The Rieske iron-sulfur cluster of plant mitochondria

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The Rieske iron-sulfur cluster, which no-one as yet has been able to identify in plant mitochondria, is identified in these mitochondria through the use of 5-*N*-undecyl-6-hydroxy-4,7-dioxobenzothiazole (UHDBT). The midpoint potential of the Rieske cluster in mung bean and potato mitochondria is +300 mV. The orientation of the Rieske cluster in the potato mitochondrial membrane is identical to its orientation in animal mitochondrial and *Rhodopseudomonas sphaeroides* membranes but differs from that in chloroplast membranes.

Mitochondria *Iron-sulfur cluster* *Cytochrome* bc^1 *complex*

1. INTRODUCTION

The Rieske iron-sulfur cluster has come to be considered an ubiquitous component of all mitochondrial, photosynthetic and bacterial membrane systems, and specifically to be essential for the operation of the cytochrome bc_1 complex in electron and proton transport [2]. It came as quite a surprise then that 6 independent and detailed investigations (unpublished, and personal communications) made it abundantly clear that the use of standard techniques of redox poising and EPR spectroscopy failed to reveal the presence of the Rieske iron-sulfur cluster in a variety of plant mitochondria and submitochondrial preparations.

The discovery [3–6] that 5-*N*-undecyl-6-hydroxy-4,7-dioxobenzothiazole (UHDBT) inhibited substrate oxidation in animal mitochondria and bacterial systems, and the conclusion that these effects were due to an interaction between UHDBT and the Rieske cluster, suggested that investigations on the action of UHDBT on plant mitochondria were in order. A preliminary account of these investigations has been published [1].

2. MATERIALS AND METHODS

Mitochondria and submitochondrial particles were prepared from potato tubers (*Solanum tuberosum*) and from the hypocotyls of 5-day-old etiolated mung beans (*Vigna radiata* L.) as in [7] and [8]. Oriented membranes were prepared from submitochondrial particles by partial dehydration onto mylar (see [9]).

Oxygen consumption was monitored in a Rank Brothers' 'oxygen electrode', the reaction medium consisted of 0.3 M mannitol, 10 mM potassium phosphate buffer, 10 mM KCl and 5 mM $MgCl_2$ (pH 7.2).

Optical measurements were obtained using a Johnson Foundation dual wavelength spectrophotometer. EPR measurements were made with a Varian E-109 spectrometer equipped with a helium flow transfer line.

Redox titrations were performed with an anaerobic vessel under a constant flow of argon as in [10] using 40 μM 2,3,5,6-tetramethylphenylenediamine ($E_{m7.4}$ + 260 mV), *N,N,N',N'*-tetramethylphenylenediamine ($E_{m7.4}$ + 240 mV), *N*-methylphenazonium methosulfate ($E_{m7.4}$ + 70 mV)

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and 2-hydroxy-1,4-naphthoquinone ($E_{m7.4}$ – 180 mV) [11].

3. RESULTS

The effects of UHDBT on potato mitochondria are shown in fig.1 where it can be seen that the oxidation of succinate is strongly inhibited (apparent $K_i = 2\ \mu M$) while the oxidation of ascorbate is virtually unaffected. Similar results were obtained with mung bean mitochondria. In both potato and mung bean mitochondria the reduction of cytochrome *c* by succinate in the presence of UHDBT is completely abolished while the reduction of cytochrome *b* is enhanced. All of the above results point to a strong interaction between UHDBT and the cytochrome bc_1 complex and perhaps more specifically, with the Rieske iron-sulfur cluster and the question is then: does UHDBT help in identifying the Rieske cluster in these plant mitochondria?

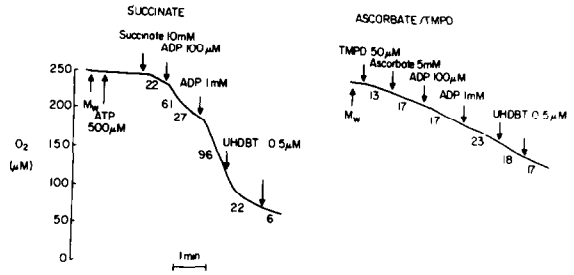

Fig.1. The effect of UHDBT on succinate and ascorbate/TMPD oxidations by potato mitochondria. The numbers by the oxygen electrode trace indicate nmol O_2 consumed · min^{-1} · mg protein^{-1}.

