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Resolution of two high-K_m ATP:D-hexose 6-phosphotransferase bands by starch-gel electrophoresis

To date four ATP D-hexose 6-phosphotransferases (EC 2 7 1 1) have been identified in rat liver supernate[1]. These have been designated I, II, III, and IV, according to their migration on starch-gel electrophoresis[2]. Types I, II, and III have been shown to be typical animal hexokinases with molecular weights of 96 000 and low K_m values for glucose[3]. Type IV, however, has previously been found only in liver extracts It has a lower apparent molecular weight than the other animal hexokinases[3,4], a higher K_m for glucose[5], and has been shown to decrease in activity with fasting and diabetes[5]

It has now been found with starch-gel electrophoresis that under well-defined conditions two type IV bands, designated IV_f (fast form) and IV_s (slow form), are seen in liver extracts (Fig. 1) If electrophoresis is performed with EDTA in the barbital buffer (Fig. 1, column 1), a typical pattern, as reported by KATZEN AND SCHIMKE[2],

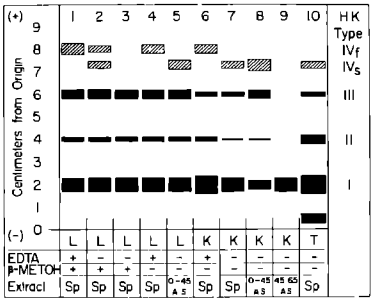

Fig 1 Diagram of the electrophoretic patterns of liver (L), kidney (K), and testis (T) extracts under a variety of conditions Homogenates of the tissues were made with buffer containing 0 15 M KCl, 0 005 M $MgSO_4$, 0 005 M EDTA, and 0 005 M 2-mercaptoethanol (β-METOH), pH 7 0 and centrifuged for 60 min at 105 000 × g (Sp) and 0–4° in a Spinco Model L ultracentrifuge $(NH_4)_2SO_4$ (AS) fractionation was performed on some of the supernatant fractions, as indicated in the diagram Samples (0 05 ml) of the supernatant and $(NH_4)_2SO_4$ fractions were subjected to vertical starch-gel electrophoresis[12] for 16 h at a current of 6 V/cm Barbital buffer (0 05 M, pH 8 6) with or without EDTA and/or 2-mercaptoethanol was used to polymerize the starch and in the buffer chambers Hexokinase bands were located on the starch gels by the method of KATZEN AND SCHIMKE[2] Column 3 is the pattern obtained when the gel is stained with 0 5 mM glucose in the staining mixture All other columns were stained using 100 mM glucose Hatched areas indicate bands which appear at 100 mM but not at 0 5 mM glucose concentrations, indicating that they are high-K_m isozymes None of the bands depicted in this figure appeared if ATP was omitted from the staining mixture

is seen. If EDTA is omitted, two bands appear in the position of type IV The appearance of the two bands is dependent upon the omission of EDTA from the electrophoresis buffer only its presence or absence in the homogenizing buffer does not affect the appearance of two type IV bands The presence or absence of 2-mercaptoethanol in the electrophoresis buffer or the homogenizing buffer was without effect on the appearance of the two type IV bands The slow form of type IV is also observed in extracts of testis and kidney

The slow form of type IV could be separated from other ATP:D-hexose 6-phosphotransferases in both liver and kidney extracts

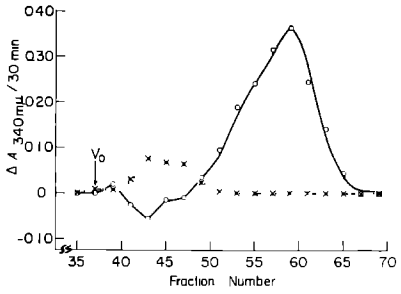

Fig 2 Sephadex G-100 gel filtration of a partially purified IV_s fraction from rat liver Rat liver 105 000 × *g* supernatant was subjected to $(NH_4)_2SO_4$ fractionation (0–45%), DEAE-cellulose chromatography, and concentration by $(NH_4)_2SO_4$ (0–45%) prior to application to Sephadex The Sephadex G-100 column (2 cm × 70 cm) was equilibrated with homogenizing buffer (Fig 1) The high-K_m IV_s (solid line) activity peak coincides exactly with that for purified IV_f, indicating that both have an apparent molecular weight of 48 000 A small amount of low-K_m hexokinase contaminant (dashed line), mostly type III, can be seen to be separated from the IV_s activity A similar elution profile from Sephadex is obtained for partially purified IV_s from kidney ATP D-hexose 6-phosphotransferase activity was assayed by the method of VINUELA, SALAS AND SOLS[13] Activity is represented by relative change in absorbance at 340 mμ The positions of the void volume (V_0), peak for low-K_m hexokinases, and the purified IV_f (as determined by previous runs on the same column) were at Fractions 37, 43, and 59, respectively

