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Respiratory Chain of Antimycin A-producing *Streptomyces antibioticus*

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An active respiratory chain system was demonstrated in sonically treated mycelium of *Streptomyces antibioticus*, the producer of antimycin A. The respiratory electron transfer from substrate to oxygen proceeded successively through flavoprotein(s), *b*-, *c*-, and *a*-type cytochromes, and terminated with the cyanide-sensitive cytochrome oxidase. The cytochrome composition of the culture was not affected by the age of the mycelium, the intensity of antimycin A production, or differences in the media. Slater factor, coenzyme Q, and vitamin K were not interposed as hydrogen carriers in the respiratory chain between flavoproteins and cytochromes. The oxidation of reduced nicotinamide adenine dinucleotide and succinate was unaffected by antimycin A. Evidence is presented in support of the absence of the antimycin A-sensitive site from the electron transport system of *S. antibioticus*.

The purpose of the present study was to clarify the pathway of hydrogen (electron) transfer to molecular oxygen in *Streptomyces antibioticus*, the producer of antimycin A, which has been found to block the electron transport chain specifically between cytochromes *b* and *c* (3, 9). Such a study provides a useful basis for more complete investigation of the physiology of the strain and contributes to the understanding of the respiratory chain of streptomycetes.

MATERIALS AND METHODS

Organism and culture conditions. The strain of *S. antibioticus* (NRRL 2838) used was obtained from the Northern Utilization Research and Development Division, Peoria, Ill. Mycelium was grown in both chemically defined (A, B) and complex (C) liquid media on a rotary shaker (240 rev/min, 2.1-inch amplitude) at 28 C. Medium A contained (grams/liter): glucose, 20.0; glycine, 7.5; L-tryptophan, 1.0; DL-alanine, 1.0; K_2HPO_4, 5.0; NaCl, 5.0; $MgSO_4 \cdot 7H_2O$, 0.5; $FeSO_4 \cdot 7H_2O$, 0.02; $ZnSO_4 \cdot 7H_2O$, 0.01, $MnCl_2 \cdot 4H_2O$, 0.008; $CoCl_2 \cdot 6H_2O$, 0.001; and $CaCO_3$, 3.0 (final *p*H, 7.2). Medium B was the same as medium A, except that K_2HPO_4 was added after 18 hr of cultivation. Medium C contained (grams/liter): glucose, 10.0; and yeast extract, 10.0 (*p*H 7.0).

Analytical methods. To examine the respiratory components, homogenates were prepared by suspending washed mycelium in 0.1 M phosphate buffer (*p*H 7.0) to form a thick slurry and by treatment for 2 min in an MSE ultrasonic oscillator (20 kc/sec). The unbroken cells and debris were removed by centrifugation. The hemeproteins in homogenates were reduced with Na-dithionite and scanned over the visible range against oxygenated blanks with a recording spectrophotometer (Unicam SP-700).

Protein content of the sonically treated mycelium was determined according to the method of Lowry et al. (11). Oxidative activities were measured at 30 C by use of standard manometric techniques (16). For the inhibitor studies 2,3-dimercaptopropanol (BAL) and fresh solutions of sodium cyanide, 5-ethyl-5-iso-amylbarbiturate (Amytal), 2-heptyl-4-hydroxyquinoline *N*-oxide (HOQNO), and antimycin A were prepared. An experiment was carried out on presence of coenzyme Q, vitamin K, and other quinones of the respiratory chain (7) in which the 24-, 48-, and 72-hr mycelium from both synthetic and complex media was investigated. The method of Gale et al. (4) was used for extraction of quinones, and that of Burrin and Beeckey (2) was used for their purification.