Fig.2 shows the effects of UHDBT on the EPR spectra of both potato and mung bean mitochondria. The mitochondria were allowed to become

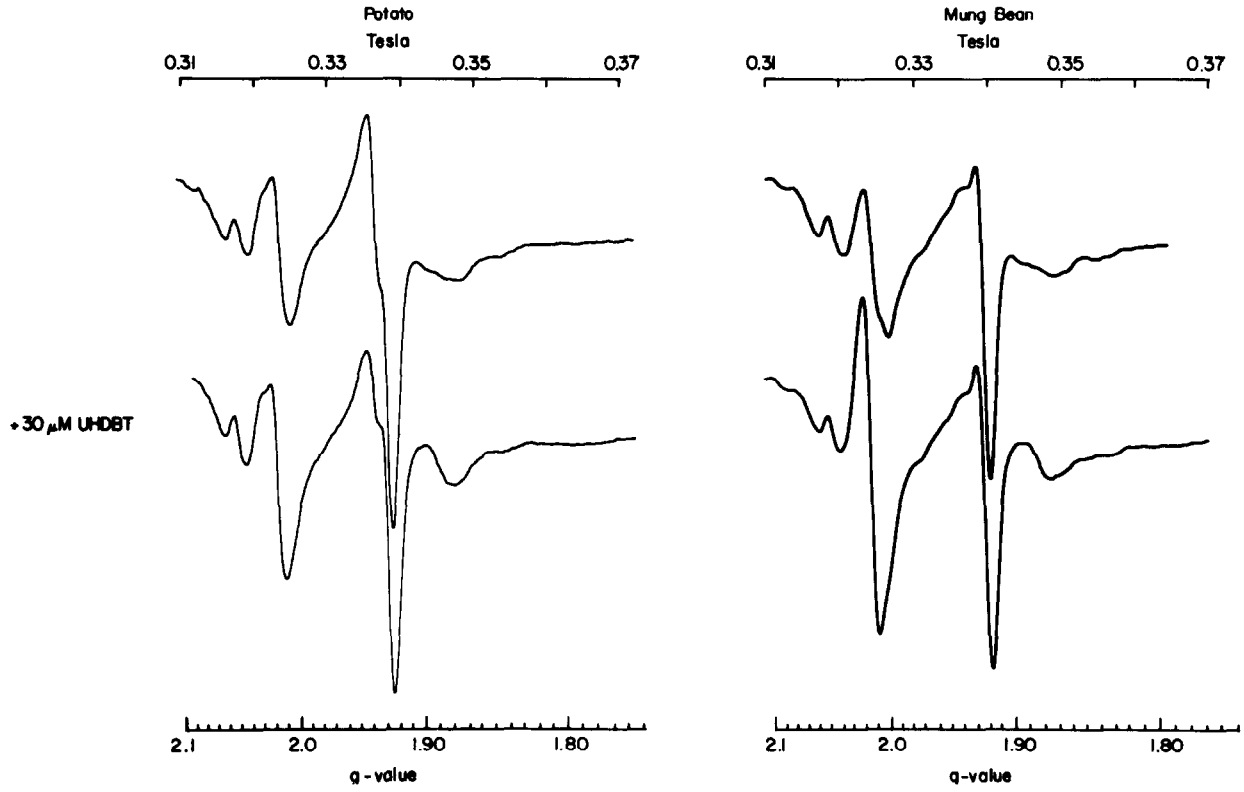

Fig.2. The effects of UHDBT on the EPR spectra of potato and mung bean mitochondria. Details in the text. EPR conditions: 21 K, 10 mW applied power, 1.25 mT modulation amplitude.

anaerobic in the presence of succinate before freezing the samples to 77 K. In the absence of the inhibitor, the EPR spectra show prominent features at g 1.94, indicative of reduced ferredoxin-like iron-sulfur clusters, but no features near g 1.90 that might be identified with the Rieske cluster. However, the addition of UHDBT reveals a significant signal centered at 1.89 which is very similar to the value of the g_y band of the Rieske cluster in UHDBT-treated photosynthetic bacteria [5]. This latter observation prompted us to measure the oxidation-reduction midpoint potential of the g 1.89 signal in order to see if it had properties consistent with an identification as a Rieske type iron-sulfur cluster. Fig.3 shows the results of these redox titrations, the $E_{m7.4}$ of the signals in both potato and mung bean mitochondria was +300 mV, a value close to the range of values measured in animal mitochondria and purple photosynthetic bacteria [5,12,13]. Fig.3 also shows a sample spectrum from one of the titrations which shows that the plant mitochondria in the presence of UHDBT have a Rieske cluster with a g_x band at g 1.79.

