ADP, ATP TRANSLOCATOR PROTEIN OF RAT HEART, LIVER AND HEPATOMA MITOCHONDRIA EXHIBITS IMMUNOLOGICAL CROSS-REACTIVITY

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1. Introduction

Differences in the content and kinetic properties of the ADP, ATP translocator in mitochondria from various sources have been demonstrated (reviewed [1,2]). Recently the translocator protein was isolated from several organs [3–5] and data were reported suggesting differences between immunological properties of this protein isolated from heart, liver and kidney mitochondria [5].

In this study a comparison of immunological properties of translocator protein of rat heart, liver and hepatoma by a radioimmunological technique revealed that the same antigenic determinant(s) related to ADP, ATP translocator protein are present in the mitochondria of all the above tissues. In addition, the data obtained suggest that antigenic properties of translocator protein isolated from different sources are unequally modified during the isolation.

2. Materials and methods

The maintenance and propagation of Zajdela hepatoma and the isolation of mitochondria from the tumor and rat liver were done as detailed in [6]. Rat heart mitochondria were isolated by a procedure similar to that used for the preparation of liver mitochondria with the exception that the isolation medium was supplemented with 10 mM KCl. Mitochondria from respective sources were loaded with carboxyatractylate (CAT) and then extracted with Triton X-100 to solubilize ADP, ATP translocator protein as CAT–protein complex [7]. The extracts obtained after centrifugation of the Triton X-100 treated mitochondria at 140 000 × *g* for 40 min were used in the radioimmunoassay. CAT–proteins were purified from these supernatant fractions by hydroxylapatite chromatography and gel filtration on Sepharose 6B as in [3,5].

The binding of ^{35}S-labeled CAT to mitochondria was measured according to [8]. SDS–polyacrylamide gel electrophoresis was performed as in [9] in the slab gels with 12–20% linear acrylamide gradient. The distribution of radioactivity in the gels was detected by autoradiography of dried [10] gel slabs.

Rabbit antisera against rat heart or rat liver CAT–protein were obtained using the immunization scheme in [5]. Immunodiffusion was done by the double diffusion agar plate technique of Ouchterlony in 0.9% agar plates. Second antibody (swine anti-rabbit IgG) was kindly supplied by Dr G. Russ.

CAT–protein isolated from rat heart mitochondria was radioiodinated to a spec. radioact. 2×10^3 cpm/ng protein using *N*-succinimidyl-3/4-hydroxy phenyl/propionate (11). In competitive inhibition radioimmunoassay a limited amount of antiserum against rat heart CAT–protein (final dilution 2×10^6), a constant amount of radioiodinated CAT–protein from heart, and then varying amounts of unlabeled CAT–proteins or Triton X-100 extracts of mitochondria from different sources were added to 0.25 ml RIA buffer (0.8% NaCl, 0.02% KCl, 0.02% KH_2PO_4, 0.92% Na_2HPO_4, 0.5% Triton X-100, 5 mM EDTA, 0.05% NaN_3, 0.1% bovine serum albumin, pH 7.4). Final volume of the mixture was 0.275 ml. The mixture was incubated 1 h at 37°C and 4 days at 4°C. Appropriate amounts of nonimmune serum and

of precipitating second antibody were then introduced into the samples and incubated 3 h at 37°C. The precipitates were washed 3-times with RIA buffer and counted in a gamma-counter.

Protein concentration was estimated according to [13] and in the presence of Triton X-100 as in [14].

3. Results and discussion

The translocator protein isolated from rat heart, liver and hepatoma mitochondria as a CAT–protein complex exhibited identical electrophoretic mobility in SDS–polyacrylamide gel electrophoresis corresponding to mol. wt 30 000. Two different antisera against heart and liver CAT–proteins were prepared. In accordance with [5], no crossreaction was detected between CAT–protein from rat liver and antibody against heart CAT–protein (fig.1) and vice versa in Ouchterlony double diffusion test. In addition, neither of the two antisera reacted with the CAT-protein isolated from rat hepatoma mitochondria

