MITOCHONDRIAL ATPase OF ZAJDELA HEPATOMA

Presence of F_1-specific antigenic determinants outside mitochondria

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1. Introduction

The properties and the content of ATPase in mitochondria of Zajdela hepatoma differ from those in rat liver mitochondria. ATPase activity of coupled Zajdela hepatoma mitochondria is insensitive to uncouplers [1] and the content of the enzyme in hepatoma mitochondria represents only about half the amount of ATPase present in mitochondria of rat liver [2]. It is conceivable that the low ATPase content in Zajdela hepatoma mitochondria may be due not to a diminished synthesis of the enzyme in tumor cells but rather to a disbalance between the formation of the soluble (F_1) ATPase which is synthesized in the cytosol (reviewed [3,4]) and its integration into the mitochondrial membrane. In an attempt to check this possibility the presence of F_1-specific antigenic determinants in postribosomal fraction of Zajdela hepatoma and rat liver has been investigated in the present study. Using competition radioimmunoassay it was found that postribosomal fraction of Zajdela hepatoma contains significant amounts of antigenically active F_1 components, whereas no F_1-specific antigenic determinants were detected in the corresponding fraction of rat liver.

2. Materials and methods

Maintenance and propagation of Zajdela hepatoma in ascitic form [5], isolation of mitochondria [5], preparation [6] and NaBr treatment [7] of mitochondrial membrane fraction were performed as detailed previously with the exception that mitochondrial membrane fraction was prepared in 0.25 M sucrose, 1 mM EDTA, 10 mM Tris–H_2SO_4, pH 7.4.

F_1 was isolated from mitochondrial membrane fraction suspended in 0.25 M sucrose, 1 mM EDTA, 1 mM ATP, 10 mM Tris–H_2SO_4 pH 7.4, by chloroform extraction [8] followed by gel filtration on Sepharose 6B [9] and precipitation with ammonium sulfate at 60% saturation. ATPase activity was determined by monitoring the oxidation of NADH in a coupled system [10].

SDS–polyacrylamide gel electrophoresis was according to [11]. Radioactivity distribution in the gel was detected by autoradiography of dried [12] gel slabs.

Postribosomal fraction was prepared from the homogenate used for the preparation of mitochondria. The supernatant after 2 min centrifugation at 20 000 × *g* was centrifuged 3 h at 150 000 × *g*. The floating lipids were removed and the supernatant containing about 7 mg protein/ml was dialyzed overnight against 0.08% NaCl, 0.002% KCl, 0.002% KH_2PO_4, 0.0917% Na_2HPO_4, lyophilized, dissolved in RIA buffer (0.8% NaCl, 0.02% KCl, 0.02% KH_2PO_4, 0.917% Na_2HPO_4, 0.5% Triton X-100, 5 mM EDTA, 0.05% NaN_3, 0.1% bovine serum albumin) to 10 mg protein/ml and used without storage. All operations were performed at 0–4°C.

Rabbit antisera against F_1 were obtained using the immunization schedule in [13]. Second antibody (swine anti-rabbit IgG) was prepared and kindly supplied by Dr G. Russ.

F_1 was iodinated with ^{125}I by the modification [14] of the chloramine T method [15]. Specific radioactivity of the labeled enzyme was about 5000 cpm/ng protein. Integrity of individual F_1

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polypeptides after radioiodination was confirmed by determining radioactivity distribution in the gel after SDS–polyacrylamide gel electrophoresis of the labeled preparation. The radioimmunoassay procedures were performed essentially according to [14]. In competition assays a limiting amount of antiserum against rat liver F_1 was incubated with varying amounts of the material analyzed or with rat liver F_1 in 0.26 ml RIA buffer for 1 h at 37°C and then overnight at 4°C. Radioiodinated rat liver F_1, 10 μl, (5000 cpm) was then added to the respective reaction mixtures and incubated 1 h at 37°C and 22 h at 4°C. Appropriate quantities of nonimmune serum and of precipitating second antibody were then added to the samples and incubated 3 h at 37°C. The precipitates were washed 3-times with RIA buffer and counted in a gamma counter. The nonimmune serum used bound <2% total radioactivity present in respective samples.

