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COMPARISON OF THE ADENINE NUCLEOTIDE TRANSLOCASE IN HEPATOMAS AND RAT LIVER MITOCHONDRIA

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Summary

Various biochemical properties of the adenine nucleotide translocase were compared with mitochondria prepared from control and host liver, and Morris hepatomas 7777, 7800 and 5123C. The transport of phospho*enol*pyruvate on the adenine nucleotide translocase was found to be three to four times more active, and inhibition of the transporter by palmitoyl-CoA and atractylate considerably less in hepatoma than in host liver mitochondria. The active transport of phospho*enol*pyruvate was associated with a greater stimulation of calcium egress from the mitochondrial matrix by the anion in the hepatoma. The diminished sensitivity of the adenine nucleotide translocase to palmitoyl-CoA in hepatoma mitochondria was associated with lower levels of long chain acyl-CoA esters in the whole tissue. A change in activation energy at 6°C for the adenine nucleotide translocase was found in host liver mitochondria while no break point in the temperature curve was observed in hepatoma mitochondria. These results are most consistent with a change in the structure-function relationship of hepatoma mitochondria due to differences in lipid composition.

Introduction

Previous work carried out in this laboratory showed that under certain conditions phospho*enol*pyruvate could be transported across the inner membrane of liver and heart mitochondria on the adenine nucleotide translocase [1,2]. Whereas at low temperatures (0—4°C) the adenine nucleotides are translocated

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at maximum rates and the transport of phospho*enol*pyruvate is relatively slow, at higher temperatures (37°C) it was noted that phospho*enol*pyruvate transport could be significantly increased [2]. This latter finding suggested that a change in the fluidity of the inner mitochondrial membrane could affect the activity and specificity of the membrane-bound adenine nucleotide translocase. Inhibition of the adenine nucleotide translocase by inhibitors such as atractylate and long chain acyl-CoA esters requires their tight binding to a specific receptor site which might also be modified by the physical state of the membrane lipids [3].

Structural alterations in membranes from tumor tissue have been reported to result from qualitative and quantitative differences in the lipid constituents [4,5]. In some cases there are associated changes in both plasma and mitochondrial membrane-bound enzymes, particularly when transition temperatures are measured [5,6]. In the present communication various biochemical properties of the adenine nucleotide translocase are examined and compared in mitochondria from normal liver and a line of hepatomas. The results show differences which are discussed in terms of the structure-function relationship of the mitochondria.

Materials and Methods

Experimental animals. Morris hepatomas maintained at the McArdle Laboratory, University of Wisconsin were implanted intramuscularly in the hind legs of the Buffalo strain male rates. Animals bearing tumors 7777, 7800 and 5123C were killed at an average of 2,4 and 6 weeks, respectively, from the time of implantation of the hepatomas. Capsular and necrotic tissues were carefully removed from the hepatomas before preparation of mitochondria.

Preparation of mitochondria. Mitochondria were isolated from liver and hepatomas by the standard procedure of Schneider [7]. All mitochondria employed in these studies exhibited an acceptor control ratio of above 3.5 with succinate.

Assay methods. [^{14}C]ADP translocation was carried out by the forward excahnge reaction essentially as previously described [8]. Radioactivity in the pellet dissolved in tissue solubilizer (Soluene) was determined in a Packard liquid scintillation spectrometer.

[^{14}C]ATP exchange with extramitochondrial ATP or with phospho*enol*pyruvate was measured by the back exchange technique of Pfaff and Klingenberg [9] and Henderson et al. [10] using approx. 100 mg of mitochondrial protein. Solubilization and preparation of the precipitate for counting were carried out as above. Any non-specific transport or leakage of adenine nucleotides across the inner mitochondrial membrane was corrected for by subtracting the values of the zero time atractylate control. The calculation of the percentage of exchange was made as described by Kleineke et al. [11].

The procedure for calcium efflux was essentially the same as that described previously [2] with calcium uptake and efflux followed by ^{45}Ca.

Extraction and determination of tissue levels of long chain fatty acyl-CoA esters was carried out in host liver and hepatoma tissue quickly frozen in liquid nitrogen by the method of Williamson and Corkey [12]. The free CoA was assayed using the phosphotransacetylase system [13].

Protein was determined by the biuret procedure [14].

Unless otherwise stated all experiments reported were carried out in duplicate at least three separate times. Data presented in the tables and figures without standard deviation represent the average values of a representative experiment. Additional information is included in the legends to the figures and tables.

