HYDROLYSIS OF ADENOSINETRIPHOSPHATE BY BLUEGILL LIVER MITOCHONDRIA

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ABSTRACT.—The hydrolysis of adenosinetriphosphate (ATP) by enzymes in bluegill (*Lepomis macrochirus*) liver mitochondria shows ATP to be the primary substrate as very little hydrolysis of adenosinediphosphate (ADP) was observed. Cadmium, zinc, manganese, magnesium, and calcium ions enhanced the hydrolysis of ATP in the order given. Sodium cyanide, sodium fluoride, and iodoacetate did not affect the hydrolysis of ATP. Sodium azide and p-chloromercuribenzoate inhibited the hydrolysis of ATP, but pentachlorophenol was less inhibitory. Sodium arsenate, sodium arsenite, and dicoumarol did not affect the hydrolysis of ATP. Gramicidin and 2, 4-dinitrophenol enhanced the hydrolysis of ATP in the presence of manganese and magnesium ions.

The hydrolysis of adenosinetriphosphate (ATP) has not been extensively investigated in fish liver mitochondria systems (Tarr 1958; Gumbmann, Brown, and Tappel 1959). An increase in the hydrolysis of ATP could result in the loss of energy to the fish, as the net energy available in the form of ATP is the composite result of two general enzyme sequences, (1) the ATP-generating sequences, and (2) the ATP hydrolyzing or utilizing sequences. The energy-producing systems of bluegill liver mitochondria are being investigated as one approach to determine the effects of pollutants on aquatic organisms. This investigation was initiated to study the hydrolysis of ATP by bluegill liver mitochondria and determine if the ATP hydrolysis may be affected by possible pollutants. The term ATPase activity as used here follows the definition of ATPase as proposed by Potter (1953), namely the release of inorganic phosphate from ATP, and does not imply the existence of a single or specific enzyme or enzymes. A preliminary report of part of the present work has been presented (Hiltibran 1964), and the effect of sodium amytal and rotenone on the bluegill mitochondrial enzyme sequences has been reported (Hiltibran and Johnson 1965).

MATERIALS AND METHODS

Native, wild bluegills were maintained in aerated aquaria at 25° C. Preparations of the mitochondria and procedures for estimating the rate of release of inorganic phosphate from ATP and for determining nitrogen content of mitochondrial preparations have previously been reported (Hiltibran and Johnson 1965). The inorganic phosphate released from the ATP was converted to micromoles of phosphate per hour per milligram of tissue nitrogen. All values have been corrected for endogenous activity. The data presented are the average values from two or more experiments.

RESULTS AND DISCUSSION

Effect of Metal Ions.—Very little hydrolysis of ATP occurred in the absence of any metal ions. One micromole of cadmium, zinc, and manganese ions per

ml of reaction medium was found to promote the maximum hydrolysis of ATP. Greater or lesser quantities of these ions resulted in less ATP hydrolyzed (TABLE 1). Maximum hydrolysis of ATP occurred with a magnesium ion concentration between 1 to 5 micromoles per ml of reaction medium, but magnesium levels greater than 5 micromoles reduced the hydrolysis. The hydrolysis of ATP increased with increased levels of calcium ions up to 15 micromoles of calcium, the highest level of calcium used. Sodium and potassium ions were not effective. The values given in TABLE 1 are the average values of 25 experiments.

Zinc and cadmium ions severely inhibited oxygen uptake by bluegill liver mitochondria, whereas manganese and calcium ions were less inhibitory. Also in the presence of these ions, there was a large increase in the inorganic phosphate content of the reaction medium, which was greater than in experiments in which oxygen uptake was inhibited by sodium cyanide or Antimycin A. It is now apparent that cadmium, zinc, manganese, and calcium ions were enhancing the hydrolysis of ATP, which resulted in the large increase observed in the inorganic phosphate of the reaction medium.