The identification of the Rieske cluster in plant mitochondria allows an estimate of the orientation of the iron-sulfur cluster in the membrane plane. Fig.4 shows spectra of oriented multilayers of potato submitochondrial particles; the magnetic field and membrane plane are parallel at 0°. The g 1.89 signal is maximal at 0°, the g 1.79 signal at 90°. Simple trigonometry predicts that the g_z signal

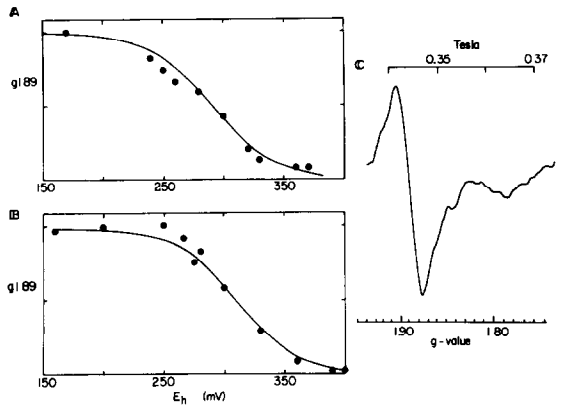

Fig.3. Redox titrations of the UHDBT induced g 1.89 EPR signal in potato (A) and mung bean (B) mitochondria. Details in the text. EPR conditions as in fig.2.

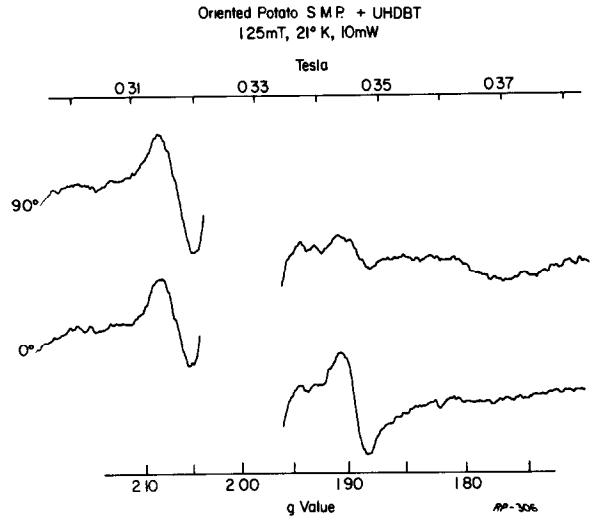

Fig.4. The EPR spectra of oriented multilayers of potato submitochondrial particles where the membrane and the magnetic field are parallel at 0°. Details in the text.

would thus be maximal at 0°. The signal of fig.4 near g 2.08 is unlikely to be part of the Rieske cluster, for both the orientation and g values are inappropriate. The Rieske clusters of animal, bacterial and chloroplast origin have g_z near g 2.03, a region that could not be examined in the samples of fig.4 due to the large free radical signal near g 2.

The Rieske cluster of animal mitochondria is a two iron-two sulfur cluster (see [16]). If the signal seen here has a similar origin, the model in [15] predicts that the g_z axis is the iron-iron axis, and fig.4 indicates that this lies in the plane of the membrane. This is identical to the orientation of the Rieske cluster in animal mitochondria [16] and *Rhodopseudomonas sphaeroides* [9]. In the latter systems it was shown that while UHDBT and 2-hydroxy-3-undecyl-1,4-naphthoquinone shifted the g values of the Rieske cluster slightly, they did not alter the orientation of the axes. The orientation is similar to that of the chloroplast Rieske cluster [17] although in this case the g 1.90 signal was orthogonal to the membrane plane instead of parallel with it as here. The significance of this finding is unclear, but it indicates that the Rieske clusters of plant mitochondria and chloroplasts are different.

Thus, in summary, the experiments reported here show that plant mitochondria contain an iron-

sulfur cluster which may be identified as a Rieske type. Its properties are very similar to those of animal mitochondria and photosynthetic bacteria, and close to those of the chloroplasts cluster.

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