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RAT BRAIN HEXOKINASES OF TWO DIFFERENT MOLECULAR SIZES

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SUMMARY

Two different molecular species of particulate hexokinase (ATP:D-hexose 6-phosphotransferase, EC 2.7.1.1) from rat brain were separable by gel filtration. The higher-molecular weight hexokinase (hexokinase-H) was converted to the lower-molecular weight enzyme by treatment with ethanol or by digestion with phospholipase A. The lower-molecular weight hexokinase (hexokinase-L) was converted to hexokinase-H by incubation with lecithin. These results indicate that hexokinase-H is an aggregate of hexokinase-L and phospholipid moieties.

INTRODUCTION

Soluble hexokinase (ATP:D-hexose 6-phosphotransferase, EC 2.7.1.1) in frog skeletal muscle apparently exists in both a lower-molecular weight and a higher-molecular weight species [1]. Recently, Craven and Basford [2] found that particulate hexokinase from rat brain, solubilized with glucose 6-phosphate, is part of a large aggregate as shown by its appearance in the void volume upon Sephadex G-200 column chromatography. Their experiments suggested that the enzyme exists as a lipoprotein complex. We have found that particulate hexokinase of rat brain also exists as two molecular species which can be separated by gel filtration. Moreover, the higher-molecular weight hexokinase (hexokinase-H) was converted to the lower-molecular weight enzyme (hexokinase-L) by treatment with ethanol or by digestion with phospholipase A.

MATERIALS AND METHODS

Enzyme preparation

The enzyme was prepared by the procedure of Baquer and McLean [3] with a slight modification. Male Sprague–Dawley rats weighing 140–150 g were used. The cerebra were homogenized in 5 volumes of ice-cold 0.02 M Tris–HCl buffer, pH 7.5, containing 150 mM KCl, 5 mM $MgCl_2$, 5 mM EDTA and 0.5 mM dithiothreitol.

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The homogenate was centrifuged at 700 × *g* for 10 min and then the supernatant was centrifuged at 105 000 × *g* for 45 min. The pellet, containing the particulate enzyme, was washed once with the same buffer solution and then suspended in the buffer solution containing 0.5 M glucose and 1 % Triton X-100 for 1 h at 25 °C with gentle stirring to solubilize the enzyme. The pellet obtained from 1 g of cerebra was suspended in 1 ml of the Tris buffer solution. The suspension was centrifuged at 105 000 × *g* for 20 min. The supernatant was used as the particulate enzyme.

Hexokinase assay

Hexokinase activity was assayed by the method of Sharma et al. [4].

RESULTS

Fig. 1 shows a typical gel-filtration pattern of particulate hexokinase of rat brain. Two peaks of hexokinase activity were consistently observed, indicating that the hexokinase existed in two molecular sizes. Hexokinase-H was eluted immediately following the void volume (V_0), while elution of hexokinase-L was retarded. Although

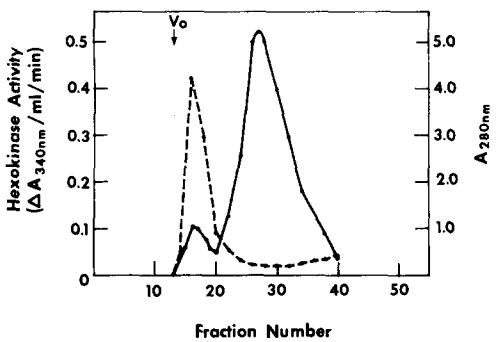

Fig. 1. Gel filtration of particulate hexokinase on Sephadex G-200. 2 ml of particulate fraction were applied to a Sephadex G-200 column (2.5 cm × 26 cm) and eluted with 0.02 M Tris–HCl buffer, pH 7.5, containing 150 mM KCl, 5 mM $MgCl_2$, 5 mM EDTA and 0.5 mM dithiothreitol. Fractions of 2.2 ml each were collected. ——, hexokinase activity; – – – –, absorbance at 280 nm.

