EVIDENCE FOR A PROTONMOTIVE Q CYCLE MECHANISM OF ELECTRON TRANSFER THROUGH THE CYTOCHROME $\underline{b}$-$\underline{c}_1$ COMPLEX

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Summary A protein named oxidation factor can be reversibly removed from succinate-cytochrome $\underline{c}$ reductase complex and shown to be required for electron transfer between succinate and cytochrome $\underline{c}$. This protein is required for reduction of cytochrome $\underline{c}_1$ and, in the presence of antimycin, for reduction of both cytochromes $\underline{b}$ and $\underline{c}_1$. These results are consistent with a protonmotive Q cycle mechanism in which the oxidation factor catalyzes electron transfer from reduced quinone to cytochrome $\underline{c}_1$ and thus liberates from reduced quinone one of two protons required for energy conservation during electron transfer through the cytochrome $\underline{b}$-$\underline{c}_1$ complex.

Introduction

The mechanism of electron transfer through the cytochrome $\underline{b}$-$\underline{c}_1$ complex is unknown. This problem is of special interest since this segment of the electron transport chain encompasses the second coupling site of mitochondrial oxidative phosphorylation. Hence, to fulfill the requisites of the chemiosmotic hypothesis (1), electron transfer through this complex must, in some currently unknown fashion, translocate protons outward across the mitochondrial membrane. Accordingly, there has been considerable effort directed toward identifying components which participate in electron transport through the $\underline{b}$-$\underline{c}_1$ complex, with particular emphasis on those which might also translocate protons.

A protein named oxidation factor was recently discovered to be an intrinsic component of the cytochrome $\underline{b}$-$\underline{c}_1$ complex (2). This protein is required for electron transfer between succinate and

cytochrome c and appears to function as a cytochrome b-c_1 oxidoreductase (2). Further details regarding the possible catalytic function of the oxidation factor are unknown.

In this communication I wish to describe results of experiments which indicate how the oxidation factor may catalyze electron transfer in the cytochrome b-c_1 complex and, at the same time liberate a proton from reduced quinone as a direct participant in the primary energy conserving step of oxidative phosphorylation.

Materials and Methods

Succinate-cytochrome c reductase complex from bovine heart mitochondria was depleted of oxidation factor by extraction with quanidine and cholate (2). The depleted complex was reconstituted with oxidation factor in the presence of phospholipids, ubiquinone, and succinate dehydrogenase (2).

Cytochrome c reductase activity and difference spectra of cytochromes were measured as described previously (3).

Results and Discussion

Succinate-cytochrome c reductase complex, a resolved membranous segment of the mitochondrial electron transport chain, catalyzes electron transfer from succinate to cytochrome c with a zero order rate of 6-8 μmole of cytochrome c reduced per min per mg (3).

If reductase complex is extracted with guanidine and cholate, the resulting membranous complex contains cytochromes b and c_1 but exhibits no succinate-cytochrome c reductase activity unless reconstituted with a protein named oxidation factor, which is removed by the extraction procedure (2). When succinate is added to reductase complex which is depleted of oxidation factor, there is rapid reduction of 75 percent of the cytochrome b and no reduction of cytochrome c_1 (Figure 1). The requirement for oxidation factor for reduction of c_1 appears to be absolute in that no

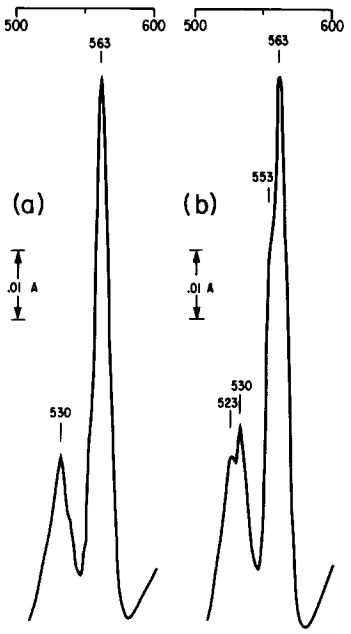


Figure 1 Difference spectra of cytochromes in depleted reductase complex reduced by succinate. The depleted reductase complex was suspended at 1 mg/ml in 0.1 M sodium phosphate-0.5 mM EDTA, pH 7.4. The spectrum in (a) shows the reduction of cytochrome b by succinate in depleted reductase complex. The spectrum in (b) is of the same sample 5 minutes after addition of 100 µg of oxidation factor. Both spectra were recorded against a reference in which the cytochromes were oxidized with ferricyanide.