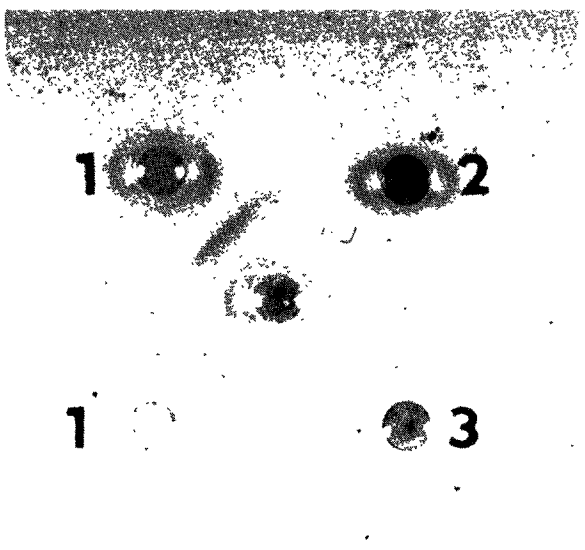

Fig.1. Immunodiffusion reaction of CAT–protein from different sources with antiserum against heart CAT–protein. Centre well, antiserum against rat heart CAT–protein. Other wells, equal concentrations of isolated CAT–protein from (1) rat heart, (2) liver and (3) hepatoma.

when tested by the same technique. The data obtained by this technique with CAT–protein isolated from heart, liver and kidney mitochondria and antibody against heart CAT–protein had been interpreted in terms of an immunological organ specificity of this protein [5]. However, such an interpretation would require a corroboration by additional immunological technique, since in several studies [15,16] a cross-reaction of antigenic determinants could be demonstrated by radioimmunoassay in case when it had not been detected by conventional immunological methods.

To examine whether the CAT–proteins from heart, liver and hepatoma mitochondria share common antigenic determinant(s) a competitive inhibition radioimmunoassay has been employed in this study. In the assay, heart CAT–protein as a labeled antigen and homologous heart CAT–protein antiserum were used. The isolated heart CAT–protein was electrophoretically pure (fig.2A), gave a single precipitin line in Ouchterlony double diffusion test (see fig.1), and after iodination retained its integrity and ability to interact with homologous antiserum (fig.2B). In the competitive inhibition radioimmunoassay it was found that the CAT–protein isolated from hepatoma was able to inhibit the binding of labeled heart CAT–protein with homologous antibody almost completely, whereas only partial (25%) inhibition was obtained with the CAT–protein isolated from liver mitochondria even at the highest concentration used (fig.3A). One of the reasons why high concentrations of isolated hepatoma and liver CAT–protein are required to demonstrate a crossreaction with CAT–protein isolated from heart might be a modification of antigenic structure of these proteins during the isolation. To restrain such a modification, Triton X-100 solubilized CAT-loaded mitochondria were used instead of isolated CAT–proteins in Ouchterlony double diffusion and in the competitive inhibition radioimmunoassay. Triton X-100-solubilized mitochondria from all the sources examined gave visible precipitin line with heart CAT–protein antibody. Yet, the use of detergent-solubilized membranes in immunodiffusion may lead to artifactual results [17]. More conclusive data can be obtained using the competitive inhibition radioimmunoassay. In this assay almost complete inhibition of binding of the labeled CAT–protein isolated from heart with

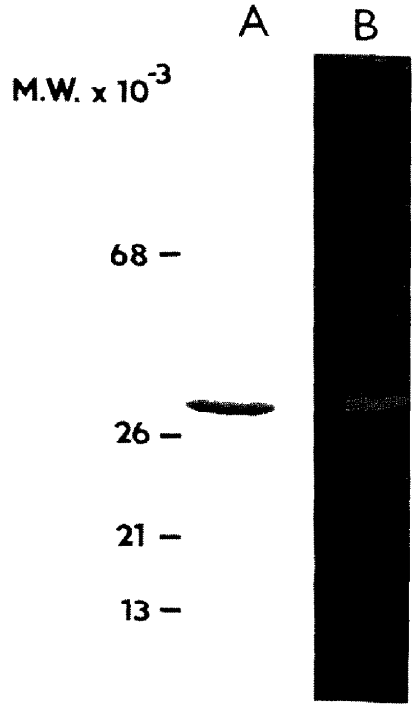

Fig.2. SDS–polyacrylamide gel electrophoresis of isolated and immunoprecipitated rat heart CAT–protein. (A) 15 μg CAT–protein isolated from rat heart mitochondria were electrophoresed and stained. (B) ^{125}I-labeled heart CAT–protein (2 × 10^6 cpm) in RIA buffer was incubated with 5 μl homologous antiserum for 5 min at room temperature. The precipitate formed after addition of second antibody was washed 5 times with RIA buffer, dissolved in 2% SDS, 8 M urea, 4% β-mercaptoethanol, 10% glycerol, and heated for 2 min at 100°C. The sample was electrophoresed in the same slab as that to be stained, dried and autoradiographed. The position of molecular weight standards is indicated; cytochrome *c* (13 000), soybean trypsin inhibitor (21 000), chymotrypsinogen (26 000), bovine serum albumin (68 000).

