A TRANSMEMBRANE QUINONE PAIR IN THE SUCCINATE DEHYDROGENASE–CYTOCHROME *b* REGION

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1. Introduction

Recently, Ruzicka et al. [1] reported an EPR signal characteristic of dipolar coupled species in beef heart mitochondria. At first this signal [2] was believed to be due to the interaction of ubisemiquinone with an iron sulfur center, but simulation of the spectra in Complex II [1] showed that a better fit could be obtained if the other species was another semiquinone. Potentiometric methods confirmed this and the second species was identified as a ubisemiquinone rather than a flavosemiquinone [3,4] which had been considered a viable alternative [1].

The orientation of the heme planes in cytochrome oxidase has recently been determined using oriented multilayers made by centrifugation and partial drying of purified cytochrome oxidase vesicles, electron transport particles [5] and mitochondria [6]. The necessary assumption is that each molecule (and therefore each heme) has a relatively fixed orientation in the direction normal to the membrane; this is borne out by experimental results.

Analysis of the EPR spectra of the coupled quinone pair in oriented multilayers made from mitochondria allow information about the orientation and relative location of the quinones in the membrane to be deduced.

2. Materials and methods

Beef heart mitochondria were prepared as described in ref. [7]. Oriented multilayers were prepared from mitochondria as described by Erecinska et al. [6]. After drying, a small amount of concentrated succinate and fumarate solutions were added to the multilayer and excess was carefully removed with absorbent paper.

It was possible to induce the signals from the semiquinone pairs (characteristic of an effective potential poise between +60 and +180 mV) without significantly affecting the orientation in the EPR spectrum of Rieske iron–sulfur center [8]. This orientation was measured by monitoring changes between parallel and perpendicular orientations of the membrane plane with respect to the magnetic field*.

3. Results and discussion

The expression for dipolar splitting at a particular orientation is

$$\Delta H = \frac{\mu_{\text{eff}}}{r^3}(1-3\cos^2\psi) = D\,(1-3\cos^2\psi)$$

where μ_{eff} is the magnetic moment of one of the interacting species, r is the distance between them, and ψ the angle between the line joining them and the applied magnetic field. The unoriented spectrum of a dipolar coupled pair of isotropic spins consists of

* The EPR signal at g 1.90 was a factor of 10 larger in the parallel direction as compared to the perpendicular direction. The orientation of the iron–sulfur centers is the subject of a forthcoming paper.

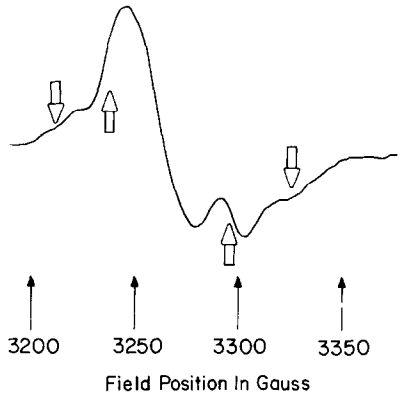

Fig.1. EPR spectrum of suspension of electron transport particles at 12°K. Protein was 20 mg/ml; potential was poised potentiometrically at 100 mV. EPR conditions were: microwave frequency, 9.18 GHz; microwave power, 5 mW; modulation frequency, 100 kHz; modulation amplitude, 10 G; scan rate, 100 G/min, time constant, 1 s. Arrows show the calculated positions of lines from the quinone pair.

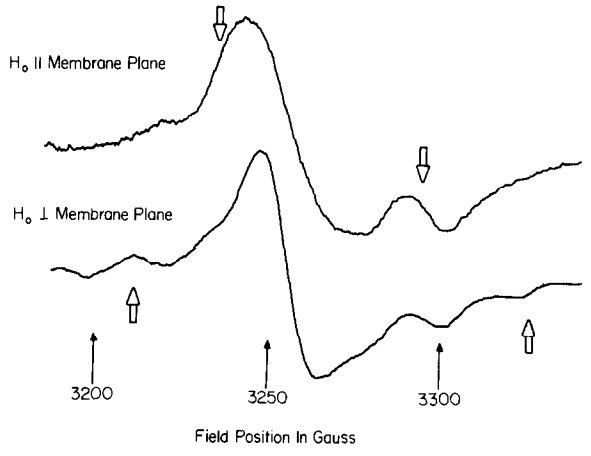

Fig.2. EPR spectra of oriented multilayers prepared from beef heart mitochondria. EPR conditions were as in fig.1; arrows show the calculated positions of the inner pair (upper arrows) and outer pair (lower arrows). (top) Magnetic field perpendicular to the multilayer. (bottom) Applied static magnetic field parallel to the plane of the multilayer.

two pairs of lines. The inner pair is displaced by the dipolar strength D to each side of the original line; the outer pair is displaced by $\pm 2D$. Ruzicka et al. [1] simulated the spectrum of the coupled semiquinones in Complex II (particulate succinate UQ reductase) using $g_{\|}$ 2.0066 (in the plane of the quinone), $g_{\perp}$ 2.0041, and $r = 7.71$ Å, and r aligned with $g_{\|}$. The small amount of g-value anisotropy causes the spectra to be slightly asymmetric.

