"KINASINE" - A GLYCOLYSIS STIMULATING PROTEIN OF HEART MITOCHONDRIA

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Received October 23, 1963

On incubation, mitochondria isolated from normal or cancer cells release a nucleotide and a protein factor into the medium. Separated from mitochondria, and added to the soluble cellular fraction (SF), these factors cause a marked intensification of glycolysis (Neifakh et al.,1961,1962,1963). Analysis has shown the nucleotide factor to be chiefly ATP with slight admixture of other coenzymes of glycolysis. The presence of this factor in a mitochondrial medium could be easily predicted. It is different in the case of the protein factor - neither its nature, origin or mechanism of action had ever been referred to in the literature.

We succeeded in obtaining this protein in pure state. As it was found to increase the activity of kinase in glycolytic oxidoreduction, the compound was given the name "kinasine".

A suspension of rabbit myocardial mitochondria was prepared in a mixture of 0.25 M mannitol - 0.05 M Tris buffer, pH 7.4, at a concentration of 15-20 mg protein per 1 ml of the mixture. It was incubated for 30 minutes at 30°C. After removal of mitochondria the supernatant liquid was run through a gel column of Sephadex G-50 (Pharmacia, Uppsala),7.5x2.5 cm.

Then it was eluted with bi-distilled water. The effluent contained two fractions: nucleotide and protein. The protein fraction (0.5-0.7 mg/ml) was dried by lyophilization. The accumulated protein (175 mg) was dissolved in 5 ml of 0.05 M medinal, pH being adjusted to 10.5 with 1 N NaOH. The transparent light-brown protein solution was placed on a column of cellulose powder and zonal electrophoresis after Porath(1956) was performed in the LKB apparatus (Stockholm). The movement of the coloured protein zone could be observed visually. The separated zones were eluted from the column and passed through "Uvicord". Three major peaks were recorded on the "Uvicord" tape, corresponding to the three protein fractions. Each fraction was tested for ability to stimulate SF glycolysis in a medium saturated with coenzymes. All the stimulating activity was found to be concentrated in the third light-brown fraction (aliquots N 54-84) (Fig.1).

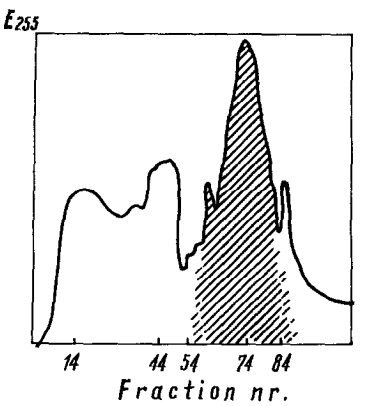

Figure 1.
Zone electrophoresis in packed column with cellulose powder. A potential gradient of 8volt/cm; veronal buffer pH 8.6; μ = 0.05, time 12 hours. Shaded peak on the "Uvicord" tape corresponds to the protein fraction stimulating glycolysis.

Specific activity of this fraction (its capacity to enhance glycolysis in terms of μg of lactate per 1 mg protein) had increased to 100 of that of mitochondria (Table 1).

Table 1

Experimental data on purification of the mitochondrial glycolysis stimulating protein ("Kinasine")

Fraction	Specific activity μg lactate/mg protein	Enrichment
Suspension of myocardial mitochondria	50	1
Mitochondrial medium after incubation	136	2.7
Protein fraction after Sephadex, lyophilized	360	7.2
Protein fraction isolated by means of electrophoresis ("kinasine")	5082	101.6

Upon ultracentrifugation (centrifuge Phywe, Göttingen) "kinasine" was found to be a perfectly homogenous protein (Fig.2).

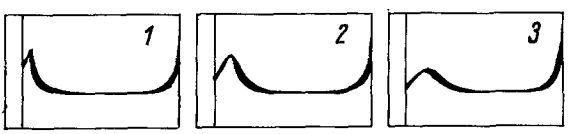

Fig.2. Sedimentation patterns of "kinasine". Protein 7 mg in 1 ml in veronal buffer pH 8.6; μ = 0.05;50000 r.p.m. Temperature 17°C. Photographs taken in 5(1), 15(2) and 35(3) minutes after attaining the maximal rate. Bar angle 40°.

