BINDING OF MAGNESIUM BY PROTEINS OF THE MITOCHONDRIAL INTERMEMBRANE COMPARTMENT

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SUMMARY: The intermembrane compartment of rat liver mitochondria contains high molecular weight compounds, most likely proteins, complexing magnesium ions. Compound/s/ of about 150 000 daltons has a low affinity /K_d 0.37 mM/ and a high binding capacity of 300 ng atoms Mg/mg protein; compound/s/ of about 100 000 daltons is characterized by a very high affinity towards magnesium /K_d of the order of magnitude of 0.001 mM/ and a low capacity of about 20 ng atoms Mg/mg protein.

It has been previously suggested /1/ that magnesium may be present in the mitochondrial intermembrane compartment in a non-diffusible form or loosely bound to either of the two membranes. This is based on the observation /1/ that about half of magnesium content of washed rat liver mitochondria can be released after disrupting the outer membrane by any of the available procedures. The present investigation shows that the intermembrane compartment contains at least two high molecular weight compounds, most likely proteins, of a high binding ability towards Mg^{2+}.

MATERIALS AND METHODS

Mitochondria were isolated from livers of albino rats by routine procedure /2/. The soluble fraction of the intermembrane compartment was isolated by the following procedure. Mito-

chondria from three livers /about 200 mg protein/ were suspended in 20 ml of 10 mM Na-K-phosphate /pH 7.4/ and incubated at 0°C during 10 min. Thereafter, the suspension was centrifuged for 15 min at 20 000 x g and then for 1 hour at 105 000 x g. The final supernatant consisted mostly of soluble proteins of the intermembrane compartment. Its contamination by soluble proteins from the matrix compartment did not exceed 5% as estimated from the activity of malate dehydrogenase. Binding of Mg^{2+} was determined by equilibrium dialysis of 2.0 ml samples /2 - 4 mg protein/ against 400 ml of 125 mM KCl - 20 mM tris--HCl /pH 7.4/ at 0°C during 24 hours.

Gel filtration was performed using Sephadex G-200 /Pharmacia, Uppsala, Sweden/. Prior to the application on the column, the intermembrane fraction was concentrated by ultrafiltration to contain 30 mg protein/ml. Dodecyl sulfate-polyacrylamide gel electrophoresis was made as described by Weber and Osborn /3/. Approximate molecular weights of the polypeptides were determined from their electrophoretic mobility using myosin subunits /4/ and cytochrome c as standards.

Protein was determined by the biuret method /5/ using crystalline serum albumin as standard, and magnesium was estimated by atomic absorption.

RESULTS AND DISCUSSION

Binding of Mg^{2+} to soluble proteins of the intermembrane compartment of rat liver mitochondria was studied using the equilibrium dialysis procedure. The Scatchard plot obtained from these experiments /Fig. 1/ shows a biphasic character, indicative for two kinds of binding: a very high affinity binding of K_d value tentatively determined as being around 2 /uM and

the capacity of about 20 ng atoms Mg/mg protein, and low affinity binding of K_d 0.37 mM and the capacity of about 300 ng atoms/mg protein.

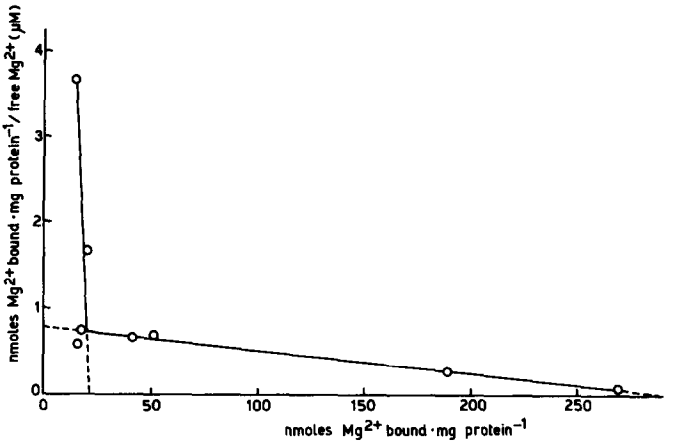

Fig. 1. Scatchard plot of magnesium binding to the intermembrane non-diffusible fraction of rat liver mitochondria.

