SIMILARITIES BETWEEN PYRUVATE KINASE FROM HUMAN PLACENTA AND TUMOURS

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1. Introduction

Pyruvate kinase (PyK) (EC 2.7.1.40) which catalyses one of the three physiologically irreversible steps in glyolysis has been studied in animals such as rat where it occurs in three different isoenzymic forms [1]. Rat liver has two PyK isoenzymes one form termed type L is under hormonal and dietary regulation whereas the other form type M_2 is not and more closely resembles the third PyK isoenzyme, type M_1 which is present in muscle and brain. The three types of PyK have distinct kinetic properties and react differently with several metabolites such as ATP, fructose 1, 6-diphosphate and amino acids. For instance, L type PyK is activated by fructose 1, 6-diphosphate and inhibited by ATP whereas type M_1 is not.

In this paper we describe the purification from human placenta of a distinct PyK isoenzyme with properties different from PyK in human liver and muscle. The placental isoenzyme of PyK has a number of features in common with PyK from some human tumours.

2. Materials and methods

2.1. *Tissues*

Human placentae were obtained immediately after vaginal deliveries, at term, from uncomplicated pregnancies; amnion and cord were discarded and the remaining tissue was exhaustively washed in cold 0.9% NaCl to remove all traces of blood. Other human tissues were operation or post-mortem specimens and after thorough washing in cold 0.9% NaCl were stored at −20°C. Biopsy speciments of diagnosed carcinoma were obtained from one male and one female with carcinoma of the lung, a male and female with stomach carcinoma and a male with carcinoma of the jejunum; these tissues were also exhaustively washed as before.

2.2. *Preparation of extracts*

For routine enzyme determinations, chromatography and electrophoresis, tissues were extracted with 1 vol of 0.1 M phosphate buffer, pH 7.4 (containing 1 mM dithiothreitol and 2 mM EDTA) in a Sorvall omnimix for 2 min at 4°C. The homogenate was then centrifuged at 35 000 *g* for 30 min and the clear supernatant was retained.

2.3. *Electrophoresis*

Horizontal-starch gel electrophoresis was used to separate PyK isoenzymes which were then detected in situ on the gels as described previously [2]. Electrophoresis was carried out in 14% starch gels prepared in 5 mM Tris–maleate buffer, pH 8.4, (containing 1 mM dithiothreitol and 2 mM EDTA). The bridge buffer was 0.1 M Tris–maleate and electrophoresis was carried out for 16 hr at 6 V/cm of gel at 4°C, as before [2].

2.4. *PyK assay*

PyK activity was measured spectrophotometrically at pH 7.4 in 0.16 M triethanolamine–HCl buffer with lactate dehydrogenase as before [2]. Enzyme activity is expressed in International Units per mg protein which was measured by the method of Lowry et al. [3].

2.5. *Reagents*

All chemicals were the purest grades available. The

amino acids were chromatographically pure samples, supplied by Calbiochem. Nucleotide phosphates were obtained from Boehringer (Mannheim) and fructose 1, 6-diphosphate from Sigma Chemical Co.

3. Results

3.1. *Purification of PyK from placenta*

All operations were carried out at 4°C. Placenta (300 g) was extracted for 2 min in a Sorvall Omnimix with an equal volume of 0.1 M phosphate buffer pH 7.4 containing 2.5 mM dithiothreitol and 2 mM EDTA. The homogenate was then centrifuged at 35 000 *g* for 30 min and solid $(NH_4)_2SO_4$, 35% (w/v) was added very slowly with shaking to the clear supernatant. After standing for 2 hr the extract was centrifuged at 12 000 *g* for 15 min. After the precipitate was discarded the supernatant was brought to 42% (w/v) with solid $(NH_4)_2SO_4$ and allowed to stand for a further 2 hr, after which it was centrifuged at 12 000 *g* for 15 min. The supernatant was discarded and the precipitate dissolved with stirring in a minimal volume of 20 mM Tris–HCl buffer, pH 7.4 (containing 1 mM dithiothreitol) and dialysed for 12 hr against several changes of the same buffer.

After dialysis, the extract was placed on a CM-cellulose column (1.5 cm × 12 cm). previously equilibrated with 0.1 M imidazole buffer, pH 5.9 (containing 0.1 M KCl and 1 mM dithiothreitol). The enzyme was eluted from the column with the same buffer.