RESULTS

The sonically treated mycelium of *S. antibioticus* contains an active respiratory chain system, i.e., capable of catalyzing the oxidation of reduced nicotinamide adenine dinucleotide ($NADH_2$) and succinate (Fig. 1). The oxygen uptake could always be inhibited by cyanide. The same is true for 2-heptyl-4-hydroxyquinoline *N*-oxide, which is known to block the flow of electrons between *b*- and *c*-type cytochromes (12). The spectrophotometric evidence (Table 1) indi-

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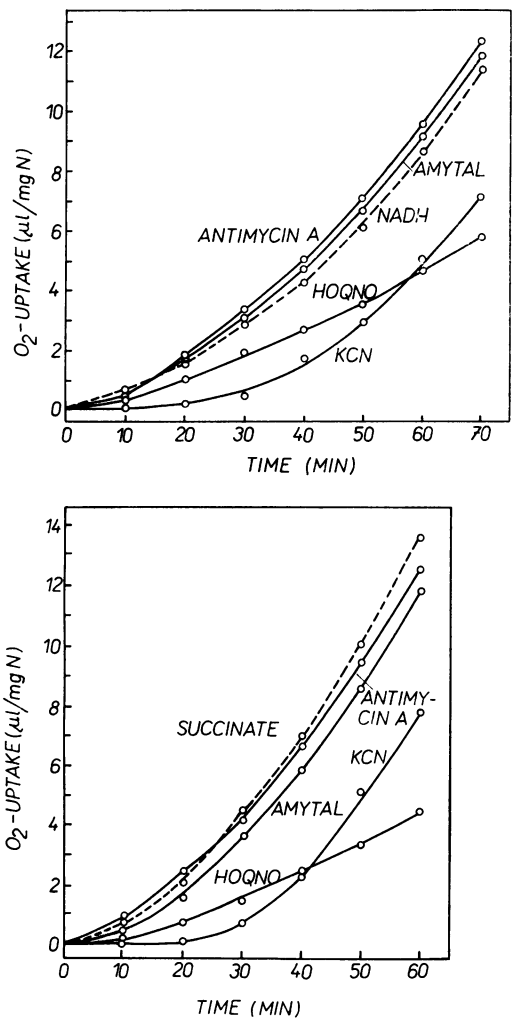

FIG. 1. *Effect of some respiratory chain inhibitors on $NADH_2$ and succinate oxidation by Streptomyces antibioticus. The reaction mixture contained: sonic extract (6.3 mg of protein) of 40-hr mycelium grown on synthetic medium; potassium phosphate buffer (pH 7.0), 250 μmoles; $NADH_2$, 0.1 μmole, or sodium succinate, 60 μmoles; antimycin A, 0.016 μmole; amytal, 0.3 μmole; sodium cyanide, 0.3 μmole; HOQNO, 0.03 μmole; and water to 3.0 ml.*

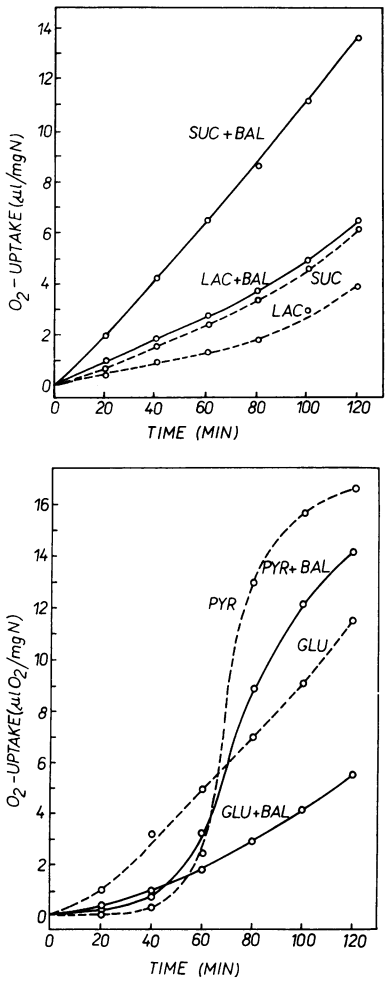

FIG. 2. *Effects of BAL on various substrate oxidation by Streptomyces antibioticus. The reaction mixture (3 ml) contained: sonic extract (3.5 mg of protein) of 40-hr mycelium grown on synthetic medium; 0.025 M substrate, 1 ml (GLU, glucose; PYR, pyruvate; SUC, succinate; LAC, lactate); 0.075 M potassium buffer (pH 7.0), 1.0 ml; 10^{-3} M BAL or paraffin oil (in control), 0.5 ml.*

