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EFFECT OF ANTIMYCIN ON THE POTATO MITOCHONDRIAL CYTOCHROME *b* SYSTEM

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SUMMARY

1. The difference spectrum anaerobic + succinate *minus* aerobic of potato mitochondria reveals bands at 552, 558 and 566 nm, probably belonging to three species of cytochrome *b*.

2. The longer-wavelength cytochrome *b* is distinguished in three respects: (1) It is much more highly reduced in State 4; (2) its degree of reduction increases on energization of the mitochondrion; (3) either the degree of reduction or the position of the α-band is affected by antimycin in the same way as cytochrome *b* in fragmented heart-muscle mitochondria. It is probably analogous to the cytochrome *b* of mamalian mitochondria.

3. The curve describing the effect of antimycin added to anaerobic mitochondria in the presence of succinate and ATP is hyperbolic. In the presence of uncoupler, the curve is strongly sigmoidal. The amount of antimycin required for a maximum effect is unaffected by the presence of uncoupler, but the maximum effect is greater.

4. It is concluded that antimycin combines preferentially with a site present in higher concentrations in energized mitochondria.

INTRODUCTION

Three absorption peaks at 552, 557 and 561 nm have been identified at 77° K in plant mitochondria treated with succinate and antimycin in the presence of air[1]. Evidence has been presented supporting the view that three separate cytochromes are responsible for the three peaks. On the basis of the inhibition of their oxidation by antimycin, it is probable that all are of the *b* type, although isolation and measurement of the position of the absorption maximum of the pyridine haemochromes derived from them is necessary to establish this. Provisionally they have been referred to as cytochrome b_{552}, b_{557} and b_{561}, respectively.

The cytochrome *b* in fragmented heart-muscle mitochondria (the Keilin and Hartree heart-muscle preparation) responds in two ways to the addition of antimycin. First, both the rate at which cytochrome *b* is reduced by succinate or NADH and the amount of cytochrome *b* reduced in the absence of oxygen are increased[2]. Secondly,

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the position of the absorption peaks of the cytochrome are displaced 1–2 nm towards the red[2]. Both effects of antimycin are described by sigmoidal antimycin concentration curves, the sigmoidicity being abolished by dispersal of the particles by cholate and restored again after removal of the cholate by dialysis[3]. This paper reports the effect of antimycin on the cytochrome *b* system in mitochondria isolated from white potatoes.

RESULTS

Fig. 1 shows the kinetics of the changes of absorbance in the region of the α-bands of the cytochromes *b* on the addition of succinate to mitochondria in the presence of ATP. There is a slow increase of absorbance which is not complete when the suspension becomes anaerobic due to consumption of O_2 by State-4 respiration. At this point, the absorbance reaches its maximum value. With the preparation used in this experiment (but not in all experiments) this was followed by a slow decrease of absorbance. When a steady state was reached, the addition of antimycin caused a further increase in absorbance. Fig. 2 shows the wavelength dependence of the slow decrease after anaerobiosis and of the effect of antimycin.

Fig. 3 gives spectra between 550 and 575 nm, obtained in another experiment in which the slow increase of absorbance after anaerobiosis was not observed, of aerobic + succinate (measured immediately before anaerobiosis) *minus* aerobic, and of anaerobic + succinate *minus* aerobic. Fig. 4 shows the spectrum of the effect of antimycin added to anaerobic mitochondria in the presence of succinate.

