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Effect of ATP on the EPR spectrum at 20°K of phosphorylating sub-mitochondrial particles

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SUMMARY

By measurements of the EPR spectrum of substrate-reduced anaerobic phosphorylating sub-mitochondrial particles at 20°K, ATP was found to bring about the oxidation of four components (two iron–sulphur proteins associated with NADH dehydrogenase, and two unidentified iron proteins) and the reduction of one component. Thus energization of the particles lowers the effective redox potential of four components and raises that of a fifth.

We have recently shown that ATP causes the oxidation of iron–sulphur proteins seen in substrate-reduced anaerobic phosphorylating sub-mitochondrial particles by EPR spectrometry at 83°K[1]. These experiments have now been extended to 20°K, where additional iron–sulphur and other iron components are visible[2–4].

Fig. 1 shows that ATP causes a decline in the intensity of the line at $g = 1.943$ (top), as was also found at 83°K[1]. Since Fe–S proteins associated with succinate dehydrogenase make only a small contribution to this line in the EPR spectrum at 20°K of NADH-reduced particles[4], under the conditions of our measurements, it is probable that the effect of ATP seen at $g = 1.943$ in Fig. 1 is mainly on components associated with NADH dehydrogenase. No effect of ATP was seen on the $g = 2.052$ line, while the trough at $g = 1.922$, associated with this line[3,4], decreased slightly in intensity, probably due to interference from the line at $g = 1.943$. ATP causes a 40% increase of the top at $g = 2.025$, due to oxidation of the component with a line at $g = 2.014$[4].

Fig. 2 shows that ATP has a greater effect on the line at $g = 2.014$ (and on the accompanying trough at $g = 2.002$) when succinate is substrate. The trough at $g = 1.990$, belonging to another component in oxidized preparations[4], is also clearly seen. Its intensity is 30% of that seen in oxidized particles. ATP also brings about a large decline in the intensity of the line at $g = 1.922$.

Abbreviation: TMPD, tetramethyl-*p*-phenylenediamine.

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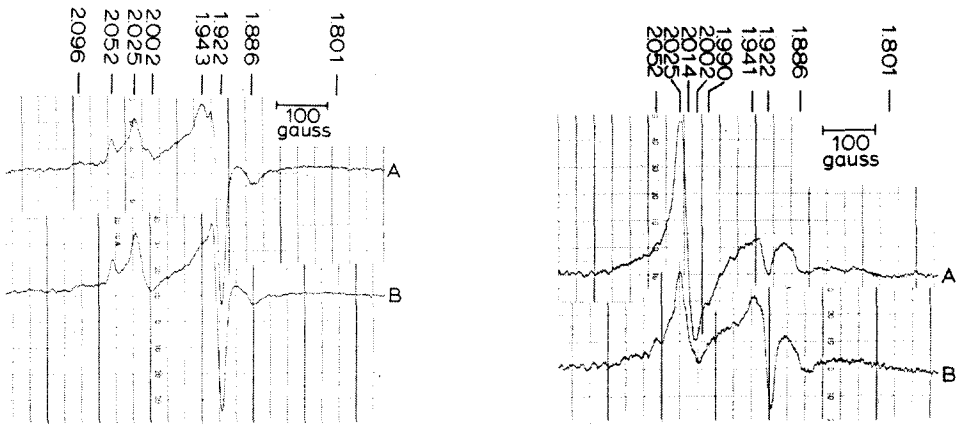

Fig. 1. Effect of ATP on the EPR spectrum at 20°K of Mg-ATP particles[5] reduced with NADH. Mg-ATP particles (40 mg/ml) in 0.25 M sucrose and 10 mM $MgCl_2$ were incubated for 2 min at 22°C with 5 mM NADH and then frozen in liquid nitrogen. 5 mM ATP was, where indicated, added after 2-min incubation with NADH and the suspension frozen in liquid nitrogen 1 min later. A, NADH alone; B, NADH + ATP. The EPR spectra were measured as in ref. 4, with modulation amplitude 5 gauss and microwave power 16 mW.

Fig. 2. Effect of ATP on the EPR spectrum at 20°K of Mg-ATP particles reduced with succinate. Experimental conditions as in Fig. 1. A, 25 mM succinate + 5 mM ATP; B, 25 mM succinate alone. The gain for trace B was 1.26 times higher than for trace A. The EPR spectra were measured at 20°K as in Fig. 1.

Fig. 3 shows that ATP also increases the intensity of the top at $g = 2.025$ and the troughs at $g = 2.002$ and 1.990 with ascorbate and tetramethyl-*p*-phenylenediamine (TMPD) as substrate. The $g = 1.990$ trough in the presence of ATP is now 60% of that seen in oxidized particles. No effect of energization was observed on the $g = 1.990$ trough at 83°K (Fig. 4), giving further support to the conclusion[4] that the trough seen at 20°K is, for a large part at least, not due to Cu(II), which is responsible for this trough at 83°K.

It may be concluded from the experiments shown in Figs. 1–3 that energy effectively lowers the redox potential of the two species responsible for the signals at $g = 2.014$ (trough at $g = 2.002$) and 1.990, respectively so that, whereas in the absence of ATP they are reducible by ascorbate (Fig. 3, Trace B), in the presence of ATP the former becomes oxidizable by NAD^+ (Fig. 1, Trace A) and the latter by fumarate (Fig. 2, Trace A).