cates the presence of three types of cytochromes in the mycelium of the investigated strain: a *b* type indicated by the 565- to 570-mμ (α) peak, 528- to 532-mμ (β) peak, and 427- to 430-mμ (γ) peak, a *c* type by the 550-mμ (α) peak and 520-mμ (γ) peak, and an *a* type by the 603-mμ (α) peak and 450 mμ (γ) peak. The 480- and 500-mμ peaks are unidentified. The decrease in absorbancy in the region around 460 mμ supports the presence of flavin. The cytochrome composition of the culture was not altered by the age of the mycelium, the intensity of antimycin A production, or by differences in the cultivation media (Table 1). The relative insensitivity of the system to amytal (Fig. 1) suggests either that different flavoproteins are involved in the system or that the site of amytal inhibition of NADH oxidase lies between the level of flavoprotein and oxygen. It is reasonable to assume that the normal respiratory electron transfer from substrate to oxygen proceeds successively through flavoprotein, *b*-, *c*-, and *a*-type cytochromes, and

TABLE 1. *Absorption maxima in different spectra of Streptomyces antibioticus cytochromes*

Medium	Mycelium		Wavelength (mμ)		
	Age (hr)	Antimycin A production[a] (μg/10 mg, dry wt)	α peaks	β peaks	γ peaks
A	42	4	603 565 550	528	520 500 480 427
	44	6	603 565 550	528	520 500 490 450 430
	66	10	600 565 550		520 500 450
B	42	26	603 565 550	532	520 500 480 450 427
	44	20	570	528	520 500 450 430
	66	4	600 570 550		500 480 450 428
C	72	0	603 565 550	528	520 500 480 430

[a] Determined by a microbiological plate method (14).

terminates with the cyanide-sensitive cytochrome oxidase.

The results of experiments carried out on the effect of BAL on the oxidation of various organic compounds are shown in Fig. 2. Glucose and pyruvate oxidation were inhibited, while succinate and lactate were BAL-insensitive. The accelerating effect on succinate and lactate is attributable to the oxidation of benzylbenzoate as compound of BAL (8). Regarding the data obtained, it can be concluded that the investigated strain does not possess the Slater factor (15) as the electron acceptor for succinate oxidation (6). The observations also show that the oxidation of both $NADH_2$ and succinate was not inhibited by antimycin A (Fig. 1). Neither coenzyme Q nor vitamin K was found in the sonically treated mycelium (Fig. 3). Quinone catalysis is in all likelihood not interposed as a hydrogen carrier in the respiratory chain between flavoproteins and cytochromes.

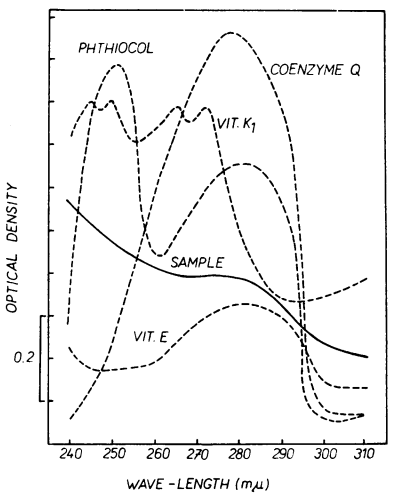

FIG. 3. *Comparison of sample of Streptomyces antibioticus with some quinones of the respiratory chain.*

DISCUSSION

The present study has shown that electron transfer in antimycin A-producing *S. antibioticus* proceeds from substrate to molecular oxygen via flavoproteins and cytochromes. The absence of coenzyme Q and vitamin K in the respiratory chain was surprising. In this respect, the investigated streptomycete resembles anaerobic gram-positive bacteria (5). It is worthwhile to note that in *S. griseus* quinone catalysis, interposed as a hydrogen carrier in the respiratory chain between the flavoproteins and the cytochromes, is represented by vitamin K (10).

In sonically treated mycelium of *S. antibioticus*, antimycin A did not block the cytochrome-linked electron transport system. This fact proved that this insensitivity of the system was not due to lack of permeability, and led to the assumption that the antimycin A-sensitive site, which ought to lie between coenzyme Q or cytochrome *b* and cytochrome c_1 (1) was absent. An opportunity now presents itself to draw attention to some similarities between *S. antibioticus* and most bacteria which are also lacking the antimycin A-sensitive site and are consequently antimycin A-insensitive as distinct from mammalian, fungal, and higher plant cells, all of which possess linked electron transport systems containing the antimycin-sensitive site (13).

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