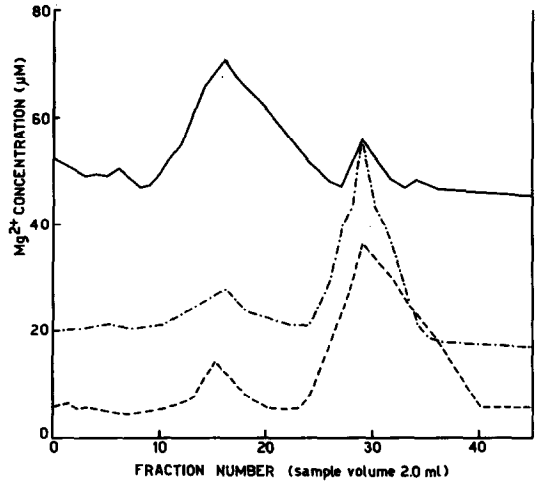

Fig. 2. Gel filtration of the mitochondrial intermembrane fraction equilibrated with various concentrations of $MgCl_2$. One ml of the concentrated fraction /30 mg protein/ml/ was applied on a column /16 x 400 mm/ of Sephadex G-200 previously equilibrated with 125 mM KCl - 20 mM tris-HCl /pH 7.4/ containing 6 uM /- - -/, 20 uM /-·-·-/ or 52 uM /———/ $MgCl_2$. The column was then eluted with the same solution. Fractions of 2.0 ml were collected.

In order to characterize the magnesium-binding proteins, the intermembrane fraction was passed through a Sephadex G-200 column equilibrated with low concentrations of $MgCl_2$. Fig. 2 shows the distribution of magnesium in the effluents from the columns equilibrated with 6/uM, 20/uM and 52/uM $MgCl_2$ respectively. Two peaks of an increased magnesium content can be observed. The height of the first peak largely depends on the concentration of Mg^{2+} in the water phase of the column, whereas that of the second peak is relatively independent on Mg^{2+} concentration in the medium. This suggests that the first peak corresponds to proteins or other compounds having a low affinity towards Mg^{2+} while the second peak includes compounds of a high affinity to magnesium. A systematic study on Mg^{2+} binding of individual fractions obtained from the columns appeared difficult because of low quantities of the material available. Nevertheless, preliminary examination by equilibrium dialysis of the fractions corresponding to the two magnesium peaks confirmed this suggestion /not shown graphically/.

A complete elution pattern of the intermembrane fraction on Sephadex G-200 column containing no $MgCl_2$ is illustrated in Fig. 3. Sixteen peaks of protein concentration can be distinguished. The first magnesium peak is relatively low, what again confirms a low binding affinity of the corresponding protein, while the second peak is well pronounced, indicative for a very tight binding.

In order to obtain a better insight into the characteristics of the intermembrane compartment proteins dodecyl sulfate--polyacrylamide gel electrophoresis was performed. In this case 17 discrete bands can be detected /Fig. 4/ which is in a rather good agreement with the separation pattern obtained from Sepha-

