MULTIPLE FORMS OF PHOSPHOFRUCTOKINASE IN RAT TISSUES AND RAT TUMORS

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SUMMARY

The phosphofructokinase (PFK) isozyme patterns of rat tissues and tumors were investigated by electrophoresis on cellulose acetate membranes. Four types of PFK were observed, which were named I, II, III and IV in order of increasing mobility to the anode. Muscle contained only PFK I and brain only PFK II. Kidney, spleen and erythrocytes had PFK II and III. Liver contained only PFK IV. Fast-growing Yoshida ascites hepatomas and Yoshida sarcomas had PFK II with or without PFK III. Slowly-growing Morris hepatoma had only PFK IV, like normal liver.

INTRODUCTION

Phosphofructokinase (PFK, ATP : D-fructose-6-phosphate 1-phosphotransferase, EC 2.7.1.11) is a key enzyme in the glycolytic pathway and is localized exclusively in the cytosol. The presence of isozymes of PFK has been noticed by several investigators. Tarui et al. reported that some patients had no muscle PFK and about half the normal level in red blood cells (1). Layzer et al. found that human skeletal muscle PFK differed from red blood cell PFK (2) and more recently detected at least four PFK isozymes in human tissues (3). Tanaka et al. (4) also demonstrated four types of PFK in rat tissues by TEAE-cellulose column chromatography of partially purified tissue extracts. Previously, we developed a quick method for separation of aldolase and hexokinase isozymes of normal and tumor tissues of rats by electrophoresis on a cellulose acetate membrane (5).

In this work, we used the same rapid method for separation of PFK isozymes from very fresh tissues of rats, which minimized possible artifacts and modifications of isozymes.

Tissue extract obtained after high speed centrifugation was directly applied to a cellulose acetate membrane and submitted to electrophoresis. Four bands of PFK isozymes were separated and visualized by staining for activity. The bands of PFK were designated PFK I, II, III and IV in order from the origin to the anode. PFK I was only present in adult muscle and PFK IV was found in adult liver and in a slowly-growing Morris hepatoma. PFK II and III were detected in other normal adult tissues. Most of the transplantable tumors investigated, possessed PFK II with or without PFK III. The patterns of PFK isozymes are discussed in relation with those of other isozymes in tumors.

MATERIALS AND METHODS

Animals and tumors. Donryu strain rats were used in most experiments. Fast-growing Yoshida ascites hepatoma and Yoshida ascites sarcoma were transplanted intraperitoneally. Buffalo strain rats were only used for experiments on slowly-growing Morris hepatoma. This tumor was transplanted intramuscularly into both hind legs. Ascites tumor cells were freed from red cells by repeated washing with low speed centrifugation. Fresh tumorous tissues were carefully collected from solid tumors.

Enzyme extracts. Tissues or packed cells were homogenized with 1 or 2 volumes of cold 25 mM glycylglycine buffer (pH 7.5) containing 25 mM glycerol-phosphate, 1 mM EDTA, 0.1 mM ATP, 0.1 mM DTT and 30 mM KF solution using a Potter-Elvehjem homogenizer for all material except muscle. Muscle was homogenized in a Vir-Tis homogenizer. The homogenate was centrifuged for 60 min at 105,000 x g and the supernatant was used as the enzyme extract. All procedures were carried out in the cold as quickly as possible.

Enzyme assay. PFK activity was measured by a modification of Kemp's method (6). One unit of PFK was defined as the amount of enzyme which converted 1 μmole of fructose-6-phosphate to fructose-1,6-diphosphate in 1 min at 30°. Protein was determined by the method of Lowry et al. (7) with bovine serum albumin as a standard.

Electrophoresis. Electrophoresis was performed at 4° on a cellulose acetate membrane (1' x 6 3/4', Gelman Instrument Co.), using buffer system consisting of 50 mM glycylglycine, 5 mM ammonium sulfate, 0.1 mM EDTA, 1 mM ATP and 10 mM 2-mercaptoethanol, pH 8.2. Several investigators claimed that PFK tended to aggregate when the PFK protein concentration was high (2, 8-14). To avoid this, enzyme extract was quickly diluted to 0.2-0.6 unit per ml and about 3 μl of this solution were applied to the center of the cellulose acetate membrane. After electrophoresis, the bands of PFK were visualized by a slight modification of Kemp's method (6). The cellulose acetate membrane was applied to a thin layer agar gel containing 0.5 % ionagar No. 2, 50 mM Tris-HCl, 10 mM sodium arsenate, 2 mM EDTA, 1 mM fructose-6-phosphate, 1 mM ATP, 4 mM $MgCl_2$, 1 mM NAD, 0.06 mg/ml diaphorase, 0.4 mg/ml nitroblue tetrazolium chloride, 0.1 mg/ml glyceraldehyde-3-phosphate dehydrogenase, 0.1 mg/ml aldolase, 5 μg/ml triosephosphate isomerase. The final pH was 8.3. Fructose-6-phosphate was omitted from the control gel. After incubation for 10-20 min at 37° in a moist chamber, the membrane and gel together were photographed with transillumination.

RESULTS

PFK activities in various tissues and tumors. The PFK activities of rat tissues and tumors are summarized in Table 1. Among the normal tissues examined, muscle possessed the highest activity and activity in the liver was quite low. These findings agree with those reported previously by Shonk et al. (15). The activities of PFK in embryonal tissues were quite low. Most tumors investigated had moderately high activities (5-25 units/wet weight gram). Morris hepatoma, 7316A, which grows slowly, had low activity.

PFK isozyme patterns on cellulose acetate membrane electrophoresis. Fig. 1 shows the isozyme patterns of adult rat tissues. PFK I was only found in muscle, where it was the only isozyme present. Brain contained only PFK II. Kidney showed PFK II with a weak band of PFK III. Spleen and erythrocytes contained PFK II and III and liver only PFK IV. PFK IV was found to be specific to liver.