Animals and reagents. Buffalo rats were purchased from Simonson Laboratories, Gilroy, CA.

Chemicals. [U-^{14}C]ADP and [U-^{14}C]ATP were purcahsed from Amersham Searle; $^{45}CaCl_2$ from New England Nuclear; palmitoyl-CoA and CoASH from P-P-L Biochemicals; atractylate, lubrol WX, phospho*enol*pyruvate, acetyl phosphate, phosphotransacetylase and dithiothreitol from Sigma. All other reagents were of the highest grade commerically available.

Results

Most of the results reported herein were obtained using the poorly differentiated hepatoma 7777. However, some studies were also carried out with the more slowly growing tumors 7800 and 5123C.

It has been peviously reported that phospho*enol*pyruvate can be transported, albeit at a slower rate than ATP and ADP, on the adenine nucleotide translocase of liver and heart mitochondria [1,2]. The present results indicate that the translocase from hepatoma mitochondria possesses similar though distinctly modified properties for transport of phospho*enol*pyruvate. The assay was run at 28°C to maximize the translocation of internal ATP with externally added phospho*enol*pyruvate. Under the experimental conditions used, it can be observed (Table I) that the transport of phospho*enol*pyruvate is 3—4-fold higher in the hepatoma mitochindria at all concentrations of phospho*enol*-

TABLE I

EFFECT OF PHOSPHO*ENOL*PYRUVATE AND PALMITOYL-CoA CONCENTRATIONS ON PHOSPHO*ENOL*PYRUVATE EXCHANGE WITH INTRAMITOCHONDRIAL [^{14}C]ATP IN HOST LIVER AND HEPATOMA 7777

[^{14}C]ATP back exchange with phospho*enol*pyruvate was carried out as described in Materials and Methods. Following preincubation of 1 mg [^{14}C]ATP-loaded mitochondrial protein with palmitoyl-CoA for 4 min, the reaction was initiated by adding phospho*enol*pyruvate and after 2 min at 28°C the reaction was stopped with atractylate.

Phospho*enol*pyruvate (mM)	Palmitoyl-CoA (μM)	Exchange (%)	
		Host liver	Hepatoma
2.5	0	6.4	29.0
	3	1.6	13.6
	5	0.5	7.4
5.0	0	11.0	36.1
	3	3.1	22.4
	5	0.8	11.6
10.0	0	12.5	45.5
	3	4.4	34.2
	5	4.5	19.7

pyruvate added. Long chain acyl-CoA esters which produce a significant inhibition of the adenine nucleotide translocase at low concentrations [15—17] are shown in the present experiment to inhibit the carrier when phospho*enol*pyruvate is transported in host liver mitochondria. By contrast, there was considerably less inhibition of phospho*enol*pyruvate transport in hepatoma mitochondria, particularly at the lower concentration of palmitoyl-CoA.

The mitochondrial transport of phospho*enol*pyruvate was further examined as a function of its ability to stimulate calcium egress from the mitochondrial matrix [18,19]. This is an interesting but imperfectly understood phenomena whereby calcium egress from the mitochondria is related to the counter transport of phospho*enol*pyruvate and ATP on the adenine nucleotide translocase [1,2]. In a related study it has been reported that the ability of mitochondria from Ehrlich ascites tumor cells to retain calcium differs markedly from that of rat liver mitochondria [20]. As shown in Fig. 1, calcium added to the reaction mixture was retained to the same extent in both host liver and hepatoma mitochondria. However, upon subsequent addition of phospho*enol*pyruvate, there was a greater stimulation of calcium egress in the hepatoma than in the host liver mitochondria. These results are consistent with a more active transport of phospho*enol*pyruvate on the adenine nucleotide translocase of hepatoma mitochondria thereby promoting the increased calcium egress.

