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On the binding of antimycin to the respiratory chain

In previous communications from this laboratory[1,2] we have reported that the antimycin-inhibited activities of succinate oxidase and succinate–cytochrome *c* reductase were almost completely restored by extraction of mitochondrial preparations with diethyl ether, providing that soluble cytochrome *c* was added. It was suggested that the reversal of antimycin inhibition was due to removal of the antibiotic from the site that was responsible for the inhibition of the electron transport, while some ether-unextractable inhibitor remained bound at another site. The effect of antimycin on preparations previously treated with antimycin and then extracted with ether has now been studied.

Materials, treatment of preparations with antimycin and its extraction, as well as assay of enzyme activities, were the same as previously described[2].

As can be seen from Fig. 1, the addition of increasing amounts of antimycin to heart-muscle preparation (or rat-liver mitochondria) followed by ether extraction makes the preparation increasingly sensitive to the subsequent addition of antimycin. Complementary experiments presented in Table I indicate that all the added antimycin may be removed by extraction with ether–ethanol. In agreement with the data of RIESKE *et al.*[3] we have also found that freezing of antimycin-treated preparations in the presence of 0.2 M guanidine releases almost all of the added inhibitor.

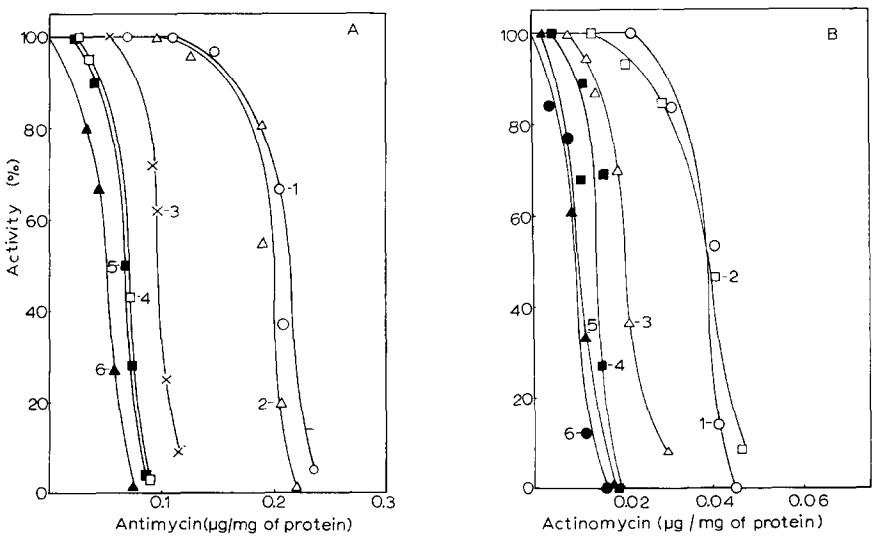

Fig. 1. Effect of antimycin on succinate oxidase activity of heart-muscle preparation (A) and rat-liver mitochondria (B) previously treated with various amounts of antimycin and then extracted with diethyl ether. The mitochondrial preparation was not centrifuged after removal of residual ether by evaporation (see ref. 2). A. Curve 1, previously untreated preparation; Curve 2, preparation previously untreated with antimycin, but extracted with ether; Curves 3, 4, 5 and 6, preparations previously treated with 0.074 μg (no inhibition), 0.148 μg (2% inhibition), 0.192 μg (57% inhibition), and 0.242 μg (97% inhibition) of antimycin per mg of protein, respectively, and then extracted with ether. B. Curve 1, previously untreated mitochondria; Curve 2, mitochondria previously untreated with antimycin, but extracted with ether; Curves 3, 4, 5 and 6, mitochondria previously treated with 0.023 μg (no inhibition), 0.031 μg (68% inhibition), 0.043 μg (92% inhibition) and 0.054 μg (100% inhibition) of antimycin per mg of protein, respectively, and then extracted with ether.

