A Comparative Study of Rat Liver, Muscle, and Hepatoma 3924A Phosphofructokinase Isozymes

George A. Dunaway, Jr., Harold P. Morris, and George Weber

Department of Pharmacology, Indiana University School of Medicine, Indianapolis, Indiana 46202 [G. A. D., G. W.], and Department of Biochemistry, Howard University College of Medicine, Washington, D. C. 20001 [H. P. M.]

SUMMARY

Two phosphofructokinase isozymes are present in rat liver representing 85 and 15% of the total activity, respectively. The single isozyme observed in muscle is distinct from the two in liver. In hepatoma 3924A three isozymes were detected that were 5, 20, and 75% of the total activity. The major liver and hepatoma isozymes were judged to be identical on the basis of the following criteria: diethylaminoethyl cellulose chromatography, starch gel electrophoresis, and kinetic and immunological properties. The major isozyme was elevated two- to threefold in hepatoma 3924A on the basis of total activity and amount of antiserum required for complete neutralization. The minor liver isozyme is probably also present in hepatoma, but in addition a nonhepatic isozyme also appears to be present which is quite similar to, but distinct from, the muscle isozyme. This nonhepatic isozyme could not be detected in embryonic liver. Although the alteration in gene expression observed with the hepatoma phosphofructokinase isozyme pattern is not as drastic a change as was found for the other two key glycolytic isozymes (adenosine 5'-triphosphate-glucose phosphotransferases and adenosine 5'-triphosphate-pyruvate phosphotransferases), the increase in total activity agrees with the prediction of the molecular correlation concept.

INTRODUCTION

It has been shown previously that the key liver glycolytic enzyme activities, pyruvate kinase (EC 2.7.1.40), glucokinase (EC 2.7.1.2), and PFK (EC 2.7.1.11) are responsive to nutritional, hormonal, pathological, and developmental states of the rat. PFK activity in diabetic rats decreases to 45% of the control values, and insulin administration produces a dose-dependent increase of this activity (26). After 6 days of starvation total PFK activity decreases to 30% of normal levels (26, 27). Also, the activity gradually increases 3-fold from 10 days postpartum to normal adult levels at 51 days postpartum (26). In the Morris hepatoma, total PFK activity is increased concomitantly with increased glycolysis and growth rate (25). A comparison of recent work concerning PFK isozymes in the Yoshida hepatomas and sarcomas will be presented under “Discussion” (12, 20).

In the fast-growing hepatoma 3924A, the total activities of all 3 key glycolytic enzymes are increased over normal liver levels (18, 24). To evaluate the cause and biological significance of the increase in total enzyme activity, extensive work with this hepatoma has been carried out by many investigators on the isoenzyme patterns of the pyruvate kinase and glucose-ATP phosphotransferase activities in normal liver and in hepatomas. The total hepatic pyruvate kinase activity is the summation of 2 distinct isozymes (21). The major isozyme (L-type) is subject to extensive regulation, whereas the minor isozyme (M-type) is less sensitive to regulatory signals (2, 10, 21). In the fast-growing hepatoma 3924A, the L-type isozyme is replaced by an isozyme that has a high affinity for phosphoenolpyruvate (23) and which is similar to but distinct from the muscle pyruvate kinase (23). An analogous situation occurs for the glucose-ATP phosphotransferase isozymes in fast-growing hepatomas (6, 16, 18). The major liver isozyme, glucokinase, which is regulated by diet and insulin and is highly specific for glucose although it has less affinity for this hexose than the hexokinases, is almost completely lost in the fast-growing, poorly differentiated hepatomas. However, maintenance of glucose phosphorylation in the hepatoma is a consequence of increased activity of the hexokinases, which are less affected than glucokinase by diet or insulin and have approximately a 10-fold lower apparent K_m for glucose than has glucokinase.

