Biochimica et Biophysica Acta, 590 (1980) 170—181

BBA 47813

OXIDATIVE PHOSPHORYLATION AND ATPase ACTIVITIES OF HUMAN TUMOR MITOCHONDRIA

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(Received July 23rd, 1979)

Key words: Oxidative phosphorylation; Mg^{2+}-ATPase; (Human tumor mitochondria)

Summary

Studies were carried out with intact mitochondria isolated from human astrocytoma, oat cell carcinoma and melanoma which were propagated in athymic mice. These human tumor mitochondria were capable of coupled oxidative phosphorylation. They also showed significant uncoupler-stimulated ATPase if defatted bovine serum albumin was included in the assay media. However, the uncoupler response curves were different and the magnitude of the ATPase activity was lower than could be obtained with mitochondria of a normal tissue, such as liver. Some of these characteristics were also exhibited by mitochondria from several animal hepatomas and Ehrlich ascites tumor. In the three tumors studied, mitochondria from oat cell carcinoma were more labile, whereas higher respiratory control ratios and greater stimulation of ATPase by uncouplers were obtained with melanoma mitochondria.

The mitochondrial ATPase was not the major cellular ATPase in any of the three tumors. This was indicated by a low inhibition of the ATPase activity of tumor cell homogenates by oligomycin. A very large fraction of the cellular ATPase activities was recovered in the microsomal fractions.

Introduction

Many fast growing malignant tumors exhibit increased glycolytic activity. The shift to this less efficient energy producing pathway in cancer cells has prompted many investigators to examine tumor mitochondria to search for

Abbreviations: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone.

lesions in mitochondrial functions which might lead to the dominance of glycolysis. The most popular model system for these studies has been the animal hepatomas since the host liver serves as the control. Much data have also been accumulated for mitochondria isolated from Ehrlich ascites tumor. Results from these studies indicated that tumor mitochondria are capable of coupled oxidative phosphorylation [1—3]. Several unusual properties of tumor mitochondria have, however, been noted. These include a general reduction in the content of mitochondria (for review see Ref. 4), and increased ability to retain calcium once it is accumulated [1,5], a high rate of ΔH^+ back decay [6], alteration of phospholipid content and composition [7], altered activity and amount of several mitochondrial enzymes, e.g. ATPase [8], cytochrome oxidase [9], adenine nucleotide translocase [10], iron-sulfur proteins and dicarboxylate transport [11]. A phenomenon which has been repeatedly observed with tumor mitochondria is the insensitivity of the latent ATPase of freshly prepared mitochondria to uncoupler stimulation [2,11—13]. These results were rather puzzling in view of the fact that other energy-linked reactions of tumor mitochondria were affected by these agents in a normal fashion. Recently, Barbour and Chan [14] have shown that the mitochondrial ATPase activity of a hepatoma and Ehrlich ascites tumor can be stimulated by dinitrophenol many fold if mitochondria were isolated in the presence of large amounts of defatted bovine serum albumin. Bovine serum albumin binds free fatty acid which appears to be more abundant in tumor tissues. Excess free fatty acids apparently induce leakage of magnesium from the tumor mitochondria and also inhibit adenine nucleotide translocase activity, thus preventing the full expression of the latent ATPase under the influence of uncouplers.

The high glycolytic activity of malignant cells has also been attributed to uncoupled cellular ATPases normally involved in energy transduction [15]. Evidence supporting this hypothesis came from experiments in which glycolysis of transformed cells could be inhibited by specific inhibitors of various ATPases [16—18]. Similar experiments also indicated that the uncoupled ATPase could be different in different cell lines. An apparent 'ATPase activity' such as that resulting from the very active mitochondrially bound hexokinase in some tumor cells [19] should also be taken into consideration.

We report here experiments describing properties of mitochondria from human tumors as part of our systematic investigation of membrane bound ATPases which are implicated in the energy metabolism of these tumor cells.

