INHIBITION OF THE DINITROPHENOL-SENSITIVE ATP-ADP EXCHANGE REACTION BY OLIGOMYCIN

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The antibiotic oligomycin inhibits the dinitrophenol, arsenate and Mg^{++}-stimulated ATP-ase activities, the ATP-P_i^{32} and $P_i(O^{18})$-H_2O exchange reactions of mitochondria and submitochondrial particles as well as phosphate-acceptor controlled respiration of tightly coupled mitochondrial preparations (Lardy et al., 1958; Huijing and Slater, 1961). These findings suggest that oligomycin inhibits oxidative phosphorylation at that reaction in the coupling mechanism where initial uptake of phosphate occurs. It was also reported (Lardy and McMurray, 1959) that oligomycin does not inhibit the ATP-ADP exchange reaction in sonic particles, a finding which we have confirmed. In addition, it has been reported (Huijing and Slater, 1961) that oligomycin does not inhibit the ATP-ADP exchange reaction of intact rat liver mitochondria. However, their data indicate that they did not measure the initial reaction rate of the exchange, but rather the amount of ADP-C^{14} incorporated into ATP at isotopic equilibrium.

In this paper it is shown that the rate of the ATP-ADP exchange reaction of fresh, tightly coupled rat liver mitochondria is in fact strongly depressed by concentrations of oligomycin

which inhibit oxidative phosphorylation. Data presented in Table I show that when the initial rate of incorporation of labeled ADP into ATP is measured in the absence of added Mg^{++}, oligomycin inhibits the ATP-ADP exchange reaction to the same extent as does dinitrophenol and arsenate. Under these test conditions less than 20% of the labeled ADP has exchanged with ATP. On the other hand, adenylate kinase activity is not inhibited by dinitrophenol, arsenate, or oligomycin in the absence or presence of added Mg^{++}. Similar studies with intact mitochondria from kidney, heart, and brain indicate comparable extent of inhibition of the ATP-ADP exchange activity by dinitrophenol, arsenate, and oligomycin at levels employed with liver mitochondria.

Arsenate, dinitrophenol, and oligomycin also inhibit the conversion of labeled ADP into ATP even when adenylate kinase is activated by the addition of Mg^{++}, as is seen in Table 1. Although the percentage inhibition of the total ATP-ADP exchange is low under these conditions, the absolute decrease is nearly the same as that measured in the absence of added Mg^{++}. It is therefore probable that the ATP-ADP exchange of intact mitochondria is catalyzed by at least two enzymes or enzyme systems of which only one is sensitive to dinitrophenol, arsenate, and oligomycin.

Table 1 also shows that the addition to the reaction system of both arsenate and oligomycin at concentrations that alone inhibit maximally, was found to result in less than complete inhibition of the exchange. On the other hand, the combination of oligomycin and dinitrophenol resulted in complete inhibition. These results suggest that arsenate and oligomycin exert a mutually

Table 1

ATP-ADP Exchange Activity of Rat Liver Mitochondria

The exchange reaction was assayed in a system containing 2.0 micromoles ADP-C^{14} (20,000 CPM), 6.0 micromoles ATP, 10.0 micromoles tris buffer, pH 7.4 and 750 micrograms mitochondrial protein in a final volume of 0.5 ml. Adenylate kinase activity was estimated in a system containing 3.0 micromoles AMP-C^{14} (25,000 CPM), 3.0 micromoles ATP, 10 micromoles tris buffer pH 7.4 in 0.5 ml final volume. Mg^{++} was present at 2×10^{-3} M when used. The reaction for both assays was initiated by addition at zero time of the nucleotide mixture to the mitochondrial reaction system containing the inhibitors or activators. The latter mixture was incubated for two minutes at 30° to insure temperature equilibration. The reaction time of both assays was 10 minutes at 30°. The reaction was stopped with perchloric acid, the system neutralized and the protein removed by centrifugation. The nucleotides of an aliquot were separated by paper electrophoresis and eluted with water and the specific radioactivity of each nucleotide determined.