In consequence, not only is there an increase in total activities of the key glycolytic enzymes in hepatoma 3924A, but there is also a change in gene expression, resulting in drastic alteration of the isozyme patterns of at least 2 key liver glycolytic enzyme activities. Thus, the purpose of this paper is to report information concerning the PFK isozyme patterns of hepatoma 3924A, liver, and muscle in order to determine whether an altered pattern exists in this hepatoma for PFK activity. Further, this paper extends an earlier report (4) that demonstrated that the major liver PFK isozyme was present in this hepatoma and was elevated 2- to 3-fold over the normal liver level.
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MATERIALS AND METHODS

Animals and Tissues. Adult tumor-bearing ACI/N male rats carried bilateral s.c. transplants of 3924A hepatomas. Livers and hind leg muscles were removed from animals weighing 180 to 200 g, and all tissues were rapidly excised from decapitated animals. The tumors were carefully dissected free of any necrotic, hemorrhagic, and nontumorous tissue.

Measurement of Enzyme Activity. One unit of PFK activity is defined as the conversion of 1 µmole of fructose-6-P to 1 µmole of fructose 1,6-diphosphate per min at 37°C. During purification, the liver, hepatoma, and muscle PFK activities were measured at 2.0 mm fructose-6-P, 1.0 mM ATP, 1.0 mM MgCl₂, 0.1 mM NADH, 3 units of aldolase, 30 units of triosephosphate isomerase, and 10 units of α-glycerophosphate dehydrogenase in a total volume of 3.0 mL. During determination of kinetic properties, ammonium sulfate was removed from the ancillary enzymes using a coarse Sephadex G-25 column equilibrated with 50 mM Tris-HCl, pH 8.0, and the eluant was then dialyzed overnight against 50 mM Tris-HCl, pH 8.0. For the kinetic assays the concentrations of substrates and cofactors were as described in the charts.

The rate of the PFK-catalyzed reaction was monitored by measuring the decrease in absorbance at 340 nm at 37°C using a Beckman DU spectrophotometer coupled with a Gilford 2000 recorder. The reaction was started by the addition of the enzyme. The protein was measured using a modified biuret method (11).

Ammonium Sulfate Fractionation of Liver, Muscle, and Hepatoma 3924A 100,000 g Supernatant Fluids. The 100,000 g supernatant fluids from 20% homogenates were prepared in 50 mM Tris-HCl, pH 8.0; 50 mM NaF; 10 mM diithiothreitol, and 1.0 mM ATP (4). All homogenizations and centrifugations were performed at 4°C. The PFK activity for all 3 tissues was precipitated with ammonium sulfate at 4°C in order to determine whether it could be concentrated by this procedure. Each fraction was resuspended in a minimum volume of extraction medium and the activity was assayed. The weight of powdered ammonium sulfate used to make each cut was determined from a nomogram developed by Dixon (3). The 0 to 25% saturated fraction contained no activity and was discarded for all tissues. Subsequent 5% cuts were made from 25 to 60% saturation in ammonium sulfate which constituted a total of over 90% of the original activity. Each cut was made by increasing the ammonium sulfate concentration by 5% in the preceding supernatant fluid and allowing the solution to remain for 60 min without stirring, followed by centrifugation for 10 min at 40,000 x g.

Isozyme Determination by DEAE-cellulose Chromatography. To determine the number of PFK isozymes that can be separated by DEAE-cellulose chromatography, the supernatant fluids prepared as described in the previous section were made 60% in solid ammonium sulfate, and the precipitate was collected as described. The following operations were performed in a cold room. The precipitate was resuspended in the chromatography starting buffer: 0.02 M Tris-HCl buffer, pH 8.0; 0.05 M NaF; 0.01 M dithiothreitol; 0.01 M ammonium sulfate; and 1.0 mM ATP. Ammonium sulfate was removed from the solution using a coarse Sephadex G-25 column equilibrated with the starting buffer. This solution was introduced onto a 0.9-x 30-cm DEAE-cellulose column. The DEAE-cellulose (0.9 mEq/g capacity, fine mesh from Sigma Chemical Co., St. Louis, Mo.) was prepared as directed in Whatman Information Leaflet IL2. The isozymes were eluted with a continuous gradient formed by mixing 75 mL of the starting buffer and 75 mL of 1.0 M Tris-HCl buffer, pH 8.0; 0.05 M NaF; 0.01 M dithiothreitol; 1.0 mM ATP; and 1.0 mM MgCl₂. In a cold room 1-ml fractions were collected, and the total isolated activity was 80 to 90% of the added activity for all 3 tissues.

