Increased content of natural ATPase inhibitor in tumor mitochondria

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The ATPase activity of Zajdela hepatoma and Yoshida sarcoma submitochondrial particles was several times lower than the enzyme activity in rat heart and rat liver submitochondrial particles. The content of F_1-ATPase in the tumor mitochondria was found not to be very different from that in mitochondria of rat liver. Immunochemical determination of the amount of the natural ATPase inhibitor revealed that the tumor mitochondria contain 2–3-times more ATPase inhibitor than control mitochondria. It is concluded that the low ATPase activity of the tumor mitochondria results from the inhibition of the enzyme activity by the natural ATPase inhibitor.

Natural ATPase inhibitor *ATPase activity* *Tumor mitochondria*

1. INTRODUCTION

Mitochondria of different tumors exhibit ATPase activity which could not be maximally activated under the conditions known to elicit the maximum ATPase activity of normal mitochondria. This could reflect (a) a defective ATPase molecule, (b) a lower content of the enzyme, or (c) an inhibition of the ATPase activity in tumor mitochondria.

F_1-ATPase isolated from Zajdela hepatoma possesses the same enzymatic, electrophoretic and immunological properties as the enzyme from rat liver [1]. The content of F_1-ATPase in Zajdela hepatoma mitochondria does not differ significantly from that in mitochondria of rat liver [2]. Yet, the maximum ATPase activity of Zajdela hepatoma mitochondria or submitochondrial particles is about the half of the enzyme activity of rat liver mitochondria or submitochondrial particles. A possible explanation of these observations is that the natural ATPase inhibitor in Zajdela hepatoma mitochondria is either more efficient or present in a larger amount compared with mitochondria of rat liver.

Here, we compared the contents of F_1-ATPase and the natural ATPase inhibitor in normal (rat liver and heart) and tumor (Zajdela hepatoma and Yoshida sarcoma) mitochondria using sensitive immunochemical methods. The results show that the low ATPase activity of tumor mitochondria results from a higher content of natural ATPase inhibitor in these mitochondria.

2. MATERIALS AND METHODS

Ascitic Zajdela hepatoma and Yoshida sarcoma were maintained and mitochondria isolated as in [3]. Sonic submitochondrial particles were prepared according to [4] at pH 9.2. The particles were depleted of the ATPase inhibitor by gel filtration [5] at alkaline pH. After elution from the column with 0.25 M KCl, 75 mM sucrose, 2 mM EDTA, 30 mM Tris–H_2SO_4, pH 9.2, the particles were centrifuged and resuspended in 0.25 M sucrose, 10 mM Mops, pH 6.5. ATPase activity was determined in a coupled system [6]. ATPase inhibitor was isolated from beef heart according to [7]. F_1-ATPase was isolated [8] and further purified [9] from rat liver. Antisera against

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Table 1

The effect of Sephadex treatment on ATPase activity of submitochondrial particles

Source of submitochondrial particles	Specific ATPase activity (μmol P_i/min per mg)		
	Submitochondrial particles	S-particles	S-particles supplemented with ATPase inhibitor
Rat heart	0.89	1.80	0.36
Rat liver	0.64	1.08	0.60
Zajdela hepatoma	0.38	1.30	0.32
Yoshida sarcoma	0.18	0.64	0.15

Submitochondrial and S-particles were prepared as described in section 2. For the reconstitution experiments with isolated ATPase inhibitor 250 μg S-particles were incubated with 6.25 μg ATPase inhibitor (25 μg ATPase inhibitor/mg S-particles) in 250 μl 0.25 M sucrose, 10 mM Mops (pH 6.5), 0.5 mM MgATP for 15 min at room temperature. 100 μg of particles were used for determining the ATPase activity

ATPase inhibitor [10] and F_1-ATPase [11] were raised in rabbits. Radioimmunoassay of the ATPase inhibitor was performed [1] using ^{125}I-iodinated [12] ATPase inhibitor as the labeled competing antigen. SDS–polyacrylamide gel electrophoresis (SDS–PAGE) [13], immunoblotting [14] and protein estimation [15] were performed according to published procedures.

3. RESULTS AND DISCUSSION

Table 1 shows the ATPase activity of submitochondrial particles from the examined sources. The ATPase activity of rat heart and rat liver submitochondrial particles was considerably higher than the enzyme activity of the particles from Zajdela hepatoma and Yoshida sarcoma. Determination of the ATPase activity in mitochondria disrupted sonically or by repeated freezing and thawing and in submitochondrial particles prepared using different sonication times indicated no preferential loss of the ATPase activity from the tumor membranes during sonication. The different ATPase activities in the types of submitochondrial particles studied could thus result either from an unequal content of F_1-ATPase or from an unequal inhibition of the ATPase activity.

The F_1-ATPase content in the examined types of mitochondria was compared by quantitative immunoblotting (fig.1). The estimated ratios of the F_1-ATPase contents in mitochondria of rat heart, liver, Zajdela hepatoma and Yoshida sarcoma were: 2.4:1:0.92:0.62. Approximately the same values also hold for the ratios of the F_1-ATPase contents in submitochondrial particles from the examined sources. It is highly unlikely that the observed small differences in the F_1-ATPase content could account for the observed variances in the ATPase activities.