Effect of Substrate.—The results of preliminary experiments indicated that adenosinediphosphate (ADP) was not utilized as a substrate by the liver mitochondria in the presence of zinc, cadmium, manganese, and magnesium ions. However, in the presence of calcium ions, apparently some hydrolysis of ADP may have occurred, but this hydrolysis contributed to less than 20 percent of the total amount of the inorganic phosphate released from either ATP or ADP. These observations were confirmed in later experiments. When either ADP or inorganic phosphate was added to the reaction medium, the total amount of inorganic phosphate released was less than in the presence of ATP alone, as would be expected since ADP and inorganic phosphate are end products of the enzyme reaction. The mitochondrial enzymes did not hydrolyze sodium pyrophosphate. It appears, therefore, that the inorganic phosphate released from ATP is the terminal phosphate group of ATP and conforms to the definition of the ATPase activity as proposed by Potter (1953).

Five micromoles of ATP per ml of reaction medium did not result in a large increase in the hydrolysis of ATP over that shown by 2.5 micromoles of ATP, indicating that 2.5 micromoles of ATP were a suitable substrate level under the conditions of the experiments. Less ATP was hydrolyzed at ATP levels of 1 and of 7.5 micromoles.

Effect of Homogenizing Medium.—The ATPase activities within mitochondria prepared in various media were investigated and are summarized in TABLE 2. Water, 0.04 M sucrose, and 0.9% sodium chloride were poor homogenizing media. Active preparations were obtained from 0.15 M potassium chloride (KCl) and from 0.08 to 0.25 M sucrose solutions. The largest amounts of ATP were hyrolyzed in the presence of cadmium and zinc ions by the mitochondria prepared in 0.25 M sucrose and by the mitochondria prepared from 0.15 M sucrose when

TABLE 1.—Effects of Metal Ions on ATPase.

Ion	micromoles per ml of reaction medium	micromoles PO_4/hr/mgN
None	..	17.4
Cadmium	1	93.4
Zinc	1	69.5
Manganese	1	50.4
Magnesium	1	27.5
Calcium	5	24.3

TABLE 2.—Effects of Homogenizing Media on ATPase.

	micromoles PO_4/hr/mgN				
	Cd	Zn	Mn	Mg	Ca
KCl (0.15M)	85.9	54.9	37.4	29.9	23.4
NaCl (0.9%)	9.9	7.4	10.4	12.3	4.6
Sucrose					
0.44MS	20.2	20.1	28.1	25.7	26.8
0.34MS	12.3	9.9	31.7	24.7	18.7
0.25MS	106.6	98.8	32.7	24.3	20.0
0.15MS	83.9	83.3	49.2	24.6	18.8
0.08MS	66.7	66.2	37.4	23.7	17.3
0.04MS	7.2	11.4	13.0	7.3	4.8
Water	8.1	6.7	9.7	6.3	5.4
0.15MS +EDTA	122.4	74.5	44.7	13.7	16.9

assayed in the presence of manganese ions (TABLE 2). The sucrose concentrations from 0.08 to 0.44 M did not appreciably alter the magnesium ATPase activity. The cadmium ATPase activity was increased approximately 46 percent and the magnesium ATPase activity was inhibited about 50 percent in the mitochondria prepared in 0.15 M sucrose containing 0.001 M ethylenediaminetetraacetic acid (EDTA). Highest calcium ATPase activity was observed in mitochondria prepared in 0.44 M sucrose.

Fractionation studies indicated that all the ATPase activity was present in the mitochondria as very little activity was found in the supernatant fractions when the mitochondria were prepared in 0.15 M sucrose.

These data indicate that the various ATPase activities were altered when mitochondria were prepared in various media. These data confirm that 0.15 M sucrose is a suitable homogenizing medium for bluegill liver mitochondria, as previously proposed (Hiltibran 1965).

Effect of Inhibitors.—The effects of several enzyme inhibitors on mitochondrial ATPase activities are summarized in TABLE 3. The levels reported are comparable to the level of the inhibitors which were utilized in the investigation of oxygen uptake by bluegill liver mitochondria in the presence of succinate (Hiltibran 1965). Sodium azide and p-chloromercuribenzoate inhibited all the ATPase activities. Cyanide, iodoacetate, and fluoride were not effective inhibitors, but fluoride was inhibitory at extremely high levels (15 micromoles).