Isozyme Determination by Starch Gel Electrophoresis. The starch gel was prepared by suspending 65 g of Hiller hydrolyzed starch (Electrostarch Co., Madison, Wis.) in 500 ml of 100 mM Tris-phosphate, pH 8.3; 1.0 mM MgSO₄; and 1.0 mM ATP. The starch was added to the buffer in a 1-liter filter flask and heated over a burner until ready for degassing with a vacuum line and trap. The degassed gel was poured into a mold and cooled for 1 hr at room temperature and a minimum of 2 hr to overnight in a cold room. After the samples had been added to each slot, the gels were sealed with liquefied petrolatum (Fisher Scientific Co., Pittsburgh, Pa.). Electrophoresis was performed in a cold room with the duration of the run and voltage varied to give the best results (usually 200 V for 24 hr). After the electrophoresis was completed, the gel was sliced longitudinally with a Buchler slicer that was set for 3-mm-thick slices. One-half of the gel was used as a control with no fructose-6-P in the assay solution. The activity in the remaining slice was developed in trays containing 50 mL of 50 mM Tris-phosphate buffer, pH 8.0; 5 mM fructose-6-P; 2 mM ATP; 2 mM MgCl₂; 10 mM NaAsO₄; 1 mM NAD⁺; nitro blue tetrazolium, 1 mg/mL; phenazine methosulfate, 0.25 mg/mL; aldolase, 100 µg/mL; triosephosphate isomerase, 10 µg/mL; and glyceraldehyde-3-phosphate dehydrogenase, 150 µg/mL. Both gels were incubated overnight in the dark at room temperature. If the gels were to be saved, they were rinsed with water, fixed with a methanol:acetic acid:water (5:1:5) solution, wrapped in Saran wrap, and stored in a refrigerator.

Purification of Rat Liver and Muscle PFK. The major rat liver PFK isozyme was purified as previously reported (4) with the following improvement. The pooled DEAE-cellulose fractions were concentrated in a collodion bag apparatus containing extraction buffer to approximately 5 mL. After concentration this solution was dialyzed against extraction buffer containing 30% saturated ammonium sulfate and 5 mM MgCl₂ with constant stirring. The majority of the activity was precipitated between the 1st and 2nd hr. This precipitate was resuspended in 1.0 mL of extraction buffer, warmed to 60°C for 1 min, stirred rapidly with a Vortex mixer, and rapidly cooled to 4°C. A more detailed description of this improved purification is published elsewhere (5).

Muscle homogenates (25%) were prepared as the liver homogenates, except that homogenization was performed with a Sorvall Omnimixer and then centrifuged at 100,000 x g for 30 min. The resulting supernatant fluid was made...
40% in ammonium sulfate, stirred to dissolve the salt, allowed to stand without stirring for 60 min, and centrifuged at 40,000 × g for 10 min. The pellet was discarded and the supernatant fluid was made 60% in ammonium sulfate. This precipitate, which was collected as previously described, was resuspended in a minimum of chromatography starting buffer, and the ammonium sulfate was removed using coarse Sephadex G-25. This solution was introduced onto a DEAE-cellulose column (0.9 x 30 cm), and the activity was chromatographed and collected in the same manner as for the liver.