Studies were carried out to examine the biochemical properties of the adenine nucleotide translocase more directly in host liver and a series of hepatomas. Because of the difference noted in effectivenss of palmitoyl-CoA as an inhibitor of phospho*enol*pyruvate transport in liver and hepatoma mitochondria, a more definitive series of experiments was carried out using the well characterized inhibitor atractylate and measuring ADP uptake directly (Table II). Although the results vary somewhat depending on the hepatoma mitochondria used, it is evident, particularly at the lower concentration (0.3 μM) of atractylate, that the adenine nucleotide translocase from hepatoma mitochon-

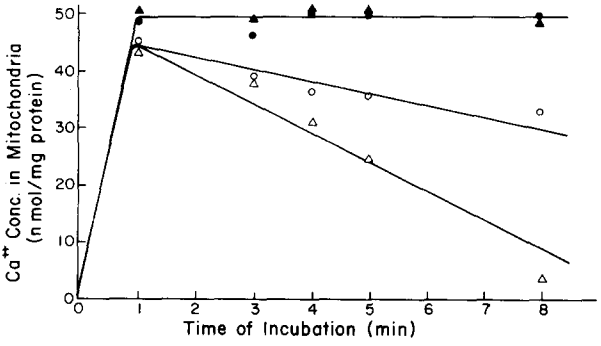

Fig. 1. Effect of phospho*enol*pyruvate on Ca^{2+} egress from mitochondria from host liver and hepatoma 7777. The basic mixture contained 75 mM KCl, 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), pH 7.4, 0.7 mM potassium phosphate, pH 7.4, 10 mM β-hydroxybutyrate and 4.0 mg mitochondrial protein. Following preincubation for 1 min at 28°C the reaction was started with the addition of 0.30 mM $^{45}CaCl_2$ (20 000 cpm). Phospho*enol*pyruvate was added 30 s after the addition of $^{45}CaCl_2$. $^{45}Ca^{2+}$ in the supernatant was measured at indicated time intervals. ●———●, host liver control; ○———○, 1.2 mM phospho*enol*pyruvate; ▲———▲, hepatoma control; △———△, 1.2 mM phospho*enol*pyruvate.

TABLE II

EFFECT OF VARIOUS CONCENTRATIONS OF ATRACTYLATE ON ADENINE NUCLEOTIDE TRANSLOCASE FROM HOST LIVER AND HEPATOMAS

Mitochondria (1.0 mg protein) were incubated with 10 μM [^{14}C]ADP for 30 s as described under Material and Methods.

Additions	Concentration (μM)	[^{14}C]ADP uptake (percent of control)			
		Host liver	7777	7800	5123C
None		100	100	100	100
Atractylate	0.1	78	97	89	90
	0.3	23	65	34	70
	0.5	17	31	21	46
	0.8	11	18	15	21

dria is more resistant to inhibition by atractylate. These results are consistent with those shown in Table I where palmitoyl-CoA was used as the inhibitor. It may be of interest that the effects of atractylate on hepatomas 7777 and 5123C, the most rapid and slowest growing of the three tumors are quite similar whereas the effect on 7800 most resembles that in host liver.

Like atractylate, long chain acyl-CoA esters are competitive inhibitors with adenine nucleotides for the adenine nucleotide translocase of liver and heart mitochondria [21,22] and, as shown in Fig. 2, this is also true for the translocase isolated from hepatoma mitochondria. However, in comparing some of the kinetic properties of the adenine nucleotide translocase in host liver and hepatoma mitochondria (Table III), hepatoma mitochondria showed slightly lower K_m values for ADP, and a higher concentration of palmitoyl-CoA was necessary for 50% inhibition of transport than in host liver mitochondria. The translocation of adenine nucleotides in mitochondria from a rat mammary tumor were found to be more rapid, and the K_m for ADP lower than in mitochondria from pregnant and lactating rat mammary glands [23].

Under certain physiological and pathophysiological conditions the concen-

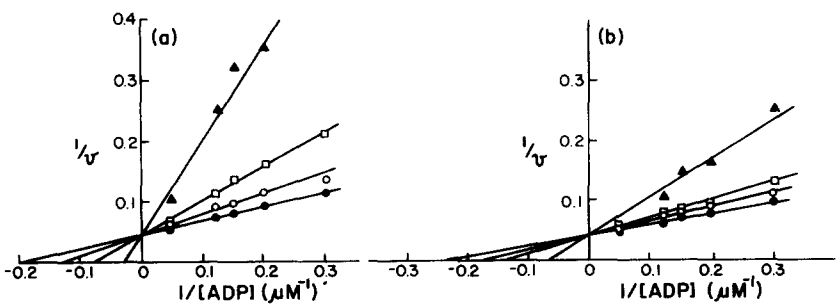

Fig. 2. Effect of ADP and palmitoyl-CoA concentration on ADP uptake by mitochondria from host liver (a) and hepatoma 7777 (b). Mitochondria were incubated with varying concentrations of [^{14}C]ADP with and without palmitoyl-CoA and assayed for adenine nucleotide translocase activity as described under Materials and Methods. ●———●, control; ○———○, 1 μM palmitoyl-CoA; □———□, 2 μM palmitoyl-CoA; ▲———▲, 5 μM palmitoyl-CoA.