Fractions from the CM-cellulose column which contained PyK were applied directly to a DEAE-Sephadex A-50 column, previously equilibriated with 0.02 M Tris–HCl, pH 7.4 (containing 0.1 M KCl, 0.1 M $MgCl_2$ and 1 mM dithiothreitol). The enzyme was eluted with the same buffer.

Fractions containing the enzyme were pooled, concentrated with solid $(NH_4)_2SO_4$ and the resulting precipitate dissolved in a minimal amount of 0.1 M phosphate buffer, pH 7.4, and dialysed for 12 hr, against several changes of the same buffer. Enzyme solution (1 ml) was applied to a column (2 × 58 cm, bed volume, 190 ml) of Sephadex G-200 and eluted with 0.1 M phosphate buffer, pH 7.4 (containing 0.9% NaCl + 2 mM dithiothreitol). Fractions containing the enzyme were pooled and stored at −20°C. As shown in table 1 the overall purification was 190-fold.

3.2. *Partial purification of PyK from other tissues*

PyK was partially purified from the following human tissues; five tumours, muscle, liver, stomach, intestine and lung by chromatography on Sephadex G-200. Each column (2 × 58 cm; bed volume, 190 ml) was equilibrated with 0.1 M phosphate buffer, pH 7.4 (containing 0.9% NaCl and 2 mM dithiothreitol). One ml of tissue extract (see section 2.2.) was applied to the column and PyK was eluted with the quilibrating buffer-mixture. A partial purification of about 40-fold was obtained in this way and small molecules such as amino acids and organic phosphates were removed. The specific activities of tumour PyK were over 3-fold greater than those of the tissues of origin of the tumours. These partially purified extracts were used in subsequent studies.

3.3. *Properties of PyK from placenta and other tissues*

3.3.1. Stability

PyK from human muscle, stomach, intestine and lung was relatively stable and could be stored for several weeks at 4°C with little loss in activity. PyK from human liver, in contrast, was highly unstable and lost up to 90% of its original activity after 15 hr at 4°C. Placental and tumour PyK were more stable than the

Table 1
Purification of pyruvate kinase from human placenta.

Stage of Purification	Volume (ml)	Total activity (I.U.)	Total protein (mg)	Specific activity (units/mg protein)	Purification (Fold)	Yield %
Original Supernatant	280	1456	364 000	0.04	1	100
$(NH_4)_2SO_4$ ppt.	38	2148	2972	0.73	18	148
CM-cellulose	30	1760	619	2.8	70	120
DEAE–sephadex	24	1512	420	3.6	91	104
Sephadex G-200	10	152	20	7.6	190	10

Table 2
Influence of various substances on pyruvate kinase from different human tissues and tumours*.

Substance	Concentration	Pyruvate kinase from: Tumours**	Placenta	Muscle	Liver	Stomach	Lung	Small intestine
Alanine	10^{-6}M	80–100% Inhibition	100% Inhibition	No effect	No effect	No effect	No effect	No effect
Phenylalanine†	10^{-5}M	84–100% Inhibition	100% Inhibition	No effect	No effect	No effect	No effect	No effect
Serine	10^{-5}M	82–305% Activation	200–300% Activation	No effect	No effect	No effect	No effect	No effect
ATP	4.2×10^{-3}M	No effect	No effect	10% Inhibition	50% Inhibition	–	–	–
Fructose	4.2×10^{-3}M	–	–	No effect	400% Activation	No effect	No effect	No effect
1,6-diphosphate	10^{-6}M	72–111% Activation	60–80% Activation	–	No effect	–	–	–

*For inhibition and activation studies, 0.01 ml of the above substances were incubated with 0.2 ml of enzyme solution in 2.5 ml 0.16 M triethanolamine buffer, pH 7.4, at 30°C. Enzyme activity was measured after 15 min.

**Because there was an overall similarity in the behaviour of pyruvate kinase from the five tumours, the results are presented under one heading and the range of inhibition or activation is given. The figures for placenta represent results of five separate experiments with different placentae. The experiment with human muscle and liver were repeated once and good agreement was obtained. The remaining values for stomach, lung and small intestine are results from one experiment.

† At higher concentrations of phenylalanine (4.2×10^{-3} M) over 90% inhibition of muscle and stomach pyruvate kinase was obtained.

liver enzyme and lost about 30% of their original activity after 15 hr at 4°C. For these studies on stability, enzyme solutions were stores in 0.1 M phosphate buffer, pH 7.4 (containing 0.9% NaCl and 2 mM dithiothreitol).