**Separation of Hepatoma PFK Isozymes.** Separation of the 3924A PFK isozymes was achieved by using the previously described methods of ammonium sulfate fractionation and DEAE-cellulose chromatography. Hepatoma 20% homogenates were prepared as described for the liver homogenates and centrifuged at 100,000 × g for 30 min. The major hepatoma isozyme was collected in the 25 to 40% ammonium sulfate fraction and the 2 minor hepatoma isozymes were collected in the 40 to 60% ammonium sulfate fraction of the 100,000 × g supernatant fluid. Ammonium sulfate was removed from each of the ammonium sulfate fractions with G-25 coarse Sephadex and chromatographed separately with a DEAE-cellulose column in the same manner as the liver PFK activity. For each peak the tubes of highest activity were pooled and the protein was collected by precipitation with 60% ammonium sulfate.

**Immunological Studies.** Male adult rabbits were immunized by the following schedule with the major hepatic PFK, which was purified as described. Four mg of protein were injected 3 times at weekly intervals with Freund’s complete adjuvant (Difco Laboratories, Inc., Detroit, Mich.) the 1st week, and thereafter with Freund’s incomplete adjuvant (Difco). In the scapular region approximately 0.5 ml of the emulsion was injected per inoculation site. One week after final inoculation the animals were bled and the immune serum was stored in 1.0-ml aliquots at −20°C.

Ouchterlony immunodiffusion studies were performed on microscope slides covered with 0.75% Noble agar (Difco) which had been prepared in the extraction media minus dithiothreitol using LKB slide frames and leveling table. Diffusion was allowed to proceed overnight at room temperature in sealed, humid chambers. Neutralization studies were performed by diluting with phosphate-buffered saline the amount of serum as shown in each figure to a total volume of 2.0 ml. The purified isozymes, which were diluted with extraction medium to about 10 units in 0.2 ml, or 0.5 ml of the 20% supernatant fluids, which is the equivalent of the activity in 100 mg of tissue, were mixed with 2.0 ml of diluted serum. These solutions were incubated 2 hr at room temperature and then centrifuged, and the amount of activity remaining in the supernatant fluid was assayed and compared with controls of phosphate-buffered saline and normal rabbit serum.

**RESULTS**

**Ammonium Sulfate Fractionation.** In Chart 1 are shown the ammonium sulfate PFK fractionation patterns for liver, hepatoma 3924A, and muscle 100,000 × g supernatant fluid. The patterns are similar but not identical for the liver and hepatoma, and they are grossly different from the pattern for muscle. The majority of PFK activity is precipitated by 60% ammonium sulfate for all 3 tissues, and PFK activity could thus be effectively concentrated by 60% ammonium sulfate precipitation for DEAE-cellulose chromatography and starch gel electrophoresis.

**DEAE-cellulose Chromatography.** Chart 2 presents 3 representative experiments utilizing DEAE-cellulose column chromatography of the 60% ammonium sulfate-precipitated liver, muscle, and hepatoma 3924A PFK activity. As can be seen, 2 separable peaks of PFK activity are eluted for the liver. The major liver PFK isozyme (L₂), which is about 85% of the eluted activity, has a combined specific activity of 1 to 2 units/mg protein when tubes of 1 or more units of activity are pooled. The remaining 15% of eluted activity, the minor isozyme (L₁), had a collected specific activity of 0.1 unit/mg protein and appears to elute not far behind the void volume, indicating that it is only weakly if at all anionic. For the muscle, 1 peak was obtained with a small shoulder. The latter has been interpreted as a transient form of the muscle enzyme (14). The specific activity of the combined fractions of the muscle isozyme
was approximately 60 units/mg protein. For hepatoma 3924A, 3 separable peaks of activity were detected. The 1st (H1), 2nd (H2), and 3rd (H3) peaks had net specific activities of about 0.1, 0.5, and 2.0 units/mg protein, respectively, and were 5, 20, and 75% of the eluted activity, respectively.

