A MITOCHONDRIAL FACTOR CONFERRING OLIGOMYCIN SENSITIVITY ON SOLUBLE MITOCHONDRIAL ATPase*

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Oligomycin is a potent inhibitor of ATPase activity in mitochondria, as well as of oxidative phosphorylation (Lardy et al., 1958). Submitochondrial particles, obtained after disintegration of beef-heart mitochondria in a sonic oscillator (S-particles), contained oligomycin-sensitive ATPase activity. After exposure of S-particles to 1.6 M urea, the particles (U-particles) exhibited little or no ATPase activity, but were capable of conferring oligomycin sensitivity on added soluble ATPase (Racker, 1962). Attempts to extract the factor in mitochondria responsible for oligomycin sensitivity with acetone were not only unsuccessful, but yielded particulate preparations of ATPase that were insensitive to oligomycin. Attempts to solubilize the factor with trypsin from U-particles were equally unsuccessful.

As shown in Table I, treatment of S-particles or U-particles with trypsin resulted in a 10-fold stimulation of ATPase activity. To eliminate all ATPase activity from S-particles, it was therefore necessary to treat first with trypsin and then with urea. The resulting particles (T-U-particles) contained neither manifest nor trypsin-activated ATPase activity, but still conferred oligomycin sensitivity (Table II, Experiment 1). However, when again treated with trypsin, the ability to confer oligomycin sensitivity was lost (Table II, Experiment 2). Since treatment with trypsin before exposure to urea had not destroyed this ability, it appeared that the factor responsible for conferring sensitivity was protected by ATPase against proteolytic

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TABLE I

EFFECT OF TRYPSIN ON ATPase ACTIVITY OF SUBMITOCHONDRIAL PARTICLES

S-particles, prepared as described previously (Racker, 1962) were suspended in 0.05 M Tris sulfate buffer, pH 8.0, at 10 mg protein/ml and exposed at 30° to 30 μg trypsin/ml for various time periods, as indicated. The reaction was stopped by addition of 100 μg trypsin inhibitor/ml. U-particles were prepared by exposure of S-particles to 2 M urea at 0° for 45 minutes, centrifugation for 30 minutes at 105,000 x g, washing, and suspending in 0.25 M sucrose. ATPase was measured as described previously (Pullman et al., 1960).

Trypsin treatment	μmoles P_i/10 minutes/mg protein	
minutes	S-particles	U-particles
0	4.8	0.26
30	43	2.8
60	50	3.9
90	56	4.1

digestion. This could be demonstrated directly by addition of soluble ATPase prior to the second trypsin treatment. As shown in Table II, Experiments 2a and 2b, there was a considerable protection of the ability to confer oligomycin sensitivity when ATPase (30 μg ATPase for 4 mg particles) was present during proteolytic digestion of T-U-particles. Cold-inactivated ATPase (30 μg) or serum albumin (300 μg) did not protect. Control T-U-particles incubated without trypsin (Table II, Experiments 2c and 2d) revealed no loss in oligomycin sensitivity. It should be noted that ATPase itself was not inactivated by trypsin (Experiment 2b).

These experiments indicated an increased accessibility of the oligomycin-sensitive factor (F_o) after treatment with trypsin and urea. Therefore, attempts were made to extract F_o from T-U-particles. Sonic oscillation of the T-U-particles for 4 minutes and centrifugation at 105,000 x g for 2 hours yielded a clear supernatant solution which was capable of conferring oligo-

mycin sensitivity on soluble ATPase (Table III). Other mitochondrial proteins, such as F_3 or F_4 (see preceding communication)* were ineffective in conferring oligomycin sensitivity. It should be noted from Table III that preparations of F_3 caused some inhibition of ATPase activity, a phenomenon

TABLE II

CONFERRAL OF OLIGOMYCIN SENSITIVITY ON SOLUBLE ATPase BY SUBMITOCHONDRIAL PARTICLES TREATED WITH TRYPSIN AND UREA

T-U-particles were prepared by exposing S-particles to trypsin for 30 minutes and then to 2 M urea, as described in Table I. In Experiment 2 the T-U-particles (8 mg/ml) were exposed to trypsin for 60 minutes, as described in Table I except that 120 μg/ml trypsin were used (Experiment 2a). In Experiment 2b, soluble ATPase (60 μg/ml) was added before trypsin. For the control experiments (2c and 2d), the T-U-particles were incubated without and with ATPase, as for 2a and 2b, but without trypsin. ATPase was added at the end of the 60 minutes to Experiments 2a and 2c, and all samples were analyzed for ATPase activity in the absence and presence of oligomycin (3.3 μg/ml).

