SEMIQUINONE Q IN THE RESPIRATORY CHAIN OF ELECTRON TRANSPORT PARTICLES

Electron spin resonance studies

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1. Introduction

The specific role of coenzyme Q semiquinone ($QH^{\cdot}$) in the respiratory chain has been discussed extensively [1–7], but few experimental data are available on this point. Though evidence for the $QH^{\cdot}$ contribution to the ESR signal at $g \sim 2.00$ in mitochondria [8], ETP [9] and bacteria [10] had been previously obtained, (for references, see [8–12]) significant interference from flavosemiquinones obscured the results of those early observations.

We have recently chosen ETP poised with the succinate/fumarate redox couple as a convenient experimental system for the $QH^{\cdot}$ ESR studies [11,12]. The rationale for this choice was that at low succinate/fumarate ratios ($E_h \sim +50$ mV) reduction of the SDH flavin ($E_m = -40$ mV [13] or -90 mV [14]) and, the more so, of the NADH-dehydrogenase flavin should be thermodynamically unfavourable while the yield of $QH^{\cdot}$ should be about maximal (E_m $QH_2/Q = +40$ mV at pH 7.4 [15]). Some data relevant to the 'protonmotive Q cycle' [4,5] are now reported.

Abbreviations: QH_2, ubiquinol; $QH^{\cdot}$, ubisemiquinone; Q, ubiquinone; ESR, electron spin resonance; ETP, submitochondrial particles; SDH, succinate dehydrogenase; TTFA, α-thenoyltrifluoroacetone; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; DMSO, dimethylsulfoxide

2. Methods

Beef-heart Mg–ATP ETP [16] were washed and resuspended in the 'basic' medium containing 100 mM sodium fumarate, 50 mM Hepes, pH 7.4 and 2 mM NAD^+. Antimycin and TTFA were used as solutions in DMSO. After appropriate additions ETP were incubated at room temperature and frozen in liquid nitrogen. ESR spectra of the frozen samples were measured at -30°C with Varian E-4 spectrometer (microwave frequency, 9.13 mHz; modulation frequency, 100 kHz; modulation amplitude, 4 G). Cytochromes *b* oxidoreduction was measured with a Hitachi-356 spectrophotometer. For more details see [12].

3. Results and discussion

3.1. *The $QH^{\cdot}$ signal in ETP poised with the succinate/fumarate couple*

The free-radical ESR signal observed in ETP poised with the succinate/fumarate couple in the anaerobic or KCN-inhibited aerobic state is shown in

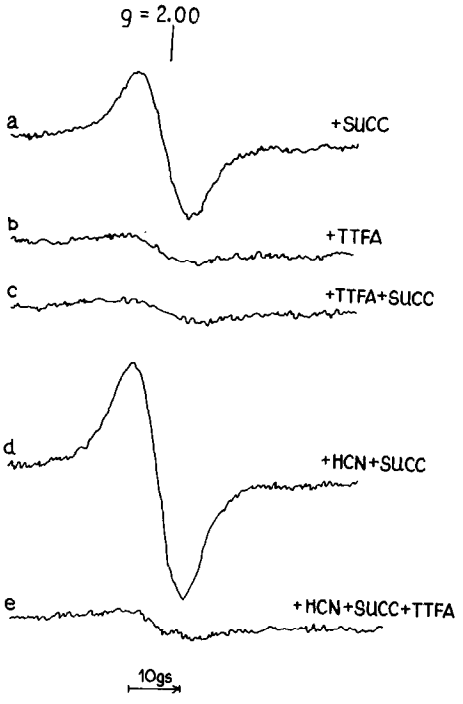


Fig.1. The free-radical ESR signal in ETP poised with the succinate/fumarate redox couple. ETP (52 mg protein/ml in the basic incubation medium) were incubated at room temperature as follows: (a) 5 min with 5 mM succinate; (b) 5 min with 5 mM TTFA; (c) 5 min with 5 mM TTFA and 5 mM succinate; (d) 10 min with 5 mM cyanide and 5 mM succinate; (e) as in (d) but TTFA (5 mM) was added 30 s before freezing the sample. Microwave power, 10 mW.

