Hypothesis

THE INTERACTION OF THE RADICALS OF UBIQUINONE IN MITOCHONDRIAL ELECTRON TRANSPORT

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1. Introduction

The main purpose of the 'protonmotive Q-cycle' which was proposed by Mitchell [1–4] is to achieve theoretically the transport of two protons from the inside to the outside of the mitochondrial membrane by the transfer of only one electron from the dehydrogenase site of ubiquinone to cytochrome c_1. Thus the cycle is thought to include loop 2 and 3 of the 'chemiosmotic hypothesis'.

The flow scheme given in fig.1 is essentially that proposed by Mitchell in [2] and represents the Q-cycle of the mitochondrial electron transport [3]. Two electrons and two protons are transferred by the diffusion of QH_2 from the inside to the outside of the membrane. Here QH_2 is oxidized to give Q which diffuses back to the inside. Only one of the two electrons is passed on to oxygen via cytochrome c_1; the other is conducted back to the inside via the *b*-cytochromes, where it is transferred to Q together with the electron donated by the dehydrogenase.

In the oxidation reaction the specific transfer of the two electrons may be achieved by the b–c_1 complex without the intermediate liberation of the quinone radical. In contrast, in the reduction of Q to QH_2 the radicals have to be liberated in order to equilibrate with Q and QH_2 via the dismutation reaction (a).

$$2\dot{Q}^- + 2H^+ \rightleftharpoons Q + QH_2 \qquad (a)$$

Abbreviations: Q, ubiquinone; QH_2, ubihydroquinone; $\dot{Q}H$, neutral radical of ubiquinone; $\dot{Q}^-$ radical anion of ubiquinone.

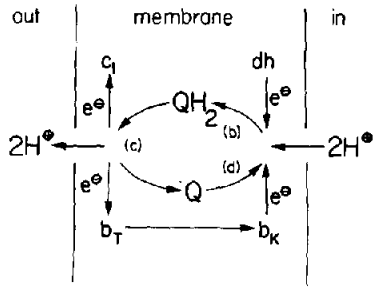

Fig.1. Flow scheme of the protonmotive Q-cycle proposed for the mitochondrial electron transport by Mitchell [2,3]. c_1, b_T and b_K designate the respective cytochromes and dh means the dehydrogenase site of Q interaction. The letters given in brackets refer to the reactions written in the text.

This is necessary for explaining the reduction of Q in the presence of antimycin [3,4]. This situation requires that cytochrome *b* reacts specificly with Q and the dehydrogenase specifically with the radical [3,4]. Hence the Q-cycle can be described by reactions (a–d). Reaction (b) represents the reduction of

$$\dot{Q}^- + 2H^+ + e^- \longrightarrow QH_2 \qquad (b)$$

$$QH_2 \longrightarrow Q + 2H^+ + 2e^- \qquad (c)$$

$$Q + e^- \longrightarrow \dot{Q}^- \qquad (d)$$

the quinone radical by the dehydrogenases, and is associated with the uptake of two protons from the inside of the membrane. The protons are liberated on the outside of the membrane in the oxidation of QH_2 by the b–c_1 complex (reaction c). One of the electrons of reaction (c) is transferred back to Q

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(reaction d) which forms the radical once again, and is thought to be inhibited by antimycin [3,4].

In this communication arguments are raised against the function of the Q-cycle in mitochondrial electron transport. These arguments are based largely on the fact that the stability constant of the quinone radical is extremely small. In addition it will be shown that a ratio of two protons translocated per electron transferred through the Q-region of the respiratory chain can be also achieved by the 'pool-function of Q' [5], and does not require the interaction of the Q-cycle.

2. The equilibrium quinone radical concentration

The rate of formation of the quinone radicals in the Q-cycle (reaction d) is equal to that of their reduction according to reaction (b). This means that the radical concentration as determined by the equilibrium of dismutation (reaction a) is unaffected by electron transport. The equilibrium constant of the dismutation as measured for duroquinone in water (about $10^{-24}M^2$) [6,7] should be similar for ubiquinone in the mitochondrial membrane [8]. With this constant at pH 7 the radical concentration is calculated to be about 0.001% of the total quinone [8]. This number is about three orders of magnitude smaller than the content of the NADH- and succinic dehydrogenase of the membrane. This extremely small concentration makes the quinone radical a very unlikely acceptor of reducing equivalents, and therefore argues against the participation of reaction (b) in electron transport. Furthermore, the reduction of Q was found to be pseudo-first order with respect to the concentration of Q [9]. This suggests that Q and not the radical is the acceptor of the reducing equivalents donated by the dehydrogenases.

3. The possible function of quinone radicals in electron transport

As proposed earlier by Kröger and Klingenberg [9], electron transport from the dehydrogenases to the cytochromes is mediated by the sequential operation of the reactions (d), (a) and (e) as illustrated in fig.2. According to this sequence quinone radicals are formed as primary products of the reduction of Q by the

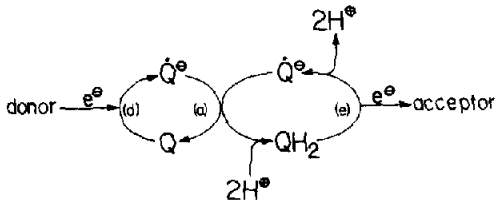

Fig.2. The interaction of Q in the mitochondrial electron transport as proposed by Kröger and Klingenberg [9]. The radical anion rather than the neutral radical of Q is assumed to be formed by the reduction of Q and the oxidation of QH_2, because the pK of the proton of the neutral radical is about 6 [10]. The dismutation is probably attained by the diffusion of the radicals in the membrane. In direct electron transport the donor represents the dehydrogenase and the acceptor the cytochrome site of the Q-pool; the opposite obtains in the energy-driven reverse of the electron transport. The letters given in brackets refer to the reactions written in the text.