TABLE I

RELEASE OF ANTIMYCIN BOUND TO HEART-MUSCLE PREPARATION BY EXTRACTION WITH DIETHYL ETHER OR ETHER–ETHANOL

Extraction of antimycin from particles with ether–ethanol (4:1, v/v) was carried out as previously[2]. The pooled extracts from each sample were evaporated *in vacuo*, and the dry residue dissolved in 10 or 25 ml of ethanol. The amount of antimycin extracted was estimated by determination of the amount required for 50% inhibition of succinate–cytochrome *c* reductase of heart-muscle preparation. In Expts. 1 and 2, 3 ml of a suspension of heart-muscle preparation (24.5 and 17.2 mg of protein per ml, respectively) were used.

Expt. No.	*Added antimycin (μg)*	*Inhibition of succinate oxidase (%)*	*Antimycin extracted by*			
			Diethyl ether		*Ethanol–diethyl ether*	
			μg	%	*μg*	%
1	8.43	0	3.56	42	7.55	90
	16.85	30	8.20	48	15.70	94
	17.95	98	7.56	43	17.10	96
2	4.83	0	2.76	53	4.61	95
	9.65	10	5.12	53	9.23	96
	13.10	100	6.62	50	13.10	100

On the other hand, extraction with diethyl ether of antimycin-treated preparations released only about 50% of the antimycin, independently of the degree of initial inhibition, even after several extractions. This shows that the increased sensitivity to antimycin of ether-extracted antimycin-inhibited preparations cannot be explained simply by a higher affinity for antimycin of an ether-resistant non-enzymic site than for an ether-extractable enzymic site.

THORN[4] and REDFEARN, WHITTAKER AND BURGOS[5] have observed translocation of antimycin from antimycin-inhibited to antimycin-untreated preparations. In the experiments described in Table II, a preparation almost completely inhibited by antimycin was used as antimycin donor, and ether-extracted heart-muscle preparation (which is inactive in the absence of added cytochrome *c*) or a preparation whose succinate oxidase activity was almost completely inactivated by *p*-aminophenylarsenoxide[6] was used as antimycin acceptor. BAL-inactivated preparations[7] gave similar results. The addition of $A_{100}E$ (100% inhibited by antimycin, followed by ether extraction) to A_{97} (97% inhibited by antimycin) resulted in 52% reactivation of succinate oxidase, presumably due to the translocation of the inhibitor from the antimycin-sensitive site of A_{97} to that of $A_{100}E$, which as already shown[2] does not contain antimycin at this site. On the other hand, when preparation A_{97} was added to preparation $A_{21}E$ the reactivation amounted to 71%, presumably because the 'enzymatically-inactive' site of the preparation $A_{21}E$ was not wholly saturated and could still bind antimycin. A similar conclusion can be drawn from the experiments in which preparation $A_{16}As$ was added to A_{88} and preparation A_{16} to $A_{88}As$.

The shape of the inhibition curve suggests that antimycin is an allosteric inhibitor of the respiratory chain. This implies that the antimycin-sensitive component of the chain is an oligomer, and that inhibition requires binding to several or all the protomers. If the protomer consists of different subunits (as in haemoglobin) it is possible that antimycin binds to one subunit in such a way that it can be extracted

TABLE II

TRANSLOCATION OF ANTIMYCIN FROM ONE PREPARATION TO ANOTHER

The following symbols are used to describe the preparations: C = untreated; CE = ether-extracted; A_{16}, A_{21}, A_{88}, A_{97} and A_{100} = antimycin-treated to produce 16%, 21%, 88%, 97% and 100% inhibition, respectively; $A_{21}E$ and $A_{100}E$ = A_{21} and A_{100}, extracted with diethyl ether; CAs = inactivated with 0.4 μequiv of *p*-aminophenylarsenoxide as described by SLATER[6]; $A_{16}As$ and $A_{88}As$ = treated with antimycin to produce 16% and 88% inhibition, respectively, and then inactivated with 0.4 μequiv of *p*-aminophenylarsenoxide. The activity of succinate oxidase was measured in the absence of soluble cytochrome *c*.

