ISOLATION OF A CYTOPLASMATIC REGULATOR OF THE TRANSPORT OF Ca^{2+} IN LIVER MITOCHONDRIA

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From rabbit liver cytoplasm a low-molecular-weight "factor" has been isolated which in low concentrations of the order of 10^{-6} M increases the capacity of mitochondria for accumulating Ca^{2+} ions. The incubation medium for the mitochondria contained 10^{-1} M KCl, 10^{-2} M succinate, 10^{-3} M phosphate, 10^{-2} M tris (pH 7.3), and rotenone (0.5 μg/ml). Rabbit liver was homogenized in 0.3 M sucrose $+5 \cdot 10^{-3}$ M tris, pH 7.5, and was centrifuged at 3000g for 20 min. The supernatant was heated at 95°C for 5 min and was then recentrifuged. After concentration in a rotary evaporator the material was deposited on a column of Sephadex G-25 (90×2.5 cm) and was eluted with distilled water. Activity was detected in the yellow peak of lowest molecular weight. The fraction containing the activity was brought to pH 9.7 and deposited on a column of DEAE-cellulose (15 × 140 mm). Activity was found in the fraction eluted from the column by 0.04 M KCl +5 mM tris, pH 7.0. When the active fraction was rechromatographed on a column [DEAE-Sephadex A-25 (15×150 ml)] it was possible to separate it into two peaks according to the optical density at 280 nm. Activity was detected in the larger peak, which was eluted from the column first when 0.04 M KCl was added.

The UV spectrum of the purified factor from the cytoplasm showed the presence of a nucleotide in the preparation (presumably an adenine nucleotide).

The purified material was shown by the biuret method to contain peptide bonds. Our factor is apparently an adenosylpeptide.

Apart from the increase in the "calcium capacity" of the mitochondria, the factor isolated from the cytoplasm increases the rate of oxidative phosphorylation of mitochondria charged with calcium and prevents the inhibition of oxidative phosphorylation induced by storing a suspension of mitochondria under conditions of hypoxia at room temperature.

Another factor from cytoplasm has been described in the literature – CMF [1]. In contrast to our factor, for which $V_E/V_O = 2.6$, CMF is eluted from a column of Sephadex G-25 immediately after the proteins, which pass through the free volume of the column. In contrast to the factor that we have described, CMF does not absorb in UV light, does not contain peptide bonds, and is unstable at room temperature. The metabolic effects of CMF and of our factor are also different. This permits the conclusion that cytoplasm contains another regulator of the function of the mitochondria besides CMF.

LITERATURE CITED

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