Fig. 2 is a schematic representation of the PFK isozyme patterns of embryonal rat tissues and rat tumors. Embryonal muscle contained predominantly PFK II unlike adult muscle. Embryonal brain and kidney had PFK II and III. Embryonal liver had PFK IV like adult liver.

As demonstrated in Fig. 2, most tumors possessed PFK II, and AH131A, LY5 and LY54 showed a distinct band of PFK III. Morris hepatoma, 7316A, had PFK IV, just like normal and embryonal livers.

DISCUSSION

Our results clearly show that four bands of PFK isozymes were detectable in various rat tissues by electrophoresis on cellulose acetate membranes. Layzer et al. (3) and Tanaka et al. (4) already reported the detection of four types of PFK isozymes from human and rat tissues, by DEAE or TEAE cellulose column chromatography and immunological techniques. Our PFK I, II, III and IV, numbered in order of increasing mobility to the anode may correspond to the PFK eluted by increasing salt concentrations from

Table 1. PFK activities of various tissues of adult and embryo rats and tumors.

Values represent averages of more than two determinations.

Tissue	Activity (units/g. wet wt.)	Tumor	Activity (units/g. wet wt.)
Adult		Ascites Hepatoma	
Muscle	81.9	AH13	15.0
Brain	12.1	AH57B	4.12
Spleen	1.41	AH66	1.16
Kidney	3.55	AH66F	18.0
Erythrocytes	2.66	AH130	25.6
Liver	1.52	AH131A	12.4
		AH7974	18.1
Embryo (18 days)			
Muscle	1.21	Morris Hepatoma	
Brain	0.48	7316A	1.34
Kidney	0.32		
Liver	0.93	Yoshida Sarcoma	
		Original strain	6.63
		Substrain LY5	8.70
		Substrain LY52	0.66
		Substrain LY54	1.93

an anion exchange cellulose column. Adult muscle PFK was eluted first and adult liver PFK was eluted last from the column (3, 4).

Summarizing the distribution of PFK isozymes in adult rat tissues detected by our electrophoretic method, PFK I and IV were rather specific to muscle and liver, respectively. Brain had PFK II and kidney, spleen and erythrocytes had PFK II and III. Embryonal muscle had PFK II while adult muscle had PFK I. Embryonal liver had PFK IV, like adult liver. The most characteristic thing about the patterns of PFK isozymes in various tumors was that PFK II was predominant, and was sometimes associated with PFK III. There was no clear cut correlation between the appearance of PFK III and the growth rate of tumors. However, Morris hepatoma, which grows very slowly and has many enzymes which are specific to liver (5, 16), had PFK IV, like normal liver.

We always subjected very fresh preparations of tissues directly to electrophoresis on

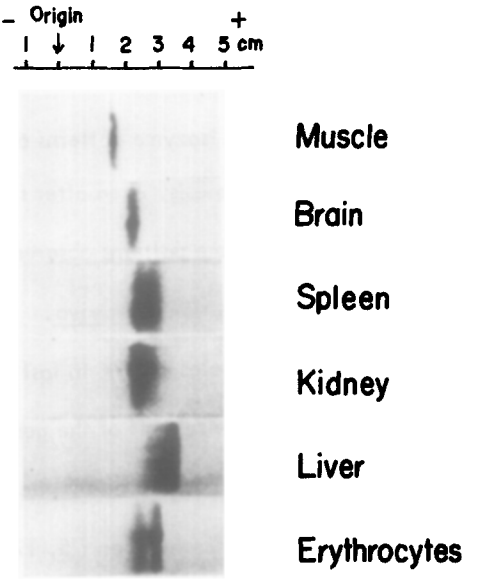

Figure 1. PFK isozyme patterns of adult rat tissues on electrophoresis.

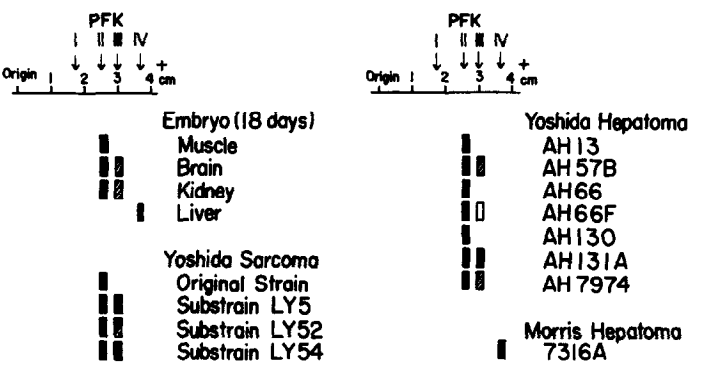

Figure 2. Schematic representation of PFK isozyme patterns of embryonal rat tissues and rat tumors. Black, hatched and open bars indicate bands staining strongly, moderately and weakly, respectively.

cellulose acetate membranes, without using any previous treatments, such as ammonium sulfate fractionation or column chromatography. Thus possible artifacts should be minimal under these conditions. We confirmed that the isozyme patterns of extracts of liver, muscle and spleen did not change on electrophoresis, even after standing the extracts at 4° for 24 hours. This suggests that the isozyme patterns observed in the present paper represent the true PFK isozymes present in these tissues in vivo.

The results indicated that the molecular species of PFK in fast growing hepatomas differed from that in normal liver, namely, a switch-off of the gene for PFK IV and switch-on of the gene for PFK II or the genes for PFK II and III occurred during hepatocarcinogenesis. This supports the idea of disdifferentiation (5, 17, 18).

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