The effects of oxidative phosphorylation uncoupling agents are summarized in TABLE 3. Arsenate, arsenite, and dicoumarol did not greatly alter any of the ATPase activities. Pentachlorophenol was somewhat inhibitory, but 2, 4-dinitrophenol (DNP) increased the hydrolysis of ATP in the presence of manganese and magnesium. Gramicidin slightly inhibited the zinc ATPase activity, but enhanced the hydrolysis of ATP in the presence of manganese and magnesium ions.

In the investigations of succinic oxidase by bluegill liver mitochondria, all the oxidative phosphorylation uncoupling agents tried except arsenate and arsenite inhibited oxygen uptake, but only arsenate, pentachlorophenol, and DNP severely altered the phosphate uptake (Hiltibran 1965). Gramicidin and dicoumarol inhibited oxygen uptake but did not greatly alter the phosphate uptake and would not be expected to affect the hydrolysis of ATP. This was confirmed. Pentachlorophenol did not alter the hyrolysis of ATP in the presence of magnesium ions and thereby must affect the synthesis of ATP.

The effect of 2,4-dinitrophenol on the succinic oxidase has been reported (Hiltibran 1965) and the

TABLE 3.—Effects of Inhibitors on ATPase.

Inhibitor	micromoles/ml reac. med.	Ave. change in micromoles PO_4/hr/mgN				
		Cd	Zn	Mn	Mg	Ca
Azide	0.15	— 40	— 49	— 34	— 17	— 6
Cyanide	0.15	+ 2	+ 1	+ 1	+ 1	— 0.3
Fluoride	0.15	± 4	± 7	+ 3	± 3	± 1
Iodoacetate	0.15	— 1	± 3	± 19	— 1	± 5
p-Chloromercuribenzoate	0.15	— 76	— 62	— 21	— 16	— 10
Arsenate	0.15	— 10	— 9	± 3	— 7	— 8
Arsenite	0.15	— 6	— 8	— 1	— 5	— 0.5
Dicoumarol	0.15	— 30	— 7	— 11	— 9	— 5
Gramicidin	0.15[1]	— 3	— 18	+ 43	+ 14	+ 0.2
Pentachlorophenol	0.15	— 47	— 34	— 26	— 4	— 3
2,4-dinitrophenol	0.15	± 6	± 16	+ 20	+ 21	± 10

[1] micrograms.

data suggest that DNP interferes with the incorporation of inorganic phosphate into ATP. The magnesium and manganese ATPase activities were increased approximately 88 and 40 percent, respectively. DNP did not greatly alter other ATPase activities. Higher and lower levels of DNP were not particularly more effective. During the investigation of effects of various homogenizing media on the ATPase activity, the effect of DNP on the various ATPase activities was also investigated at levels of 1.5 micromoles.

The cadmium and zinc ATPase activities of mitochondria prepared in 0.25 M sucrose and 0.15 M KCl and the magnesium and manganese ATPase activities of mitochondria prepared in 0.15 M sucrose were increased as well as were the zinc, cadmium, manganese, and magnesium ATPase activities from mitochondria prepared in 0.15 M sucrose containing 0.001 M EDTA. These data suggest that in the presence of magensium ions, the hydrolysis of ATP is increased, and thereby contributes to the uncoupling effect previously noted. Even though ATP hydrolysis in the presence of magnesium ions is increased about 80 percent, this does not appear to account for the large increase in inorganic phosphate content of the reaction medium in the investigations of succinic oxidase of mitochondria. Even though oxygen uptake was reduced about 70 percent, some incorporation of inorganic phosphate would be expected, and apparently dinitrophenol interferes with this incorporation of inorganic phosphate, and effect previously demonstrated by Eisenhardt (1964).

The importance of the cadmium, zinc, manganese, and calcium ATPases is not known. However, since these ions inhibit oxygen uptake and affect the hydrolysis of ATP, these effects on total energy production by fishes would supplement each other by decreasing energy production and promoting the energy loss. Cadmium and zinc ions are relatively toxic to fish, and these data suggest that the toxic effect of these ions might be the result of their effect on energy production.

Of all the inhibitors investigated, only the dinitrophenol effect on the magnesium ATPase activity appears to contribute to the previously reported effect on phosphate uptake. Higher levels of DNP were not any more effective, either on the magnesium ATPase, or on phosphate uptake in the presence of succinate.

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