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The effect of ATP on the apparent mid-point potentials of cytochrome *b* and cytochrome *c* in beef-heart mitochondria

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SUMMARY

1. In the presence of uncoupler, beef-heart mitochondria contain two potentiometrically distinguishable species of cytochrome *b* with apparent mid-point potentials of 154 mV and 28 mV, contributing about 20% and 80%, respectively, to the absorbance at 562 nm. Spectroscopic analysis further resolves the low-potential species into two components.

2. Energization by addition of ATP lowers the apparent mid-point potential of the high-potential component to 118 mV and raises that of half of the low-potential components to 270 mV (the other half is slightly increased to 48 mV).

3. Spectral analysis at different wavelengths shows that ATP increases the potential of one-half of both b_{562} and b_{565}.

4. ATP also lowers the apparent mid-point potential of much of the cytochrome *c* to below 170 mV.

Wilson and Dutton[1] have reported that rat-liver mitochondria contain two cytochrome *b* species, differing in mid-point potential by 90 mV at pH 7.3, and that addition of ATP raises the potential of the lower-potential species from −55 mV to 245 mV. Simila results have been reported in pigeon-heart mitochondria by Chance *et al.*[2] and in beef-heart mitochondria by Dutton *et al.*[3]. Since the addition of ATP to succinate-reduced mitochondria results in an increased reduction of a long-wavelength *b* species (b_{565})[4,2], Chance *et al.*[2] assume that it is this species and not b_{562} whose potential is increased by

Abbreviation: FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone.

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ATP. Since, however, b_{562} with a mid-point redox potential of 77 mV at pH 7 (ref. 5) is in equilibrium with the succinate–fumarate system in the absence of ATP, and is almost completely reduced under these conditions, an increase in the mid-point redox potential of this species by addition of ATP would not be detected with succinate as substrate. In order to determine whether the mid-point redox potential of b_{562} is also increased by ATP, it is necessary to determine the effect of ATP on the spectrum at high potentials.

Potentiometric titrations carried out by the method of Wilson and Dutton[1] have shown that beef-heart mitochondria, in the presence of uncoupler, contain a *b* species with potential about 150 mV (*cf.* ref. 3), that is sensitive to antimycin, as well as a low-potential component whose potential is not affected by antimycin[6] (see also Table I). The low-potential components may be separated spectrally, b_{562} having a higher potential than b_{565} (with shoulder at 558 nm).

Fig. 1 shows a potentiometric redox titration of beef-heart mitochondria at the wavelength pair 562–575 nm in the presence of ATP. The logarithmic plot shown in Fig. 2A reveals three components that have been resolved in Fig. 2B by the procedure of Wilson and Dutton[1]. Three species are present with mid-point redox potentials at 48, 118 and 270 mV, contributing about 40, 20 and 40%, respectively, to the $\Delta A_{562-575\ nm}$ (Table I). The position of its absorption maximum and the amount present makes it likely that the 118-mV species is derived from the 154-mV species seen in the absence of energy. Thus, it appears that both antimycin[6] and ATP lower the potential of this species.

TABLE I

POTENTIALS OF CYTOCHROME *b* SPECIES IN BEEF-HEART MITOCHONDRIA

*In the presence of uncoupler**			*In the presence of ATP***		
E'_o *at pH 7.2 (mV)*	λ_{max} *(nm)*	*Relative concentration(%)*	E'_o *at pH 7.2 (mV)*	λ_{max} *(nm)*	*Relative concentration(%)*
154	562	21	118	562	20
28	562,565	79	48	563	38
			270	563	42

*From ref. 6.
**From Fig. 1.

As summarized in Table I, about one-half of the lower-potential species is raised in potential by adding ATP. The spectra of the cytochrome *b* reduced at different potential increments in the presence of uncoupler and in the presence of ATP are shown in Fig. 3A. In Fig. 3B the difference spectra ATP *minus* uncoupler are plotted. On lowering the potential from 310 to 200 mV a rather broad band with peak at 563 nm is seen in Fig. 3B, indicating that both b_{562} and b_{565} are reduced. On lowering the potential from 100 mV to 0 mV, both b_{565} and b_{562} are reduced, while the shoulder at 558 nm is also clearly visible (Fig. 3A). In the presence of ATP the amount of both b_{562} and b_{565} reduced in this potential range is halved, the other half already having been reduced at the

higher potential. It is concluded that about a half of both b_{562} and b_{565} (including the 558-nm component) is changed in apparent mid-point potential on adding ATP, suggesting that both b_K and b_T (energy-transducing)[2], contain b_{562} and b_{565}.