Protein was determined according to [16].

3. Results and discussion

F_1 preparations from rat liver and Zajdela hepatoma displayed identical electrophoretic properties in SDS–polyacrylamide gel (fig.1), exhibited approximately equal enzymatic activity (of 20 μmol P_i/min/mg), and gave line of identity in Ouchterlony double diffusion test (fig.2). Three largest subunits of F_1 were identified in the immune complex after reaction of cold-dissociated F_1 with antiserum against the rat liver enzyme (fig.3); preparations from both sources examined gave identical results in this test. F_1 from rat liver and Zajdela hepatoma are thus electrophoretically, enzymatically, and immunologically equivalent. Therefore, only the rat liver enzyme and corresponding antiserum were employed in radioimmunoassay procedures.

Using competition radioimmunoassay it was found that the postribosomal fraction of Zajdela hepatoma contains significant amounts of antigenically-active F_1 components. Under identical conditions F_1-specific antigenic determinants in the postribosomal fraction of rat liver were not detected (fig.4). Several methods [17] were employed to isolate from postribosomal fraction of Zajdela hepatoma specific immune complexes with IgG from anti-F_1 antiserum. Considerable non-specific binding of protein components with mol. wt 30 000–60 000 of hepatoma

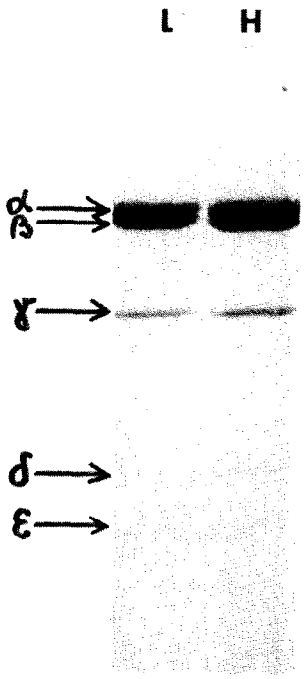


Fig.1. Electrophoretograms of F_1 of rat liver and Zajdela hepatoma mitochondria. F_1 of respective sources was isolated and separated in SDS–polyacrylamide gel electrophoresis as in section 2. The position of F_1 subunits in the gel is indicated by the arrows. (L) F_1 of rat liver; (H) F_1 of Zajdela hepatoma.

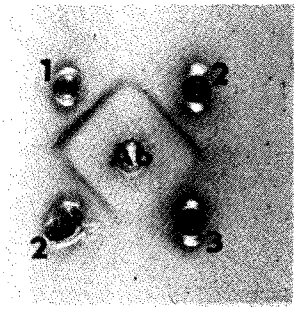
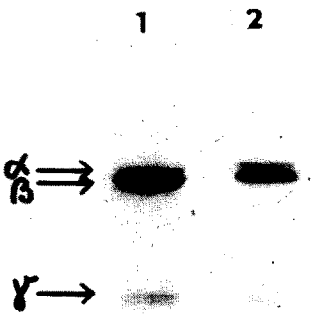


Fig.2. Immunodiffusion reaction of rat liver and Zajdela hepatoma F_1 with antiserum against rat liver F_1. (Ab) antiserum against rat liver F_1; (1) F_1 of rat liver; (2) F_1 of Zajdela hepatoma; (3) Triton extract from NaBr-treated submitochondrial particles of rat liver.



postribosomal fraction to IgG from nonimmune sera prevented to determine directly the nature of F_1-specific antigenic determinants in the tumor postribosomal fraction. However, the extent of competition and the slope of the titration curves for purified F_1 and for hepatoma postribosomal fraction were almost identical. This allows to suppose that essentially

Fig.3. Autoradiogram of electrophoretically separated immunoprecipitate of radioiodinated F_1. Radioiodinated cold-dissociated F_1 of rat liver (10^6 cpm) in RIA buffer was incubated with 10 μl anti-F_1 or control serum. The precipitates formed after the addition of second antibody were 5-times washed with RIA buffer, dissolved in 2% SDS, 8 M urea, 4% β-mercaptoethanol, 10% glycerol, heated 2 min at 100°C and electrophoresed in SDS–polyacrylamide. In the presence of control serum the precipitate contained only insignificant amount of radioactivity. The position of three major F_1 subunits is indicated by the arrows. (1) immunoprecipitated F_1; (2) whole F_1.