Its sedimentation constant S_{20} = 2.8 was almost invariable in the 3-7 mg/ml range of protein concentration, which indicates the absence of protein interaction in the solution. The diffusion constant D_{20} determined with the aid of a polarization interferometer was $2.5 \cdot 10^{-7}$ cm^2/sec. The molecular weight was calculated to be approximately 97000 assuming a partial specific volume of 0.72. Electrophoresis of "kinasine" in the gradient of sucrose concentration according to Svensson (1960) revealed the presence of two components with almost similar electrophoretic mobility (Fig.3).

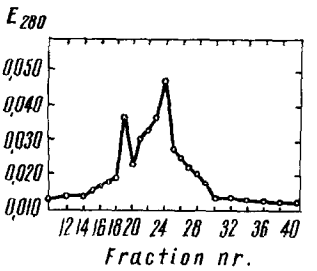

Figure 3.

Electrophoresis patterns of "kinasine" in the sucrose gradient. Protein 2.5 mg/ml in veronal buffer pH 8.6; $\mu = 0.05$. Sucrose gradient from 0 to 20%. The mean potential gradient = 7 volt/cm, time of electrophoresis 2 hours. Volume of each fraction 0.6 ml.

A similar result was obtained from a study of moving boundary electrophoresis after Tiselius in a Hilger apparatus (London) (Fig.4).

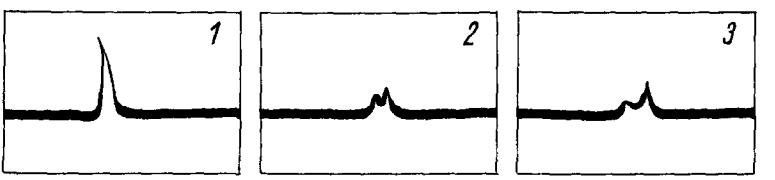

Fig.4. Electrophoresis patterns of "kinasine" after the method of moving boundary. Protein 2.5 mg/ml in veronal buffer pH 8.6; $\mu = 0.09$. The potential gradient = 8.5 v/cm. Photographs taken in 15(2) and 30(3) minutes after electrophoresis was started. Bar angle 35°.

The electrophoretic mobility of the protein U = $= 6.7 \cdot 10^{-5} cm^2 sec^{-1} volt^{-1}$. The protein solution stains light blue with Sudan black. Paper electrophoresis of "kinasine" shows only one protein band, stained with bromphenol blue and showing the same mobility as the band stained with Sudan. Consequently, "kinasine" proves to be a lipoprotein. The lipid content of "kinasine", determined by isooctane extraction was found to be about 200 μg/mg protein.

Owing to this, "kinasine" is poorly soluble in water, but after alkalinizing of the medium it dissolves more readily and at pH 10.5 a solution can be prepared with a protein concentration of about 3.5%. The absorption spectrum of "kinasine" has a characteristic maximum in the 410 mμ range. On

the addition of dithionite to the solution this maximum shifts to the 425 mμ region with a small peak appearing at 550 mμ The addition of ferricyanide results in a considerable decrease in the 425 mμ maximum, while the 550 mμ maximum disappears completely.

At first sight some of the properties displayed by "kinasine" appear similar to those of the structural protein of heart mitochondria discovered by Criddle et al.(1962). However molecular weight, electrophoretic mobility and spectrum of adsorption indicate that "kinasine" is not identical to any of the proteins hitherto detected in mitochondria.

On the intact cell, when the ATP level is high, "kinasine" appears to be linked to actomyosin of mitochondrial membranes and is not likely penetrate into hyaloplasm (Neifakh and Kazakova,1963). Reduction of ATP level may change the configuration of mitochondrial actomyosin, disrupting link between actomyosin and "kinasine", The latter is them released into hyaloplasm, where it accelerates glycolysis.

References

Criddle R.S., Bock R.M., Green D.E., Tisdale H., Biochemistry, 1, 827 (1962).
Neifakh S.A., Kazakova T.B., Melnikova M.P., Turovski V.S., Dokl.Akad.Nauk SSSR, 138, 227 (1961).
Neifakh S.A., Gaitskhoki V.S., Kazakova T.B.,Melnikova M.P., Turovski V.S., Dokl.Akad.Nauk SSSR, 144, 449 (1962).
Neifakh S.A., Gaitskhoki V.S., Kazakova T.B., VIII Internat. Cancer Congress, Moscow, July 1962.
Neifakh S.A., Kazakova T.B., Nature (Lond.),197,1106 (1963).
Porath J., Bioch.Biophys.Acta, 22, 151 (1956).
Svensson H., A lab.manual of analyt.methods of protein chemistry (ed.P.Alexander and R.J.Block), Vol.1, p.195, (1960).