CHANGES IN HEPATIC PHOSPHOENOLPYRUVATE CARBOXYKINASE ACTIVITY AND ITS INTRACELLULAR DISTRIBUTION DURING BULLFROG METAMORPHOSIS

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1. Introduction

Of many biochemical alterations observed during amphibian metamorphosis, changes in carbohydrate metabolism have not been extensively investigated. It has been reported that glycogen is stored in the liver prior to tail resorption and catabolized during the resorptive processes [1–3]. Blood glucose concentration [4], activities of hepatic glucose 6-phosphatase [5] and glycogen synthetase [6] have been observed to increase during metamorphosis of frogs. Studies with thyroidectomized toads [7] have shown that thyroxine appeared to lower the content of hepatic glycogen and increase blood glucose, liver glycolysis and glucose 6-phosphatase activity.

The purpose of this study is to survey changes in PEP carboxykinase (EC 4.1.1.32) activity during metamorphosis of the bullfrog, *Rana catesbeiana*. This enzyme is a marker for gluconeogenesis. In this paper, we report that i) bullfrog hepatic PEP carboxykinase activity increases transiently during both spontaneous and T_3-induced metamorphosis; ii) the elevated activity is found in the supernatant fraction; iii) the increases in cytosolic PEP carboxykinase activity are due to fasting and metamorphosis itself; iv) metamorphic variations in the activity of this enzyme in the mitochondrial fraction are observed.

2. Methods and materials

The monosodium salt of PEP, the trisodium salt of IDP and pig heart malate dehydrogenase were obtained from Böhringer Mannheim GmbH. T_3 and NADH were from Sigma Chemical Company and Oriental Yeast Co., Ltd., respectively. [^{14}C]Sodium bicarbonate was purchased from Daiichi Pure Chemicals Co., Ltd. Tadpoles and adult bullfrogs collected in the surroundings of Tokyo, were commercially obtained.

Cell fractionation was performed according to the method of Schneider [8], using 4 vol of 0.25 M sucrose and about 10 tadpole livers. Particulate material was resuspended in 0.25 M sucrose prior to assay.

PEP carboxykinase activity was determined by incorporation of bicarbonate into malate in the presence of NADH and malate dehydrogenase. Reaction mixture contains 0.27 M imidazole HCl buffer pH 6.3, 13.4 mM PEP, 2.7 mM IDP, 10 mM mercaptoethanol, 10 mM $MnCl_2$, 64 mM $NaHCO_3$ (0.3 μCi), 10 mM NADH, 4 μg of malate dehydrogenase and the appropriate amount of the enzyme solution in a total volume of 0.05 ml. After a 5 min incubation at 37°C, the reaction was terminated by addition of 0.05 ml of 1 N HCl. An aliquot (0.05 ml) was applied on a paper disc and the acid stable radioactivity was determined by using a toluene base scintillator and an Aloka liquid scintillation counter model 201. One unit of enzyme activity represents the formation of 1 nmole of malate per min.

Transcaudal injection of 0.2 ml of a saline solution containing 5 μg of T_3 was made into the intraperitoneal cavity of tadpoles at the premetamorphic stage. As a control, tadpoles were similarly treated with 0.2 ml of a saline solution. The animals were maintained at 20°–22°C without feeding. The treated tadpole metamorphosed to the froglet at the 9th day.

Abbreviations:
PEP, phosphoenolpyruvate; T_3, 3,3',5 Triiodo-L-thyronine.

3. Results

3.1. Increases in PEP carboxykinase activity during spontaneous metamorphosis

Table 1 indicates the results of studies on changes in PEP carboxykinase activity in liver subcellular fractions during spontaneous metamorphosis. The following points are apparent: i) The activity detected in the homogenate begins to increase at stage XIX–XX and the maximal increase (2.5-fold) is observed at stage XXIII. Thereafter, the activity decreases. ii) PEP carboxykinase activity is mainly present in the cytosol fraction. The activities found in the homogenate and cytosol fractions increase almost simultaneously. The percentage of the cytosolic activity to the total activity is 65% at stage X–XI and 90% at stage XXIII. iii) The PEP carboxykinase activity located in the mitochondrial fraction decreases continuously during metamorphosis to about 20% of the activity detected in stage X–XI. The relative activity found in the mitochondrial fraction is 17% at stage X–XI and 1.5% at stage XXIII.

