Hypothesis

A MODEL FOR THE CYTOCHROME *b* DIMER OF THE UBIQUINOL : CYTOCHROME *c* OXIDOREDUCTASE AS A PROTON TRANSLOCATOR

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1. Introduction

Complex III of the respiratory chain is a unique multiprotein complex consisting of 9 polypeptide subunits [1]. Four of them possess electron-transferring redox centers: the two cytochromes *b*, the Rieske Fe/S-protein, and cytochrome c_1 [2]. Electrons are transferred from ubiquinol to cytochrome *c*, associated with a proton translocation across the mitochondrial inner membrane [3]. This segment of the electron-transfer chain is also termed the energy conservation site II. The mechanism of the electrogenic H^+ transport is still under investigation. As a hypothetic mechanism (a) a ubiquinone-mediated hydrogen transport via a so-called protonmotive redox loop is proposed [4,5]; as an alternative (b) a proton transport by certain, as yet undefined protein components by means of a redox-linked electrogenic proton pump is suggested [6,7]. The cytochrome *b* dimer proton translocator to be described follows mechanism (b) [8]. At site II, 4 $H^+/2\ e^-$, but only 2 charges/2 e^-, are transferred across the membrane [9,10]. The hydrogen carrier ubiquinone, in its classical arrangement, releases 2 $H^+/2\ e^-$ to the outside upon oxidation; the remaining 2 H^+ and 2 charges may be translocated by a proton pump. The existence of a heme-linked proton pump seems to be established at site III [11]. An analogous mechanism may function at site II. A partial model for such a mechanism has already been proposed [12].

2. Types of cytochrome *b*

The two cytochromes *b* of complex III are hydrophobic, integral membrane proteins, both of which show M_r 30 000 in SDS gel electrophoresis, but they form a dimer with M_r 60 000 when mild detergents are used for their solubilization [13,14]. Attempts to find a difference between the two monomers have failed so far [15]. Protein–chemical studies, for instance, partial amino acid sequence analysis, gave no evidence for the existence of two heterogeneous monomers [14]. Accordingly, genetic studies revealed the existence of only one single mosaic structural gene coding for a cytochrome *b* apoprotein of M_r ~30 000 [16,17].

In contrast to these findings, the existence of two functionally different cytochromes *b* in complex III has generally been postulated on the basis of spectrophotometric, kinetic and potentiometric studies carried out with cytochrome integrated in the mitochondrial membrane and on the isolated cytochrome bc_1 complex [2]. About half of the cytochrome *b* dimer has an α-absorbance band with a λ_{max} at 562 nm, and a half-reduction potential of $E_{m,7}$ = +50 mV; the other half has an α-absorbance band with a λ_{max} at 566 nm, and a half-reduction potential of $E_{m,7}$ = −50 mV. Cytochrome *b*-566 can be reduced by succinate only if a high electrochemical proton gradient is adjusted. The phenomenon has to be attributed to the numerous energy-linked reduction reactions of cytochrome *b* [18].

3. Protonation:deprotonation reactions of cytochromes *b*

An analysis of the published redox titration experiments reveals the sequence of protonation and reduction of cytochromes *b*-562 and *b*-566. The two possible sequences, first protonation and then reduc-

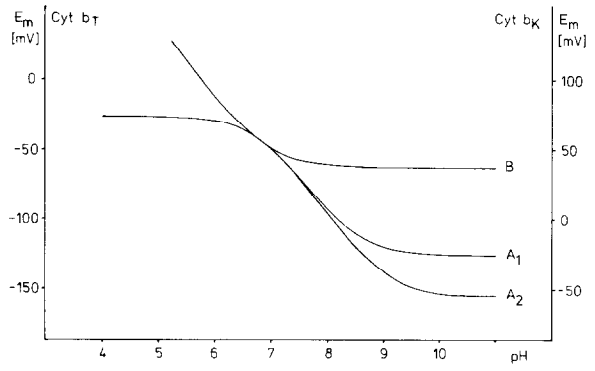

Fig.1. Dependence of the apparent half-reduction potential E_m of cytochromes *b* on pH. Curves A represent calculated plots for cytochrome b_T (left ordinate) and cytochrome b_K (right ordinate) assuming pK_a 6.8 for the oxidized state and pK_a 8.5 (curve A_1) and 9.0 (curve A_2) for the reduced state, respectively. For curve B pK_a 6.8 for the reduced state and pK_a <5.0 for the oxidized state have been assumed.

tion (cases A_1,A_2), or first reduction and then protonation (case B) can be distinguished by a plot of the apparent half-reduction potential (E_m) versus the pH value adjusted during redox titration. Curves A of fig.1 indicate that the H^+ is accepted before the e^-, according to the following reaction sequence:

$$b_{ox}R \overset{H^+}{\rightleftharpoons} b_{ox}RH^+ \overset{e^-}{\rightleftharpoons} b_{red}RH^+ \quad (1)$$