Materials and Methods

Human tumor materials. The three human tumors used in this study were an astrocytoma, an oat cell carcinoma and a melanoma. The original tumor samples came from patients in the San Diego area in 1974-1976 and have been maintained by serial transplantation in the Balb/nu athymic mice ever since as described by Reid et al. [20]. The astrocytoma used in this study were from the 24th to the 35th passage. The oat cell carcinoma were from the 11th to 15th passage. Melanoma used were from tumors obtained from injecting cultured cells into athymic mice. The cells came originally from a melanoma of the 15th passage and were subcultured in Dulbecco's MEM/F-12 media for more than 60 passages.

Isolation of tumor mitochondria and other subcellular fractions. After cervical dislocation of the animals, tumors were removed and placed in ice-cold buffer containing 0.225 M mannitol-0.075 M sucrose-2 mM Hepes, pH 7.4-0.1 mM EDTA and 1 mg defatted bovine serum albumin per ml. Necrotic and hemorrhagic tissues were removed with surgical blades. The remaining tissue was rinsed with isolation buffer, blotted dry, weighed and minced finely using scissors. The minced tissue was homogenized with 2.5 volumes of isolation buffer in a Potter-Elvejhem homogenizer. In the case of melanoma, an additional 1.5 volumes of isolation buffer was added at this point. The tumor homogenates were filtered through two layers of cheesecloth before centrifugation at 700 × *g* for 10 min. The pellet was resuspended in the original volume of isolation buffer and centrifuged again at 700 × *g* for 10 min. The pellet consisted mainly of nuclear material. The supernatant solution from the two centrifugation steps were combined and centrifuged at 7700 × *g* for 10 min. This mitochondrial pellet was washed twice with isolation buffer of 1/2 and 1/4 the homogenate volume. The pellet obtained after the final washing consisted of a light layer which poured off rather easily and a heavy layer of mitochondria.

The post-mitochondrial supernatant was centrifuged at 60 000 × *g* for 90 min to obtain the microsomal fraction.

Respiratory control and P/O ratio determination. Respiratory control (or acceptor control) ratios and P/O ratios were determined polarographically as described by Chance and Williams [21]. Respiration measurement was carried out with 1.5—6 mg mitochondrial protein at room temperature (20°—23°C) in a closed reaction vessel (1.8 ml capacity) equipped with a Clark oxygen electrode. In addition to the isolation buffer, the respiration medium contained 15 mM potassium phosphate, pH 7.4 and 0.5 mM EDTA. Substrates (succinate or glutamate) were 5.6 mM when added.

ATPase assays. ATPase of coupled mitochondria was determined in a reaction mixture containing 50 mM Tris-HCl, pH 7.4, 50 mM sucrose, 75 mM KCl and 1 mM EDTA. Mitochondria (250—500 μg) were preincubated in this medium with uncouplers at 22°C for 5 min before the reaction was initiated by the addition of 5 μmol ATP. After incubation for 10 min at 37°C, the reaction was terminated by the addition of 0.1 ml of 50% trichloroacetic acid. After removal of denatured protein by centrifugation, inorganic phosphate was determined on an aliquot of the supernatant solution using the procedure of Lohmann and Jendrassik [22].

ATPase activity of frozen and thawed tumor mitochondria and other subcellular fractions was determined in a reaction mixture containing 50 mM Tris-HCl, pH 7.4, 5 mM $MgCl_2$ and 5 mM ATP. Reactions were carried out at 37°C for 10 min.

Succinate-cytochrome c reductase assay. Succinate-cytochrome c reductase activity was determined according to King [23] at 37°C.

Materials. Bovine serum albumin (Fraction V from Sigma) was defatted according to Chen [24]. All other chemicals were obtained from Sigma Chemical Co., St. Louis, MO.

