SHORT COMMUNICATIONS

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The electron transport systems of heart muscle and yeast: site of inhibition by chloramphenicol

Recently, much interest has been evoked by the demonstration of the inhibitory effects of chloramphenicol on mitochondrial protein synthesis in *Saccharomyces cerevisiae*[1-3]. As shown by CLARK-WALKER AND LINNANE[3], chloramphenicol inhibits the synthesis of cytochromes *a*, a_3, *b*, and c_1, and its effects result in the production of yeasts which resemble the respiratory deficient mutants previously described by SLONIMSKI[4]. More recently, FREEMAN AND HALDAR[5] have shown that chloramphenicol also has a direct inhibitory effect on the DPNH oxidase activity (but not the succinate oxidase activity) of isolated rat liver mitochondria. The present paper describes the inhibitory effects of chloramphenicol on purified preparations of electron transport particles and DPNH dehydrogenase from heart muscle, and electron transport particles from various yeasts, and demonstrates that the site of inhibition in heart preparations is located between the flavin component (FMN) of the DPNH oxidase and cytochrome *b*.

Electron transport particles were prepared from beef heart and from *Saccharomyces cerevisiae* and *Candida utilis* as described previously[6-8]. DPNH dehydrogenase was prepared from beef heart as described by MACKLER[9]. Assays for DPNH oxidase and succinate oxidase activities were performed at 38° as described previously with an oxygen polarograph. DPNH dehydrogenase activity with ferricyanide and 2,6-dichlorophenolindophenol (DCIP) as electron acceptors was determined spectrophotometrically at 38° as described previously[9,10]. For studies of the effects of chloramphenicol or seconal on enzymatic activity, the inhibitor was added directly to the reaction cuvette and was not preincubated with the enzyme preparation. Spectra were recorded on a sensitive recording spectrophotometer similar to that described by YANG ET LEGALLAIS[11]. Protein was determined by the method of LOWRY *et al.*[12]. Chloramphenicol was obtained as a gift from Parke, Davis and Co.

Table I shows the effects of chloramphenicol on the activities of preparations of electron transport particles from heart muscle, *Candida utilis* and *Saccharomyces cerevisiae*. As shown in Table I, chloramphenicol (2 mM) inhibited the DPNH oxidase activity of heart muscle electron transport particles over 80%, and the DPNH oxidase and succinate oxidase activities of the electron transport particles from *C. utilis* to a lesser degree (40%), but did not affect the succinate oxidase activity of heart electron transport particles or the activities of the electron transport particles from *S. cerevisiae* even at higher concentrations. The pattern of inhibition resembles closely the results obtained previously with seconal inhibition of heart and yeast preparations[8,9]

Abbreviation: DCIP, 2,6-dichlorophenolindophenol.

TABLE I

EFFECTS OF CHLORAMPHENICOL ON THE ACTIVITIES OF THE ELECTRON TRANSPORT PARTICLES FROM HEART MUSCLE AND FROM *C. utilis* AND *S. cerevisiae*

All values are the average of determinations on 3 preparations. Average specific activities of the preparations were as follows: DPNH oxidase activity: heart electron transport particles, 2.9; *C. utilis* electron transport particles, 1.2; *S. cerevisiae* electron transport particles, 3.1. Succinate oxidase activity: heart electron transport particles, 0.33; *C. utilis* electron transport particles, 0.49; *S. cerevisiae* electron transport particles, 0.80. Specific activity is expressed as μmoles substrate oxidized per mg enzyme protein per min.

	Inhibition (%) of activity		
	DPNH oxidase 2 mM chloramphenicol	*Succinate oxidase*	
		2 mM chloramphenicol	*10 mM chloramphenicol*
Heart electron transport particles	81.0	0.0	0.0
Electron transport particles from *C. utilis*	40.0	40.0	—
Electron transport particles from *S. cerevisiae*	0.0	0.0	0.0

and adds further evidence to support the previously reported finding that electron transport particles from heart and *C. utilis* contain an active component (probably non-heme iron), in the DPNH dehydrogenase region, not found in the electron transport particles from *S. cerevisiae*[8].