The possibility that the unequal ATPase activity of the preparations with similar F_1-ATPase content reflects a different inhibition of the enzyme by the natural ATPase inhibitor was further examined. The ATPase activities of submitochondrial particles devoid of the inhibitor (S-particles) were determined. The ATPase activity of submitochondrial particles from rat heart and rat liver increased 1.5–2-times, whereas that of the tumor particles increased 3–4-times upon removal of the inhibitor (table 1). The addition of an excess of the ATPase inhibitor to rat liver and tumor S-particles resulted in a decrease in the ATPase activity to values close to those found in the submitochondrial particles prior to the removal of the inhibitor. The ATPase activity of rat heart S-particles decreased below the activity of the original particles. This indicates a partial release of the ATPase inhibitor from rat heart mitochondria during preparation of sonic submitochondrial particles by the methods used.

The above data strongly suggest that the low ATPase activity of tumor submitochondrial particles is due to the inhibition of the enzyme activity

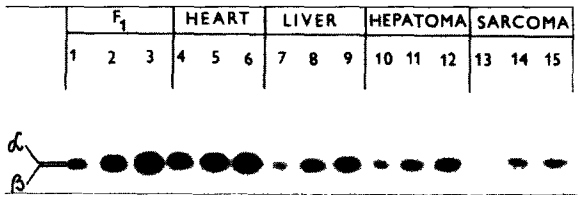

Fig.1. The content of F_1-ATPase in rat heart, rat liver, Zajdela hepatoma and Yoshida sarcoma mitochondria. Lanes 1–3, isolated F_1-ATPase, 0.25, 0.5 and 1 μg; lanes 4–6, rat heart; lanes 7–9, rat liver; lanes 10–12, Zajdela hepatoma; lanes 13–15, Yoshida sarcoma. The amounts of mitochondrial protein used were 5, 7.5 and 10 μg. Relative contents of F_1-ATPase in respective types of mitochondria were estimated by measuring the radioactivity.

by the natural ATPase inhibitor. Therefore, the amount of the ATPase inhibitor in the studied types of mitochondria was compared using competition radioimmunoassay. The reliability of the assay was confirmed by determining the content of the inhibitor in beef heart mitochondria, which in accordance with the published data [16] was found to be in the 1:1 stoichiometry with F_1-ATPase. Since the antiserum against beef heart ATPase inhibitor and the labeled competing antigen from the same source were used in the assay, the absolute amounts of the inhibitor in rat mitochondria could not be evaluated. However, as all of the compared types of mitochondria were of rat origin it is reasonable to assume that the antiserum used was equally cross-reactive with all of them, and that the relative values for respective rat mitochondria are valid. The relative contents of the ATPase inhibitor in submitochondrial particles of rat heart, liver, Zajdela hepatoma and Yoshida sarcoma were 4:1:1.9:4 whereas in whole mitochondria they were 18.5:1:2.2:3 (table 2, fig.2). The decrease in the relative amount of the inhibitor in rat heart mitochondria upon the conversion to submitochondrial particles is in accord with the conclusion drawn from the reconstitution experiment with S-particles and isolated ATPase inhibitor. The differences in the content of ATPase inhibitor in submitochondrial particles from the sources examined practically disappear after the conversion of submitochondrial particles to S-particles.

Our data show that the low ATPase activity of at least the tumor mitochondria examined results

Table 2

Relative content of the ATPase inhibitor in submitochondrial particles prior and after the Sephadex treatment

Source of mitochondria or particles	Relative content of ATPase inhibitor in:		
	Mitochondria	Submitochondrial particles	S-particles
Rat heart	18.5	4	1.12
Rat liver	1	1	1
Zajdela hepatoma	1.9	2.2	1.3
Yoshida sarcoma	3	4	1.2

The amount of ATPase inhibitor was detected using competition radioimmunoassay [1]

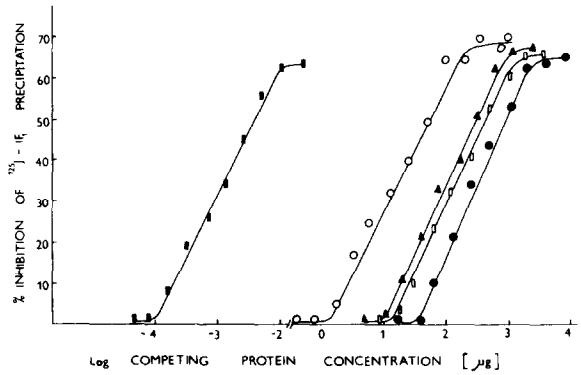

Fig.2. Detection of ATPase inhibitor in mitochondria from rat heart, rat liver, Zajdela hepatoma and Yoshida sarcoma by competition radioimmunoassay. (■—■) Isolated ATPase inhibitor, (○—○) rat heart, (●—●) rat liver, (□—□) Zajdela hepatoma, (▲—▲) Yoshida sarcoma mitochondria.

from the inhibition of the ATPase activity by the natural ATPase inhibitor. Moreover, they suggest that the inhibition is due to the elevated mitochondrial content of the inhibitor rather than to a more efficient inhibition of the ATPase activity in the tumor mitochondria. Recently, it has been shown that in Zajdela hepatoma mitochondria the respiratory chain components are greatly enriched, whereas the content of F_1-ATPase is not increased compared to mitochondria of rat liver [2]. It appears that in these mitochondria the content of the ATPase inhibitor follows the tendency of other in-

ner membrane components to increase rather than to remain in a constant stoichiometry with the ATPase complex.

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