3.3.2. Influence of ATP, amino acids, etc. on PyK.

ATP, fructose 1,6-diphosphate, alanine and phenylalanine are known to influence the activities of PyK isoenzymes in rat tissues [1]. The effects of these compounds on PyK from various human tissues are shown in table 2. In common with PyK in similar tissues from rat, PyK from human liver was inhibited by ATP and activated by fructose 1,6-diphosphate whereas the muscle PyK was not affected by the latter and only slightly inhibited by ATP. In contrast, PyK from placenta reacted in a different manner with these aforementioned compounds and showed a striking resemblance to tumour PyK (table 2).

Likewise, phenylalanine and alanine inhibited both placental and tumour PyK while serine activated the isoenzyme from both of the latter tissues. These amino acids, at concentrations of 10^{-5} or 10^{-6} M, had no effect on PyK from human liver, muscle, stomach, intestine and lung (table 2).

3.3.3. Electrophoresis

A zymogram of PyK from various human tissues is shown in fig. 1. Placental and tumour PyK had the same electrophoretic mobility which was quite distinct from PyK in other human tissues including the tissues of origin of the tumour. The electrophoretic mobility of the cationic isoenzyme of PyK was 10 mm from the origin in tissues such as lung, stomach, muscle, liver and small intestine whereas the comparable figure for tumours and placenta was 16 mm. This result was repeated on at least five occasions.

3.3.4. Molecular weight

The apparent molecular weight of PyK from placenta and human muscle was determined with Sephadex G-200 calibrated with the following: cytochrome *c* (12 400), ovalbumin (45 000), bovine serum albumin (67 000), yeast hexokinase (96 000), lactate dehydrogenase (rabbit muscle, 135 000), catalase (248 000), R-phycocyanin (270 000) and R-phycoerythrin

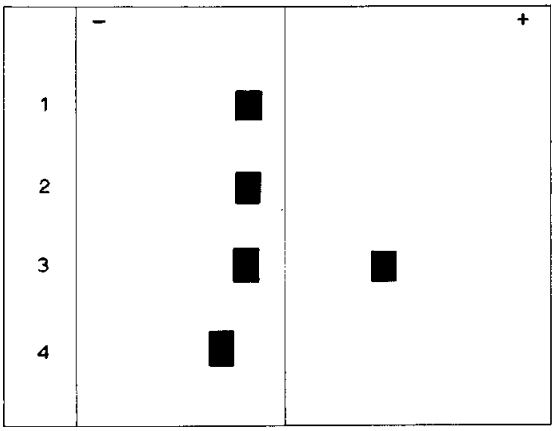

Fig. 1. Starch gel electrophoresis patterns of pyruvate kinase from various human tissues. For details see text: 1 = Stomach, small intestine, lung, heart, brain; 2 = muscle; 3 = liver; 4 = placenta and tumour.

(291 000). Human placental PyK had a lower molecular weight (126 000) than either PyK from human muscle (202 000) or rabbit muscle (230 000).

4. Discussion

These studies represent, to our knowledge, the first description of the purification and properties of PyK from term placenta, a tissue where the Embden–Meyerhof pathway accounts for the vast majority of glucose metabolised [4]. The present results have also shown that based on a number of different criteria, human placental PyK is different from other human tissues including liver and muscle. The latter two organs contain between them the full complement of PyK isoenzymes present in rat tissues [1]. The placental PyK isoenzyme may represent another example of the synthesis of a specific isoenzyme by this organ which elaborates, for instance, a distinct heat stable alkaline phosphatase [5]. The similarity between placental PyK and tumour PyK (table 2 and fig. 1) is also interesting in view of the elaboration by human tumours of placental proteins including the aforementioned alkaline phosphatase [6]. Many animal tumours contain isoenzymes with distinct properties from the tissue of origin of the tumours [7]. In this regard it is of interest that rat hepatomas contain a distinct PyK isoenzyme quite different from PyK isoenzymes in liver and muscle [8,9]. Also a recent preliminary communication has suggested, on the basis of electrophoretic and kinetic data, that a distinct PyK isoenzyme exists in human tumours [10].

Although additional studies are necessary to further characterize placental PyK, these results, in our opinion, do provide evidence for the presence of a distinct PyK isoenzyme in human placenta. They also indicate that a considerable degree of similarity exists between placental PyK and PyK from five human tumours. The presence of a distinct PyK isoenzyme in placenta and tumours could have important diagnostic applications especially if this isoenzyme escapes from these tissues into the circulation.

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