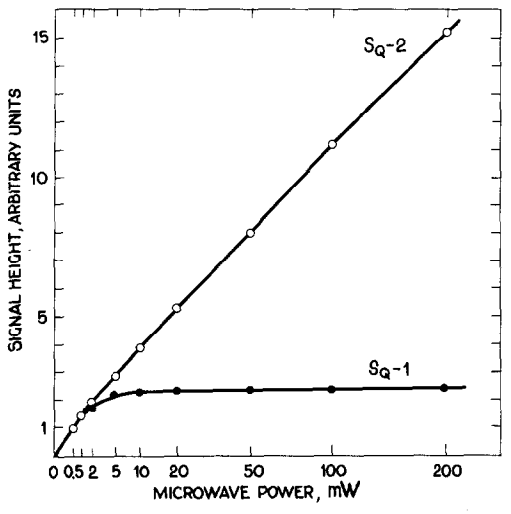


Fig.2. Saturation of the free-radical ESR signals S_Q-1 and S_Q-2 with microwave power. Saturation curves (amplitude of the signal versus $\sqrt{\text{microwave power}}$) are given for the samples shown in fig.1a (S_Q-1) and fig.4a (S_Q-2). The amplitudes of both signals at 0.5 mW were arbitrarily assumed to be 1.

fig.1 (a,d). This signal termed previously S_Q-1 [11,12] has a peak-to-trough width (ΔH_m) of 9 G and is both prevented and abolished by TTFA (fig.1, b,c,e) and ethanol [12]. A relatively high redox potential, a small width, and sensitivity of the signal to TTFA all indicate that S_Q-1 belongs to ubisemiquinone (cf. ref. [8–10]) rather than to any flavosemiquinone [17], as had been discussed in detail elsewhere [12]. In particular, S_Q-1 differs from the SDH flavosemiquinone signal which has a ΔH_m of 12 G [17], and is not suppressed by TTFA [12,18] and ethanol [12]. Below 5 mW S_Q-1 could be monitored under non-saturating conditions (fig.2). Under the close-to-equilibrium conditions of our experiments, S_Q-1 magnitude was fairly reproducible corresponding to ca. 1 pmol QH·/mg ETP protein; this value is accounted for by the QH· dismutation equilibrium constant of $\sim 5 \times 10^{-8}$. Importantly, a similar concentration of QH· was determined in ETP at room temperature [12].

3.2. *Effect of antimycin + oxygen on the QH· ESR signal*

Antimycin has been long known to increase the apparent midpoint potentials of cytochromes *b* when added in the presence of oxygen or another oxidant (see [19] for review). It was proposed by Wikström and Berden [20] that, in the antimycin-inhibited respiratory chain, the redox potential of cytochrome(s) *b* is governed by that of the $QH_2/QH\cdot$ couple. Under these conditions, antimycin-insensitive oxidation of QH· to Q upon addition of oxidant could increase the $[QH_2]/[QH\cdot]$ concentrations ratio and, consequently, will bring about extra-reduction of cytochromes *b*. It was of interest to test this hypothesis directly, measuring the effect of antimycin + oxygen on the free-radical signal of QH·.

Figure 3A shows a typical pattern of the antimycin + oxygen-induced extra-reduction of cytochromes *b* in ETP poised with the succinate/fumarate

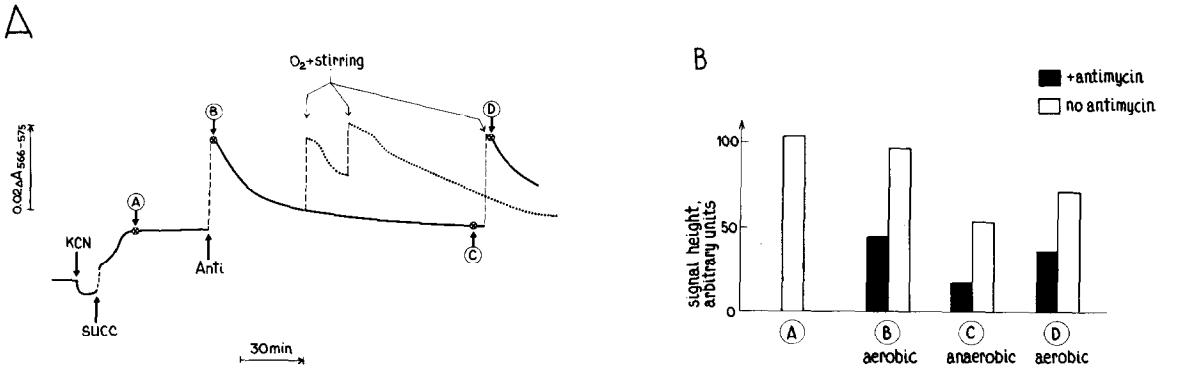
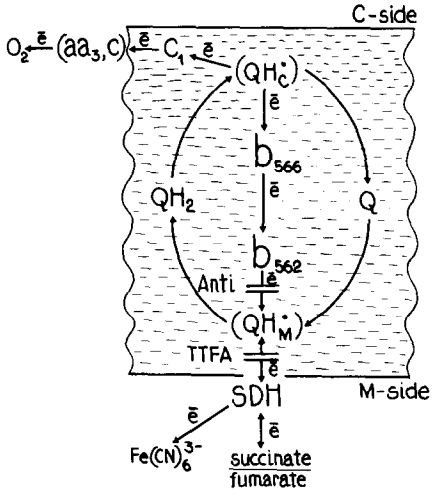


