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Binding of Calcium Ions with Mitochondrial ATPase Preparations

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One of the unsolved problems of energy exchange in mitochondria is the mechanism of Ca^{2+} ion active transport. According to the most common opinion Ca^{2+} ions interact in mitochondria with translocase which performs Ca^{2+} transfer across the hydrophobic region of membrane. Isolation from mitochondria of a translocase system evidently would favour the investigation of the mechanism and reconstruction of active transport. The specific property of translocase is its ability to complex with Ca^{2+} ions, which may be used for an identification of carriers isolated from the membrane. Recently Lehninger [1] isolated from mitochondria of the soluble Ca^{2+} binding factor by the exposing rat liver mitochondria to osmotic shock. Some experimental data discussed in our previous report [2] suggested that mitochondrial ATPase plays the role of Ca^{2+} ion translocase. In connection with this, in the present study the Ca-binding with various preparations of the mitochondrial ATPase was investigated.

Methods

ATPase was prepared from acetone powder of mitochondria and from submitochondrial particles. The rat liver mitochondria were washed by 10^{-3}M Tris buffer at first and then by 10^{-1} M KCl solution for removing the outer membrane and cytochrome C. Washed fragments of the mitochondria were acetone treated by Selwyn's procedure [3]. Acetone powder was extracted by 10^{-3} M Tris buffer at 20° C and

centrifuged at 150,000 *g* for an hour. There were no cytochromes in the supernatant but more than 90% of flavoproteins and nearly total ATPase activity. The protein fraction precipitated from the extract between 40-50% saturation with $(NH_4)_2SO_4$ was used to investigate the Ca^{2+} binding ability.

For preparation of submitochondrial particles, the washed fragments of mitochondria (40 mg of protein/ml) were treated with 0.7% Triton X-100 and centrifuged at 150,000 *g* for 1 h. The precipitate was suspended in the medium containing 0.5% Triton X-100 and 0.2 M Na_2SO_4 so that the concentration of protein would be 30 mg/ml and then this suspension was centrifuged at 150,000 *g* for 1 h again. More than 50% of the ATPase activity and some amount of flavoproteins were found in the supernatant. Binding of Ca^{2+} ions to ATPase took place during gel filtration on Sephadex G-200. The columns with Sephadex ($l = 250$ mm, $d = 16$ mm) were equilibrated with solution containing 10^{-3} M tris-buffer and 10^{-6} M $^{45}CaCl_2$ with specific activity 1.9 mc/mM. Elution was carried out by the same buffer. The sample of ATPase was applied in 0.7 ml to the top of the column. The eluate was collected in 2 ml fractions. The protein content determined by method of Lowry *et al.* [4], the binding of ^{45}Ca and ATPase activity were estimated in the effluent fractions. At the same time the flavin-containing compounds were determined by fluorometry since they were present in the preparations of ATPase.

Results

Figure 1 shows the Ca^{2+} binding to the preparation of ATPase isolated from acetone powder of liver mitochondria. As is seen, the maximum content of protein, of the Ca^{2+}-binding and of ATPase activity are found in the same fraction, maximum content of flavins being discovered in the following one.

The character of protein and ^{45}Ca distribution in fractions indicates that there are more than one Ca^{2+}-binding components in the investigated preparations.

Some characteristics of the Ca-protein complex in the fraction with maximum ATPase activity were investigated such as the number of the Ca^{2+}-binding sites and dissociation constant. The concentration of calcium in the equilibrating buffer may be regarded as a content of free calcium in the effluent fraction of ATPase preparation accepting that the volume of the column is big enough. Total Ca^{2+} content in the effluent fraction would be equal to the sum of free and bound calcium. The amounts of bound calcium have been determined in the range of free Ca^{2+} concentration from 2.10^{-7} M to 2.10^{-6} M. The calculation of