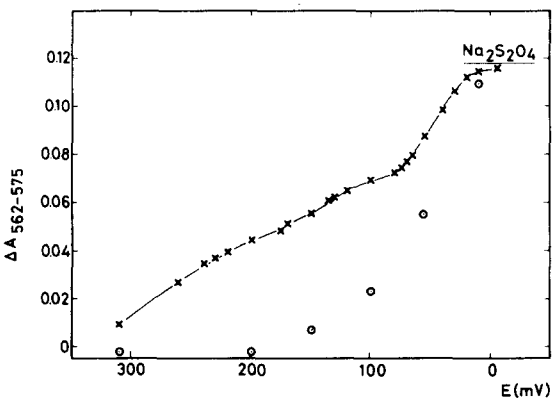

Fig. 1. Potentiometric redox titration of the *b* cytochromes in freshly prepared beef-heart mitochondria. Beef-heart mitochondria (2.0 mg/ml) were suspended in a medium containing 0.25 M sucrose, 0.05 M Tris–HCl buffer (pH 7.2), 1 mM EDTA, 30 μM diaminodurene, 20 μM phenazine ethosulphate, 50 μM duroquinone, 4 μM pyocyanine, 25 μM 2-hydroxy-1, 4-naphthoquinone, 25 μM anthraquinone-1, 5-disulphonate and 5 μM rotenone. Ascorbate was added to bring about anaerobiosis and the potential of the system brought to +310 mV by addition of ferricyanide. After adding 4.5 mM ATP the potential was lowered by the action of endogenous substrate and by adding small amounts of 200 mM NADH. When the titration was completed FCCP was added and the potentials were made more positive with ferricyanide. ×——×, in the absence of FCCP; ⊙ , in the presence of FCCP. The apparatus used is described in ref. 6.

Figs. 3A and 3B show that between 310 and 150 mV less cytochrome $c + c_1$ is reduced in the presence of ATP than in the presence of uncoupler. The cytochrome $c + c_1$ not reduced in this potential range becomes reduced between 150 and 100 mV. The spectrum in Fig. 3B of the $c + c_1$ reduced between 150 and 100 mV shows no interference of the $c + c_1$ spectrum due to changes in the redox state of cytochrome *b*. In freshly prepared beef-heart mitochondria, the amount of cytochrome *c* reducible only at lower potentials is much larger. Fig. 4 shows that, in these freshly prepared mitochondria, about one half of the cytochrome $c + c_1$ is not reduced at 170 mV when ATP is added. The spectrum of the component reduced in the presence of ATP (Fig. 4, Curve 2), with peak at 554 nm, and that oxidized by addition of ATP (Fig. 4, Curve 3) with peak at 551 nm, suggest that it is mainly the cytochrome *c* whose apparent mid-point potential is affected by ATP. The effect of ATP on the apparent mid-point potential of cytochrome *c* is much more sensitive to mitochondrial damage than that on that of cytochrome *b*, since storage of the mitochondria at −20°C leads to almost complete loss of the effect on cytochrome *c* whereas the effect on cytochrome *b* remains almost unchanged.

The ATP-induced oxidation of cytochrome *c*, in the presence of redox mediators

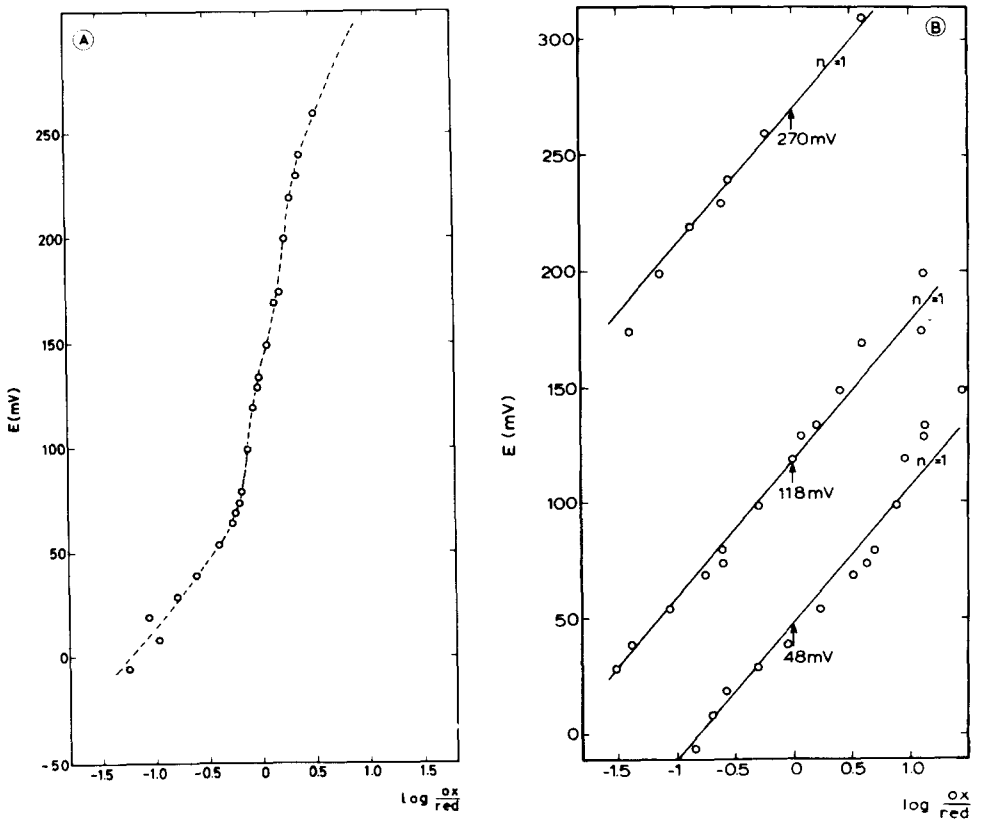

Fig. 2. Redox potentials of cytochrome *b* in beef-heart mitochondria. (A) Absorbance changes between 310 and −10 mV, measured in the experiment of Fig. 1, in the absence of FCCP, are presented as the logarithm of the ratio oxidized/reduced cytochrome *b*. The absorbance change reached with dithionite was assumed to correspond to 100% reduction. (B) The curve given in (A) is resolved into its component parts, assuming that the different components contribute 38, 20 and 42%, respectively, to the total $\Delta A_{562-575\ nm}$.

