TWO DIFFERENT FORMS OF PYRUVATE KINASE IN RAT KIDNEY CORTEX

Luis JIMÉNEZ DE ASÚA, Enrique ROZENGURT and Héctor CARMINATTI

Instituto de Investigaciones Bioquímicas "Fundación Camponar" and Facultad de Ciencias Exactas y Naturales, Obligado 2490, Buenos Aires 28, Argentina

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1. Introduction

The regulation of pyruvate kinase activity has an important role in gluconeogenic tissues [1]. Liver and kidney, which are the main gluconeogenic organs in mammals, contain at least two different forms of pyruvate kinase [2–5] as shown by electrophoresis in different systems. In rat liver the level of the main component (type L) is under hormonal and dietary regulation and its activity is allosterically modulated by metabolites [2, 4, 6–11]. The other hepatic isoenzyme (type M) appears to be formed constitutively [2] and its activity is not affected by fructose-1,6-diphosphate (FDP) or ATP but is strongly inhibited by several amino acids [12]. The kinetic behaviours of the different forms of kidney pyruvate kinase have not been investigated. Furthermore, previous studies, carried out with crude extracts, disagree regarding the activating effect of FDP [4, 6, 13].

This communication presents evidence showing that extracts from rat kidney cortex contain two forms of pyruvate kinase. One of them, pyruvate kinase type I (PK I) is the major component and displays a kinetic behaviour clearly different from that of all the other pyruvate kinases of rat tissues so far studied. The other, pyruvate kinase type II (PK II), has kinetic properties very similar to that of pyruvate kinase (type L) from liver.

2. Materials and methods

Wistar rats maintained on a laboratory diet and water ad libitum were used throughout. The animals were killed by decapitation. The kidney cortex was dissected and homogenized with 1.5 volumes of cold 15 mM tris-HCl buffer, pH 7.5, 250 mM sucrose, 5 mM EDTA, 1 mM dithiothreitol in a glass homogenizer. The extract was centrifuged at 100,000 *g* for 60 min. The technical details for the separation, purification and assay of both isoenzymes are described in the legends to the figures. All other materials and methods were as described in an earlier paper [10].

3. Results and discussion

A typical elution pattern from chromatography of the 100,00 *g* kidney cortex supernatant on DEAE-cellulose is shown in fig. 1. The first peak (PK I) is eluted from the column with the equilibrating buffer and represents the major component (70% of total enzymatic activity). This fraction can be completely separated from the second peak (PK II), which elutes at 120 mM KCl. In the inserts of fig. 1 it can be observed that only PK II is activated by FDP.

The kinetic characterization of the two forms of pyruvate kinase is depicted in fig. 2. The data are plotted in terms of the Hill equation. It can be observed that the kinetic behaviour of PK II is quite similar to that of the L isoenzyme from liver [9, 11]. At pH 7.5 it exhibits a homotropic cooperative effect with respect to the substrate, phosphoenolpyruvate (PEP). The modifiers (ATP, alanine or FDP) have a marked heterotropic effect on the cooperativity of PEP (see fig. 2 and table 1). Furthermore the allosteric properties of this enzyme are dependent on pH. At pH 6.8 the homotropic cooperative effect of PEP is almost abolished (see fig. 2).

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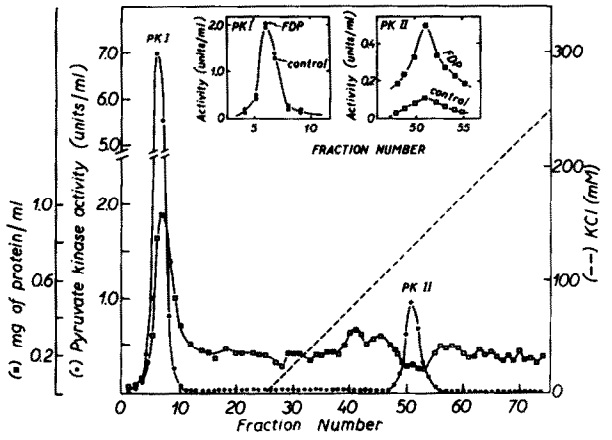

Fig. 1. Chromatographic fractionation of pyruvate kinases from rat kidney cortex. The 100,00 *g* supernatant fluid was passed through a Sephadex G-25 column equilibrated with 15 mM tris-HCl buffer, pH 7.5, 250 mM sucrose, 1 mM EDTA and 1 mM dithiothreitol. The preparation (22 mg of protein with a specific activity of 1.8 units/mg) was applied to a DEAE-cellulose column (12 × 150 mm) previously equilibrated with the same buffer mixture. The column was washed with 50 ml of the above mentioned buffer and then a linear gradient of KCl from 0 to 250 mM in the equilibrating mixture was applied. Two-milliliter fractions were collected; the flow rate was 2 ml/min, and the experiments were carried out at 4–6°. Pyruvate kinase activity was measured by the colorimetric assay procedure previously described [8] except in the determinations depicted in the inserts in which a low level of PEP was used (0.8 mM) and 0.1 mM FDP was added as indicated. The specific activity of the enzyme expressed as units/mg is as defined elsewhere [10].