The three cytochromes, observed previously by low-temperature spectropho-

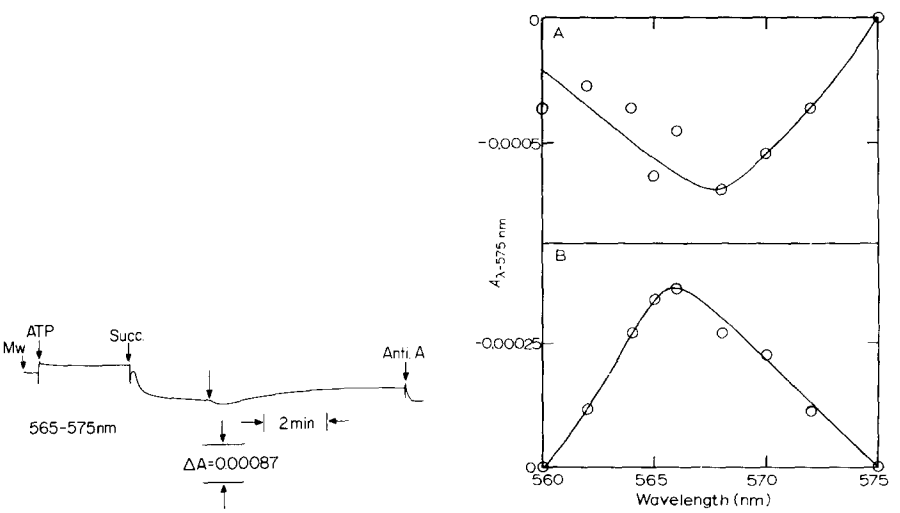

Fig. 1. Kinetics of changes of $A_{565-575\ nm}$ (an increase of $A_{565-575nm}$ is plotted downwards), measured in a dual-wavelength spectrophotometer, on adding succinate to potato mitochondria in the presence of ATP. Mitochondria (1.21 mg protein per ml) suspensed in 0.3 M mannitol, 5 mM $MgCl_2$, 10 mM phosphate buffer (pH 7.2), 10 mM KCl; 0.2 mM ATP added at second arrow, followed by 8 mM succinate. The fourth arrow gives the point at which the suspension became anaerobic. At the fifth arrow, 3.3 μg antimycin (1.6 μmoles/g protein) were added.

Fig. 2. Spectra of the slow absorbance changes after anaerobiosis (A) and of the effect of antimycin (B). Measured as in Fig. 1. The reference wavelength was 575 nm.

tometry[1], are all revealed in Fig. 3, with peaks at 552, 558 and 566 nm, respectively. The first is very largely oxidized in the aerobic State 4, the last largely reduced. Antimycin has no effect on either the degree of reduction or on the position of the absorption maximum of the two shorter-wavelength cytochromes, but does affect the longer-wavelength cytochrome. The available data do not allow a definite conclusion as to whether antimycin increases the amount of cytochrome reduced, or

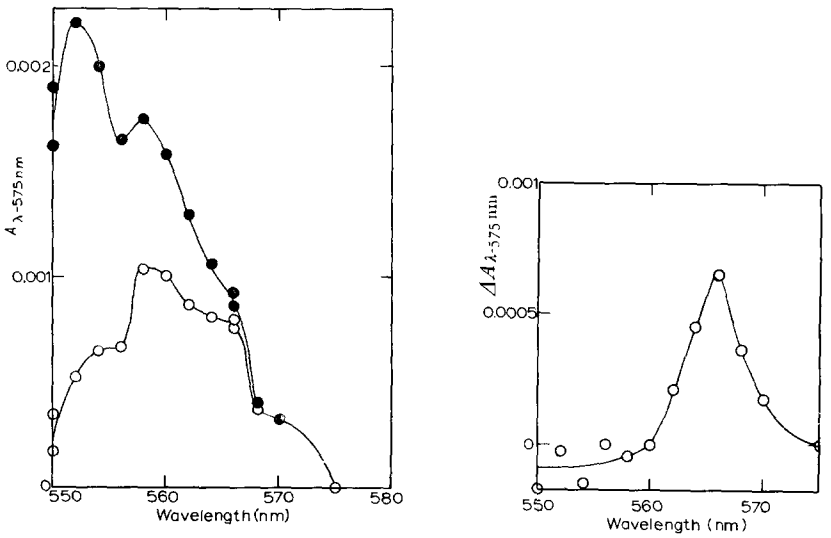

Fig. 3. Spectra obtained by adding succinate to potato mitochondria in the presence of ATP, and allowing the suspension to go anaerobic. 1.7 mg protein per ml. Suspension medium as in Fig. 1. The reference wavelength was 575 nm. ●—●, anaerobic + succinate *minus* aerobic; ○—○, aerobic + succinate *minus* aerobic.