	Additions	ATPase activity μmoles P_i cleaved/10 minutes		Inhibition
		- oligomycin	+ oligomycin	
Exp. 1	T-U-particles (680 μg)	0.16	0.14	
	" " + ATPase (3 μg)	1.54	0.3	80 %
Exp. 2a	T-U-particles (400 μg) after trypsin treatment + ATPase (3 μg)	1.52	1.40	8 %
2b	T-U-particles (400 μg) after trypsin treatment in the presence of ATPase	1.55	0.48	69 %
2c	Control for 2a, without trypsin	1.47	0.1	93 %
2d	Control for 2b, without trypsin	1.52	0.14	91 %

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TABLE III

CONFERRAL OF OLIGOMYCIN SENSITIVITY ON SOLUBLE ATPase BY AN EXTRACT FROM SUBMITOCHONDRIAL PARTICLES AFTER TREATMENT WITH TRYPSIN AND UREA

The extract containing F_o was prepared by exposing T-U-particles in 0.25 M sucrose to sonic oscillation for 4 minutes in a 10 Kc Raytheon oscillator and by centrifugation for 2 hours at 105,000 x g. The supernatant solution was carefully pipetted off and used as a source for F_o. Preparations of F_3 and F_4 were as described in the preceding communication.* Oligomycin was added to a final concentration of 3.3 μg/ml.

Additions	μmoles P_i cleaved/10 minutes		Inhibition
	- oligomycin	+ oligomycin	
ATPase (3 μg)	1.0	1.06	0
ATPase (3 μg) + F_o (320 μg)	1.18	0.16	86 %
" " + F_3 (1.2 mg)	0.73	0.71	0
" " + F_4 (725 μg)	1.19	1.20	0

which is currently under investigation. The oligomycin-sensitive factor (F_o) was very heat-labile and sensitive to trypsin. It did not inhibit ATPase activity itself, but became slightly inhibitory after heat inactivation. Solutions of ATPase containing 4 mM ATP added to F_o afforded partial protection against heat inactivation (2 minutes at 65°) or exposure to trypsin.

Solutions of F_o could be dialyzed for 3 hours against 0.25 M sucrose with little loss in activity. In the presence of salt (e.g., 0.8 M ammonium sulfate) F_o precipitated. It was difficult to resolubilize, but was active in conferring sensitivity to oligomycin on soluble ATPase. Since ATPase and oligomycin were not precipitated at 0.8 M ammonium sulfate, the insolubility of F_o was useful for studies on the interaction of these components. The addition of ammonium sulfate (0.8 M final concentration) to 100 μg of soluble ATPase in the presence of 1.3 mg F_o and 10 mM $MgSO_4$ resulted in the precipitation of 90% of the ATPase activity which was inhibited 57% by oligomycin.

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If the precipitation of F_o and ATPase was carried out in the presence of 1.65 μg of oligomycin, the precipitated complex (washed with 0.8 M ammonium sulfate) had only 25% of the activity of the control complex prepared in the absence of oligomycin. This indicated a rather firm attachment of oligomycin to F_o in the presence of ATPase. F_o reacted with oligomycin even in the absence of ATPase, but less effectively than in its presence. On the other hand, treatment of ATPase with oligomycin, followed by re-isolation after precipitation at 2.0 M ammonium sulfate, resulted in fully active ATPase, both in the presence and absence of F_o.

Some of the properties of F_o, particularly its firm attachment to mitochondria and its insolubility at low salt concentrations, resembled those of a structural protein isolated from mitochondria by Criddle et al. (1962). However, structural protein prepared as described by these authors did not confer oligomycin sensitivity on soluble ATPase.

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