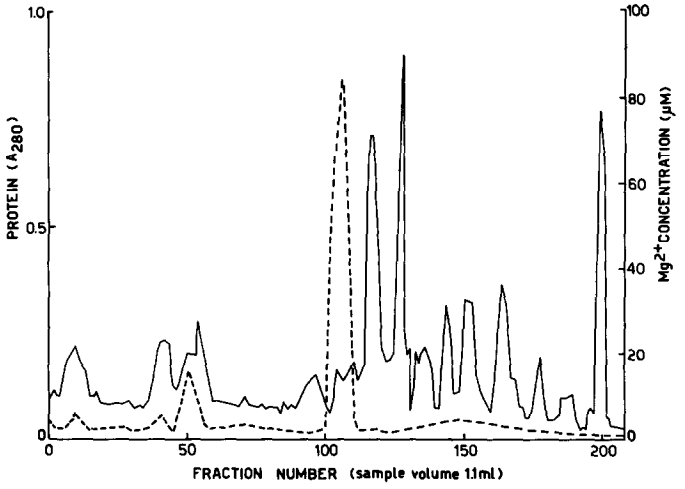

Fig. 3. Elution pattern of mitochondrial intermembrane proteins. Gel filtration was performed as described in Fig. 2, except that the eluent contained no $MgCl_2$. The column was 30 mm in diameter and 450 mm in length of the gel bed. Protein concentration /———/ was expressed as absorbance at 280 nm. Magnesium concentration /- - - -/.

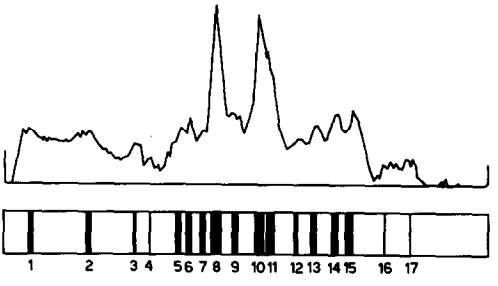

Fig. 4. Polyacrylamide gel electrophoresis of mitochondrial intermembrane proteins. Electrophoresis was performed in blocks of 5 mm in diameter and 80 mm in length of 5% polyacrylamide gel containing 0.1% sodium dodecyl sulfate; 28 μg protein was applied. Electrophoregrams were stained with Commassie Brilliant Blue. The lower part of the Figure represents schematically the distribution of the bands while the upper part is a densitogram. Approximate molecular weights corresponding to the bands were estimated as follows: 1, 230 000; 2, 195 000; 3, 160 000; 4, 146 000; 5, 130 000; 6, 120 000; 7, 105 000; 8, 88 000; 9, 80 000; 10, 67 000; 11, 65 000; 12, 55 000; 13, 40 000; 14, 23 000; 15, 20 000; 16, 13 000; 17, 5 000.

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dex filtration. Moreover, quantitative distribution of proteins between fractions obtained by gel filtration and those from

polyacrylamide electrophoresis is strikingly similar. This provides a strong suggestion that the two main electrophoretic bands /Fig. 4/ correspond to the two major peaks from gel filtration /Fig. 3/ and gives a basis to attribute the latter peaks to proteins of molecular weight of 88 000 and 67 000. On the same basis the two magnesium peaks can be estimated as corresponding to the molecular weight of 150 000 and 100 000.

It can be thus concluded that the intermembrane compartment of rat liver mitochondria contains at least two distinct high molecular weight compounds, most likely proteins, able to bind Mg^{2+}. The compound of 150 000 daltons forms a loose magnesium complex which is in equilibrium with external free Mg^{2+}. On the contrary, the compound of 100 000 daltons binds magnesium firmly and is responsible for a part of the non-diffusible magnesium of the intermembrane compartment /1/.

The role of both magnesium-binding proteins is not known. Neither of them is identical with either adenylate kinase or the calcium-binding glycoprotein isolated by Sottocasa et al. /6, 7/ since both these proteins have a much lower molecular weight /6, 7/. The calcium-binding glycoprotein can also bind Mg^{2+} /7/, but its affinity towards this ion has not been determined. It is also to be noted that the affinity to Mg^{2+} of the 100 000 dalton protein as determined in the present investigation /K_d of the order of 10^{-6}M/ is almost as high as the affinity to Ca^{2+} of the glycoprotein of Sottocasa et al. /K_d around 10^{-7}M, ref. 6 and 7/.

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