Fig.3. Effect of antimycin + oxygen on the redox state of cytochromes *b* (A) and the ubisemiquinone ESR signal (B) in ETP. ETP, 52 mg protein/ml in the basic incubation medium. Additions: KCN, 5 mM; succinate, 5 mM; antimycin, 1.8 γ/mg protein; DMSO, 1% v/v. (A) Oxidoreduction of *b*-cytochromes in the undiluted suspension of ETP was monitored in the 2 mm optical pathlength cuvette. The response of cytochromes *b* to a repetitive aeration of the sample in the presence of antimycin (measured in the separate experiment) is shown on same trace by a dotted line. The encircled letters indicate the moments at which the samples were frozen for ESR assay in the parallel experiment. (B) The heights of the columns show the amplitude of the QH· ESR signal S_Q-1 (at microwave power 10 mW) in ETP at the moments indicated at fig.3A by the corresponding encircled letters. In Ⓑ, Ⓒ, and Ⓓ, open columns refer to the control samples to which DMSO was added instead of antimycin.

redox couple. This experiment was repeated under identical conditions except that incubation was carried out in the ESR sample tubes. At the moments indicated in fig.3A by encircled letters the samples were frozen and assayed for the QH· ESR signals. The results are given in fig.3B. It may be seen that addition of antimycin to ETP diminishes S_Q-1 approx. two-fold. Although the sign of the effect is that predicted by the Wikström-Berden's scheme [20], the magnitude of the effect (ca. 18 mV decrease of the $QH_2/QH\cdot$ E_h) cannot account for the ~100 mV shift in the cytochromes *b* redox state observed under those conditions [20–22]. More importantly, this two-fold decrease of S_Q-1 is observed both in the absence and in the presence of O_2 and therefore is probably not related to the oxygen-dependent extra-reduction of cytochromes *b*. As for the effect of oxygen on S_Q-1, it is just opposite to that predicted by the discussed hypothesis. Thus it seems that the Wikström-Berden's model in its original form [20] is not confirmed by our observations.

It is however compatible with our results as a part of Mitchell's 'protonmotive Q cycle' [4,5] which postulates QH· compartmentalization between the M- and C-sides of the coupling membrane (Scheme 1). Only $QH\cdot_C$ should be involved in the effect of anti-



Scheme 1. The protonmotive Q cycle (after Mitchell [5]) Proton transfer is not considered. Abbreviation: Anti, antimycin; others as in the footnote on the first page of the paper. Only $QH\cdot_C$ should be involved in extra-reduction of cytochromes *b* in the presence of antimycin and oxygen (fig.3). The non-saturating free radical signal S_Q-2 (fig.4) is proposed to arise from the $QH\cdot_M$ bound to the high-potential iron–protein of SDH, the complex being sensitive to TTFA.

mycin + oxidant on the redox state of cytochromes *b*.

If $QH^{\cdot}_C$ does contribute to S_Q-1, decrease of this signal upon anaerobiosis and increase upon aeration (fig.3B) paralleled by the cytochromes *b* respective oxidation and reduction (fig.3A) could fit the Wikström-Berden-Mitchell's model provided that the coenzyme Q redox couple equilibrating with cytochromes *b* at the C-side of the coupling membrane were $QH^{\cdot}/Q$ rather than $QH_2/QH^{\cdot}$.

