

The second part of the mechanism is the repletion of hydrogen on the resultant phenoxy radical (tyrosine 67). This step may proceed rapidly after, or simultaneously with the electronic shifts depicted in Fig. 1. It is proposed that the phenoxy radical abstracts hydrogen from a hydrogen-donating residue (reductase) presented to the appropriate locus during the interaction. This presumption of a proximate hydrogen donor is similar to the proposal of an electron donating group near this locus in the electronic mechanism (1), while the proposal for a deprotonating function (B^-:, Fig. 1) is parallel to the proposal for an oppositely-functioning residue in the electronic mechanism (1). The overall process in the proposed mechanism is one-electron reduction.

The reduction mechanism avoids implausible steps, predicting a hydrogen transfer of low activation energy. The (neutral) phenoxy radical proposed as intermediate cannot be discounted on energetic grounds, while the hydrogen donor to this species may be one of several residues, like tyrosine, capable of hydrogen donation to an oxidant. Repletion of hydrogen on this donor may be effected by a reaction similar to the proposed reduction mechanism (in reverse). Stepwise hydrogen transfer between tryosine residues would constitute a free radical chain mechanism, which would not suffer the energetic difficulties (1) of the equivalent electron transfers (2). The proposed mechanism focuses on the creation of a hydrogen abstracting function, the tyrosine-threonine hydrogen bond playing an important role. If the displacement of the ferric-stabilizing anion provides the major driving force for the electronic shifts proposed, then the hydrogen bond can be regarded as a proton drain, bridging the space between the point of radical formation and external water. The hydrogen

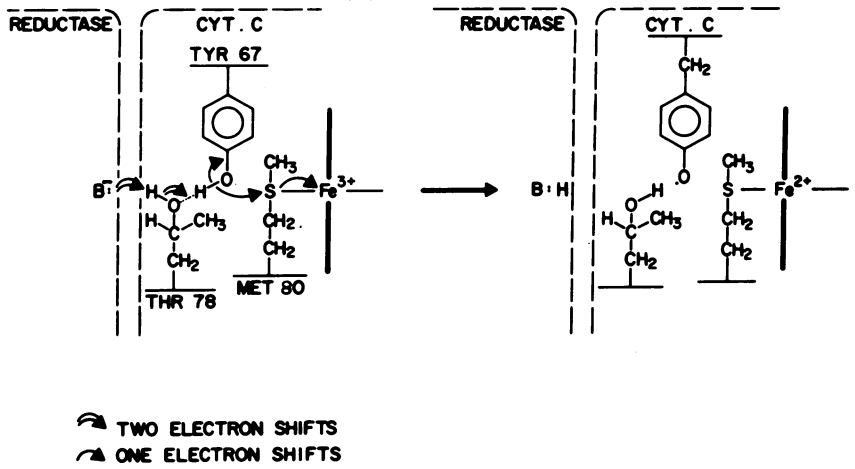

FIG. 1. Proposed electronic shifts leading to transition state in reduction of cytochrome *c*. B^-: represents a nucleophilic function on the reductase. The mechanism is proposed to occur simultaneously with displacement of the stabilizing anion.

bond, in this mechanism, acts in concert with the heme group, allowing a concerted separation of charge resulting in the transfer of (neutral) hydrogen through a short radical chain. Such a chain process, if induced in this way, may be impossible to detect, yet appears to be an attractive possibility because of a low activation energy and its avoidance of charged groups in an unfavorable environment.