TABLE III

COMPARISON OF K_m ADP AND PALMITOYL-CoA REQUIRED FOR 50% INHIBITION OF ADENINE NUCLEOTIDE TRANSLOCATION IN LIVER AND HEPATOMA MITOCHONDRIA

The data represents the average values and standard deviations from six animals.

	K_m ADP (μM)	Palmitoyl-CoA (μM) added for 50% inhibition of translocation
Control liver	5.3 ± 0.14	0.7 ± 0.06
Host liver	5.0 ± 0.34	0.7 ± 0.16
Hepatoma 7777	3.8 ± 0.21 **	1.6 ± 0.11 **
Hepatoma 7800	3.5 ± 0.10 **	1.2 ± 0.20 *

Statistical significance against control liver was measured by Student's *t*-test: * $P < 0.05$, ** $P < 0.01$.

tration of long chain fatty acyl-CoA esters and liver and heart tissue are inversely correlated with the rate of adenine nucleotide translocation [8]. In order to determine whether a similar correlation might exist in hepatomas, the tissue concentration of long chain fatty acyl-CoA esters was compared in hepatomas, control and host livers (Table IV). The concentration of long chain fatty acyl-CoA esters was similar in both control and host liver whereas it was approx. 50% lower in the 7800 and 5123C hepatomas. In the more rapidly growing 7777 hepatoma a slight decrease in the long chain acyl-CoA content of host liver was also noted along with a more significant drop in the level in the hepatoma tissue. Similar findings for the 7777 hepatoma have been reported by Halperin et al. [24]. It appears that in hepatomas, the decreased sensitivity of the adenine nucleotide to inhibition by long chain acyl-CoA esters in vitro is associated with a lower in vitro concentration of the esters.

Discontinuities in the activation energies of enzymatic activities or transport processes have been found to depend on the fatty acid composition and cholesterol content of the membranes [5]. It has been reported that in hepatoma mitochondria where lipid alterations occur, the discontinuities of activation energies of a large number of membrane-bound enzymes are slight or absent. By contrast Arrhenius plots of most membrane-associated enzymes in normal mitochondria give a bi-phasic curve with a break point at a specific temperature [5]. The adenine nucleotide translocase activities of mitochon-

TABLE IV

CONCENTRATION OF LONG CHAIN FATTY ACYL-CoA ESTERS IN HOST LIVER AND HEPATOMAS

Determinations were carried out as described under Materials and Methods. The data represents the average values and standard deviations from six animals.

	Acyl-CoA (nmol/g wet tissue)	
	Host liver	Hepatoma
Control	91.3 ± 3.9	—
Hepatoma 7800	97.0 ± 11.7	52.2 ± 5.7
Hepatoma 5123C	93.5 ± 9.0	51.8 ± 5.4
Hepatoma 7777	72.8 ± 2.7	24.6 ± 4.4

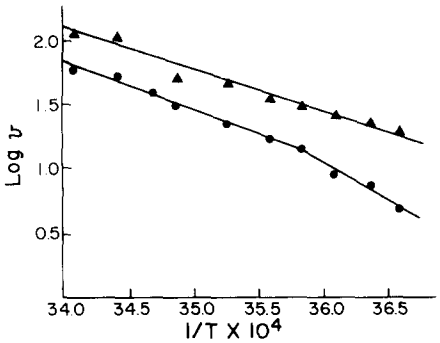

Fig. 3. Arrhenius plot for ATP exchange in mitochondria from host liver (●———●) and hepatoma 5123C (▲———▲). Mitochondria were loaded with [^{14}C]ATP and the back exchange was carried out with 100 μM ATP as described under Materials and Methods. The reaction was run at three time intervals between 20 and 60 s to obtain each point on the curves.

dria from host liver and hepatoma were compared at various temperatures (Fig. 3). Whereas there is a change in activation energy at 6°C in host liver mitochondria, hepatoma mitochondria do not show a discontinuity in the slope. A change in the activation energy at 8°C for the adenine nucleotide translocase of normal liver mitochondria has been reported [25] whereas it has been shown that the translocase from yeast mitochondria does not show a break in the slope with temperature change [26].