Results

ATPase profiles of human tumors. Table I shows the distribution of Mg^{2+}-activated ATPase activities among the subcellular fractions of tumor homogenate and their sensitivity to oligomycin, a specific inhibitor of mitochondrial ATPase. We have not attempted to tailor different separation procedures for the individual tumor tissues and it is obvious that there is some cross contamination of the various membrane fractions. The data are presented here to illustrate several points: (1) The mitochondrial ATPase constitutes a relatively small portion of the total cellular ATPase in all three human tumors. The ATPase in the tumor homogenate is only inhibited about 20% by oligomycin. This low sensitivity is in marked contrast to that observed with liver, a tissue rich in mitochondrial ATPase. In liver homogenates, the oligomycin sensitivity of the total ATPase can be as high as 80%. (2) All three tumors possess a large fraction of Mg^{2+} activated ATPase which sediments in the microsomal fraction. (3) The partial inhibition of the tumor mitochondrial ATPase by oligomycin is attributed to contamination by the microsomal fractions. Although the separation of these two membrane fractions can be achieved by centrifugation through discontinuous Ficoll or sucrose gradients, the resulting purified mitochondria are completely uncoupled and are useless for the study of energy-linked functions.

Oxidative phosphorylation of human tumor mitochondria. Mitochondria from all three human tumors are well coupled. Fig. 1 shows that the respiration of tumor mitochondria (astrocytoma) with either succinate or glutamate as substrate are regulated by ADP and can be uncoupled by the addition of dinitrophenol. Table II summarizes the results of several experiments carried

TABLE I

DISTRIBUTION OF ATPase ACTIVITIES IN SUBCELLULAR FRACTIONS OF HUMAN TUMOR TISSUES

Subcellular fractions	Astrocytoma			Oat cell carcinoma			Melanoma		
	S.A.	%	% inhib. by olig.	S.A.	%	% inhib. by olig.	S.A.	%	% inhib. by olig.
Homogenate	87.6	100	16.7	131	100	20.7	135	100	26.5
Nuclei	74.6	22.3	27.0	82.7	34.2	21.5	156	41.5	46.7
Mitochondria									
Heavy	301	5.9	45.5	328	4.3	55.0	302	3.7	68.3
Light	295	10.8	10.2	235	4.0	40.0	403	11.0	38.8
Microsomes	288	39.9	4.0	481	55.5	4.1	258	35.7	9.3
Supernatant	7.4	4.0	0	11.1	4.4	0	0	0	0

Subcellular fractions were obtained from homogenate (28% homogenate for astrocytoma and oat cell carcinoma and 20% for melanoma) by the procedure described in Methods and Materials. ATPase was determined with fractions which had been frozen at —60°C and then thawed. Specific activity (S.A.) is expressed in nmol P_i released per min per mg protein. ATPase was determined at 37°C for 10 min in a reaction mixture containing 50 mM Tris-HCl, pH 7.4, 5 mM $MgCl_2$, 5 mM ATP and 2 μg oligomycin when present.

TABLE II

RESPIRATORY CONTROL AND ADP/O RATIOS OF ISOLATED MITOCHONDRIA FROM HUMAN TUMORS

Oxidation of succinate or glutamate was assayed polarographically. Respiratory control ratio (RCR) is defined as the ratio of the rate of oxygen consumption in the presence of ADP to the rate of oxygen consumption after ADP is exhausted. Oxidase activity is the rate of oxygen consumption in the presence of ADP (55—270 μM) or 0.1 mM dinitrophenol. Data include the range of values observed and the average value (in parentheses).

Mitochondria from	No. of experiments	Substrates	RCR	ADP/O	Oxidase (nmol/min/mg)
Astrocytoma	6	Succinate	2.0—2.8 (2.36)	1.5—2.0 (1.72)	46—114 (66)
	4	Glutamate	2.6—3.9 (3.55)	1.8—2.5 (2.19)	19—61 (33)
Oat cell carcinoma	4	Succinate	1.5—2.1 (1.90)	1.3—2.0 (1.63)	51—117 (80)
	4	Glutamate	2.3—4.2 (3.36)	2.1—2.7 (2.42)	18—78 (42)
Melanoma	6	Succinate	2.0—4.8 (2.7)	1.3—2.0 (1.67)	29—83 (62)
	6	Glutamate	2.7—8.0 (4.0)	1.9—2.7 (2.31)	18—67 (36)
Liver	1	Succinate	4.1	1.72	141
	1	Glutamate	4.6	2.38	51.7