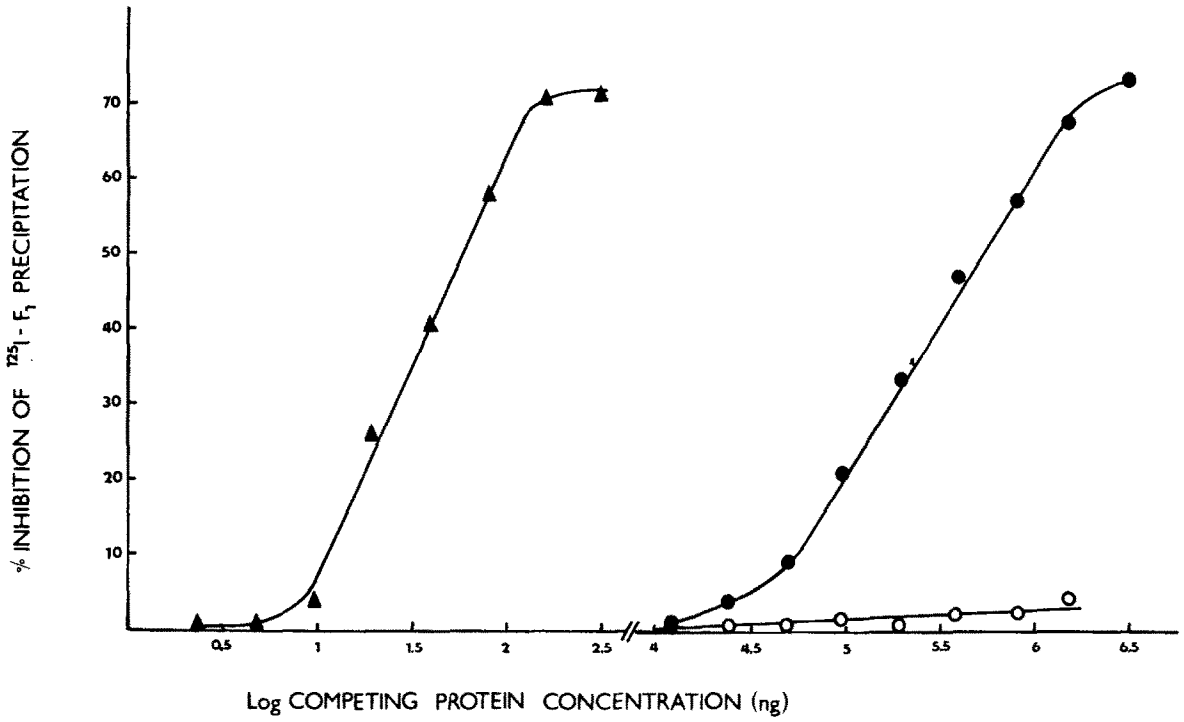


Fig.4. Detection of F_1-specific antigenic determinants in postribosomal fraction of rat liver and Zajdela hepatoma by competition radioimmunoassay. The assays were performed as in section 2. (-▲-) F_1 from rat liver; (-●-) postribosomal fraction of Zajdela hepatoma; (-○-) postribosomal fraction of rat liver were used for the competition.

all antigenic determinants involved under the conditions used in the formation of F_1–antibody complex, i.e., at least the antigenic determinants of α, β and γ subunits of F_1 are present in the tumor postribosomal fraction.