The overall mechanism calls on no more assumptions (in terms of the number and nature of groups involved) than the electronic mechanism, but also fulfills the elegant proposal made by Salemme *et al.* (1) that ferricytochrome c_2 may represent an analogue of a transition state in cytochrome *c* reduction. This outcome of the proposed mechanism is illustrated in Fig. 2 in terms of the species lost and gained and the stabilization of the transition state of cytochrome *c*. As comprehensively discussed by Salemme *et al.* (1), cytochrome c_2 lacks the ferric stabilizing anion possessed by cytochrome *c*, but achieves stabilization of ferric state by interaction of the iron, through the methionine 91 (ligand) sulfur, with a negatively charged hydrogen-bonding grouping of three residues (tyrosine 70, tyrosine 52, and serine 89) near the heme. Cytochrome *c*, conversely, possesses a full complement of (two) protons in the analogous threonine-tyrosine structure. The simultaneous loss of a proton (Figs. 1 and 2), and anion displacement (Fig. 2) results in a transition state equivalent, in the terms considered, to the structure of ferricytochrome c_2. Furthermore, the residue in cytochrome c_2 (tyrosine 70), which occupies an analogous position to phenoxy radical (tyrosine 67) proposed in Fig. 1, has been represented in the ferric cytochrome as being partially deprotonated, and as having transferred some of its resultant negative charge to the heme iron in the stabilization function mentioned above (1). This suggests that the ferric form of this cytochrome is analogous to a structure in the cytochrome *c* reduction mechanism which just proceeds the completion of the electronic shifts in Fig. 1; i.e., after deprotonation but before heme reduction. It also follows that (cytochrome c_2) tyrosine 70 may accept hydrogen in a mechanism parallel to that proposed for cytochrome *c*. A parallel reduction mechanism was proposed (1), and is required, to explain the comparative kinetic data of Davis *et al.* (8). It is again stressed that the mechanisms as represented in Fig. 2 are consistent with the observed redox behavior of the two cytochromes; cytochrome *c* is a net electron acceptor, cytochrome c_2 a net hydrogen acceptor.

While Salemme *et al.* (1) have proposed that cytochrome *c* oxidation occurs through the reverse of their proposed reduction mechanism, Takano *et al.* (2) favor the right channel (tyrosine 97, phenylalanine 10) as the path of the electron in oxidation. Either structure involved in these proposals could mediate a hydrogen transfer to cytochrome oxidase, with simultaneous proton uptake, in a reaction similar to the reverse of the reduction mechanism. Reverse electron transfer through cytochrome *c* (9) indicates that both reactions must be freely reversible. This has been substantiated for the reduction reaction by the work of Yu *et al.* (10).

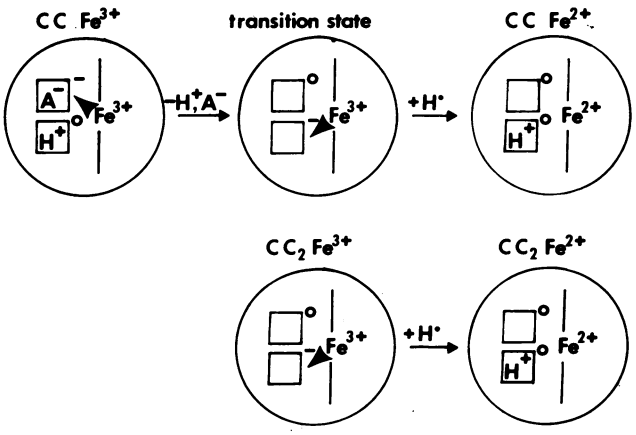

FIG. 2. Representation of proposed reduction mechanism of cytochrome *c* in terms of species lossed and gained. The *lower boxes* in each circle represent the hydrogen bonding systems in each cytochrome, as referred to in the *text;* the *upper boxes* represent the location of the ferric-stabilizing anion (absent in ferricytochrome c_2). The overall charge on each structure is indicated, the *arrowheads* representing charge interaction with the heme. Loss of a proton and the stabilizing anion from ferricytochrome *c* gives a proposed transition state equal, in the terms considered, to the structure of ferricytochrome c_2; internal reduction and hydrogen transfer occur through parallel reactions in each cytochrome.

