PYRUVATE KINASE ISOENZYMES IN TISSUES OF THE HUMAN FETUS

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1. Introduction

Four major isoenzymes of pyruvate kinase (EC 2.7.1.40) have been identified in various animal tissues which, based on increasing mobility, have been labelled PK4* (alternatively M_2), PK3 (alternatively M_1), PK2 and PK1 (alternatively liver L) [1–7].

The presence of pyruvate kinase isoenzymes in tissues of the fetal rat [8–13] and fetal guinea pig [14] has been described. The fetal rat liver possesses little PK1 activity particularly early in gestation while we found that PK1 represented a major portion of the total pyruvate kinase activity of the fetal guinea pig liver. Moreover Balinsky et al. [15] recently demonstrated that PK1 may represent a major portion of total activity in the 20 week human fetal liver.

In addition to the 4 major isoenzymes fetal guinea pig tissues were found to possess additional pyruvate kinase isoenzymes of mobilities intermediate between PK3 and PK4 [14].

The present work describes further studies on the pyruvate kinase isoenzymes in fetal tissues. Pyruvate kinase activity has been measured in tissues from human fetuses and the distribution of isoenzymes determined by electrophoresis and chromatography. PK1 and a previously unidentified isoenzyme have been found in substantial amounts in the fetal liver. The pattern in the other tissues is discussed in relation to the changes observed during development.

* *Abbreviations*: PK1, PK2, PK3, PK4 – pyruvate kinase isoenzymes; FDP, fructose 1,6-diphosphate; PEP, phosphoenol pyruvate.

2. Methods

Five human fetuses (6–28 g, 11–16 weeks) were obtained after hysterotomy. The fetuses were immediately placed on ice and the fetal tissues homogenised (30%, w/v) in 10 mM Tris–HCl, pH 7.5, containing 0.1 mM FDP, 1 mM $MgCl_2$ and 0.1 mM dithiothreitol within 1 hr of the operation. Homogenates were centrifuged at 180 000 *g* for 45 min at 2°C and the supernatant was used for all experiments. Pyruvate kinase was assayed at 25°C by a coupled assay system similar to that described by Bücher and Pfleiderer [16], the final reaction mixture contained: 100 mM Tris–HCl, pH 7.5, 50 mM KCl, 10 mM $MgCl_2$, 0.5 mM PEP, 0.15 mM NADH, 0.1 mM FDP, 5 U/ml lactate dehydrogenase and 1 mM ADP. The reaction was started with ADP.

Pyruvate kinase isoenzymes were separated by DEAE cellulose (Whatman DE 52) chromatography. Samples (about 0.4 ml) of the liver supernatant were applied to a 1.5 × 15 cm column equilibrated in 20 mM Tris–HCl, pH 7.5, containing 1 mM KCl, 1 mM $MgCl_2$, 0.1 mM EDTA and 0.1 mM dithiothreitol. Initially 15 ml of the equilibrating buffer was run through the column after sample application followed by 55 ml of a linear 0–0.4 M KCl gradient. Fractions of 2 ml were collected and a flow rate of about 25 ml/hr was maintained.

A 10% (w/v) starch gel was made in 10 mM Tris–HCl, pH 7.8, containing 10% (w/v) sucrose, 5 mM $MgCl_2$, 5 mM KCl, 1 mM EDTA and 0.2 mM dithiothreitol and horizontal electrophoresis of tissue extracts carried out at 2°C for 10 hr at 10–15 V/cm using a Mini 68 Pherograph (Hormuther-Vetter, Heidelberg, Wieslock). After electrophoresis sliced gels were stained for pyruvate kinase activity with a solution

containing 100 mM Tris–HCl, pH 7.5, 0.15 mM NADH, 0.5 mM PEP, 0.1 mM FDP, 1 mM ADP, 10 mM $MgCl_2$, 50 mM KCl and 5 U/ml lactate dehydrogenase. After incubation for 30–60 min at 30°C the activity was made visible under UV light and photographed. All bands of activity described were dependent on the presence of ADP.

Results are expressed as means ± SD with the number of observations in parentheses. Enzyme activities refer to μmoles of pyruvate produced/min at 25°C.

3. Results

The mean activity of pyruvate kinase in the fetal liver was 8.9 ± 1.5 (5) μmol/g wet wt/min. Chromatography of crude liver extracts on DEAE cellulose produced two peaks of activity. The first peak did not bind to the column and is probably the isoenzyme PK4 (lung activity was eluted in the same position), while the second peak was eluted at about 0.1 M KCl and is probably the isoenzymes PK1 together with PK2 and an unknown isoenzyme (fig.1). The recovery of total activity was 32.8 ± 14.4 (5)% of which 52.2 ± 11.4 (5)% was the isoenzyme PK4.