Table II shows the effects of chloramphenicol on the activity of a purified preparation of DPNH dehydrogenase from heart muscle. As shown in Table II, both chloramphenicol and seconal inhibited the activity of the dehydrogenase when indophenol was used as acceptor, but had little or no effect on the activity with ferricyanide as acceptor. The difference in effect of the inhibitors on the reactions with DCIP and ferricyanide is probably not due to differences in rate limiting reactions for the two acceptors, since the enzymatic activity with ferricyanide is more rapid than with DCIP (specific activities with ferricyanide and DCIP are 65 and 50 μmoles of DPNH oxidized per min per mg of protein, respectively). More likely, the difference in effect of the inhibitors would appear to be due to a difference in the pathways of electron transport for the two acceptors as has been suggested previously by RAO

TABLE II

EFFECTS OF CHLORAMPHENICOL AND SECONAL ON THE ACTIVITIES OF HEART MUSCLE DPNH DEHYDROGENASE

All values are the average of determinations on 3 preparations. Average specific activity of the preparations was 50.0 and 26.0 μmoles of DPNH oxidized per mg of enzyme protein per min for ferricyanide and DCIP, respectively.

Acceptor	*Inhibition (%) of activity*	
	Chloramphenicol (5 mM)	*Seconal (2 mM)*
Ferricyanide	<5.0	0.0
DCIP	35.0	20.0

et al.[10], with the site of reduction of ferricyanide before the site of action of the inhibitors in the enzymatic pathway.

In order to locate the site of action of chloramphenicol in the pathway of electron transport in beef heart electron transport particles, difference spectra (reduced *minus* oxidized), were recorded for preparations of heart electron transport particles in the presence of a concentration of chloramphenicol (5 mM) sufficient to inhibit the DPNH oxidase activity over 90%. As shown in Fig. 1, DPNH in the presence of chloramphenicol produced reduction of almost all of the flavin in the heart electron transport particles as demonstrated by the decrease in absorbance in the 465-mμ region of the spectrum, but only small amounts of cytochromes *b*, *c*, c_1 and $a+a_3$ were reduced as shown by the lack of absorption maxima of any magnitude at 550, 552, 562 or 605 mμ.

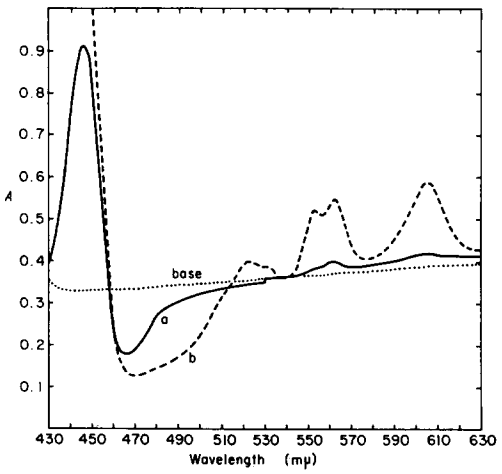

Fig. 1. Difference spectra (reduced *minus* oxidized) of electron transport particles from beef heart. The difference spectrum after: (a) addition of 2 mg of solid DPNH to the sample cuvette; (b) 1 mg of solid dithionite to the sample cuvette. The sample cuvette contained chloramphenicol (5 mM) and both sample and control cuvettes contained 4.5 mg of enzyme protein in 3 ml of 0.05 M phosphate buffer of pH 7.6.

The difference spectrum which was recorded following reduction of the electron transport particles with dithionite represents 100% reduction of the flavin and cytochrome components. Therefore, it would appear that the site of inhibition by chloramphenicol of DPNH oxidase activity in heart electron transport particles is located at or between the flavin component (FMN) and cytochrome *b*, and is of the same general location as is ascribed to the sites of action of seconal and rotenone[8], other inhibitors of heart muscle DPNH oxidase activity.

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