reduction of c_1 is evident even 30 minutes after addition of succinate. If oxidation factor is subsequently added to the depleted complex in which the b has been reduced by succinate, the endogenous c_1 is completely reduced, as indicated by the appearance in the absorption spectrum of a shoulder at 553 nm and the β absorption band of c_1 at 523 nm (Figure 1b). It would appear that oxidation factor is not required for electron transfer between succinate dehydrogenase and cytochrome b since the same amount of b is reduced by succinate in the parent reductase complex and the complex depleted of oxidation factor (cf Figure 1 and reference 3).

That oxidation factor is involved in the reduction of cyto-

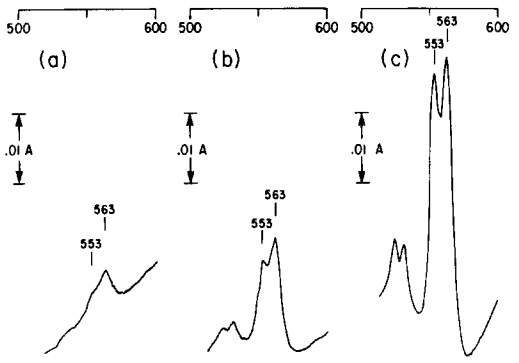
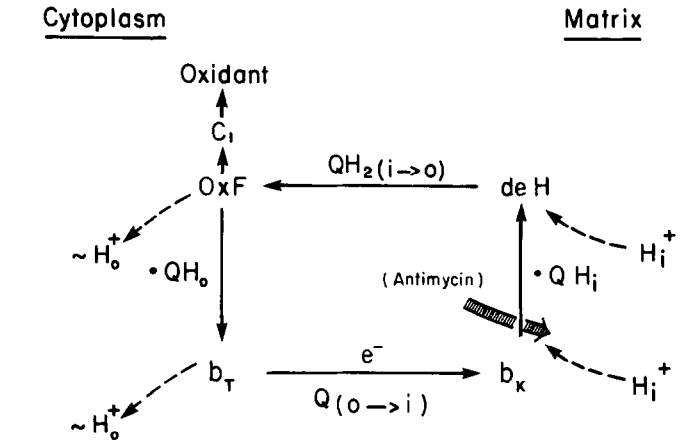


Figure 2 Difference spectra of cytochromes in depleted reductase complex reduced by succinate in the presence of antimycin. Depleted reductase complex was suspended at 1 mg/ml and incubated with 5 µg of antimycin prior to addition of succinate. All spectra were recorded 8 minutes after addition of 15 mM succinate against a reference in which the cytochromes were oxidized with ferricyanide. The spectrum in (a) is of the depleted reductase complex. The spectra in (b) and (c) are of reductase complex after reconstitution with 50 and 150 µg of oxidation factor respectively.

chrome b by succinate can be demonstrated by examining the reduction of cytochromes b and c_1 in the presence of antimycin, a fungicide which prevents reduction of cytochrome c_1 while allowing reduction of cytochrome b in mitochondria in the aerobic steady state (4). When succinate is added to the depleted complex in the presence of antimycin, there is virtually no reduction of either cytochrome b or c_1 (Figure 2a). If oxidation factor is added to the depleted reductase complex after addition of succinate and antimycin, both cytochromes b and c_1 undergo reduction (Figures 2b and 2c). The small amount of b which is reduced in the depleted reductase in the presence of antimycin (Figure 2a) is consistent with the slight amount of cytochrome c reductase activity exhibited by the depleted complex. In addition, as more oxidation factor is restored to the complex there occurs progressively more reduction of both cytochromes b and c_1 (Figures 2b and 2c).