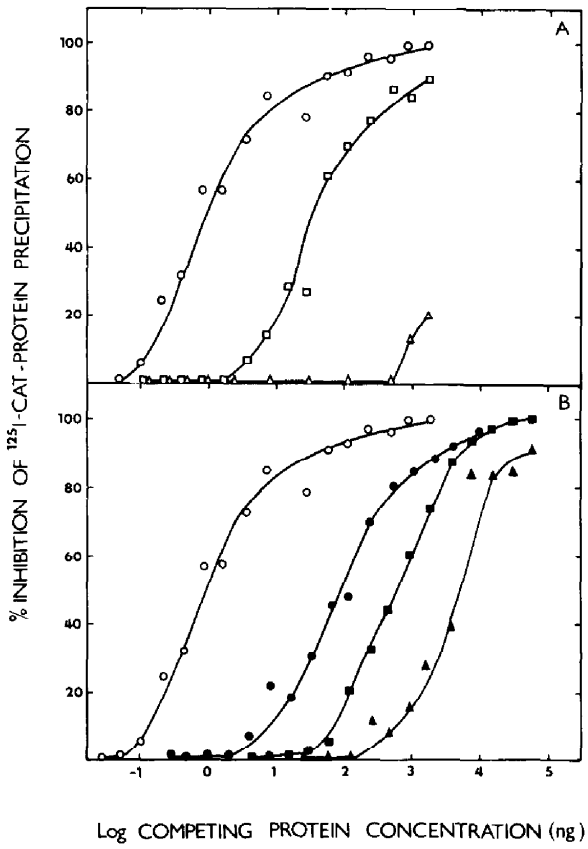

Fig.3. Immunological cross-reactivity between CAT–proteins from rat liver, heart and hepatoma mitochondria. Competitive inhibition radioimmunoassay was performed as in section 2 with ^{125}I-labeled heart CAT–protein (5 × 10^4 cpm/tube) and antiserum against heart CAT–protein (final dilution 2 × 10^6). In the competition isolated CAT–protein from (–○–) rat heart, (–△–) liver and (–□–) hepatoma mitochondria or Triton X-100-solubilized mitochondria from (–●–) rat heart, (–▲–) liver and (–■–) hepatoma were used.

homologous antibody was obtained with detergent-solubilized mitochondria of all the sources examined (fig.3B). The extent of inhibition and the slope of the competition curves for purified heart CAT–protein and for solubilized mitochondria of heart, hepatoma and liver were almost equal. This indicates that all heart CAT–protein specific antigenic determinants involved in the radioimmunoassay under the conditions used, are present in the solubilized mitochondria of heart, liver and hepatoma.

The use of solubilized mitochondria instead of isolated CAT–protein in the assay resulted not only in the complete inhibition of binding but also in the mutual approaching of competition curves for respective competing antigens. Lateral displacement of identical competition curves reflects a modification of antigenicity of all antigenic determinants involved in the assay [18]. Thus the differences in the antigenicity of the CAT–proteins present in the respective solubilized mitochondria are smaller than those of corresponding isolated CAT–protein preparations. While the ratio of the competing protein

concentration required for 50% inhibition was 1 : 40 : >1000 for isolated CAT–proteins, the ratio of 1 : 5 : 50 was found for solubilized mitochondria of heart, hepatoma, and liver respectively. The amounts of CAT–protein in these mitochondria which bind to antibody raised against heart CAT–protein are proportional to the capacities of the mitochondria to bind ^{35}S-labeled CAT. It was found that Zajdela hepatoma mitochondria bind 0.35 μmol, rat liver – 0.15 μmol, and rat heart mitochondria – 1.1 μmol ^{35}S-labeled CAT/gram protein. Thus the differences in the antigenicity of the competing antigens present in the respective mitochondria after Triton X-100 solubilization are actually smaller than those indicated by the mutual distances between the competition curves.

The differences in the ratios of the competing protein concentrations required for 50% inhibition using isolated antigens and solubilized mitochondria demonstrate an unequal attenuation of CAT–protein antigenicity during the purification of the antigen (hydroxylapatite chromatography and gel filtration) from respective solubilized mitochondria. It can be extrapolated that a similar, through less pronounced effect takes place also in the course of the solubilization of mitochondria. A lack of cross-reactivity between CAT–proteins isolated from different organs in Ouchterlony double diffusion test thus probably reflects an unequal modification of CAT–protein antigenicity by the solubilization and isolation.

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