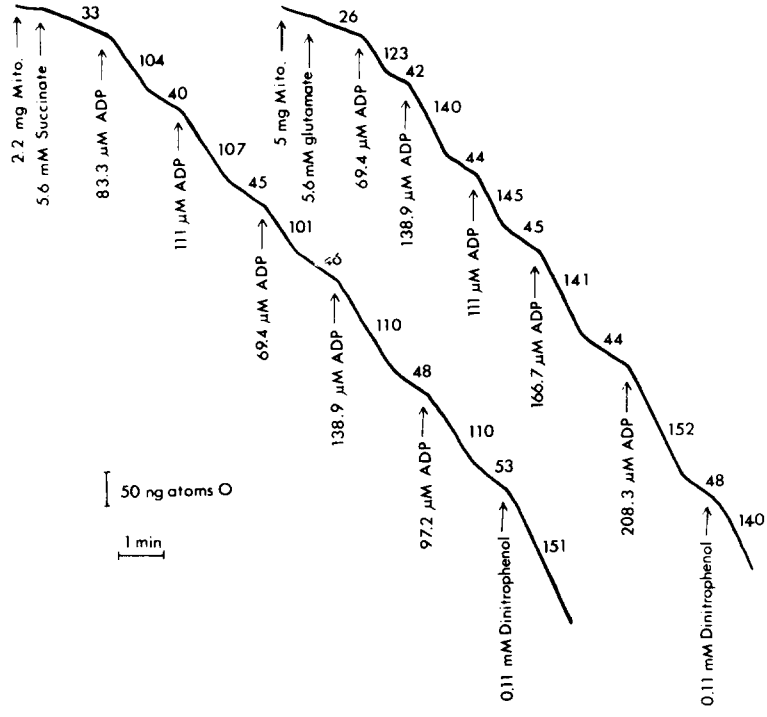

Fig. 1. Oxidative phosphorylation properties of mitochondria from human astrocytoma. Assay conditions were as described in Methods and Materials. Respiration rates in ngatoms oxygen/min/mg protein are indicated by the numbers next to the oxygraph traces.

out with mitochondria from the three tumors. Data with liver mitochondria are included for comparison as this is the most extensively studied system. It can be seen from Table II that the ADP/O ratios of tumor mitochondria vary slightly, yielding values close to 1.5 with succinate as substrate and 2.5 with glutamate as substrate. The respiratory control ratios are low in comparison with liver mitochondria, especially with succinate as substrate. However melanoma mitochondria consistently exhibit higher respiratory control ratios. Oxidase activities of all three tumor mitochondria are less than that obtained with liver mitochondria reflecting the impurity of these fractions.

Effect of uncouplers on the latent mitochondrial ATPase. Both dinitrophenol and CCCP stimulate the latent ATPase of the human tumor mitochondria to varying degrees. Table II shows that the stimulation is rather small in comparison to liver mitochondria. Under the assay conditions described in the legend of Table III, mitochondria from oat cell carcinoma occasionally shows no stimulation by the uncouplers and inhibition of ATPase was observed at

TABLE III

UNCOUPLER STIMULATED ATPase OF HUMAN TUMOR MITOCHONDRIA WHEN ASSAYED IN THE ABSENCE OF ALBUMIN

In each experiment, the mitochondrial ATPase activities were determined at several uncoupler concentrations. The maximal activity observed was recorded for this table and the % stimulation was calculated from the maximal activity and the activity obtained in the absence of any uncouplers. Maximal ATPase activities of tumor mitochondria were usually elicited by 20—100 μM dinitrophenol and 0.05—0.2 μM CCCP. Liver mitochondrial ATPase was stimulated maximally by 300—500 μM dinitrophenol and 0.2—0.5 μM CCCP.