3.3. *Free-radical ESR signal in 'oxidized' ETP*

We were somewhat surprised to observed routinely an intensive resonance at $g \sim 2.00$ in the ESR spectrum of ETP incubated aerobically in the absence of added succinate (fig.4a). This free-radical signal, termed previously SII [11] or S_Q-2 [12], was slightly broader than S_Q-1 (ΔH_m 10–11 G) and could not be saturated with microwave power as high as 200 mW (fig.4a; fig.2). S_Q-2 was completely abolished by TTFA (fig.4b), while malonate, cyanide, and several iron chelators including bathophenanthroline–sulfonate and EDTA exerted only small effects on the signal. Like S_Q-1, S_Q-2 was highly sensitive to ethanol [12].

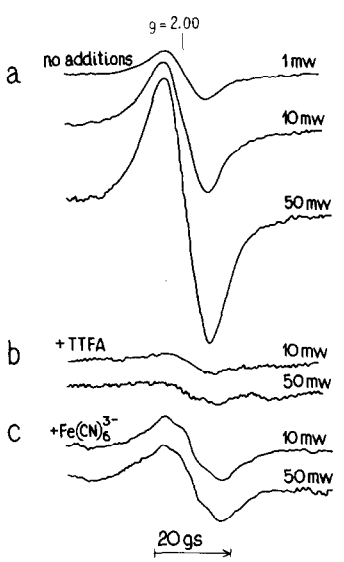


Fig.4. The free-radical ESR signal in the 'oxidized' ETP. ETP (52 mg protein/ml in the basic incubation medium) was incubated at room temperature as follows: (a) 14 min without additions; (b) 9 min without additions and 6 min with 5 mM TTFA; (c) 9 min without additions and 6 min with 1 mM ferricyanide.

When ferricyanide was added to 'oxidized' ETP (fig.4c), the signal observed was greatly reduced in size and differed from S_Q-2 in shape, width, and saturation behaviour; thus S_Q-2 was largely abolished by ferricyanide.

There are two interesting points about the non-saturating free-radical signal S_Q-2 ascribed previously to $QH^{\cdot}$ [11,12]. First, why this $QH^{\cdot}$ is not oxidized by oxygen via the respiratory chain but reacts readily with ferricyanide. A simple explanation might be provided by Mitchell's 'protonmotive Q cycle' (Scheme 1). One can visualize that, upon aerobic oxidation of the 'last' molecule of QH_2, there will be one molecule of $QH^{\cdot}$ trapped in the 'oxidized' ETP at the M-side of the membrane. This $QH^{\cdot}_M$ would be inaccessible to oxidation by oxygen via cytochromes, c_1, c, aa_3; ferricyanide, on the other hand, could withdraw an electron from $QH^{\cdot}_M$ either directly, or via SDH.

Another interesting feature of S_Q-2 is its saturation curve (fig.2). This one is unusual for free semiquinone and may indicate the $QH^{\cdot}$ localization in the close proximity to some paramagnetic centre [23]. Evidence for $QH^{\cdot}$ interaction with the high-potential iron–protein of SDH (Centre S-3) has recently come from ESR studies of the mitochondrial iron–sulfur centres at liquid helium temperatures [24–28]. Our observation of the non-saturating free-radical ESR signal S_Q-2 could be another proof of this interaction; specific sensitivity of S_Q-2 to TTFA, which is known to inhibit Centre S-3 oxidation by coenzyme Q [18,29] is probably of particular interest at this point.

In conclusion, we propose that S_Q-2 arises from the ubisemiquinone trapped at the M-side of the 'oxidized' ETP and stabilized there in a complex with the high-potential iron–protein of SDH, all in agreement with the 'protonmotive Q cycle' hypothesis of Mitchell.

Addendum

A correlation has been recently found between the occurrence of the free-radical ESR signal S_Q-2 in ETP frozen at –30°C and the presence of the split signal with g=1.99 and g=2.04 in the ESR spectra of the same samples cooled to 12°K; simultaneous disappearance of both signals was observed upon

addition of TTFA, ferricyanide, ethanol and benzohydroxamate (Tichonov, A. N., Burbaev, D., Grigolava, I. V., Konstantinov, A. A., Ksenzenko, M. Yu. and Ruuge, E. K. (1977) Biophysics (USSR) 22, issue 4).

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