A New Pyruvate Kinase Isozyme In Hepatomas*

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Multiple forms of pyruvate kinase (EC 2.7.1.40) have been observed in various tissues (Tanaka et al., 1967; Koler et al., 1964; Pogson, 1968; Bailey et al., 1968; Bailey and Walker, 1969). Cellulose acetate electrophoresis revealed one major and one minor form in rat liver and kidney and a single third form in rat skeletal muscle and heart (Pogson, 1968; Susor and Rutter, 1968). All other rat tissues studies had only one of these three forms upon cellulose acetate electrophoresis. Starch block zonal electrophoresis yielded four electrophoretic pyruvate kinase isozymes from rat liver (Tanaka et al., 1965). Although one of these four forms was identical with the muscle form in electrophoretic mobility, the antibody for the muscle form showed no cross reactivity with the major liver enzyme (Tanaka et al., 1967).

The liver and muscle enzymes have been purified and characterized. Liver isozyme B is allosterically activated by fructose 1,6-diphosphate, whereas liver isozyme A is not (Bailey et al., 1968). Pyruvate kinase from both muscle and liver are inhibited in the forward direction by ATP (Reynard et al., 1961). Liver pyruvate kinase activity is adaptive to diet and hormones (Krebs and Eggleston, 1965; Weber et al., 1965), but the muscle enzyme doesn't seem to be adaptive to diet or hormonal stimuli

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(Weber et al., 1965; Tanaka et al., 1967). The major pyruvate kinase isozymes from rat liver and muscle also differ in their reactivity to p-chloromercuribenzoate, in molecular weight, and in their neutralization by liver pyruvate kinase anti-serum (Tanaka et al., 1967).

Using DEAE cellulose column chromatography, Farina et al., 1968 and Lo et al., 1968; reported an increase in the non-liver isozyme and a decrease in the liver isozyme during increased tumor growth rates and degree of dedifferentiation. In the present paper I report the occurrence of a new species of pyruvate kinase in well and poorly differentiated rat liver tumors.

This form of pyruvate kinase is easily discernable using the new technique of pH isoelectrofocusing. The method is described by the LKB Instruments Inc., and was the same as that reported by Criss et al., 1969, for separating of adenylate kinase isozymes.

Methods

Normal Sprague Dawley male rats, purchased from the Holtzman Co., Madison, Wisconsin, were maintained on a commercial stock diet and were sacrificed when they were about 2 1/2 months old and weighed 180 to 240 gms. The Morris Hepatomas were kindly supplied by Dr. H. P. Morris. These tumors were transplanted in Washington into Buffalo rats, sent to Philadelphia, and maintained in our laboratories prior to sacrifice. The Novikoff Hepatomas were transplanted in our laboratory into Sprague-Dawley rats. At the time of sacrifice, the animals were decapitated and exsanguated. The liver and hepatomas were removed and placed into cold 0.25 M sucrose. After cooling several minutes, the tumor was cleaned of necrotic tissue. The liver or tumor was then homogenized in cold 0.25 M sucrose containing 1 mM mercaptoethanol. A section of tumor was routinely taken for histologic examination. The homogenate was centrifuged for one hour at 2^{o} at 100,000xg in a Spinco Model L

Centrifuge. An aliquot of the supernatant fraction was mounted in the center of a 440 ml electrofocusing column and electrofocused for 60 hrs. Elution was begun and one hundred and fifty 3 ml fractions were collected and assayed for pyruvate kinase activity. The assay system contained 100 mM triethanolamine-HCl buffer (pH 7.5), 0.13 M KCl, 3.2 mM $MgSO_4$, 0.38 mM NADH, 0.68 mM phosphoenolpyruvate and 50 units of lactate dehydrogenase to a total volume of 3 ml. The reaction was started by adding 1.0 mM ADP. It was monitered at 25^{o} by following the decrease in absorbancy at 340 mμ on a Beckman DU Spectrophotometer equipped with a Gilford automatic multiple absorbance recorder.

Results and Discussion

The results are illustrated in Fig. 1. Normal rat liver has four electrophoretically distinct pyruvate kinase forms, with respective isoelectric points of pHs 5.50, 5.75, 6.00 and 6.75. Only one isozyme was found in skeletal muscle with an isoelectric point of 6.75. The highly differentiated Morris Hepatoma 9618A showed a pyruvate kinase isozyme pattern similar to that found in liver. In the well differentiated Morris Hepatoma 9633, the predominant liver isozymes were greatly diminished and a new pyruvate kinase species was revealed having an isoelectric point of 7.28. A predominance of this new pyruvate kinase isozyme was observed in the poorly differentiated Novikoff Hepatoma. Studies on liver supernatants from diabetic, fasted, carbohydrate refed, fetal, and regenerating rat liver did not reveal this new enzyme species. Therefore, a new pyruvate kinase isozyme, absent from liver tissue, appears in liver tumors and increases with loss of differentiation.

Weber (1969) has partially purified the pyruvate kinase from the poorly differentiated Morris Hepatoma 3924A and found this enzyme to have several properties which distinguish it from the major liver and muscle forms. It is thermally labile, has lower Ki's for ATP and GTP, and it not stimulated by fructose 1,6-diphosphate. It is likely that this enzyme is the

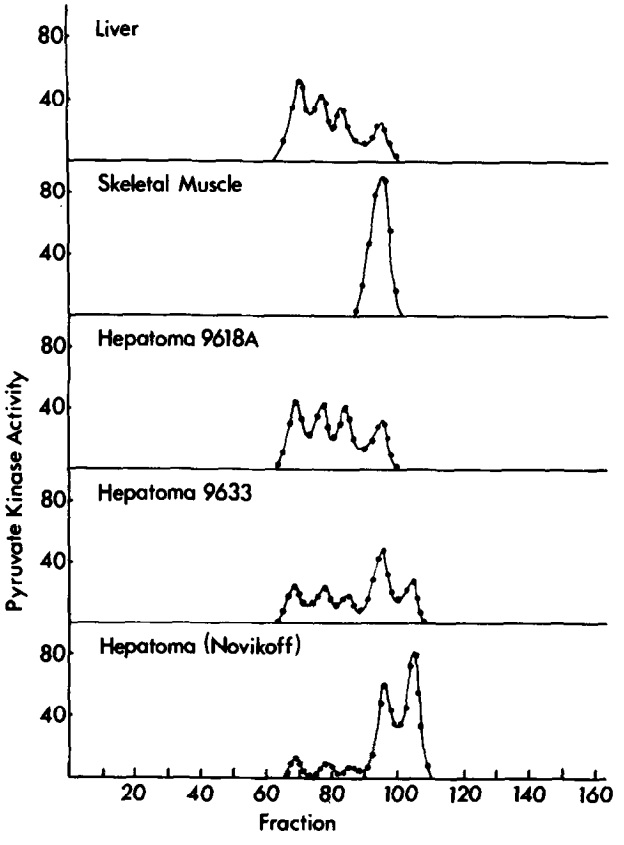

Fig. 1. Pyruvate kinase activity fractions obtained by electrofocusing the cytosols from rat liver and muscle, Morris Hepatomas 9618A and 9633, and the Novikoff Hepatoma. The method of enzymatic assay is described in the text and the method of electrofocusing is described by Criss et al., 1969. The pyruvate kinase activity is expressed as recorder squares per minute.

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same one as reported in this study. Further characterization is underway.

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