**Starch Gel Electrophoresis.** Chart 3 illustrates the results of a typical starch gel electrophoresis experiment for the DEAE-cellulose separated major liver and hepatoma isozymes and the 60% ammonium sulfate-precipitated PFK activities from liver, muscle, and hepatoma 3924A supernatant fluid. For the hepatoma 2 distinct bands and a faint 3rd band were located. For the liver there were 2 distinct bands, while with muscle only 1 band was located. It seems that the 2 liver isozymes are distinct from the muscle PFK. Comparison of the electrophoretic mobilities of the separated major liver (L2) and hepatoma (H3) isozymes indicates that they are identical with each other and also to the fastest migrating bands of the 60% ammonium sulfate-precipitated liver and tumor PFK activity. A comparison of the weakly migrating liver band and the corresponding hepatoma band indicates that they have similar electrophoretic mobilities. The mobilities of the intermediate migrating hepatoma band and the muscle-type band are similar, but the muscle-type band (11 mm) appears to migrate slightly less than the intermediate migrating hepatoma band (15 mm).

**Comparison of Kinetic Properties and Their Modulation for the Liver, Muscle, and Hepatoma PFK Isozymes.** The major liver and muscle PFK isozymes were purified as described in “Materials and Methods.” The muscle isozyme had a specific activity of approximately 60 units/mg protein and, as determined by sodium dodecyl sulfate-gel electrophoresis, it was not homogenous. The L2 isozyme was purified to homogeneity with a specific activity of 95 units/mg protein (5). The L1 isozyme was partially purified in essentially the same way as the minor hepatoma isozymes and had a specific activity of about 0.1 unit/mg protein. The hepatoma PFK isozymes were separated as described in “Materials and Methods” and were used without further purification. A comparison of kinetic properties and the effect of low-molecular-weight modulators is shown in Table 1. It is evident that the L1, H1, L2, and H3 isozymes have essentially identical kinetic properties. Although H2 and M have similar kinetic properties, the apparent Km for fructose-6-P for the muscle enzyme was affected only at inhibitory levels of ATP, whereas for H2 it was affected at both nonsaturating and inhibitory levels of ATP. Also, it is evident that L1 and H1, L2 and H2, M or H3 are kinetically distinct isozymes. For example, the affinity of L2, H2, M, and H3 for fructose-6-P is dependent on ATP concentration; however, L1 and H1 are insensitive to ATP. The affinity of all isozymes for fructose-6-P can be increased by AMP or ammonium sulfate, although in this case L1 and H1 are less sensitive. Apparently, the Km for ATP for all of the isozymes is similar and relatively insensitive to fructose-6-P concentration, AMP, or ammonium sulfate. ATP appears to be a more effective inhibitor of H2 or M than L2 and H3; moreover, L1 and H1 are even less inhibited by ATP than any of the other isozymes. Although ATP inhibition for nearly all the isozymes is partially relieved by increasing fructose-6-P, AMP, or ammonium sulfate, it is evident that ATP inhibition is only slightly relieved by increasing fructose-6-P concentration and unaffected by AMP or ammonium sulfate. Partial relief of citrate inhibition by increasing fructose-6-P concentration, AMP, or ammonium sulfate is a property of all of these isozymes. However, there is a variability in the sensitivity of these isozymes to citrate inhibition. For example, L1 and H1 are the least sensitive, L2 and H3 are moderately sensitive, and H2 or M are extremely sensitive to citrate inhibition.

**Immunological Studies.** In Chart 4 are shown characteristic results obtained from neutralization and immunodiffusion studies of liver, muscle, and hepatoma supernatant fluids with antiserum developed against L2. The immunodiffusion studies demonstrate the presence of L2 in the liver and hepatoma supernatant fluids and the lack of its presence in the supernatant fluid from muscle. The neutralization curves confirm that this antiserum was ineffective against PFK activity in the muscle supernatant fluid and that L2 is the major isozyme in liver and hepatoma 3924A.

The neutralization curves for liver and 3924A PFK isozymes in Chart 5 indicate that this antiserum completely neutralized L2 and H3 activities, and only partly neutralized the minor liver and the 2 minor hepatoma isozymes. This probably explains the inability of the antiserum completely to remove PFK activity from the liver and 3924A supernatant fluids with this antiserum. Also, L1 and H1 had quite similar neutralization curves.