Figure 1 shows the EPR spectrum of suspension of beef heart mitochondria at 12°K. The strong central resonance is due to HiPIP-type iron–sulfur center S-3. A small amount of g_z signal from Rieske's iron–sulfur center overlaps near 3340 G. The arrows indicate the positions of the quinone signals.

Figure 2 (bottom) shows the spectrum of the oriented multilayers with the field perpendicular to the membrane plane. The outer pair of lines is clearly visible despite some overlap from Center S-3 signals.

Figure 2 (top) shows the spectrum of the same sample with the field parallel to the membrane plane. The outer lines have disappeared and been replaced by the inner pair of lines; the low field member of the inner pair is heavily overlapped by the signals of Center S-3 [9,10] and a small amount of signal from Rieske's center. The spectrum of these two iron–sulfur centers is also dependent on the orientation of the multilayer in the magnetic field.

Observation of the outer pair in the lower spectrum of fig.2 allows us to immediately deduce the quinone–quinone geometry relative to the membrane plane. $|1-3\cos^2\psi| \sim 2$, so ψ must equal zero to within the disorder of the sample. Therefore the 'dipolar axis' (quinone–quinone direction) is approximately perpendicular to the membrane plane. That is, the quinones are arranged in a *trans*-membrane fashion.

It should also be possible to discern the orientation of the quinone rings with respect to the membrane plane. The anisotropic g values of a quinone are generally oriented such that the oxygen–oxygen direction within the quinone ring is the maximal g direction (2.0066 for the present case), and the smallest g value (closest to the free electron value) oriented normal to the quinone ring [11]. Computer simulations of the spectra presented in this paper and the better resolved results of Ruzicka et al. [1] show best fits with the quinone–quinone direction parallel to the largest g value direction. Thus we may conclude that the quinone rings are perpendicular to the membrane plane with the oxygen–oxygen directions probably oriented essentially perpendicular to the membrane plane.

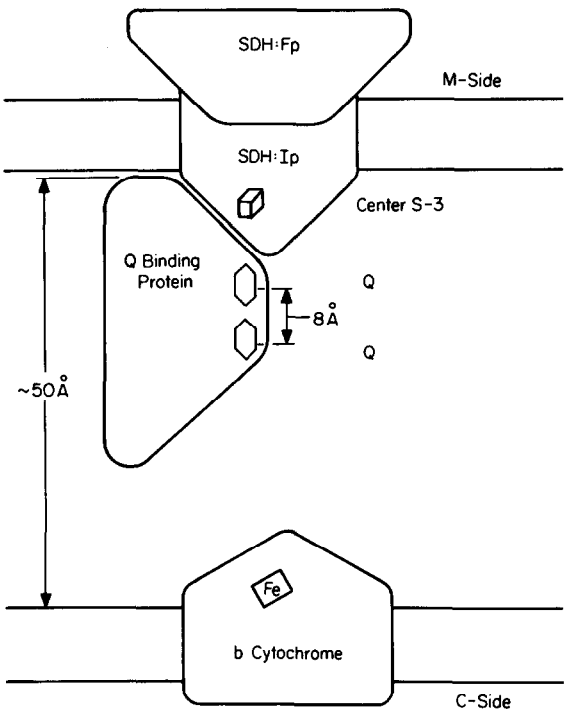

Fig.3. Schematic diagram of a portion of the mitochondrial electron-transport chain. Reducing equivalents from succinate leave succinate dehydrogenase through Center S-3, and move deep into the membrane via the fixed quinone pair. It is possible that other bound quinones complete a hydrogen transfer path to a *b* cytochrome.

The EPR spectra observed both in suspension and especially in oriented multilayers indicate a degree of order which is unexpected for free quinone. Recently Yu et al. [12] have described a hydrophobic quinone-binding protein isolated from succinate cytochrome *c* reductase. A protein of this sort capable of binding a quinone pair would provide an obvious explanation for the high degree of order necessary to produce the observed spectra.

It has previously been suggested from several lines of evidence that these quinones function as electron acceptors from succinate dehydrogenase [2,3,13,14]. Figure 3 shows a hypothetical schematic of the arrangement of electron carriers in the succinate dehydrogenase–cytochrome *b* segment of the respiratory chain. The quinone pair must be held specifically apart in a fixed orientation, presumably by incorporation into a 'quinoprotein'. In this orientation the two quinones may form part of an electron and proton transferring chain. If more than one such quinoprotein exists in this region, protons may be translocated across the membrane solely by non-diffusible quinones. The stability of the free radical form of the quinones observed in this work must be a result of changes in the E_m values of the two one-electron reduction steps due to binding to a protein; a free quinone would be expected to have an unstable free radical at any reasonable pH [15]. Other quinoproteins, if they exist, might have quite different effects on the half reduction potentials and hence on radical stability; pK values might also be affected.

The experimental data does not, of course, rule out of the participation of diffusible quinones in proton translocation. However, the localization of at least one rigidly bound quinone pair in a conformation suggestive of a hydrogen transfer chain is strong evidence that proton transfer takes place at least to some extent by strings of bound hydrogen carriers.

The data are also consistent with loop schemes [15,16] if minor modifications are introduced. Loops can be constructed using non-diffusible quinones arranged as 'bucket brigades', and binding of quinones at various sites in the membrane could explain the differences in thermodynamic properties necessary for the function of some loop schemes.

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