Avian Heart Fructose 1,6-Bisphosphatase - An Embryonic Enzyme [1]

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Summary

Embryonic chick heart contains a specific FbPase that is similar to the FbPase in other vertebrate tissues. The adult chicken heart does not contain a specific FbPase. It is not known whether the enzyme is in an inactive form in the adult or whether the synthesis of the enzyme in the adult heart has ceased.

Fructose 1,6-bisphosphatase (FbPase) (EC3.1.3.11)[5] is one of the key enzymes necessary for the formation of glucose from lactate, glycerol and from gluconeogenic amino acids (1). During the past several years, the enzyme has been isolated from and investigated in a variety of different species (2). In vertebrates, studies on the enzymes have been limited to the liver, muscle and kidney of the adult. Embryonic tissues and the adult heart have been neglected. In this communication we are reporting results of a study which show that FbPase is present in the avian heart during embryonic development but is not present in the adult chicken heart.

Methods

Fertile eggs were obtained from a local hatchery; they were incubated in a Leahy incubator. Ten day embryos were removed from

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[5]Abbreviations used: Fructose 1,6-bisphosphatase, FbPase; Fructose 1,6-bisphosphate, FbP.

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Table 1. The effect of Mg^{++}, EDTA and 5' AMP on 10 day chick heart FbPase activity pH 7.5 and 9.3.

Reaction Component		Specific Activity	
		pH 7.5	pH 9.3
Complete		10.00	5.00
"	$-Mg^{++}$	1.50	2.80
"	-EDTA	3.00	2.70
"	$-Mg^{++}$ and EDTA	1.00	0.80
"	+1.0 mM 5' AMP	0.00	0.00

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 distilled water 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 enzymes 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 four-fold purification.

The enzyme was assayed spectrophotometrically according to the procedure of Rosen et. al. (3) by measuring the rate of reduction of $NADP^+$ at 340 nm. The complete reaction mixture contained .02mM $NADP^+$, 0.2mM FbP, 5 ug phosphoglucose isomerase, 5 ug glucose-6-phosphate dehydrogenase, 1.0mM MgCl, 0.1mM EDTA, appropriate amounts of enzyme extract. The reaction was started by adding FbP after equilibration of reaction mixture. The sensitivity of the recorder was set such that full scale expansion was 0.10 optical density unit. Protein concentration was estimated by the method of Lowry et. al. (4). Specific activity is expressed as mumoles of F-6-P produced/ 3 min/mg protein.

Results and Discussion

FbPase with a low specific activity can be measured at pH 7.5 and 9.3 in enzyme preparations from embryonic chick heart. If either Mg^{++} or EDTA is not present at pH 7.5 the activity is greatly reduced. The presence of 10.mM 5'AMP inhibits the activity at neutral pH and at

Table 2. The effect of Mg^{++}, EDTA and 5' AMP on adult heart FbPase activity at pH 7.5 and 9.3.

Reaction Component		Specific Activity	
		pH 7.5	pH 9.3
Complete		1.60	2.0
"	$-Mg^{++}$	1.30	1.8
"	-EDTA	1.30	1.8
"	$-Mg^{++}$ and EDTA	1.50	1.2
"	+1.0 mM 5' AMP	1.50	2.0

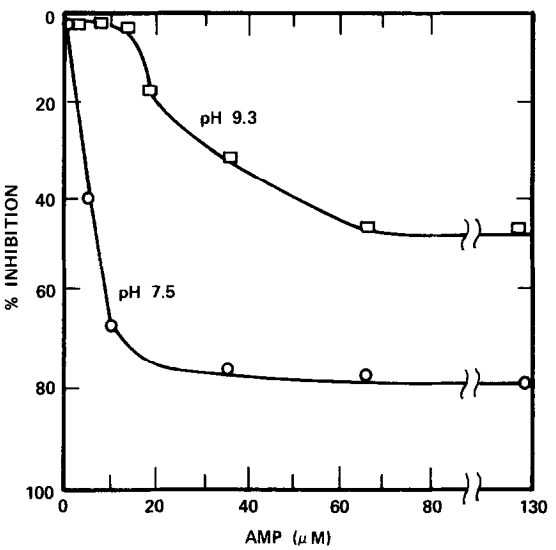

Figure 1. Effect of 5'AMP on 10 day chick heart FbPase at pH 7.5 and 9.2. The standard conditions for spectrophotometric assay as described in Materials and Methods was used except that the enzyme and AMP were incubated at room temperature in a cuvette for 3 minutes prior to the addition of assay mixture.

pH 9.3. The enzyme activity at pH 9.3 is not as dependent on Mg^{++} and EDTA as it is at pH 7.5. (Table 1). In some enzyme preparations from adult heart there was a measurable enzyme activity which was not sensitive to Mg^{++} and EDTA at either pH 7.5 or 9.3 and this activity was not inhibited by 5'AMP (Table 2).

Table 3. Effectiveness of homogenization procedure on release of soluble enzymes from chicken hearts. Pyruvate kinase is used as a marker.

Stage of Development	Specific Activity	
	Pyruvate Kinase [a] (umoles/min/mg protein)	FbPase [b] (mumole/3 min/mg protein)
7 day embryo Heart	3.12	12.0
14 day embryo Heart	1.50	7.0
Adult Heart	0.93	0.00

a) Pyruvate Kinase was assayed spectrophotometrically according to established methods.
b) FbPase was assayed as described in materials and methods. Specific activity of pyruvate kinase is expressed as mumoles/min/mg protein.

Kinetic experiments indicate that the embryonic enzyme is sensitive to low levels of 5'AMP at pH 7.5 (Fig. 1). While approximately 70% of the activity is lost in the presence of 5'AMP at 10 micromolar concentration, complete inhibition requires 5'AMP at about 1.0 mM. This is a much higher concentration of 5'AMP than is needed to completely inhibit purified chicken liver FbPase (7).

Thus, embryonic chicken heart FbPase shares in common with the specific FbPase from other organisms a requirement for a cation and EDTA for maximal activity at pH 7.5 and is inhibited by 5'AMP. We are not certain as to how early in development the enzyme appears but it could not be detected after 16 to 18 days of development. It is not known whether the enzyme is in an inactive form in the adult or whether the synthesis of the enzyme in the adult heart has ceased. Adult chicken heart tissue is difficult to homogenize because of its high degree of musculature. To determine whether we were homogenizing the tissue sufficiently to release enzyme, we always tested for the

presence of a soluble enzyme pyruvate kinase (EC 2.7.1.40) (Table 3). Kuroda and Nagatani (5) detected FbPase with a high specific activity in embryonic chick heart from day 6 to hatching and at 3 weeks post-hatching. Their assays were done at pH 9.5 by determining the release of Pi. They did not provide any data on the Mg^{++} and EDTA requirement nor on 5'AMP sensitivity. We have repeated their study and found that the activity measured by this procedure was not influenced by Mg^{++} and EDTA at neutral pH nor was the activity sensitive to 5'AMP (data not shown). They were probably measuring high levels of non specific phosphatase.

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