Mitochondria from	Maximal % stimulation by		Maximal uncoupler induced ATPase (nmol/min/mg)	
	Dinitrophenol	CCCP	Dinitrophenol	CCCP
Astrocytoma				
15	280	283	133	134
16	109	118	135	146
17	165	136	112	98
24	250	224	113	101
31	193	170	83	72
Oat cell carcinoma				
2	— *	— *	166 *	
4	208	177	133	113
5	— *	— *	80 *	
6	147	170	89	98
7	131	132	137	138
9	141	145	131	135
Melanoma				
2	370	420	73	84
3	287	283	109	108
7	454	419	146	129
8	310	247	172	137
Liver	856		580	
	846	763	487	440
	1245	844	912	618
	848		609	

* In these experiments, no stimulation was observed at any uncoupler concentrations.

TABLE IV

EFFECT OF DEFATTED BOVINE SERUM ALBUMIN IN THE ASSAY MEDIUM ON UNCOUPLER STIMULATED MITOCHONDRIAL ATPase OF HUMAN TUMOR MITOCHONDRIA

Uncoupler stimulated ATPase of intact mitochondria was determined at several dinitrophenol concentrations (0—200 μM) in the absence and presence of 1 mg defatted albumin per ml. The dinitrophenol concentrations which caused maximal stimulation and the highest ATPase activities were recorded in this table.

Mitochondria from	Albumin	Dinitrophenol (μM)	ATPase activity (nmol/min/mg)
Astrocytoma	—	0	55.8
		40	107
	+	0	26.2
		40	113
Oat cell carcinoma	—	0	94
		5	131
	+	0	47
		15	149
Melanoma	—	0	55
		15	172
	+	0	19.4
		40	191

all uncoupler concentrations studied. In contrast to oat cell carcinoma, the ATPase of the melanoma mitochondria is stimulated 3—4-fold by the uncouplers.

Effect of bovine serum albumin on uncoupler-stimulated ATPase. Stimula-

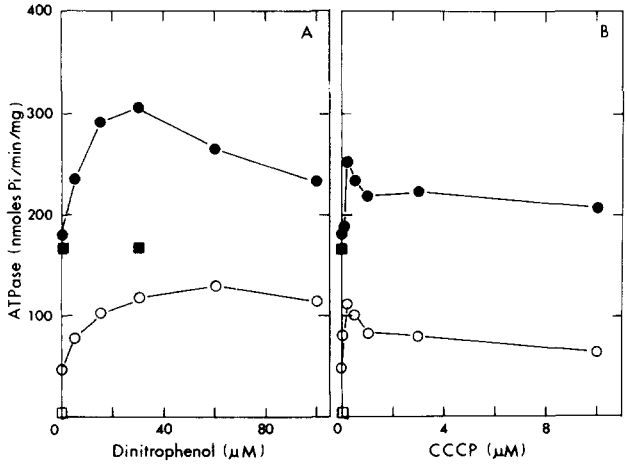

Fig. 2. Uncoupler-stimulated ATPase of astrocytoma mitochondria. ATPase activity of intact astrocytoma mitochondria was determined in a reaction mixture containing 50 mM Tris-HCl, pH 7.4, 50 mM sucrose, 75 mM KCl, 1 mM EDTA and 1 mg/ml albumin in the absence (○) and presence (●) of 5 mM $MgCl_2$. ATPase activity was also determined in the presence of 2 μg oligomycin with (■) and without (□) Mg^{2+}. Usually, the ATPase activities obtained in the presence of oligomycin was the same whether uncoupler was present or not. Uncoupler used was dinitrophenol (A) and CCCP (B).