The mean total activities measured in the other fetal tissues were as follows: brain, 21.3 ± 5.0 (5); heart, 46.5 ± 6.8 (5); kidney, 18 ± 8.1 (5); lung, 13.2 ± 6.3 (5); skeletal muscle, 16.3 ± 7.4 (4) μmol/g wet wt./min.

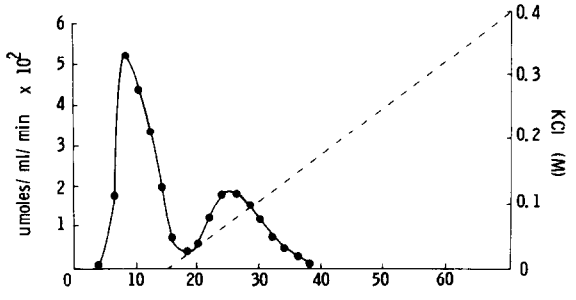

Fig.1. DEAE-cellulose chromatography of a crude extract of human fetal liver. Chromatography was performed with a linear KCl gradient and pyruvate kinase activity determined as described in the Methods. (○), pyruvate kinase activity; (– – –) KCl gradient.

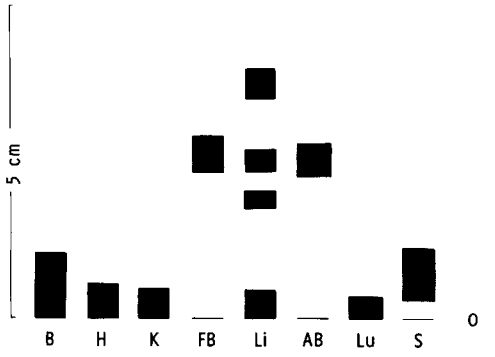

Fig.2. Starch gel electrophoresis pattern of pyruvate kinase isoenzymes from human fetal tissues and adult blood. Electrophoresis was performed as described in the Methods for 10 hr at 10–15 V/cm. The following tissues were studied: B, brain; H, cardiac muscle; K, kidney; FB, fetal blood; Li, liver; AB, adult blood; Lu, lung; S, skeletal muscle.

Up to five bands of pyruvate kinase activity were detected in the fetal tissues (fig.2). All tissues except the liver and blood had only the isoenzymes with low mobility that is comparable with PK3 and PK4 isoenzymes detected in other species. The separation between the bands of low mobility was poor, the brain probably containing both isoenzymes, skeletal muscle having the isoenzyme of higher mobility (PK3) and the other tissues having only the isoenzyme of the lowest mobility (PK4). The liver had 4 bands of activity, one was the band of lowest mobility (PK4) also detected in the other tissues except skeletal muscle. The band of highest mobility is probably PK1 followed by a band of activity which was also detected in adult and fetal umbilical vein blood and is probably PK2. The band of activity lying between PK2 and PK4 in the liver is an unidentified isoenzyme.

4. Discussion

The isoenzyme pattern for human fetal kidney and lung with probably exclusively PK4 and skeletal muscle with predominantly PK3 is comparable with the picture observed by Imamura et al. [17] for the adult tissues. The major isoenzyme in adult human brain and cardiac muscle is PK3 [17] while the fetal brain had both PK3 and PK4 and the fetal heart had

largely PK4. The human fetal isoenzyme pattern is comparable with that observed in the guinea pig [14] and the rat [10] and supports the view that in the brain and heart there is change from predominantly PK4 to PK3 during development.

The electrophoresis pattern for human fetal liver demonstrates the presence of substantial PK1 activity early in gestation as found in the guinea pig. This argues against the view that the fetal liver like tumour tissues has little or no PK1 activity [12].

The proportion of PK1 activity in human fetal liver determined by ammonium sulphate precipitation has been reported as 77% of total [15]. However the electrophoresis pattern shown above demonstrates substantial PK2 activity in the fetal liver, probably from blood or erythropoietic tissue, that will be precipitated by ammonium sulphate [18,19]. In addition to PK1 and PK2 the human fetal liver contained an isoenzyme not previously described. Adult human liver has just 2 bands of activity: PK1 and PK4 [17]. The production of pyruvate kinase isoenzyme hybrids has been described [20,21] and the possible existence of such hybrids naturally occurring in developing tissues of the fetal guinea pig has been discussed [14]. Thus the isoenzyme in the human fetal liver of mobility intermediate between that of PK2 and PK4 may have arisen as a result of hybridization between the subunits of either PK4, PK2 or PK1. It is also possible that it represents a completely independent isoenzyme only produced in developing liver.

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