Karpatkin [1] reported that the two molecular species of soluble hexokinase in frog skeletal muscle were interconvertible with changes in ionic strength, we did not observe a change in the relative distribution of hexokinase-H and -L when the ionic strength of the buffer was decreased by using 0.01 M potassium phosphate, pH 7.5, containing 10 mM glucose, 2 mM EDTA and 0.5 mM dithiothreitol instead of the usual Tris buffer solution. When the particulate enzyme solution was dialysed against the Tris buffer solution to remove Triton X-100, the elution profile was not altered. Furthermore, as can be seen in Fig. 2, the elution pattern of the soluble hexokinase fraction, obtained from the first high-speed supernatant which did not contain Triton X-100, showed the same profile as that of the particulate enzyme. These results suggest that two molecular species of hexokinase exist in rat brain cells and are not an artifact of solubilization of the enzyme.

The fractions containing hexokinase-H were milky in appearance. Moreover,

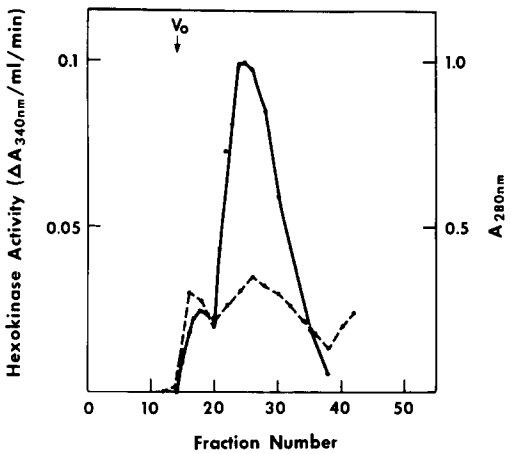

Fig. 2. Gel filtration of soluble hexokinase on Sephadex G-200. 2 ml of soluble fraction were applied to a Sephadex G-200 column. The conditions were the same as those described in the legend to Fig. 1. ——, hexokinase activity; - - - -, absorbance at 280 nm.

after electrophoresis of hexokinase-H fractions on 5% polyacrylamide gel, hexokinase activity remained at its origin. These observations suggested that hexokinase-H was associated with lipid. To investigate this possibility, several types of experiments were done. In the first, the hexokinase-H fractions of several columns were combined and treated with cold 95% ethanol, in an ethanol to hexokinase solution ratio of 1:2 (v/v), in the presence of 4 mM ATP and 0.1 M glucose. This solution was mixed vigorously on a cyclo-mixer for 10 s and then centrifuged at 12 000 × *g* for 15 min. The resulting precipitate was dissolved in the Tris buffer solution containing 4 mM ATP. It was found that treatment with ethanol alone inactivated the enzyme, but that the presence of ATP (4 mM) protected the enzyme from inactivation. The insoluble material was removed by centrifugation at 3000 × *g* for 10 min. The ethanol-treated hexokinase-H was chromatographed on a Sephadex G-200 column. The results obtained are shown in Fig. 3 which shows the elution pattern before (A) and after (B) treatment with ethanol. It is clear that treatment of hexokinase-H with ethanol resulted in the disappearance of activity in the fraction corresponding to the original hexokinase-H and a concomitant appearance of activity in the elution position of hexokinase-L. The activity of the hexokinase-L thus recovered was 40% of the original hexokinase-H activity.

In a second type of experiment to investigate whether hexokinase-H was associated with lipid, 4 mg hexokinase-H was incubated with 10 μg of phospholipase A (from Viper russelli, Sigma Chemical Co.) for 2 h at 37 °C in the presence of 0.05 M Tris buffer (pH 7.5), 25 mM $CaCl_2$ and 5 mM $MgCl_2$. After incubation, the mixture was fractionated by means of a Sephadex G-200 column. The elution pattern of hexokinase-H after digestion with phospholipase A was almost exactly the same as that obtained after ethanol treatment (Fig. 3). The activity of the enzyme in the elution position of hexokinase-L following this procedure was 85% of the original hexokinase-H activity.