(*A*) *Ether-inactivated preparation*				(*B*) *p-Aminophenylarsenoxide-inactivated preparation*			
Components	*Amount of protein in sample (mg)*	*Activity*		*Components*	*Amount of protein in sample (mg)*	*Activity*	
		nmoles/min	%			*nmoles/min*	%
C	0.48	204	100	C	0.43	194	100
CE	0.48	0	0	CAs	0.43	11	6
A_{21}	0.48	160	79	A_{16}	0.43	164	84
$A_{21}E$	0.47	0	0	$A_{16}As$	0.43	10	5
A_{97}	0.48	7	3	A_{88}	0.43	24	12
A_{100}	0.48	0	0	$A_{88}As$	0.43	9	5
$A_{100}E$	0.49	0	0				
C	0.24	178	87	C	0.215	169	87
$+A_{97}$	0.24			$+A_{88}$	0.215		
CE	0.48	151	74	CAs	0.43	151	78
$+A_{97}$	0.48			$+A_{88}$	0.43		
A_{21}	0.24	89	44	C	0.43	169	87
$+A_{97}$	0.24			$+A_{88}As$	0.43		
$A_{21}E$	0.47	145	71	A_{16}	0.215	106	55
$+A_{97}$	0.48			$+A_{88}$	0.215		
$A_{100}E$	0.49	106	52	$A_{16}As$	0.43	115	59
$+A_{97}$	0.48			$+A_{88}$	0.43		
				A_{16}	0.43	115	59
				$+A_{88}As$	0.43		

by ether, and to the other in an ether-inaccessible manner. The increased sensitivity to antimycin of antimycin-treated and ether-extracted preparations could be explained according to the allosteric model if removal of once-bound antimycin does not result in reversal of the conformation change previously induced by binding of the antimycin.

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The requirement of ubiquinone-10 for an ATP-forming system and an ATPase system of chromatophores from *Rhodospirillum rubrum*

Chromatophores from *Rhodospirillum rubrum* show ATPase (EC 3.6.1.3) activity and ATP–P_i exchange activity. It was reported earlier that, in darkness, both activities originate at least in part from the reversibility of the energy-conversion system coupled to photosynthetic or oxidative electron transport[1].

RACKER and his associates[2] extracted and purified from mitochondria a protein capable of catalyzing an ATPase activity, and they reported that phosphorylating activity by impaired mitochondria was stimulated by adding the enzyme. However, all attempts so far made in this laboratory for extraction of such an enzyme from chromatophores, by, *e.g.*, sonic disruption[1], treatment with various detergents or illumination with ultraviolet light, have resulted in a nearly parallel loss of photosynthetic ATP-forming activity, and ATPase and ATP–P_i exchange activities in darkness.

It is known that chromatophores contain a considerable amount of ubiquinone-10 (Q-10)[3], and that the level of oxidation–reduction state of quinone(s) present in chromatophores is changed when chromatophores are illuminated[4–6]. RUDNEY[7,8] found that chromatophores prepared from cells grown in the presence of diphenylamine contained a decreased amount of Q-10 and were less active in photosynthetic ATP formation, and that ATP formation was significantly stimulated by adding Q-10; he concluded that the stimulation was merely caused by adjusting the oxidation–reduction potential of the reaction medium in favor of the maximum coupling[9] of photosynthetic ATP formation.

Chromatophores were prepared from light-grown cells of a blue-green mutant (G-9) of *R. rubrum*, kindly supplied by Dr. J. W. NEWTON, and ATP-forming activity in the light and activities for ATPase and ATP–P_i exchange in darkness were assayed according to the methods described previously[1,10]. It was found that the rate of most of the ATPase activity and almost all of the ATP–P_i exchange activity was influenced by adding 2,6-dichlorophenolindophenol (DCIP) (Fig. 1). Chromatophores could reduce a part of DCIP without addition of electron donors. The rate of ATP–P_i exchange decreased with increasing concentration of DCIP and was negligible at $6.7 \cdot 10^{-5}$ M. On the other hand, the rate of ATPase increased with increasing concentration of DCIP, reaching a maximum at $6.7 \cdot 10^{-5}$ M, and then decreasing at higher concentrations. The activities of ATPase and ATP–P_i exchange, which had been depressed in the presence of $6.7 \cdot 10^{-4}$ M DCIP, were restored when ascorbate was also added;

Abbreviations: Q-10, ubiquinone-10; DCIP, 2,6-dichlorophenolindophenol.