The EPR spectrum at 83°K, with ascorbate–TMPD (Fig. 4), shows that ATP causes an increased intensity of lines at $g = 2.026$ (top), 2.002 (middle), 1.896 (middle) and 1.801 (trough). (The line at $g = 1.896$ has a trough at $g = 1.886$). The line at $g = 2.002$ is due to free radical. The other three lines are also seen with succinate as substrate, in the presence of ATP, but not with NADH. With the latter, but not with the other substrates, a shoulder at $g = 1.895$ (trough) is seen. Rotenone prevents the appearance of this signal. The signal with g values of 2.026, 1.896 and 1.801 is also seen in Complex III reduced with ascorbate–TMPD and measured at 83°K under the same conditions.

A possible explanation of these findings is that the protein responsible for the trough at $g = 1.895$ with NADH is the same species, but in another conformation, as that

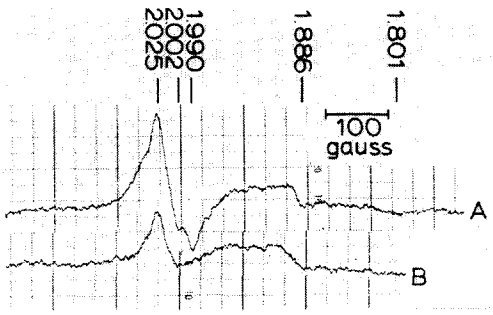

Fig. 3. Effect of ATP on the EPR spectrum at 20°K of Mg-ATP particles reduced with ascorbate plus TMPD. Mg-ATP particles (40 mg/ml) in 0.25 M sucrose and 10 mM $MgCl_2$ were incubated with 18 mM ascorbate (pH 7.0) and 0.1 mM TMPD for 2 min at 22°C. Additions were made as indicated below and after a further minute at 22°C the mixture was frozen in liquid nitrogen, A, 5 mM ATP; B, 5 mM ATP + 25 μM carbonylcyanide *p*-trifluoromethoxyphenylhydrazone (FCCP). The EPR spectra were measured at 20°K as in Fig. 1.

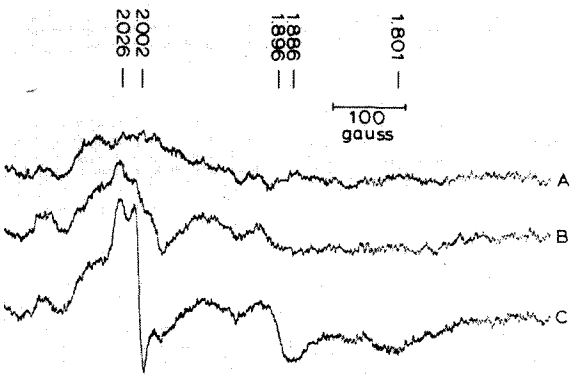

Fig. 4. Effect of ATP on the EPR spectrum at 83°K of Mg-ATP particles reduced with ascorbate plus TMPD. The samples used for Fig. 2 were measured at 83°K. A, base line recorded with water; B, ATP + carbonylcyanide *p*-trifluoromethoxyphenylhydrazone (FCCP); C, ATP alone. The EPR spectra were measured as in ref. 1 with modulation amplitude 12.5 gauss and microwave power 80 mW.

giving the signal at $g = 2.026$, 1.896 and 1.801. According to this interpretation, the former signal is derived from a low-energy, low-potential protein reducible only by NADH in non-energized particles and not energized by ATP in the reduced state. ATP raises its effective potential so that it is now reducible by succinate or ascorbate–TMPD, yielding a reduced protein in another conformation. The iron–sulphur protein involved in these reactions may well be the same as that identified by Rieske *et al.*[6].

In contrast to the findings at 83°K ATP had no effect on the trough at $g = 1.886$ at 20°K, with either succinate or ascorbate–TMPD as substrate (Figs. 2 and 3). ATP did, however, reveal a distinct trough at $g = 1.801$, the line that was associated with that at $g = 1.886$ at 83°K. Since, in the absence of ATP no trough is visible at $g = 1.801$ at 20°K, the trough at $g = 1.886$ under these conditions must be derived from a different species from

that in the presence of ATP. Indeed, it is quite clear in Fig. 3 that the line at this g value is much narrower in the presence of ATP. In Fig. 2, this would be masked by the large effect of ATP on the line at $g = 1.922$ (trough). Since the trough at $g = 2.002$ is lacking in Fig. 3, Trace B, the peak at $g = 2.025$ is not due to the ferric iron component which also has a line at this g value. It probably belongs to the same species as is responsible for the trough at $g = 1.886$ in this trace (*cf.* ref. 4).

In summary, ATP has been shown to have the following effects: (1) it causes oxidation by NAD^+ of Fe–S centre 1 (ref. 3) of NADH dehydrogenase that gives in the reduced form an EPR signal with a line of $g = 1.943$ (top); (2) it causes oxidation by fumarate of Fe–S centre 2 (ref. 3) of NADH dehydrogenase that gives in the reduced form an EPR signal with a line at $g = 1.922$ (trough); (3) it causes oxidation by NAD^+, fumarate or dehydroascorbate of a component that gives in the oxidized form an EPR signal with g values of 2.014 (middle) and 2.002 (trough); (4) it causes oxidation by fumarate or dehydroascorbate of a component that gives in the oxidized form an EPR signal with a g value of 1.990 (trough); (5) it causes reduction by succinate or ascorbate of a component that gives in the reduced form an EPR signal with g values of 2.026 (top), 1.896 (middle) and 1.801 (trough). Thus energization causes the effective redox potential of two iron–sulphur proteins associated with NADH dehydrogenase and of two unknown iron species to be lowered, and that of another species, probably an iron–sulphur protein, to be raised. There are two ways in which it might do this. First, energization might cause a structural change in the iron protein, leading to a change in redox potential. Secondly, the change in effective redox potential might be indirect, due to an energy-induced change of redox potential of another component of the respiratory chain, with which the iron species is in equilibrium.

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