Discussion

Membrane organization is related in large part to lipid-protein interactions within the membrane structure, and the interrelationship between the physical state of membrane lipids and enzymatic and transport activities have been well documented [27–29]. The formation of different lipid clusters in functional areas of the mitochondrial membrane has been proposed to explain differences in the activity transitions of the enzymes, and there does not appear to be any involvement of the bulk of the lipids in regulation of membrane-bound enzymes [5].

Mitochondria isolated from the Morris hepatoma 7777 were shown to have a markedly different phospholipid composition from those of control mitochondria both with respect to the amounts of the various types present and the fatty acid composition [30]. The level of polyunsaturated fatty acids in the mitochondrial lipids was lowered whereas there was an increase in the level of monounsaturated fatty acids. A significant increase in the cholesterol content in mitochondria from Morris hepatoma 5123 has been reported [4]. In general the observations made to date strongly indicate that there are alterations in mitochondrial phospholipid metabolism which influence the fatty acid composition of the mitochondrial phospholipids [30].

Certain differences in the biochemical properties of the adenine nucleotide translocase from hepatoma and host liver mitochondria are apparent from the present studies. In particular, the higher rate of phospho*enol*pyruvate transport, decreased effectiveness of the inhibitors atractylate and palmitoyl-CoA

and lack of a transition temperature change in the tumor mitocbondria are the most obvious differences. It is of interest that these properties are associated with a lower K_m for ADP as well as a slight increase in V. It is possible that the binding of the inhibitor ligands and adenine nucleotides to the translocator from hepatoma mitochonria are altered. Alternatively more carrier sites may be accessable which, in turn, would necessitate a higher concentration of inhibitor for saturation. For the most part, these findings are compatable with structural alterations in the inner mitochondrial membrane resulting from qualitative and/or quantitative differences in the lipid constituents of the tumor membranes. Based on certain physical studies it has been suggested that the existence of a break temperature is a consequence of a liquid crystalline to gel phase transition in the membrane lipid as the temperature is decreased [28]. This is apparently modified in the hepatoma mitochondria which contain an increase in cholesterol and a decrease in polyunsaturated fatty acids [4,30].

Halperin et al. [24] have provided evidence for differences in the mitochondrial citrate transporter in host liver and hepatoma 7777 mitochondria which is similar to that reported here for the adenine nucleotide translocase. The K_m for citrate transport in tumor mitochondria was 3-fold less than that in host liver mitochondria and added palmitoyl-CoA inhibited citrate transport more pronouncedly in host liver mitochondria than in hepatoma mitochondria. In addition, the concentration of long chain acyl-CoA esters was considerably lower in the hepatoma than host liver in both the fed and fasted state. Whereas there is a loss of normal dietary control of the de novo pathway for fatty acid biosynthesis in the cytosol of hepatomas, the acetyl-CoA carboxylase of the tumors is inhibited by palmityol-CoA to the same extent as the host liver enzyme [31]. Thus, there appear to be differences in the effector control of the soluble and membrane-bound enzymes in hepatoma tissue.

In general, it appears that energy metabolism of minimal deviation hepatomas is very similar to that of normal liver [32]. Whereas there is evidence that the mitochondrial content of tumor cells is lower than that of normal tissue [33], structural and metabolic differences in tumor mitochondria are less apparent [34]. Energy-linked activities such as oxidative phosphorylation, ATP-P_i exchange and ATP-supported calcium uptake in the Morris hepatoma 7800 are catalyzed at normal or near normal rates [35—38]. In a number of tumors examined there apears to be ample evidence that the transport proteins involved in the malate-aspartate shuttle are sufficiently active to account for the transfer of reducing equivalents of all of the cytosol NADH equivalent to the pyruvate formed by glycolysis and oxidized to completion [39,40].

At the present time it is not possible to conclude from these and related studies whether any of the differences from normal observed in hepatoma mitochondrial membrane proteins bears any causal relationship to tumor growth. The decreased sensitivity of the hepatoma adenine nucleotide translocase and citrate carrier to plamitoyl-CoA in vitro may reflect in vivo regulatory effects which could influence metabolism of the tumor. In a comprehensive review by Wallach [41] strong consideration was given to the possibility that defective mitochondrial membrane-mediated control mechanisms were responsible for the high aerobic glycolysis which is still the most widely reported metabolic abnormality of tumors.

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