In liver, IV_s is found with the low-K_m hexokinases in the 0–45% satd. $(NH_4)_2SO_4$ fraction, IV_f precipitates only in the 45–65% satd. fraction Liver IV_s was purified as described in the legend for Fig. 2 The final gel filtration separates the contaminating low-K_m hexokinases from IV_s. The IV_s form, like IV_f, has an apparent molecular weight of 48 000 by the Sephadex filtration method (Fig 2) The IV_s form, like IV_f, also has a high K_m for glucose: after starch-gel electrophoresis neither band is stained when the glucose concentration in the staining mixture is reduced to 0 5 mM (Fig. 1, column 3).

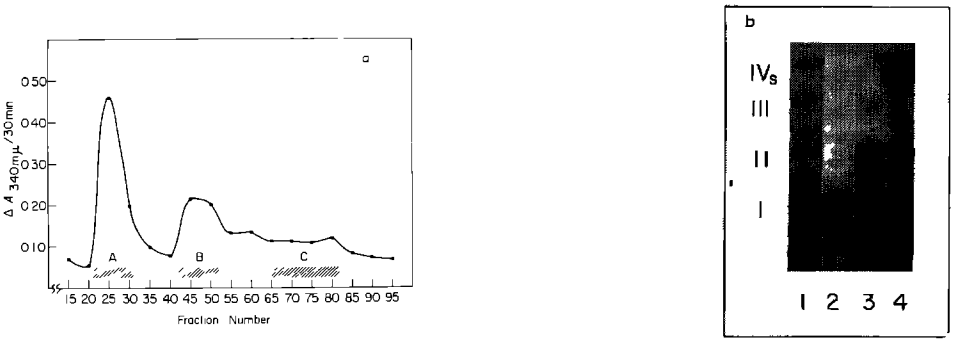

Fig 3 a DEAE-cellulose chromatogram of rat kidney supernatant The kidney extract, prepared as described in Fig 1, was applied to a DEAE-cellulose column (15 cm × 3 cm) and eluted with a linear KCl gradient (0 02–0 6 M) Fractions were assayed and activity represented as in Fig 2 Area A was pooled as representing the hexokinase I peak, area B as the type II peak, and area C as the area containing types III and IV_s The pooled fractions were concentrated by precipitation with $(NH_4)_2SO_4$ (0–45%) and resolubilization in a minimum volume of homogenizing medium These concentrated fractions were then electrophoresed on starch gel and stained for hexokinase activity the photograph of resultant patterns is seen in b When compared to a supernatant extract (column 1) area A (column 2) contains only type I, area B (column 3) contains types I, II and III, and area C (column 4) contains mainly types I and IV_s with types II and III as minor contaminants

In kidney, no IV_f is seen in the original extract in the absence of EDTA (Fig. 1, column 7) The IV_s was separated from the low-K_m hexokinases (Figs 3a and 3b) It is of interest that DEAE-cellulose chromatography does not resolve a clearly defined IV_s peak Nonetheless, if the region where liver IV_f normally elutes from DEAE-cellulose (Fig 3a, area C) is subjected to starch electrophoresis, it contains IV_s as the predominant form (Fig. 3b, column 4). The K_m for glucose, the molecular weight, and the salt solubility of kidney IV_s are the same as those of liver IV_s

A significant difference between the two forms is that the slow form is not inhibited by a specific antibody made to the purified fast form from rat liver which was shown not to cross-react with any of the low-K_m hexokinases[6]

The physiological significance of the two type IV forms is far from clear. S. WEINHOUSE (personal communication) has also observed two type IV bands in liver and some hepatomas. We have previously shown that there is a preferential disappearance of the IV_f band in fasting and diabetes, type IV_s being apparently unchanged[7]. Other investigators have demonstrated the appearance of a variable number of hexokinase bands depending on the extraction and/or electrophoretic procedures employed[8,9]. The lactate dehydrogenase isozyme system also exhibits extensive sub-banding[10,11]

In order to investigate the nature and function of such multiple forms it is necessary to isolate each and study its chemical and physical properties With these goals in mind we have been able to separate IV_s and show that it differs from IV_f in terms of salt solubility, electrophoretic mobility, antigenicity, and adaptive characteristics, while exhibiting a similar K_m and molecular weight. This evidence suggests that IV_s and IV_f are different molecular forms

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