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ISOLATION OF A CYTOPLASMIC INHIBITOR OF THE TRANSPORT OF CALCIUM IONS IN HEART MITOCHONDRIA

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The transport of calcium ions from the myoplasm into the mitochondria is one of the mechanisms regulating the concentration of ionized calcium in the myocardium [1, 2]. Our aim was the isolation from the heart cytoplasm of a thermostable factor inhibiting the permeability of the internal membrane of heart mitochondria for calcium ions.

The mitochondria were isolated from rat heart in an isolation medium containing 0.3 M sucrose, 1 mM EDTA, and 5 mM Tris, pH 7.4. The thermostable fraction of the cytoplasm was obtained by centrifuging a rabbit heart homogenate (1 g/1 ml of incubation medium containing 0.12 M KCl and 5 mM Tris, pH 7.4) at 30,000 g for 20 min. The supernatant was heated at 97°C for 7 min and the denatured proteins were then centrifuged off.

The thermostable fraction of the cytoplasm obtained from 40 g of rabbit heart was concentrated in a rotary evaporator to 1.5 ml and was deposited on a column of Sephadex G-25 (25 × 400 mm), which was equilibrated with and eluted by 0.1 M KCl. The optical density at 260 nm and the activity of the electrogenic transport inhibitor with respect to its action on the swelling of deenergized rat heart mitochondria in an isoosmotic solution of $Ca(NO_3)_2$ (80 mM $Ca(NO_3)_2$, 10^{-4} M 2,4-DNP, 0.7 μg/ml of rotenone, 10 mM Tris, pH 8.1, 0.5 mg of protein/ml of mitochondria) were measured in the fractions obtained.

Activity was detected in fractions of relatively high-molecular-weight proteins and in a low-molecular-weight fraction (V_E/V_0 = 2.15) (Fig. 1). It was concluded that the inhibitor present is in the cytoplasm both in the free form and in a protein-bound state.

The low-molecular-weight fraction containing inhibitor activity was diluted 20-fold, brought to pH 6.0, and deposited on a column of CM-cellulose (10 × 100 mm) equilibrated with 2 mM Tris, pH 6.0. The fractions containing activity were eluted from the column with 100 ml of 0.1 M KCl.

It was shown by the method of gel filtration and ion-exchange chromatography that the inhibitor of the electromagnetic transport of Ca^{2+} into heart mitochondria is an alkaline

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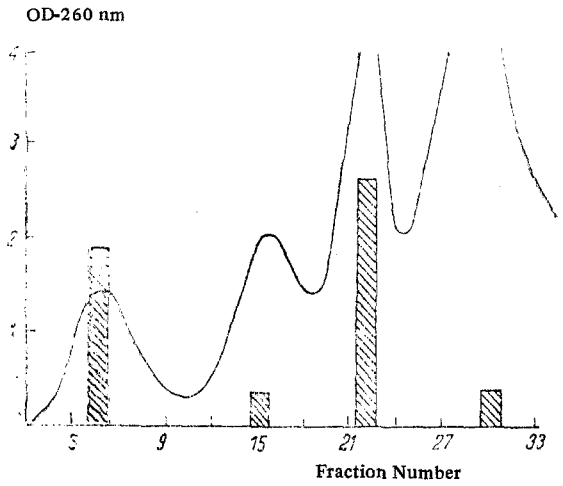

Fig. 1. Gel filtration of the thermostable fraction of rabbit heart cytoplasm on a column of Sephadex G-25. V_0 = 80 ml; 5-ml fractions. The hatched columns indicate inhibitor activity.

glycopeptide with a molecular weight of 1500-2000 daltons (protein/carbohydrate ratio approximately 3:1) the active concentration of which is 3–10 μg/ml.

It is assumed that the inhibition of the electrogenic transport of Ca^{2+} from the myoplasm into the mitochondria by the glycopeptide that we have isolated is one of the mechanisms ensuring an increase in the concentration of ionized calcium in the myoplasm on contraction of the myocardium.

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