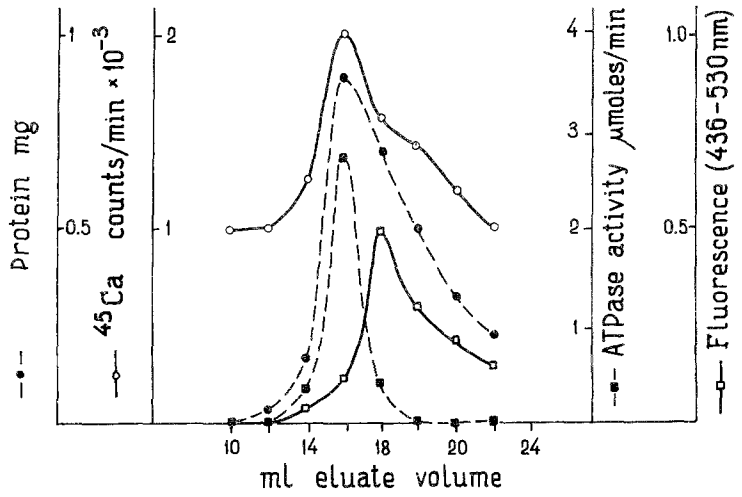

Figure 1. ATPase activity and content of protein, ^{45}Ca and flavins in ATPase preparations, obtained from acetone powder.

constants according Scatchard plot [5] gives the value of 4.10^{-6} M/g of protein for the number of high affinity sites and 2.10^{-6} M for dissociation constant.

Taking into consideration the total amount of extracted protein possessing both ATPase and Ca^{2+} binding activity the net content of high affinity Ca^{2+} binding sites in the original mitochondria is about 5.10^{-10} mol/mg of mitochondrial protein.

Figure 2 shows the Ca^{2+} binding in the fractions of extract obtained from submitochondrial particles. The most content of ^{45}Ca, flavins and the largest ATPase activity are found in the same fraction. This fraction gives one main protein band upon electrophoresis in polyacrylamide gel.

Since the pyrophosphate bond of FAD is a potential Ca^{2+} binding group, the hardly separated from ATPase flavin may play a significant role in the observed Ca^{2+} binding.

As is seen from Fig. 2 in the detergent extracted ATPase preparations the second maximum of protein content and Ca^{2+} binding is present. This low-molecular weight compound binds much more ^{45}Ca/g of protein than ATPase and has no enzyme activity. Similar compounds are in the acetone powder extract. The function of Ca^{2+}-binding protein with low molecular weight in mitochondria is unknown yet.

Thus the results of the present investigation indicate that preparation of ATPase isolated from the inner mitochondrial membrane contain the components which are able to form strong complex with Ca^{2+} ions. These compounds perhaps may take part in the active transport of Ca^{2+}

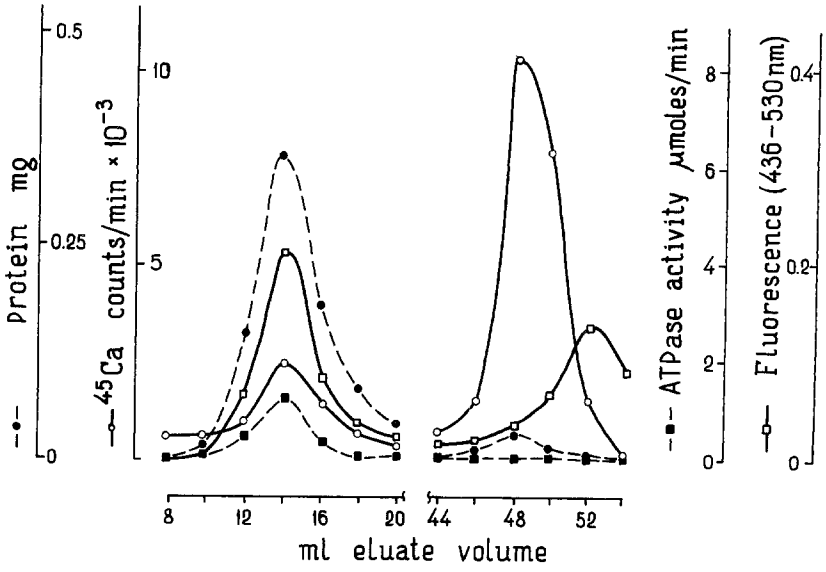

Figure 2. ATPase activity, content of protein, ^{45}Ca and flavins in detergent extracted ATPase preparations.

ions across the mitochondrial membrane and in Ca^{2+} activated ATP hydrolysis in mitochondria. Found Ca^{2+} binding components distinguish evidently from the Ca^{2+} binding factor described recently by Lehninger [5] because the investigated preparations of ATPase were obtained from mitochondria exhausted of Lehninger's factor.

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