A quantitative evaluation of the titration curves for purified F_1 and for the postribosomal fraction of Zajdela hepatoma revealed that if the tumor fraction contained all the protein components of F_1 in a proper stoichiometry, their content would be 154 ng/mg protein of postribosomal fraction. This amount represents about 1/50 of the F_1 content in mitochondria of Zajdela hepatoma expressed per milligram of mitochondrial protein. With respect to the limit of detection of the method used, this value for rat liver should be lower than 1/20 000.

The incubation of labeled postribosomal fraction of Zajdela hepatoma with rat liver NaBr-treated submitochondrial particles capable of binding isolated F_1 did not result in an appearance of oligomycin-sensitive ATPase activity or labeled F_1 subunits in the particles. This may indicate that F_1-specific antigenic determinants in the postribosomal fraction of Zajdela hepatoma are not assembled in F_1 complex capable of association with specific attachment sites on the mitochondrial membrane.

It may be argued that the F_1-specific antigenic determinants found in the postribosomal fraction of Zajdela hepatoma had been released from tumor mitochondria in the course of the preparation of the postribosomal fraction. Therefore, the effect of mechanical treatment on the release of F_1 from rat liver and Zajdela hepatoma mitochondria was compared. Mitochondria isolated from the two sources were sonicated or drastically homogenized in a Potter-Elvehjem homogenizer and the amount of F_1 released was determined by competition radioimmunoassay (table 1). About 2 times higher amount of antigenically-active F_1 was released from tumor mitochondria than from liver mitochondria by the treatment used. It is evident that this difference could not account for the at least 200-fold higher content of antigenically-active F_1 components in the postribosomal fraction of Zajdela hepatoma. It should be taken into account that the conditions of the mechanical treatment of mitochondria were much more drastic in this particular experiment than were those of whole cell homogenization employed for preparation of mitochondria or postribosomal fraction. The possibility that mitochondrial population in Zajdela hepatoma is heterogenous as for the susceptibility to mechanical treatment and that a fraction of mitochondria releases F_1 even during mild cell homogenization does not seem very likely but cannot be excluded at present.

Table 1
Effect of mechanical treatments of isolated mitochondria on the release of F_1 from the mitochondrial membrane

Source of mitochondria	Treatment	μg F_1/mg total protein released
Rat liver	Homogenization	2.25
	Sonication	2.93
Zajdela hepatoma	Homogenization	3.90
	Sonication	5.91

Isolated mitochondria (40 mg) were suspended in 2 ml 0.3 M mannitol, 1 mM EDTA, pH 7.4. Aliquots, 1 ml, were either homogenized by 25 strokes in glass–Teflon Potter-Elvehjem homogenizer (clearance 0.1 mm) or sonicated for 25 min in cleaning bath of 120 W MSE Ultrasonic Desintegrator and centrifuged 3 h at 120 000 × g. F_1 was determined in the supernatant by competition radioimmunoassay. All operations were performed at 0–4°C.

It can be speculated that a larger portion of extramitochondrially synthesized mitochondrial proteins (e.g., F_1 components) occurs in the cytosol in a stage following the formation and preceding the integration into membrane in cells with higher rate of protein synthesis. Yet, under the conditions used, the rate of in vivo amino acid incorporation into proteins of postribosomal fraction of Zajdela hepatoma was only about 7-times higher than that into proteins of corresponding fraction of rat liver. The higher rate of protein synthesis in Zajdela hepatoma comparing with rat liver thus could represent only one of the elements contributing to the difference in the content of antigenically-active F_1 in postribosomal fraction of rat liver and Zajdela hepatoma.

The presence of F_1-specific antigenic determinants in the postribosomal fraction of Zajdela hepatoma may be related to the lower content of ATPase in hepatoma mitochondria as compared with rat liver mitochondria [2] reflecting an impairment in the assembly of mitochondrial ATPase in this tumor.

Either an imbalance in the formation of respective components of mitochondrial ATPase system or a defect in integration of F_1 into mitochondrial membrane may be involved.

The results of the present study might have a bearing to the proposal [18] that F_1 outside mitochondria may be one of the factors contributing to the persistent alteration of intracellular pH and consequently to the appearance and maintenance of malignant phenotype.

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