Fig. 4. Spectrum of the effect of antimycin added to anaerobic potato mitochondria in the presence of succinate and ATP. Same experiment as in Fig. 3. The reference wavelength was 575 nm.

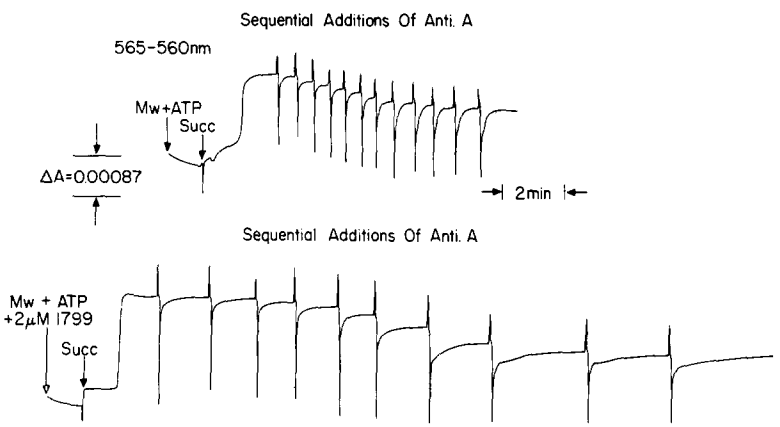

Fig. 5. Kinetics of changes at $A_{566-560\ nm}$ on adding succinate to potato mitochondria in the presence of ATP. Mitochondria (3 mg protein per ml) suspended in same medium as in Fig. 1. Sequential additions of 0.1 μg antimycin (0.02 μmole/g protein) were added as indicated. In lower trace 2 μM 1799 was present.

causes a shift in its α-band. In disrupted heart-muscle mitochondria[1,3], the α-band shift is revealed by an increase in $A_{566-560nm}$, since heart-muscle ferrocytochrome *b*, in the absence of antimycin, absorbs equally at 566 and 560 nm. Antimycin also causes an increase in $A_{566-560\ nm}$ in potato mitochondria, but it is not known whether the $A_{566-560\ nm}$ of the longer-wavelength cytochrome *b* of potato mitochondria changes on reduction.

The effect of antimycin concentration was studied using the wavelength pair 566–560 nm. On addition of succinate to State-4 mitochondria in the presence of ATP, there is a decline of absorbance measured at these wavelengths (*cf.* Fig. 2). This decline occurs in three stages: a fast decline, a short-lived increase, followed by a slower decline, which increases greatly on approaching anaerobiosis (see Fig. 5). Antimycin now causes an increase in absorbance.

When antimycin is added to an anaerobic suspension of mitochondria, air is also admitted. In the presence of antimycin, the previously fully reduced cytochromes *c*, c_1 and aa_3 will become oxidized. This is revealed as a transient increase of $A_{566-560\ nm}$. However, the complete reduction of these cytochromes is soon restored, as illustrated by the lack of any effect at 550 nm in Fig. 4, presumably *via* antimycin-resistant pathways. The duration of the transient increase varied considerably from preparation to preparation, presumably reflecting variations in the activity of the antimycin-resistant pathways.

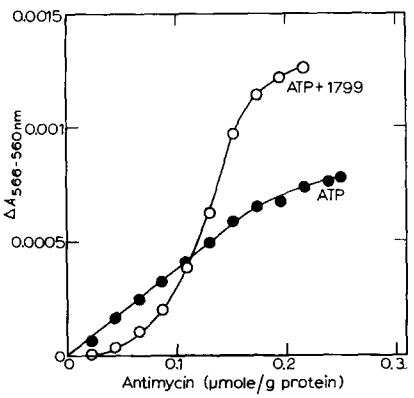

Fig. 6. Antimycin-effect curves plotted from the experiment shown in Fig. 5.