To further characterize the enzyme converted from hexokinase-H by treatment with ethanol, it was subjected to polyacrylamide gel electrophoresis. It was found

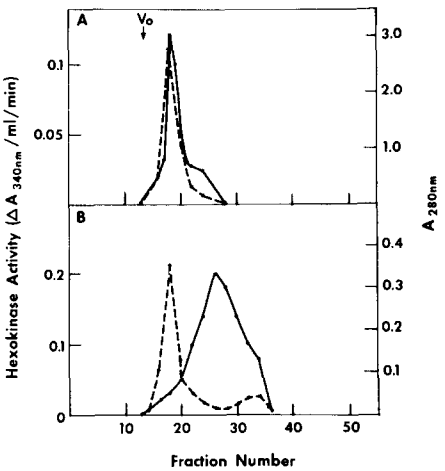

Fig. 3. Conversion of hexokinase-H to hexokinase-L by treatment with ethanol. (A) 10 ml of hexokinase-H fractions eluted from a Sephadex G-200 column as in Fig. 1 were placed in cellophane dialysis tubing and concentrated 6-fold in the presence of 0.5 M glucose by immersion in polyvinylpyrrolidone powder. No substantial loss of enzyme activity occurred. The concentrated solution was then passed through a Sephadex G-200 column. The conditions were the same as those described in the legend to Fig. 1. (B) Hexokinase-H fractions prepared as in (A) were treated with ethanol and 2 ml were applied to a Sephadex G-200 column (see text for details). ——, hexokinase activity; – – – –, absorbance at 280 nm.

that the converted enzyme migrated to the same position as that of original hexokinase-L.

Finally, the ethanol extract of hexokinase-H was subjected to thin-layer chromatography with a solvent system chloroform–methanol–distilled water (65:25:4, by vol.). Only phosphatidylcholine was found to be present in the extract. Taken together, these results indicate that hexokinase-H is a complex of hexokinase-L and phosphatidylcholine molecules.

To determine whether hexokinase-L can be converted to hexokinase-H,

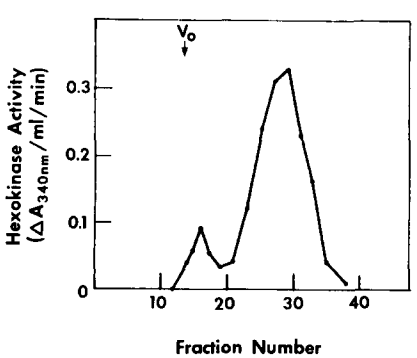

Fig. 4. Elution profile of hexokinase-L after incubation with lecithin. Partially purified hexokinase-L (1.5 ml, 1.5 mg protein) was incubated with 5 mg of lecithin at 0 °C for 16 h. The mixture (2 ml) was applied to a Sephadex G-200 column (2.5 cm × 26 cm). The conditions were the same as those decribed in the legend to Fig. 1. ——, hexokinase activity.

partially purified hexokinase-L was prepared by Triton X-100 solubilization and Sephadex G-200 column chromatography. The hexokinase-L thus prepared was incubated with lecithin (Sigma Chemical Co.) at 0 °C for 16 h. It can be seen in Fig. 4 that incubation of hexokinase-L with lecithin resulted in the appearance of a fraction corresponding to hexokinase-H. Thus, it has been demonstrated that hexokinase-H and -L are interconvertible.

DISCUSSION

Wilson [5] has recently reported that rat brain hexokinase solubilized with glucose 6-phosphate has a molecular weight of approx. 100 000 and contains bound phospholipid which apparently is required for the interaction of the enzyme with the mitochondrial membrane. If the partially purified hexokinase-L solubilized in the present work with Triton X-100 also contained phospholipid, it is possible that hexokinase-L was converted to hexokinase-H by interactions between bound phospholipid and other phospholipid molecules. Moreover, it seems likely that the small difference in the molecular weights of the soluble and mitochondrial forms of hexokinase reported by Teichgräber and Biesold [6] resulted from additional interactions of the phospholipid bound to the soluble hexokinase with substances such as phospholipid and lipid.

The present data demonstrate clearly that hexokinase-H is composed of hexokinase-L and phospholipids. Although the physiological significance of hexokinase-H has yet to be clarified, our results support the view of Craven and Basford [2] that hexokinase-H exists in the rat brain cell as an aggregate of hexokinase-L and phosphatidylcholine.

ACKNOWLEDGMENT

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