$$QH_2 \longrightarrow e^- + \dot{Q}^- + 2H^+ \qquad (e)$$

donor (reaction d) and of the oxidation of QH_2 by the acceptor (reaction e). In contrast to the Q-cycle, however, it was suggested that the radicals only dismutate (reaction a), but do not react with other respiratory carriers. The dismutation as well as the redox reactions of Q and QH_2 are probably facilitated by the diffusion of Q in the lipid space of the mitochondrial membrane [5,9]. In direct electron transport the donor would be the dehydrogenase and the acceptor the cytochrome site of the Q-pool; the opposite would occur in the energy-driven reverse of electron transport.

As the pK of the dissociation of the proton of the neutral radical according to reaction (f) is about 6 [10], the radical anion rather than the neutral radical is probably the primary product of the redox reactions of Q. This assumption has the interesting consequence, that electron transport is associated with

$$\dot{Q}H \rightleftharpoons \dot{Q}^- + H^+ \qquad (f)$$

the uptake of two protons in the dismutation (reaction a) and the liberation of two protons in the oxidation reaction (e), (fig.2). On the basis of the chemiosmotic hypothesis this would effect the transport of two protons across the membrane per electron transferred from the donor site of Q to cytochrome c_1, provided

that the protons are taken up on the inside and liberated on the outside of the membrane.

This may serve to demonstrate that the translocation of two protons per electron transferred through the Q-pool does not necessarily require the assumption that the quinone radical interacts with other components as acceptor (or donor) of reducing equivalents as postulated by the Q-cycle. It is pointed out, however, that there is no conclusive experimental evidence either in support of this or of any other chemiosmotic proton-translocating mechanism.

4. The quinone radical concentration in the steady state

The concentration of the quinone radicals in the steady state is a function of the activity of the electron transport according to the mechanism set out in fig.2. Hence the radical concentration is estimated from the velocities of electron transport and of the dismutation reaction and compared with the results obtained from the observation of the reactions of Q in the mitochondrial membrane [9,12]. This comparison may be regarded as a test of the probability of function of the mechanism given in fig.2.

The velocity of electron transport which causes the formation of the radicals (two radicals per electron) is assumed to be 4×10^{-2} M sec^{-1}. This is equivalent to a respiratory activity of 1200 μatoms O per gram of protein per min on the basis that 1 gram of protein corresponds to 1 ml of the lipid phase of the membrane as the solvent of Q. With this assumption the total concentration of Q in the membrane is 5 mM. The rate constant of dismutation of the radical anion according to reaction (g) has been measured with duroquinone in aqueous solution and is $4.6 \cdot 10^6$ M^{-1} sec^{-1} [11]. This value is assumed to hold also for Q

$$2\dot{Q}^- \longrightarrow Q + Q^{2-} \qquad (g)$$

in the membrane [8]. In the steady state where the rate of formation of the radicals is equal to the rate of dismutation, the radical concentration can be calculated as the ratio of the velocity of electron transport and the rate constant of dismutation. The radical concentration so obtained amounts to about 2% of the total Q. This number is consistent with the results obtained from the investigation of the reactions of Q as a function of electron transport and of oxidative phosphorylation [9,12]. In these studies only the apparent conversion of Q to QH_2 and the reverse reaction were noticed, and the radical concentration was estimated to be smaller than 5% of the total Q. From this it was concluded that the dismutation of the radicals proceeds much faster than their possible formation by electron transport, and this conclusion is in agreement with the result of the above calculation.

Acknowledgements

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References

[1] Mitchell, P. (1975) FEBS Lett. 56, 1–6.
[2] Mitchell, P. (1975) FEBS Lett. 59, 137–139.
[3] Mitchell, P. (1975) in: Electron Transfer Chains and Oxidative Phosphorylation (E. Quagliariello et al. eds.) North-Holland, p. 305–316.
[4] Mitchell, P. (1976) J. Theoret. Biol., in the press.
[5] Kröger, A. and Klingenberg, M. (1973) Eur. J. Biochem. 39, 313–323.
[6] Michaelis, L., Schubert, M. P., Reber, R. K., Kuck, J. A. and Granick, S. (1938) J. Am. Chem. Soc. 60, 1678–1683.
[7] Baxendale, J. H. and Hardy, H. R. (1955) Trans. Faraday Soc. 49, 1433–1437.
[8] Kröger, A. (1975) in: Genetics, Biogenesis and Bioenergetics of Mitochondria (F. Kaudewitz et al. eds.) de Gruyter and Co, Berlin, in the press.
[9] Kröger, A. and Klingenberg, M. (1973) Eur. J. Biochem. 34, 358–368.
[10] Bridge, N. K. and Porter, G. (1958) Proc. Roy. Soc. Ser. A 244, 276–288.
[11] Bensasson, R. and Land, E. J. (1973) Biochim. Biophys. Acta 325, 175–181.
[12] Kröger, A. and Klingenberg, M. (1966) Biochem. Z. 344, 317–336.