The effect of concentration of antimycin on the $A_{566-560\ nm}$ is shown in Fig. 6. In the absence of uncoupler the antimycin-effect curve is essentially hyperbolic, in the presence of uncoupler it is strongly sigmoidal. The amount of antimycin required for a maximum effect is unaffected by the presence of uncoupler, but the maximum effect is greater. The uncoupler has other effects, *viz.* it abolishes both the short-lived increase of absorbance and the subsequent slow decline observed soon after addition of succinate, and it slows down the restoration of the anaerobic equilibrium after addition of antimycin and air, presumably by inhibiting the antimycin leak (see Fig. 5).

DISCUSSION

The longer-wavelength cytochrome *b* is distinguished from the other two described by LANCE AND BONNER[1] in the following respects: (1) It is much more highly reduced in the aerobic State 4. (2) Its degree of reduction increases on energization of the mitochondria more rapidly than that of other components. This is revealed by the short-lived increase of $A_{566-560\ nm}$ before the slower decrease caused by reduction of the components absorbing more at 560 nm than at 566 nm. (3) Either the degree of reduction or the position of the α-band is affected by antimycin in the same way as cytochrome *b* in fragmented heart-muscle mitochondria. In this respect, the longer-wavelength cytochrome *b* in plant mitochondria is analogous to the cytochrome *b* of mammalian mitochondria.

BRYŁA *et al.*[3] explain the sigmoidal antimycin effect curves in terms of the allosteric model of MONOD *et al.*[4]. They propose that in particulate preparations the antimycin-sensitive segment of the respiratory chain exists in two enzymically active conformation states, the R and the T, both oligomeric (or polymeric), in equilibrium:

$$R \leftrightharpoons T$$

They further propose that, in the non-energized preparations used, the position of equilibrium lies towards the T state, and that antimycin combines more firmly with the R state.

Since, according to this explanation, the equilibrium lies far to the right, R, the antimycin-sensitive state, is an energized state. One might expect, then, that energization of the mitochondria would shift the equilibrium to the left, with, as consequence, hyperbolic antimycin-effect curves. The demonstration of a hyperbolic curve with potato mitochondria in State 4, changing to sigmoidal in the presence of uncoupler, is then good support for the explanation given by BRYŁA *et al.*[3].

According to this view, antimycin may be considered as a probe for an energized state of the mitochondrial respiratory chain.

The uncoupler 1799 (1,5-dihydroxy-1,5-tetra)(trifluoromethyl)pentanone-3) was kindly supplied by Dr. P. G. Heytler.

METHODS

Mitochondria were isolated from white potatoes (*Solanum tuberosum* L. var. Katahdin) by the method of BONNER[5]. The tissue was ground 20 sec with a Moulinex hand mixer. Protein was determined by a modified Lowry method[6]. Spectrophotometric measurements were made in a double-beam spectrophotometer. Antimycin (A, Type III) was obtained from Cal. Biochem. The concentration of an ethanolic solution was determined from its absorbance at 320 nm, using an absorption coefficient of 4.8 $mM^{-1} \cdot cm^{-1}$ (ref. 7).

REFERENCES

1 C. LANCE AND W. D. BONNER, JR., *Plant Physiology*, 43 (1968) 756.
2 B. CHANCE, *J. Biol. Chem.*, 233 (1958) 1223.
3 J. BRYŁA, Z. KANIUGA AND E. C. SLATER, *Biochim Biophys. Acta*, 189 (1969) 317.
4 J. MONOD, J. WYMAN AND J. P. CHANGEUX, *J. Mol. Biol.*, 12 (1965) 88.
5 W. D. BONNER, JR., in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. 10 (R. W. ESTABROOK AND M. E. PULLMAN, Eds.), Academic Press, New York, 1967, p. 126.
6 G. L. MILLER, *Anal. Chem.*, 31 (1959) 964.
7 F. M. STRONG, J. P. DICKIE, M. E. LOOMANS, E. E. VAN TAMELEN AND R. S. DEWEY, *J. Am. Chem. Soc.*, 82 (1960) 1513.