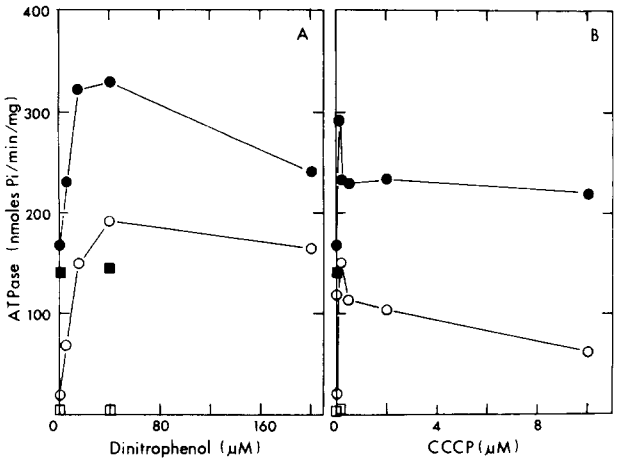

Fig. 3. Uncoupler-stimulated ATPase of melanoma mitochondria. Conditions were the same as described in the legend of Fig. 2.

tion of ATPase by uncouplers can be further enhanced if albumin is also included in the assay medium. Table IV shows that albumin lowers the ATPase activity in the absence of any uncouplers. These results indicate that in the absence of albumin, the mitochondrial ATPase is already partially unmasked without the addition of exogenous uncouplers. In the presence of albumin, the latent ATPase of astrocytoma and oat cell carcinoma mitochondria is stimulated 3—4-fold while the stimulation of melanoma mitochondrial ATPase is as high as 10-fold.

Effect of Mg^{2+} on the uncoupler stimulation of mitochondrial ATPase. The tumor mitochondria preparation is contaminated with microsomes which contain an active Mg^{2+}-ATPase. For this reason, the stimulation of mitochondrial ATPase by uncouplers was determined in the absence of Mg^{2+}. If Mg^{2+} were

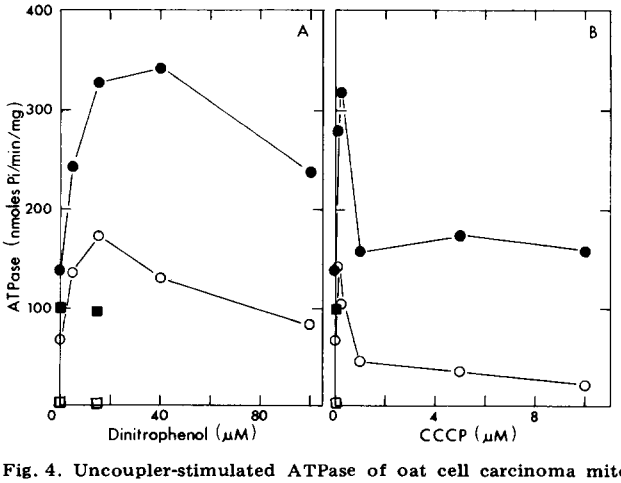

Fig. 4. Uncoupler-stimulated ATPase of oat cell carcinoma mitochondria. Conditions were the same as described in the legend of Fig. 2.

lost from the mitochondria during their isolation or under the conditions of assay, the measured ATPase values will be an underestimate of the true value. When the uncoupler effect was determined in the presence of Mg^{2+} the results shown in Figs. 2—4 were obtained. As expected, the background ATPase was elevated in the presence of Mg^{2+}. However, when the oligomycin insensitive ATPase was subtracted, the maximal activities for uncoupler stimulation were the same for mitochondrial ATPase of astrocytoma and melanoma (Figs. 2 and 3). This was not the case for mitochondria from oat cell carcinoma. The uncoupler stimulated ATPase activity was higher when measured in the presence of Mg^{2+}. From Fig. 4A, the oligomycin sensitive ATPase activity was 240 nmol/min/mg in the presence of Mg^{2+} and 170 nmol/min/mg in the absence of Mg^{2+}.