Since this antiserum effectively precipitated L2, the relative increase of L2 in the hepatoma can be estimated (see Chart 6). The isozymes were purified from equivalent amounts of tissue as described for L2, and the activity isolated from the hepatoma was 2- to 3-fold greater than that from the liver. As can be seen for the hepatoma 3924A, the increased PFK activity measured spectrophotometrically (Chart 6, ordinate) agrees with an increased serum amount (Chart 6, abscissa) required for complete neutralization. Furthermore, immunodiffusion and the slopes of the neutralization curves also demonstrate that these isozymes are serologically identical.

Having established that 2 of the 3 hepatoma 3924A isozymes were the hepatic-type isozymes, we studied embry...
Table 1

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*L_1, L_2, H_1, H_2, H_3, H_4, and M, the 1st eluting liver, 2nd eluting liver, 1st eluting hepatoma, 2nd eluting hepatoma, 3rd eluting hepatoma, and muscle PFK isozymes, respectively.

* Subsaturating level of substrate.
* Lowest optimal level of substrate.
* Inhibitory concentration of ATP.
* Excess concentration of fructose-6-P.
* ATP concentration was 1.0 mM.

DISCUSSION

This paper presents kinetic, electrophoretic, immunological, and chromatographic data that suggest that 2 different PFK isozymes are present in adult and embryonic rat liver and that another type exists in the muscle which is distinguishable from the 2 found in the liver. Other work (12, 20, 22) has shown that muscle PFK was not present in the liver, but it has not demonstrated the existence of a minor hepatic isozyme. Further, in the hepatoma 3 different PFK isozymes have been detected by these methods. Comparison of the kinetic, electrophoretic, chromatographic, and immunological properties of L_1 and L_2 indicates that they are the same as the hepatoma isozymes H_1 and H_2, respectively. It can be concluded that the hepatic isozymes are retained in hepatoma in addition to the expression of a nonhepatic isozyme. Comparison of the properties of the nonhepatic hepatoma PFK isozyme (H_3) with the muscle-type isozyme indicates that their properties are not identical although in some cases they are very similar; e.g., (a) the DEAE-cellulose fractions of highest activity and starch gel electrophoretic migration were 27 and 15 mm for the muscle and 23 and 11 mm for H_3; (b) although kinetic properties were similar, it was found that the affinity of the muscle enzyme for fructose-6-P was affected only at inhibitory levels of ATP, whereas the affinity of H_3 for fructose-6-P was altered at both inhibitory and subsaturating levels of ATP; and (c) H_3 was partially neutralized by the major liver PFK isozyme antiserum, but the muscle-
type PFK was only weakly if at all cross-reactive with this antiserum. Thus, H₂ and muscle isozymes may be similar but distinct. Further, only the hepatic isozymes, and not H₁, were present in embryonic liver. A more extensive examination of fetal, postnatal, and adult tissue is necessary to demonstrate when and in what tissues H₂ is expressed.

At least 2 other reports have been published comparing PFK isozymes in rat tissues and tumors (12, 20). Tanaka et al. (20), using triethylaminoethyl-cellulose chromatography and immunological techniques, showed 4 isozymes in rat tissues and tumors which were later confirmed by electrophoresis (12). Both reports observed distinct isozymes in the liver and muscle which we confirm with the exception that we found an additional minor hepatic isozyme. Perhaps the minor hepatic isozyme was overlooked because of its weak anionic properties. Tanaka et al. (20) reported 3 isozymes, including the liver type, for the Yoshida ascites hepatoma AH130; however, Kurata et al. (12) found for this hepatoma only 1 isozyme, a nonhepatic type. Seemingly, when compared, our paper and the other 2 papers (12, 20) have conflicting results. Since the Morris hepatomas are solid tumors originating from s.c. transplants and the Yoshida (AH) hepatomas are transplanted into the peritoneal cavity and maintained in the ascites form, this difference in environment and mode of growth might explain the pheno-
typic differences observed for the PFK isozymes, or perhaps the use of a different rat strain can explain these differences. Further, phenotypic differences in hexokinase patterns also have been observed for Morris hepatomas (see Ref. 18; Table 3) and Yoshida ascites hepatomas (see Ref. 16; Chart 1).