Additions	ATP-ADP Exchange mμmoles ATP (C^{14})		Adenylate Kinase mμmoles ADP (C^{14})	
(Fresh Mitochondria)	-Mg	+Mg	-Mg	+Mg
None	400	1500	30	1300
DNP (1×10^{-4} M)	10	1200	30	1200
Arsenate (1×10^{-3} M)	20	1200	30	1300
Oligomycin (1 μg/cc)	10	1200	35	1200
" (0.5 μg/cc)	50	1200	30	1300
" (0.1 μg/cc)	280	1400	30	1200
" (1 μg/cc) plus Arsenate (1×10^{-3}M)	280			
" (1 μg/cc) plus DNP (1×10^{-4} M)	10			
(Aged Mitochondria)				
8 hours, 2°	350			
" plus oligomycin(1μg/cc)	100			
" plus DNP (1×10^{-4} M)	75			
20 hours, 2°	280			
" plus oligomycin(1μg/cc)	200			
" plus DNP (1×10^{-4} M)	125			

interfering effect on some specific step or site in the coupling mechanism, and that arsenate and dinitrophenol exert their uncoupling effects at different sites. Estabrook (1961) has reached a similar conclusion from other evidence.

Phosphorylating submitochondrial particles prepared by the digitonin method (Devlin and Lehninger, 1958) show an entirely different response to oligomycin (Table 2). In such phosphorylating particles the ATP-ADP exchange activity is still inhibited by dinitrophenol and arsenate, as shown before (Wadkins and Lehninger, 1958), however it is completely insensitive to oligomycin. A second point of difference is that the presence of oligomycin removes the sensitivity of the ATP-ADP exchange reaction to dinitrophenol as well as to arsenate. The ATP-ase activity of these particles and its stimulation by dinitrophenol or arsenate is completely inhibited by oligomycin, as is the case in sonic particles (Lardy et al., 1958). In digitonin particles oligomycin thus acts like azide, which also inhibits ATP-ase

Table 2

ATP-ADP Exchange Activity of Digitonin Submitochondrial Particles

The ATP-ADP exchange rate was assayed by methods described in text and in Table 1 except that 200 micrograms enzyme protein and 20 minute reaction time at 30° were used.

Additions	ATP-ADP Exchange mμmoles ATP (C^{14})
None	300
DNP (1×10^{-4} M)	120
Arsenate (1×10^{-3} M)	190
Oligomycin (2 μg/ml)	340
" (1 μg/ml)	350
" (1 μg/ml) plus DNP (1×10^{-4} M)	330
" (1 μg/ml) plus Arsenate (1×10^{-3} M)	280

activity and decreases the sensitivity of the ATP-ADP exchange reaction to dinitrophenol and arsenate (Wadkins and Lehninger, 1958).

It is difficult to rationalize all of these facts in terms of present conceptions of the mechanism of oxidative phosphorylation. However, a possible common denominator useful for rationalizing these points may exist in the finding that upon aging, the ATP-ADP exchange activity of intact mitochondria loses its sensitivity to dinitrophenol and to oligomycin, during which time there is loss of respiratory control by ADP (Table 1). This property is consistent with the finding that phosphorylating submitochondrial particles prepared by sonic treatment of rat liver mitochondria, which show little or no respiratory control, catalyze an oligomycin-insensitive ATP-ADP exchange reaction (Lardy et al., 1958), and with the data in Table 2 showing that digitonin particles also catalyze an oligomycin-insensitive ATP-ADP exchange. Aged mitochondria therefore possess properties similar to the loosely coupled submitochondrial particles i.e. high Mg^{++}-stimulated ATP-ase activity, low respiratory control, and low sensitivity of the ATP-ADP exchange activity to dinitrophenol, arsenate, and oligomycin. Cooper has recently reported that submitochondrial particles prepared in his laboratory catalyze an ATP-ADP exchange activity whose sensitivity to oligomycin is approximately equal to its sensitivity to pentachlorophenol (Cooper and Kulka, 1961).

These results suggest that the ATP-ADP exchange enzyme may be involved not only in the terminal transphosphorylation reaction

of oxidative phosphorylation but also in some earlier reaction of the coupling sequence in such a way that certain inhibitors of oxidative phosphorylation can influence its availability to catalyze maximally the ATP-ADP exchange reaction in tightly coupled phosphorylating preparations. The development of "leaks" in the coupling sequence upon aging or upon alteration of the membrane structure during preparation of submitochondrial particles thus leads to loss of respiratory control which is linked functionally to decreased sensitivity of the ATP-ADP exchange reaction to certain specific agents such as arsenate, dinitrophenol and oligomycin.

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