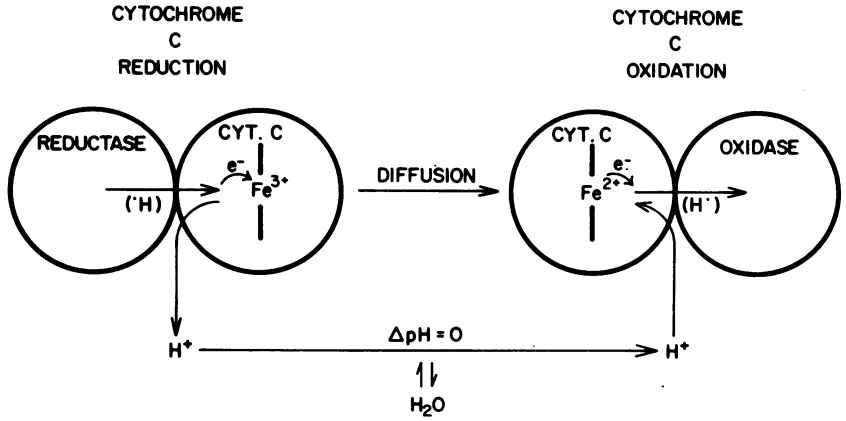

FIG. 3. Proposed hydrogen transfer function of cytochrome *c*. Net proton balance in the overall cycle is zero. The proton balance in the individual oxidation-reduction reactions depends on the source and fate of the hydrogens transferred.

The proposed oxidoreduction mechanism, considered together, imply a hydrogen transfer role for cytochrome *c*, as depicted in Fig. 3. In that the source and fate of the coupled protons has not been considered, it may be argued that the hydrogen transfer is only semantically different from the accepted electron transfer role of the cytochrome. This, however, requires that the mechanistically linked protons attain the same chemical potential that they possessed before the redox reactions, a situation which may not pertain. Yu *et al.* (10) have reported pH dependency of the equilibrium constant for the redox reaction between cytochrome *c* and c_1.

These workers have also concluded that the reduction of cytochrome *c* by ferrocytochrome c_1 involves more than a "mere redistribution of electrons" while Kihara *et al.* (11) have reported a deuterium isotope effect for the rates of the oxidation–reduction reactions of cytochromes in mitochondrial membrane fragments; these findings could well be functions of the proposed mechanisms.

Since a hydrogen transfer can also be argued for coenzyme Q, it is tempting to speculate that the observed charge separations in mitochondrial respiration may arise specifically across each of the multimolecular complexes, a conclusion forwarded primarily by Mitchell (6), with further proposals. Since it has been suggested that these charge separations are linked to energy coupling reactions, mechanisms similar to that proposed for cytochrome *c* reduction, but under different energetic constraints, and in different environments, could account for many of the phenomena observed in energy-coupling systems.

1. Salemme, R. F., Kraut, J. & Kamen, M. D. (1974) *J. Biol. Chem.* **248**, 7701–7716.
2. Takano, T., Kallai, O. B., Swanson, R. & Dickerson, R. E. (1973) *J. Biol. Chem.* **248**, 5234–5255.
3. Williams, R. J. P. (1969) in *Current Topics in Bioenergetics* ed. Sanadi, D. R., (Academic Press, New York), Vol. III, p. 79.
4. Green, D. E. & Ji, S. (1972) in *The Molecular Basis of Electron Transport*, eds. Schultz, J. & Cameron, B. F. (Academic Press, New York), pp. 557–576.
5. Williams, R. J. P. (1966) in *Hemes and Hemoproteins*, eds. Chance, B., Estabrook, R. & Yonetani, T. (Academic Press, New York), pp. 557–576.
6. Mitchell, P. (1968) *Chemiosmotic Coupling and Energy Transduction* (Glynn Research Ltd., Bodmin).
7. Mitchell, P. (1972) in *Biomembranes: Molecular Arrangements and Transport Mechanisms* (North Holland/American Elsevier, Amsterdam, London, New York), *Febs. Symp.* **XXVIII**, pp. 353–370.
8. Davis, K., Hatefi, Y., Salemme, F. R. & Kamen, M. D. (1972), *Biochem. Biophys. Res. Commun.* **49**, 1329–1335.
9. Slater, E. C. (1972) in *Biomembranes: Molecular Arrangements and Transport Mechanisms* (North Holland/American Elsevier, Amsterdam, London, New York), *Febs. Symp.* **XXVIII**, pp. 133–146.
10. Yu, C. A., Yu, L. & King, T. E. (1973) *J. Biol. Chem.* **248**, 528–533.
11. Kimara, T. & McCray, J. A. (1973), *Biochim. Biophys. Acta* **292**, 297.