Some simple approximations can be made that may offer insight into the extent of alteration of PFK isozymes occurring in the hepatoma. Since very little of the PFK activity is lost during the DEAE-cellulose chromatography, the total tissue activity contributed by each isozyme can be estimated by considering its percentage of the total activity that was separated by DEAE-cellulose chromatography. The results of these calculations indicate that the major liver isozyme is approximately 85% of the total isolated activity, and consequently the minor isozyme is 15%. A similar comparison for the hepatoma indicates that the approximate values are 5% for the 1st eluting isozyme, 20% for the 2nd eluting isozyme, and 75% for the major isozyme. Total supernatant PFK activity (units/g) of liver and hepatoma was found to be 4.6 ± 0.4 and 13.6 ± 2.0, respectively (data compiled from many livers and hepatomas). Thus the major liver PFK isozyme in the hepatoma (0.75 × 13.6) is estimated to be increased 2.6-fold greater than the level in the liver (0.85 × 4.6). This compares favorably with the 2.8-fold greater amount of asperger required to neutralize L2 in the hepatoma compared to the liver, when isolated from equivalent amounts of tissue. Similar calculations indicate that the amount of the minor liver isozyme is relatively unchanged from hepatic levels in the hepatoma (0.7 unit/g tissue). Therefore, the major changes in PFK isozymes for hepatoma 3924A are an increased level of the major liver PFK isozyme as well as the appearance of a PFK isozyme that was not detectable in either the adult or embryonic liver or the muscle. Considering the low level of the nonliver enzyme in the hepatoma relative to the major enzyme and its kinetic properties, it is difficult to rationalize any significant biochemical advantage that the presence of this isozyme conveys to the tumor.

At the present time a great deal of information is available concerning isozymatic alterations in hepatomas (for review, see Ref. 1), and one should be able to speculate on their contribution to the alteration of glycolysis. It is well established that there is an increased lactate production by this hepatoma which cannot be explained merely by increased glucose phosphorylation (19). Probably, the increased lactate production is largely due to a rechanneling of intermediary metabolism towards lactate production which could be accomplished by a combination of alterations, e.g.: a large increase in the key glycolytic enzymes (24); a decrease of key gluconeogenic enzyme activities (24, 27); a change in liver isozyme patterns that are more favorable to glycolysis, such as an increase in FDP utilizing capacity of aldolase activity (17) replacement of liver-type pyruvate kinase activity with a different pyruvate kinase that is less regulated (10, 21, 23), and an increase in M form of lactate dehydrogenase activity that is less subject to lactate inhibition (15); an increase of activators/inhibitors of key glycolytic/gluconeogenic enzymes; and possibly a diminished number of mitochondria (8, 9, 13), which would both allow a larger amount of pyruvate to be converted to lactate and allow the already increased pyruvate kinase activity to utilize more of the available ADP since less is utilized by oxidative phosphorylation.

For the previously examined key glycolytic enzymes, the pattern of alteration of gene expression in the fast-growing, poorly differentiated hepatoma has been an increase in their total activity and a significant decrease of the major liver type isozyme (6, 10, 18, 21, 23). The increase in activity may be attributed to an increase in minor isozymes present in the liver, which ultimately become the major isozymes in the fast-growing hepatomas, or to the appearance of unique isozymes. Evidently, this type of alteration of gene expression does not occur for PFK activity in this hepatoma. Considering that a minor nonhepatic isozyme does appear in hepatoma 3924A and that the major liver isozyme is increased 2- to 3-fold relative to the liver, the most striking alteration of genetic expression in hepatoma 3924A is an increase in the amount of the major liver PFK isozyme.

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