Discussion

Propagation of human tumors. Biochemical studies of human tumors have been hampered by the fact tha reproducible and conveniently large quantitites of tumor material have been difficult to obtain. This problem has been alleviated by the use of athymic mice. Due to their immunologic deficiency, these animals are able to accept xenografts. Human tumors can be maintained over an extended period of time in these animals. The three human tumors chosen for this study originated from a grade IV astrocytoma, an oat cell lung carcinoma and a melanoma. These three tumors have fairly fast growth rate in the athymic mice, and are suitable for biochemical studies where isolation of cellular components is required. The tumors usually contain a certain amount of stromal material from host. From lactate dehydrogenase isozyme determination by starch gel electrophoresis, it can be concluded that the stroma represents less than 10% of the tissue. We feel, therefore, that it is safe to conclude that the characteristics of the biochemical reactions we are studying are representative of the human tumors.

In our investigation of the three human tumors, we hope to achieve a better understanding of the mode of ATP synthesis and utilization in cancer cells, as well as an indication of possible candidates of uncoupled ATPases which have been suggested to be responsible for the high glycolytic rates of malignant cells. The results reported in this paper summarize our findings on the mitochondrial functions of the human tumors.

Purity of tumor mitochondria. The yield of mitochondria from the three human tumors was very low. The protein recovered in the mitochondrial fraction was 2—4% of the homogenate for astrocytoma and oat cell carcinoma and approximately 7% for melanoma. It should be quite clear from the text that the tumor mitochondria preparation was not entirely pure. Although a similar isolation and centrifugation scheme was used for hepatoma and Ehrlich ascites tumor, these investigators did not encounter such difficulty in their mitochondria isolation. It is noted, however, that the mitochondria from Novikoff hepatoma are smaller than liver mitochondria and are not sedimented free of smaller particles [25]. This appears to be the reason behind the incomplete separation of mitochondria and microsomes in this study. Further purification through sucrose or Ficoll gradients usually resulted in loss of respiratory

control, therefore the tumor mitochondria were generally used immediately after isolation. The purity of the mitochondria ranged from 30 to 60%.

Lability of human tumor mitochondria. The human tumor mitochondria share a characteristic with the various hepatoma mitochondria in that they are labile. In the case of rat hepatomas, the lability is sometimes indicated by low P/O ratios [26,27] but is most often expressed as a low magnitude response of the mitochondrial ATPase to uncouplers [2,11—14,27—29], probably because P/O ratio determinations are usually carried out at lower temperatures, with larger amount of mitochondrial protein and usually with albumin in the assay medium, conditions conducive to the stability of the mitochondria. The ATPase determination is usually performed at higher temperatures with smaller amounts of mitochondrial proteins and no albumin in the assay medium.

The human tumor mitochondria are capable of coupled oxidative phosphorylation although the respiratory control ratios are slightly lower than that observed with the liver mitochondria, especially with succinate as substrates. The lability of the human tumor mitochondria is more obvious when the uncoupler stimulated ATPase is examined in detail.

Uncoupler stimulated ATPase of human tumor mitochondria. As mentioned preivously, reports from many laboratories showed mitochondria from several hepatomas and Ehrlich ascites tumors have had a reduced capacity to hydrolyze ATP in the presence of uncouplers. Pedersen and Morris [2] showed that the tumor mitochondrial ATPase from Morris hepatomas 7800, 16 and 9618A was stimulated to a small extent at very low concentrations of dinitrophenol ($\sim$10 μM) but the activity was inhibited at higher uncoupler concentrations. Recent reports showed that the inhibition of the mitochondrial ATPase of hepatoma 7800 at high uncoupler concentrations could be abolished if ATP was added before dinitrophenol [3,4]. On the other hand, Barbour and Chan [14] have found that the deficiency of uncoupler stimulation of the mitochondrial ATPase of hepatoma BW7756 and Ehrlich ascites tumor could be remedied by the isolation of the tumor mitochondria in media containing 2 mg/ml albumin to quench endogenous uncouplers.

We found that the human tumor mitochondria also showed a small response to uncouplers (Table III) which could be enhanced by the addition of albumin to the assay media (Table IV). The effect of albumin here appeared to counteract some endogenous uncouplers which are produced at the assay temperature (37°C).