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\begin{array}{lll}
(1) & \cdot deH + \cdot QH_i + H_i^+ \longrightarrow & deH + QH_{2,i} \\
(2) & QH_{2,i} \xrightarrow{OxF} & QH_{2,o} \\
(3) & QH_{2,o} + C_1 \longrightarrow & \cdot QH_o + \cdot C_1 + H_o^+ \\
(4) & \cdot QH_o + b_T \longrightarrow & Q_o + \cdot b_T + H_o^+ \\
(5) & \cdot b_T + b_K \longrightarrow & b_T + \cdot b_K \\
(6) & Q_o \longrightarrow & Q_i \\
(7) & \cdot b_K + Q_i + H_i^+ \longrightarrow & b_K + \cdot QH_i \\
\hline
 & \cdot deH + C_1 + 2\,H_i^+ \longrightarrow & deH + \cdot C_1 + 2\,H_o^+
\end{array}
$$

Figure 3 A protonmotive Q cycle mechanism of electron transfer through the cytochrome b-c_1 complex showing the proposed catalytic function of the oxidation factor. The essential feature of the mechanism is that as one electron is transferred from the dehydrogenase to cytochrome c_1, two protons are actively transported from the matrix to the cytoplasm region of the inner mitochondrial membrane. It is proposed that oxidation factor (OxF) liberates a proton from reduced quinone while transferring an electron from reduced quinone to cytochrome c_1. The subscripts o and i designate the location of components outside and inside of the membrane. Thus, $\cdot QH_o$ refers to the semiquinone form of coenzyme Q, localized in the outside region of the inner mitochondrial membrane.

The function of the oxidation factor and a mechanism of electron transfer through the cytochrome $\underline{b}$-$\underline{c}_1$ complex can be described by a protonmotive Q cycle as shown in Figure 3, which is based on the Q cycle which was first proposed by Mitchell (5) but differs therefrom in two respects. The present mechanism specifies that reduced quinone (QH_2), and not semiquinone ($\cdot QH$), is the reductant for cytochrome $\underline{c}_1$ (cf 5). And, on the basis of the results described above, it is proposed that the oxidation factor catalyzes electron transfer between reduced quinone and cytochrome $\underline{c}_1$.

According to this mechanism the net transfer of one electron from dehydrogenase to cytochrome $\underline{c}_1$ results in translocation of two protons from the matrix to the cytosol side of the inner membrane as follows. An electron is transferred from dehydrogenase to the semiquinone form of coenzyme Q with the uptake of one proton from the matrix side of the membrane. The reduced quinone thus formed carries two electrons and two protons across the membrane. Oxidation factor transfers an electron from reduced quinone to cytochrome $\underline{c}_1$, thus liberating a proton and generating the semiquinone reductant for cytochrome $\underline{b}_T$. Transfer of an electron from the semiquinone to cytochrome $\underline{b}_T$ liberates a second proton and generates oxidized quinone. The quinone moves to the matrix side of the membrane and an electron is transferred from cytochrome $\underline{b}_T$ to $\underline{b}_K$. Reduced $\underline{b}_K$ is then oxidized by quinone with uptake of a second proton, thus regenerating semiquinone on the matrix side of the membrane.

If the oxidation factor functions in electron transfer in the cytochrome $\underline{b}$-$\underline{c}_1$ complex as proposed here, then several considerations follow.

First, the oxidation factor should act as a hydroquinone-

cytochrome $\underline{c}_1$ oxidoreductase to catalyze electron transfer from reduced quinone to cytochrome $\underline{c}_1$. If such is the case, it follows that the oxidation factor catalyzes an electron transfer reaction which generates the reductant for cytochrome $\underline{b}$ and which liberates from reduced quinone one of two protons required for energy conservation at the second coupling site according to the chemiosmotic hypothesis.

Second, the oxidation factor should be located on or near the cytoplasmic surface of the inner mitochondrial membrane in proximity to cytochrome $\underline{c}_1$.

Third, since the difference in midpoint potentials between the dehydrogenase and cytochrome $\underline{c}_1$ is sufficient to span the components of the second coupling site, the oxidation factor must participate in the electron transfer reaction which is the driving reaction for the entire Q cycle.

And finally, in the absence of the oxidation factor the Q cycle can run in reverse from dehydrogenase to cytochromes $\underline{b}_K$ and $\underline{b}_T$, but not through to cytochrome $\underline{c}_1$.

The mechanism of electron transfer proposed here satisfies the requisites of the protonmotive Q cycle as recently outlined in a general formulation by Mitchell (6). In addition, the present mechanism predicts a specific sequence of electron transfer between cytochromes $\underline{c}_1$ and $\underline{b}_T$ and also explains the function of the oxidation factor.

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