3.2. Changes in PEP carboxykinase activity during T_3-induced metamorphosis

As shown in fig. 1A, a transient increase in PEP carboxykinase activity in liver homogenates is detected between 6 to 10 days after T_3-treatment. Fig. 1A also shows that the activity of the control tadpoles which were not fed during the experimental period increases in parallel with that of the treated tadpoles until the 6th day. At the 8th day, about 20 and 10 units per mg liver are found in T_3-treated and control tadpole liver homogenates, respectively. These results indicate that there are two factors responsible for the increase in the PEP carboxykinase activity of T_3-treated tadpoles; one is fasting, the other is action of T_3 itself.

Changes in subcellular distribution of PEP carboxy kinase during T_3-induced metamorphosis (fig. 1B) are essentially the same as those of spontaneous metamorphosis, shown in table 1. Fig. 1B also indicates that the activity in the mitochondrial fraction decreases continuously after T_3 injection. The activity in the mitochondrial fraction at the first day is 0.5 unit/mg liver and 7% of the total activity. However, at the 15th day, only 0.06 unit/mg liver and 0.5% are detected. Although not shown in fig. 1B, the activities in nuclear and microsomal fractions do not change and are about 1.5 units/mg and 0.1 unit/mg liver, respectively, throughout the experiments.

Fig. 1C. shows an intracellular distribution of hepatic PEP carboxykinase activity of control tadpoles. The increases in the cytosol fraction of PEP carboxykinase activity during fasting explain the elevation of total activity. In this case, the activity in the mitochondrial fraction (about 0.5 unit/mg) liver) does not change.

4. Discussion

In this paper, we have shown that hepatic PEP carboxykinase activity increases in the cytosol fraction and decreases in the mitochondrial fraction during both spontaneous and T_3-induced metamorphosis of bullfrogs. Elevation of the activity in the cytosol fraction has been observed with fasting, diabetic and variously treated animals such as glucagon, cortisone and 3′,5′ cyclic AMP [10–16]. This is the first report showing similar increases during metamorphosis.

Table 1
Subcellular distribution of PEP carboxykinase activity in tadpole liver at various metamorphic stages.*

Metamorphic stage†	X–XI	XII–XV	XVI–XVIII	XIX–XX	XXI	XXII	XXIII	Froglet
Nuclear fraction	2.7	3.3	3.4	3.9	3.6	2.7	4.6	6.5
Mitochondrial fraction	2.1	1.3	1.1	0.7	0.8	0.3	0.4	0.4
Microsomal fraction	0.3	0.3	0.4	0.4	0.4	0.3	0.9	0.5
Cytosol fraction	7.4	8.2	7.5	11.5	12.5	17.2	24.6	17.6
Homogenate	11.6	11.6	10.6	15.8	18.8	21.8	26.7	21.8

These experiments were carried out in July and August, 1972.
* Activity is expressed as nmoles of malate produced per min per mg liver using 10 pooled tadpole livers.
† Metamorphic stages were classified according to the method of Taylor and Kollros [9].

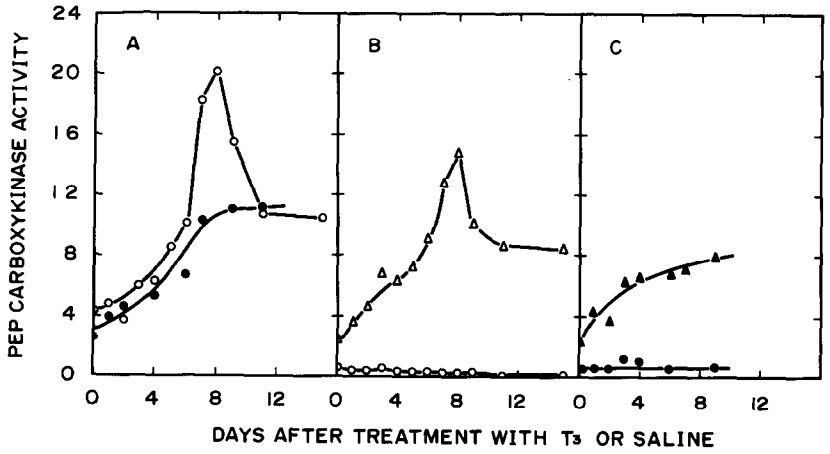

Fig. 1. Changes in tadpole hepatic PEP carboxykinase activity by treatment with T_3 or saline. The ordinate numbers indicate enzyme activity expressed as units/mg liver. These experiments were carried out in September, 1972. A) Changes in PEP carboxykinase activity in liver homogenates of T_3-induced metamorphosing tadpoles (○—○—○) and of saline-treated tadpoles (●—●—●). B) Changes in PEP carboxykinase activity of liver cytosol (△—△—△) and mitochondrial fraction (○—○—○) from T_3-induced metamorphosing tadpoles. C) Changes in PEP carboxykinase activity from liver supernatant (▲—▲—▲) and mitochondrial fraction (●—●—●) of saline-treated tadpoles.