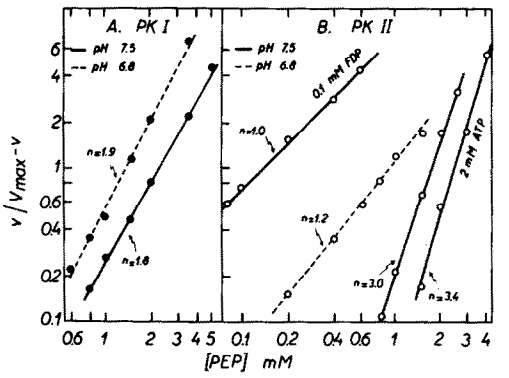

Fig. 2. Hill plots of PK I and PK II activity as a function of PEP concentration at two different pH values, in the absence or presence of 0.1 mM FDP or 2 mM ATP. The 100,000 *g* supernatant fluid was purified by ammonium sulfate fractionation between 30–45% (PK II) and 55–70% (PK I). The two isoenzymes were further purified by chromatography on DEAE- or CM-cellulose columns, respectively, as previously described for the liver pyruvate kinases [10]. The enzyme activity was measured spectrophotometrically by coupling the system with lactate dehydrogenase [15]. The incubation mixture was as follows: 100 mM tris-maleate–tetramethylammonium hydroxide buffer at pH 6.8 or 7.5 as indicated, 10 mM $MgCl_2$, 100 mM KCl, 2.5 mM ADP, 0.2 mM NADH, excess of commercial lactate dehydrogenase and different concentrations of PEP, 2 mM ATP or 0.1 mM FDP were added as indicated on the curves. The incubaton was carried out at 30°.

Table 1
Effect of alanine and FDP on the PEP saturation curve of PK I and PK II.

	PK I		PK II	
	$S_{0.5}$ (mM)	nH	$S_{0.5}$ (mM)	nH
Control	2.1	1.72	1.7	2.98
+ alanine	3.6	1.96	3.4	3.7
+ alanine + FDP	3.6	1.96	0.13	1.30

Assay conditions were as described in fig. 2. The pH value was 7.5 and the concentrations of alanine were 0.2 mM and 1 mM for PK I and PK II, respectively. These levels of alanine produce 50% inhibition at the $S_{0.5}$ values of PEP for each isoenzyme. $S_{0.5}$ is the substrate concentration giving half-maximum velocity and nH the Hill coefficient. When FDP was added, its concentration was 0.1 mM.

After further purification by CM-cellulose chromatography, PK I was free from enolase. The enzyme exhibits homotropic cooperative effects with respect to PEP (fig. 2A).

Two relevant kinetic features should be pointed out which clearly differentiate both kidney cortex isoenzymes. One is that the addition of the allosteric effectors (ATP or FDP) had no significant effect on the PK I activity. The other is that a change in the pH value within the physiological range does not influence the sigmoidal kinetics of PK I with respect to PEP.

Several amino acids were tested to see whether they would modify PK I activity. It was found that alanine is a strong inhibitor. This amino acid, at low concentrations, has only a slight effect on the PEP cooperativity, but increases the $S_{0.5}$ values for this substrate (see table 1). In contrast to PK II, FDP does not counteract the alanine inhibition. Preliminary

results indicate that threonine, proline, tryptophan and valine are also inhibitors of PK I. Therefore, the behaviour of PK I toward amino acids differs from that of the L isoenzyme of liver, but it is quite similar to that of the other hepatic M isoenzyme [12].

The most interesting finding reported in this communication refers to the distinctive kinetic properties of one of the two types of pyruvate kinase of kidney cortex (PK I) which shares properties of the two hepatic isoenzymes. In fact PK I, which is the major component, displays a cooperative effect with respect to PEP, like the L isoenzyme from liver, but its activity is not affected by FDP or ATP. Furthermore its kinetic behaviour is independent of pH within the physiological range (6.8–7.4) [14]. On the other hand its activity is inhibited by several amino acids like the M isoenzyme from liver. Some of these results are in good agreement with those obtained by Llorente et al. [13] with a crude extract of kidney cortex.

Other kinetic properties of both kidney isoenzymes of pyruvate kinase are under investigation.

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