The uncoupler effect curves of the mitochondrial ATPase of all three human tumors toward dinitrophenol and CCCP differ from that obtained with liver mitochondria but are quite similar to that obtained with hepatoma mitochondria [2]. The ATPase is stimulated maximally at very low dinitrophenol (25—50 μM) and CCCP (0.2—0.5 μM) concentrations which do not elicit maximal activity in liver mitochondria. The uncoupler-stimulated ATPase of all three tumors is usually inhibited at higher uncoupler concentrations. This is more noticeable with CCCP (Fig. 2B, 3B and 4B). CCCP is considered to be a more effective uncoupler, yet the inhibition of ATPase at high CCCP concentrations is not observed with liver mitochondria under similar assay conditions. The stimulated ATPase of liver mitochondria is sustained up to CCCP concentration

of 100 μM. As the Mg^{2+}-ATPase of freeze-thawed mitochondria is not inhibited by high concentrations of CCCP, this phenomenon is a characteristic of the ATPase of intact tumor mitochondria. In one experiment with astrocytoma mitochondria, we found that this response to uncouplers was not altered by adding ATP prior to uncouplers nor was it altered significantly by inclusion of albumin in the assay media.

When we compared the maximal activity obtained by uncoupler stimulation and the oligomycin sensitive Mg^{2+}-ATPase after the mitochondria were disrupted by freezing and thawing, we found there was good agreement between the two values. These results imply that the adenine nucleotide translocase functions properly in freshly prepared tumor mitochondria and is not rate limiting for the overall ATPase reaction. The activities of the oligomycin sensitive ATPase of the three tumor mitochondria are low (150—250 nmol/min/mg), so are the succinate-cytochrome *c* reductase activities. In view of the fact that the tumor mitochondrial preparations were not pure and data on the mitochondrial ATPase of the normal, control tissues were not available, we cannot be certain if these tumor mitochondria have lower ATPase activities than their normal counterparts as was found for many tumor mitochondria [8,14].

Differences among the tumor mitochondria. Thus far, we have emphasized the common features shared by the mitochondria from the three human tumors and hepatomas. However, the mitochondria from the three human tumors clearly differ in their lability. Melanoma mitochondria exhibit higher respiratory control ratios and stimulation of ATPase by uncouplers, but, oat cell carcinoma mitochondria show a small leakage of magnesium even when albumin is present in both isolation and assay media. If the lability is contributed solely by endogenous free fatty acids, these results suggest that oat cell carcinoma has unusually active lipid degradation. An abnormally high concentration of phosphorylglycerylcholine, a degradation product of phosphatidylcholine, has been observed in the perchloric acid extracts of astrocytoma as determined by ^{31}P-NMR [29].

The observation that the mitochondrial ATPase is a minor component of the cellular ATPases of the three human tumor cells probably reflects the property of the cell from which the tumor was derived. For obvious reasons, data from such controls are not obtainable. Nevertheless, it was noted that in some hepatomas [25—27], the number of mitochondria was reduced in comparison to the host liver cells. We emphasize that although some obvious defects of the tumor mitochondria can be remedied by precautions taken during isolation or measurement of activities, there is no doubt that the tumor mitochondria are more labile and the mitochondrial ATPase may be uncoupled in vivo in certain tumors cells.

A most interesting finding which resulted from this study was the large ATPase activity detected in the microsomal fractions of the three tumors. The microsomal fraction is actually a mixture of plasma membrane, endoplasmic reticulum and other minor membrane components. Our preliminary experiments have shown that this ATPase was activated by Ca^{2+} as well as Mg^{2+}. This loss of selectivity between Ca^{2+} and Mg^{2+} has previously been noted by Novikoff in the microsomal ATPase of a hepatoma [25]. The nature and the role of this ATPase is currently under investigation.

Acknowledgements

This research was supported by grants from U.S. Public Health Service (CA-11683, GM-17702) and a grant from American Cancer Society (BC-60). We also wish to thank the Athymic Mouse Facility Grant (CA-23052) for support.

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