It has been previously suggested that glycogenolysis causes the high blood glucose concentration during metamorphosis [4]. Based on the following evidences, we suggest that at least part of the increased blood glucose level in metamorphosis is due to the gluconeogenic pathway: i) Increased hepatic PEP carboxykinase which functions in gluconeogenesis. ii) Increases glucose 6-phosphatase during metamorphosis [5]. It seems, however, that further evidence is required to support our hypothesis.

Our experiments (fig. 1) show that two factors are involved in the increase of cytosolic PEP carboxykinase during T_3-induced metamorphosis. One factor is fasting which occurs up to about the 6th day and the other is T_3-dependent and occurs from the 6th to 10th days. Elevated PEP carboxykinase due to fasting has been previously noted [10–12, 15, 16]. During spontaneous metamorphosis, we cannot measure the specific contribution of fasting to increased PEP carboxykinase because the metamorphosing tadpole ceases to take food. However, since fasting causes parallel increased hepatic PEP carboxykinase in control and T_3-treated tadpoles up to about day 6, we conclude that fasting also contributes to increased cytosolic PEP carboxykinase during spontaneous metamorphosis. Mitochondrial PEP carboxykinase activity decreases during T_3-induced metamorphosis (fig. 1B) and remains constant during starvation (fig. 1C). The decrease in mitochondrial activity during metamorphosis corresponds to a similar decrease described in the newborn rat [17]. The slow response to fasting shown in fig. 1C is due to the slow utilization of intestinal contents in the tadpole.

The differences of this enzyme's activities in the premetamorphic tadpole livers between table 1 and in fig. 1 may come from the season when the experiments were performed. The physiological roles of the seasonal variations of PEP carboxykinase activities remains to be answered. Similar seasonal variations have been observed in blood glucose level [18–21], fat and carbohydrate content [18, 19]. The systematic studies on the seasonal changes of PEP carboxykinase are now in progress.

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References

[1] S. Bilewicz, Biochem. Z. 297 (1938) 379.
[2] R. Gosh, A.K. Medda, C. Deb and G.C. Bhattacharya, Sci. Cult. (Calcutta) 31 (1965) 538.
[3] E.S. Spiegel and M. Spiegel, Exptl. Cell. Res. 67 (1971) 222.
[4] B.E. Frye, J. Exptl. Zool. 155 (1964) 215.
[5] E. Frieden and H. Mathews, Arch. Biochem. Biophys. 73 (1958) 107.
[6] Y. Goto, Seikagaku J. Jap. Biochem. Soc. 43 (1971) 1042.
[7] M.T. Rinaudo, C. Giunta, R. Bruno, A. Guardabassi, M. Olivero and P. Clerici, Comp. Biochem. Physiol. 29 (1969) 1079.
[8] W.C. Schneider, J. Biol. Chem. 176 (1948) 259.
[9] A.C. Taylor and J.J. Kollros, Anat. Rec. 94 (1964) 7.
[10] D.C. Johnson, K.A. Ebert and P.D. Ray, Biochem. Biophys. Res. Commun. 39 (1970) 750.
[11] E. Shrago, H.A. Lardy, R.C. Nordlie and D.O. Foster, J. Biol. Chem. 238 (1963) 3188.
[12] A.J. Garber and R.W. Hanson, J. Biol. Chem. 246 (1971) 589.
[13] P.H. Taylor, J.C. Wallace and D.B. Keech, Biochim. Biophys. Acta 237 (1971) 179.
[14] W.D. Wicks, W. Lewis and J.B. McKibbin, Biochim. Biophys. Acta 264 (1972) 177.
[15] A.J. Garber and R.W. Hanson, J. Biol. Chem. 246 (1971) 5555.
[16] R.C. Nordlie, F.E. Varricchio and D.D. Holten, Biochim. Biophys. Acta 97 (1965) 214.
[17] F.J. Ballard and R.W. Hanson, Biochem. J. 104 (1967) 866.
[18] S. Mizell, J. Cell Comp. Physiol. 66 (1965) 251.
[19] C.L. Smith, J. Exptl. Biol. 29 (1950) 412.
[20] P.A. Wright, Endocrinology 64 (1959) 551.
[21] B. Hermansen and C.B. Jørgensen, Gen. Comp. Endocrinol. 12 (1969) 313.