Studies on Avian Heart Pyruvate Kinase During Development[1]

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Summary

During early embryonic development chicken heart pyruvate kinase exhibits kinetic properties similar to the mammalian type L isozyme. Pyruvate Kinase in the adult heart exhibit kinetic properties similar to the mammalian type M isozyme. The embryonic isozyme is probably the K isozyme of fetuses and the adult isozyme is probably the M isozyme. The changeover from isozymes K to M can be monitored kinetically during development.

Mammalian tissues contain three electrophoretically distinct isozymes of pyruvate kinase (EC 2.7.1.40) that are not interconvertible (1-7). They are the type K, M and L isozymes.[3] Type K is the only isozyme in fetuses; it is also found in various adult tissues. Type M is found in striated muscle and brain. Type L is found in liver, intestine, kidney and erythrocytes (8). Only two electorphoretic forms of pyruvate kinase have been found in chicken tissues (9). Type M isozyme was found in breast muscle, thigh muscle and heart ventricular muscle; type K isozyme was seen in spleen, lungs, erythrocytes, kidney, liver and jejunum. Type K and L isozymes have sigmoidal kinetics with PEP[4] and FbP; type M on the other hand has hyperbolic kinetics and is not activated by FbP.

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[3]Isozyme designation is that used by Strandholm, et. al. (8).
[4]Abbreviations used: Phosphoenol pyruvate, PEP; Fructose 1,6-bisphosphate, FbP.

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In this communication we want to show that during development the chicken heart has a pyruvate kinase whose kinetic properties are of the K type and before hatching the kinetic properties become those of the M type, indicating a changeover from isozyme K to M during development.

Materials and Methods

Fertile eggs were obtained from a local hatchery; they were incubated in a Leahy incubator. Embryos were removed from the egg and the heart was dissected out, and washed in ice cold isotonic KCl. Hearts were removed from KCl and frozen in aluminum foil at -20°C until homogenization. Hearts were homogenized in 1:3 W/V of 0.15 M KCl in a Virtis 23 homogenizer with semi micro adaptor for small quantities of tissue. Tissues were homogenized for 90 seconds and remaining lumps were broken with a hand homogenizer. The homogenate was centrifuged at 30,000 XG for 15 minutes, the precipitate was discarded and the supernatant was dialyzed against 3 one liter changes of 0.15 M KCl for three hours. A heavy precipitate formed during dialysis was removed by centrifugation. As result of the precipitation of inert proteins the specific activity of the enzyme increased. The dialyzed enzyme was stored at -20°C overnight or for one week without activity loss. A precipitate always formed upon storage which was removed by centrifugation. There was a further loss in protein, which in combination with the protein loss above resulted in a fourfold purification.

The enzyme was assayed spectrophotometrically by measuring the rate of $NADP^+$ reduction at 340 nm according to the procedure of Pogson (1968). Enolase activity was determined by the procedure of Bücher (13). Protein was determined by the method of Lowry et. al. (12).

Results and Discussion

The 10 day heart has a pyruvate kinase isozyme that gives sigmoidal kinetics with PEP. The adult isozyme exhibits hyperbolic kinetic with PEP (Fig. 1). FbP activates the embryonic enzyme at low PEP concentrations and changes the shape of the PEP saturation curve.

FbP is a potent activator of embryonic chick heart pyruvate kinase during the early days of development, i.e., up to about 12 days of embryonic development. During the latter half of the developmental period the activation by FbP decreases drastically(Fig. 2). In this respect the time of changeover from isozyme embryonic to adult in the developing chick heart resembles that which occurs in chicken thigh muscle (9). Mammalian type L isozyme is strongly inhibited by ATP and the

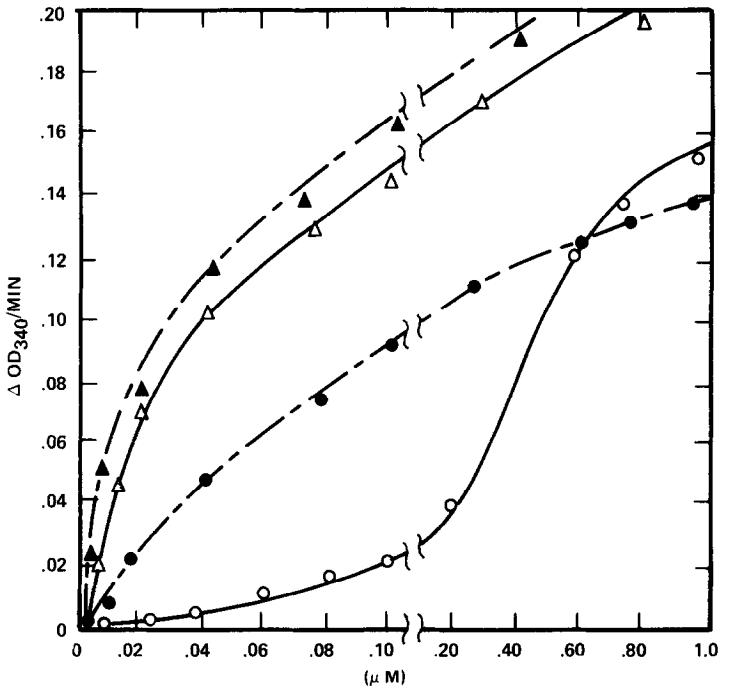

Figure 1. Activity of pyruvate kinase from 10 day embryonic chicken heart (o o o) and adult chicken heart (△ △ △). Embryonic heart in the absence FbP (o—o); in the presence of FbP (● ●). Adult heart in the absence of FbP (△—△) and in the presence of FbP (▲ ▲).

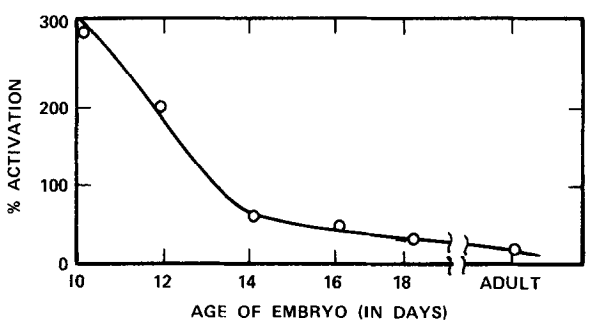

Figure 2. Effect of FbP on the stimulation of chick heart pyruvic kinase during embryonic development.

Table 1. The effect of metabolites on the activity of embryonic and adult chicken heart pyruvate kinase.

Metabolite	Conc (mM)	Percent Inhibition: Embryo (10 day)	Percent Inhibition: Adult
Phenylalanine	3.0	24	48
	1.0	14	10
Alanine	3.0	58	20
	1.0	40	17
Pyrophosphate	1.00	26	25
	.50	12	21
5' ATP	1.00	34	16
	.50	38	10

M type is inhibited by phenylalanine (10). The adult chicken heart isozyme is mildly inhibited by ATP and the isozyme from the embryonic chick heart is also inhibited by ATP (Table 1). Strong inhibition by phenylalanine further suggest that the adult chick heart pyruvate kinase is comparable to mammalian type M. Alanine which moderately inhibits the adult heart enzyme strongly inhibits the embryonic chick heart pyruvate kinase.

Thus the chicken heart undergoes an almost complete changeover in pyruvate kinase isozymes during development. On the basis of their kinetic properties and the electrophoretic data of other avian tissues from other laboratories (9, 10), the embryonic isozyme is the K type and the adult is the M type. The changeover in isozyme pattern can be easily detected by monitoring the kinetic properties in partially purified enzyme preparations.

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