The redox reaction obeys a Nernst equation, which does not involve a pH dependence. However, the E_m values of cytochromes *b* are determined by a combined spectroscopic potentiometric method which does not allow a spectral distinction between $b_{ox}RH^+$ and $b_{ox}R$. Therefore E_m is determined according to:

$$E_m = E^h + \frac{RT}{F} \ln \frac{b_{red}RH^+}{(b_{ox}RH^+ + b_{ox}R)} \quad (2)$$

Substitution of $b_{ox}RH^+ \cdot K_a/H^+$ for $b_{ox}R$ gives:

$$E_m = E_o - RT/F \ln (1 + K_a/H^+) \quad (3)$$

The equation indicates that E_m differs from E_o if the H^+ concentration is $\leqslant K_a$ of group R. In the same way, curve B of fig.1 is obtained for a reaction sequence where reduction occurs prior to protonation according to:

$$E_m = E_o + RT/F \ln (1 + H^+/K_a)$$

In this case E_m is dependent on the pH if the proton concentration is $\geqslant K_a$. The redox titrations of cytochromes *b*-562 and *b*-566 reflect case A, with p*K* values of ~6.8 [19,20], but the occurrence of both cases has been described, e.g., for different cytochromes *b* of *Escherichia coli* [21]. Reaction sequence (1) does not take into account the possibility of a deprotonation reaction of $b_{red}RH^+$. The simplification seems to be admissible, since a deprotonation of this group with a p*K* >8.5 would be negligible at physiological pH.

4. The cytochrome *b* dimer proton translocator

The model assumes the existence of a cytochrome *b* homo-dimer although the possibility of a heterodimer has not been excluded, due to minor differences between the two subunits originating from a post-translational modification. It is presumed that cytochromes *b*-562 and *b*-566, also referred to as b_K and b_T, are connected in parallel in the sequence of the electron transport components, and represent two functional states of the same molecular moiety, i.e., the 30 000 monomer can interconvert between a b_T and a b_K state. The dimer functions cooperatively, so that the monomers can only be present in opposite conformational states. The model is based on the assumption that a group R of an amino acid residue, which is subject to a p*K* change in dependence on the redox state of the heme *b* center, is moved from an inner (i) to an outer (o) position by a conformational change of the protein.

The details of the model are discussed, going through one turn of the reaction cycle of one monomer (see fig.2). It consists of an ordered sequence of 6 reactions. At physiological pH values the protonation:deprotonation reactions 1/I and 5/I precede and succeed, respectively, the reduction:oxidation reactions 2/I and 4/I of cytochrome *b* when it is in b_T or b_K state, as group R possesses pK_a ~6.8 in the oxidized cytochrome *b*, and pK_a >8.5 in the reduced cytochrome *b*. The model implies that cytochrome *b* in b_T state is reduced by the presumptive electron donor ubiquinol and that cytochrome *b* in b_K state is oxidized by the presumptive electron acceptor, the Fe/S-protein. Reaction 3/I, the conformational

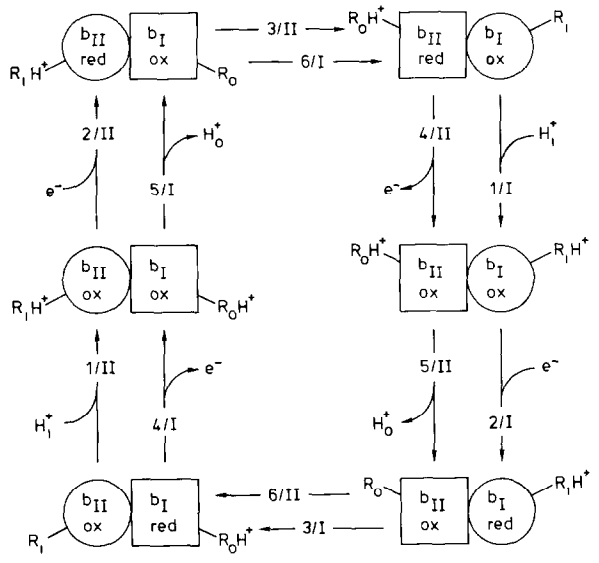

Fig.2. Scheme of the reaction cycle of the cytochrome *b* dimer proton translocator. The circles represent the b_T state, the squares the b_K state of the two cytochrome *b* monomers. The two monomers of a dimer are marked by the indices I and II. The different orientations of the R groups are indicated by the symbols i and o, representing inner (matrix) and outer (cytosolic) position, respectively. The same symbols apply to the polarity of proton uptake and release.

change from b_T to b_K state, involves a shift of group R in protonated state from i to o position, the back reaction 6/I from b_K to b_T state, involves a back-shift of group R in deprotonated state from o to i position. The shift of the amino acid residue R through probably only a few Å would be sufficient to cover the distance between two proton channels, which provide a connection to the matrix and cytosolic faces of the mitochondrial inner membrane. These proton channels may be formed by α-helical structures [22,23] of cytochrome *b*, as well as by α-helical structures of other polypeptide subunits of complex III. The shift of group R is driven by the redox reactions. The cycle yields a strict 1 H^+/e^- stoichiometry for each electron transferred from ubiquinol to cytochrome *c*.

