OCCURRENCE OF A PHOSPHOLIPID-DEPENDENT ADENOSINETRIPHOSPHATASE IN MITOCHONDRIA OF CERTAIN TUMOURS*

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During the course of a study on the effect of phospholipase A on mitochondrial metabolism of normal and tumour tissues, it became apparent that phospholipase A activated the dephosphorylation of ATP in fresh mitochondria from normal rat tissues and completely inhibited this activity in mitochondria separated from Yoshida Sarcoma cells and certain other tumours.This is a brief report of experiments showing the presence of a Ca^{++} activated adenosinetriphosphatase(ATPase) in tumour mitochondria which is firmly bound to the particles and has the properties of a phospholipoprotein. An ATPase having similar properties could not be detected in mitochondria isolated from a variety of normal rat tissues.

METHODS

Rat liver mitochondria were isolated according to Schneider (1948).The ascitic fluid containing Yoshida Sarcoma cells was withdrawn from the intraperitoneal cavity of rats three to five days after transplantation with this tumour. The tumour cells were freed from the blood cells according to McKee et al (1953) and homogenised in 0.25 M sucrose. The procedure for separating the mitochondria was the same as that employed for liver. The mitochondrial fraction contained 80% of the cytochrome oxidase activity of the homogenate. The concentration of RNA was low and DNA content was negligible.

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RESULTS

Experiments designed to explore the effect of phospholipase A more directly on the ATPases of mitochondria required the use of preparations in which the mitochondrial structures had been disrupted. These were obtained by freezing and thawing a suspension of mitochondria and then dialysing it against water at 0 to 2°C for 6 hours.

The ATPase activity of damaged mitochondrial preparations was determined according to Potter et al (1953) in presence of various supplements. The results obtained are given in table 1. The effects of Ca^{++} and Mg^{++} in normal

Table 1

ATPase activity of damaged mitochondria from rat liver and Yoshida Sarcoma Cells

Supplement added	μmoles P liberated	
	Liver	Yoshida Sarcoma
None	0.60	0.56
Mg^{++}	2.09	1.70
Ca^{++}	0.69	1.56
Mg^{++} + phospholipase A	2.05	1.67
Ca^{++} + phospholipase A	0.69	0.21
Mg^{++} + Ca^{++}	-	1.71
Mg^{++} + Ca^{++} + phospholipase A	-	1.76

System: 0.004M ATP, 0.02 M Tris buffer pH 7.4, liver mitochondria (4 mg. protein) or Yoshida Sarcoma mitochondria (15 mg. protein). Supplements: 0.005 M Mg^{++}; 0.001 M Ca^{++}; 1 μg. phospholipase A. Total volume = 1.0 ml. Temp. = 30°C. Period of incubation = 10 minutes.

rat liver mitochondria are in agreement with the properties of damaged mitochondria reported previously (Chance and Williams, 1956). Phospholipase A

prepared from Cobra venom (Suzuki et al 1958)had no effect on normal liver mitochondria. This is expected from results reported previously which have shown that phospholipase A inhibits various enzymic activities in fresh mitochondria by disrupting mitochondrial structure (Aravindakshan and Braganca). In contrast to the effects produced on normal tissue, the ATPase activity of preparations from Yoshida Sarcoma was activated by Ca^{++}. The effect of Ca^{++} was completely inhibited by 1 µg of crystalline phospholipase A. Mg^{++} also activated the ATPase in tumour preparations but as found with normal liver, the activation produced by Mg^{++} was unaffected by phospholipase A. It is also seen that Mg^{++} inhibit the Ca^{++} activated ATPase. This is evident from results which show that in systems containing Mg^{++} and Ca^{++} the activity was similar to the one obtained with Mg^{++} alone. In addition, phospholipase A had no effect on the system containing the two ions together.

The destructive action of phospholipase A on Ca^{++} activated ATPase of tumour preparation suggested that it may be a phospholipoprotein. In conformity with this view, the Ca^{++} activated enzyme was found to be firmly bound to mitochondrial fragments. Washing the sediment three times with Tris (hydroxymethyl) aminomethane (TRIS) buffer was ineffective in extracting the activity in the aqueous phase. The enzyme was solubilised by using organic solvents such as n-butanol under conditions generally employed for extracting lipoprotein (Basford and Green, 1959). The procedure consisted in suspending the washed mitochondrial sediment in a mixture of sucrose and desoxycholate. The dispersion obtained was treated with n-butanol. The two phases were separated and dialysed against water at 0 - 2°C. The ATPase activities in n-butanol and in the aqueous layer are given in table 2. The Ca^{++} activated ATPase present in tumour mitochondria is found entirely in the n-butanol extract and none could be detected in the aqueous phase. A 13 fold increase in specific activity was found in the final extract. The activity was completely inhibited by 1 µg. of phospholipase A. ATPase stimulated by Ca^{++} is totally absent in extracts obtained from normal liver. An ATPase activated by Mg^{++} was detected in n-butanol extracts prepared from

normal liver as well as from tumour mitochondria. As expected this enzyme was not inhibited by phospholipase A. The specific activity of the Mg^{++}-activated ATPase in n-butanol extract increased by a factor of 4. Although n-butanol extraction did not effect a separation of the two ATPases found in mitochondria from Yoshida Sarcoma, there was an increase in the concentration of the Ca^{++} stimulated enzyme in relation to the other.

Table 2

ATPase activity of n-butanol extract prepared from mitochondria

Enzyme preparation	Specific ATPase activity (μmoles P/mg.protein			
	Liver		Yoshida Sarcoma	
	Mg^{++}	Ca^{++}	Mg^{++}	Ca^{++}
Mitochondria (aged)	0.55	Nil	0.13	0.11
n-butanol extract	3.20	Nil	0.42	1.45
Aqueous layer	Nil	Nil	Nil	Nil
n-butanol extract + phospholipase A	3.16		0.46	Nil

System same as in Table 1.

The Ca^{++} activated ATPase present in n-butanol extract from Yoshida Sarcoma cells could not dephosphorylate uridine triphosphate, adenosine-monophosphate (muscle), adenosine diphosphate and ∝-glycerophosphate.

DISCUSSION

The findings reported here demonstrate that mitochondria separated from Yoshida Sarcoma cells contain an ATPase firmly bound to particles, which is stimulated by Ca^{++}. This enzyme is presumably in combination with a phospholipid component whose integrity is essential for its activity. These properties could explain the inactivation brought about by small amounts of phospholipase A and also its solubility in n-butanol. A similar enzyme was detected in rat hepatomas induced by feeding dimethyl-aminoazobenzene as well as in a spontaneous mammary tumour from dba(Bar)

mice. A phospholipid-dependent ATPase that was Mg^{++} activated has been described by Kielley and Meyerhof (1950) in muscle homogenate preparations. In contrast, mitochondria separated from normal rat liver, kidney and brain, as well as heart sarcosomes failed to show the presence of Ca^{++} stimulated ATPase. Mg^{++} activated ATPase of the type described by Kielley and Kielley (1953) is apparently present in both normal and tumour mitochondria. Earlier studies on ATPases of normal and tumour tissues have indicated certain differences. Novikoff et al (1951) working with homogenates observed that the ATPase in tumour could be activated by Mg^{++} and Ca^{++} whereas in normal tissues only Mg^{++} were effective. These observations can well be explained on the basis of the mitochondrial ATPases described here.

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