may be seen as a 'cross-over' between cytochrome *c* and the mediator. Grimmelikhuijzen and Slater[7] have reported a cross-over between tetramethyl-*p*-phenylenediamine and cytochrome *c* in rat-liver mitochondria in the presence of antimycin and high concentrations of azide.

The effect of ATP on the apparent mid-point potential of cytochrome *c* in freshly prepared mitochondria shown in these experiments is at variance with the conclusions of Dutton *et al.*[8], who reported no effect. It is possible that the cytochrome *c* in intact mitochondria is more readily accessible to the mediators *via* an ATP-sensitive component of the chain and that, in aged mitochondria, when the ATP sensitivity is lost, the mediators act directly with the cytochrome *c*. It also remains to be proved that the real E'_o of b_{562} and b_{565} is raised by ATP. That is why the term 'apparent mid-point potential' is used in this paper.

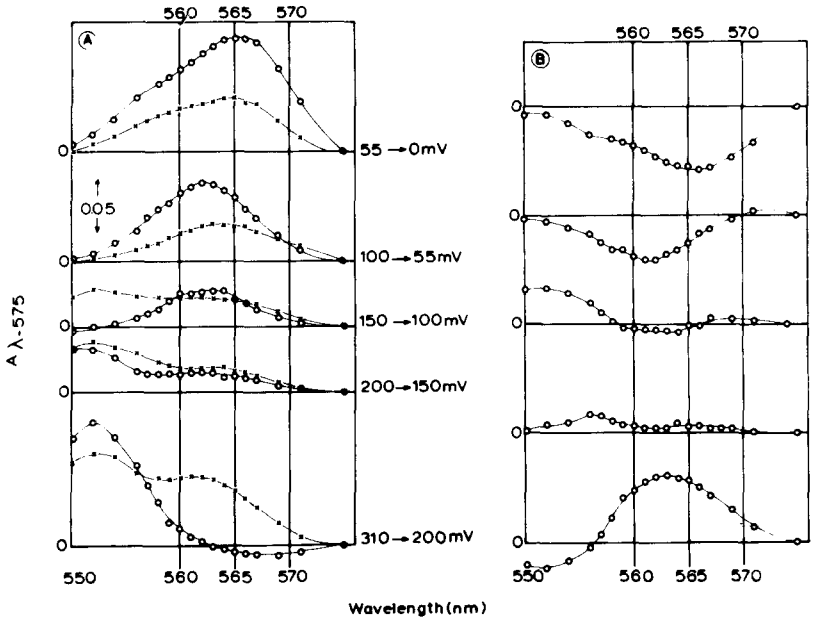

Fig. 3. Difference spectra of cytochrome *b* in beef-heart mitochondria. (A) From the *absolute* spectra ($A_{575\ nm}$ was set at zero), measured during the titration given in Fig. 1, difference spectra (spectrum at one potential *minus* spectrum at another potential) were calculated, both in the presence of ATP (×) and of FCCP (0). (B) From the spectra given in A the difference spectra ATP *minus* FCCP were calculated.

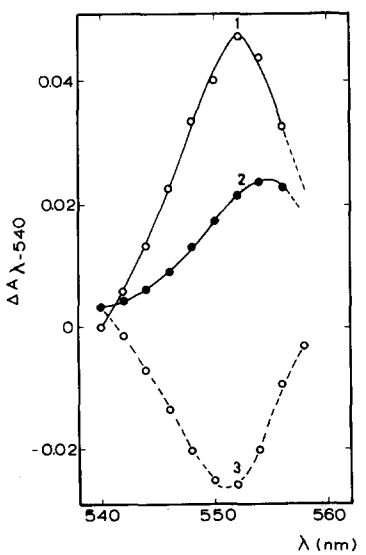

Fig. 4. Effect of ATP on cytochromes *c* and c_1 in freshly prepared beef-heart mitochondria. Freshly prepared beef-heart mitochondria (2.3 mg/ml) were suspended in the medium described in the legend to Fig. 1, with the exception that 50 μM $FeSO_4$ was present instead of anthraquinone-1,5-disulphonate. At 320 and 170 mV spectra were measured in the presence of ATP or FCCP. Curve 1, difference spectrum between 320 and 170 mV in the presence of FCCP; Curve 2, difference spectrum between 320 and 170 mV in the presence of ATP; Curve 3, the effect of ATP at 170 mV.

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