The second monomer passes through the same cycle as the first monomer, but with a compulsory coupling of reaction 3/II with 6/I, and 6/II with 3/I, since the two *b*-monomers cannot exist in the same conformational state.

The equilibria of the protonation:deprotonation reactions 1 and 5 are influenced by the $\Delta\tilde{\mu}_{H^+}$. A high phosphorylation potential increases the H^+ gradient with an increase of the H^+ concentration at the outer face and a decrease of the proton concentration at the inner face of the mitochondrial membrane. Thus the equilibria of reactions 5 and 1 are shifted from product to substrate side during control and reversal of electron flow.

6. Discussion

Models for redox proton pumps have been postulated for complex III [6] and for cytochrome oxidase [11]. A common requirement of these pumps is a pK_a change at an electron-transferring chain component linked with the change of the redox state of the reaction center. Complex III possesses 3 redox components with apparent pH dependence of the half-reduction potentials, viz., with a pK_a shift of a group R of an amino acid residue depending on the redox state of this component. One of these 3 redox centers, the iron–sulfur protein, seems to be unqualified as a proton translocator, as its pK_a shift is beyond physiological pH range [24], whereas the two cytochromes *b* possess an appropriate p*K* shift and are therefore believed to form the proton translocator of the proton pump, which is able to transduce redox energy into an electrochemical membrane potential. As the two cytochromes seem to be identical both from genetic and protein–chemical studies they should have the same, or at least a closely related function. In the isolated cytochrome *b* dimer the subunits have lost their spectral differences, but retain their different half-reduction potentials. Agents interfering in protein–protein interactions, for instance urea, abolish the potential differences of the two monomers (unpublished results). Thus the spectral characteristics of the cytochrome *b* monomers seem to be influenced by polypeptide subunits of the complex which are dissociated from the cytochromes *b* in the course of isolation, whereas their potentiometric behaviour seems to be affected by their cooperative interaction. These features seem to be more compatible with a parallel than with a serial arrangement of the two cytochromes *b* in the electron transport chain. The two cytochromes *b* should therefore be arranged in the complex in a fashion that allows the reaction with the presumptive electron donor ubiquinol as well as with the presumptive electron acceptor, the Fe/S-protein [25].

The model fits the 4 H^+/2 e^- and the 2 charges/2 e^- stoichiometry measured for site II, on condition that the 2 H^+ originating from the oxidation of ubiquinol are released to the cytoplasm. It should be mentioned, however, that a sequential arrangement of cytochromes *b*, analogous to the proposed arrangement of cytochromes *a* and a_3, would also fit the stoichiometries, assuming a release of the two ubiquinol protons to the mitochondrial matrix and a transfer of 1 H^+/1 e^- by each of the cytochromes *b*.

The classical vectorial redox Bohr mechanism assumes a functioning of group R with different p*K* values on i and o side of the membrane. The assumption is correct if at one side protonation precedes reduction, at the other side reduction precedes protonation. Such an asymmetric sequence has also been proposed recently in a scheme for the cytochrome oxidase proton translocator [11]. However, the two cytochromes *b* function with only one pK_a of 6.8 over pH 6–8.5, as proved experimentally. Apart from this result, a symmetric sequence with protonation prior to reduction on both sides of the membrane has an advantage over an asymmetric sequence, as pK_a 6.8 is unproblematic with regard to the reversibility of the reactions.

Taking into consideration a half-reduction potential E_m = −50 mV at pH 7.0, and pK_a 6.8 for oxidized cytochrome b_T, a standard redox potential of E_o = −30 mV is calculated for the protonated state. Assuming a pK_a 9.0 for reduced cytochrome b_T, a standard redox potential of E_o = −155 mV is calculated for the deprotonated state (see fig.1). These thermodynamic data indicate that reduction of cytochrome b_T by ubiquinone ($E_{m,7}$ = +40 mV) is favoured when b_T is in its protonated state (see fig.2).

We speculate that the mechanism deduced for the cytochrome *b* dimer proton translocator may also apply for other redox-linked proton pumps, such as those acting at energy-conserving sites I and III of the mitochondrial respiratory chain, as well as at the energy-conserving regions of the photophosphorylating electron transfer chain, for example in the cytochrome b_6–*f* region, which has analogies with the mitochondrial complex III [26,27]. In these cases the cooperative function of a dimer arranged in parallel in the electron-transfer chain may not be essential, but a monomer may pass through the described reaction cycle.

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