Fructose 2,6-bisphosphate and glucose 1,6-bisphosphate in erythrocytes during chicken development

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In contrast to mammalian erythrocytes, chicken erythrocytes contain fructose 2,6-bisphosphate at levels (0.5 nmol/10^9 cells) similar to those of 2,3-bisphosphoglycerate (1.2 nmol/10^9 cells) and slightly lower than those of glucose 1,6-bisphosphate (5.2 nmol/10^9 cells). In chick embryo erythrocytes the levels of both fructose 2,6-bisphosphate and glucose 1,6-bisphosphate are much lower. They begin to increase at hatching and reach the levels in chicken in a few days.

Erythrocyte *Glycolysis* *Fructose 2,6-bisphosphate* *Glucose 1,6-bisphosphate* *Phosphofructokinase*

1. INTRODUCTION

Fructose 2,6-bisphosphate (Fru-2,6-P_2) and glucose 1,6-bisphosphate (Glu-1,6-P_2) have been implicated in the control of carbohydrate metabolism [1,2]. Fru-2,6-P_2 is a very powerful activator of phosphofructokinase and an inhibitor of fructose-1,6-bisphosphatase. It seems to play a prominent role in hormonal and nutritional regulation of hepatic glycolysis and gluconeogenesis, although its role in other tissues is as yet unclear [1]. Glu-1,6-P_2 acts as an activator of phosphofructokinase and pyruvate kinase, and an inhibitor of hexokinase and fructose-1,6-bisphosphatase. It could be involved in the regulation of muscle glucose metabolism in several hormonal, physiological and pathological conditions [2].

Although phosphofructokinase from human erythrocytes is sensitive to stimulation by Fru-2,6-P_2, the bisphosphorylated hexose has not been detected in mammalian erythrocytes [3]. In contrast, mammalian erythrocytes are rich in Glu-1,6-P_2 [4], which has been found to affect erythrocyte hexokinase, pyruvate kinase and phosphofructokinase similarly to the enzymes from other tissues [5]. This paper shows the presence of Fru-2,6-P_2, Glu-1,6-P_2 and the enzymes involved in their synthesis in chicken erythrocytes, and reports the variations of their levels during chick embryo development.

2. MATERIALS AND METHODS

White Leghorn fertile chicken eggs and mature chickens were obtained from a poultry farm. The eggs were incubated at 37.8°C and showed normal development of the embryos. Blood samples were collected from the chorioallantoic vascular system of the embryos and from the jugular vein of the chicken, and drawn into 1 vol. ice-cold 150 mM NaCl, containing 15 mM sodium citrate and 5 mM glucose, pH 7.2. Red blood cells were quickly washed 3 times with the same medium without citrate at 0–3°C.

For the determination of Fru-2,6-P_2, washed erythrocytes were extracted with 5 vols 0.1 M NaOH and heated for 30 min at 80°C. After centrifugation, the supernatant was neutralized with 0.5 M acetate buffer, pH 4.0, and Fru-2,6-P_2 was measured as in [6]. For the determination of Glu-1,6-P_2, the erythrocytes were extracted with 2.25 vols 0.7 M $HClO_4$, neutralized with 3 M

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$KOH-KHCO_3$ and heated for 30 min at 90°C in 0.1 M NaOH. Glu-1,6-P_2 was determined as in [7]. 2,3-Bisphosphoglycerate (2,3-BG) was measured [8] in neutralized perchloric extracts. The enzymatic activities were determined in hemolysates [9] by the following methods: 6-phosphofructo-2-kinase (PFK-2) (pH 8.5) [10], Glu-1,6-P_2 synthesizing activities [11], hexokinase, 6-phosphofructo-1-kinase (PFK-1) and pyruvate kinase [9].

3. RESULTS AND DISCUSSION

Fig.1 shows the levels of Fru-2,6-P_2 and Glu-1,6-P_2 in chick erythrocytes during embryonic development and after hatching. For comparison, the changes in the levels of 2,3-BPG and inositol pentaphosphate (IP_5), phosphorylated metabolites which modulate the affinity of hemoblobin for oxygen, are included. In contrast with mammalian erythrocytes, chicken erythrocytes contain Fru-2,6-P_2 at slightly lower levels (0.5 nmol/10^9 cells) than those of Glu-1,6-P_2 (5.2 nmol/10^9 cells) and similar to those of 2,3-BPG (1.2 nmol/10^9 cells). Fru-2,6-P_2 and Glu-1,6-P_2 are present in chick embryo erythrocytes at much lower concentrations than in chicken erythrocytes. They begin to increase at hatching and accumulate progressively to reach adult levels. Similar changes to those found in the levels of Fru-2,6-P_2 and Glu-1,6-P_2 during embryo development have been reported for the concentration of IP_5. In contrast, the changes in 2,3-BPG concentration follow a biphasic pattern. The amount of 2,3-BPG increases in the erythrocytes from the 14-day embryo to a maximum in the 16- or 17-day embryo, and then drops suddenly immediately after hatching [12].

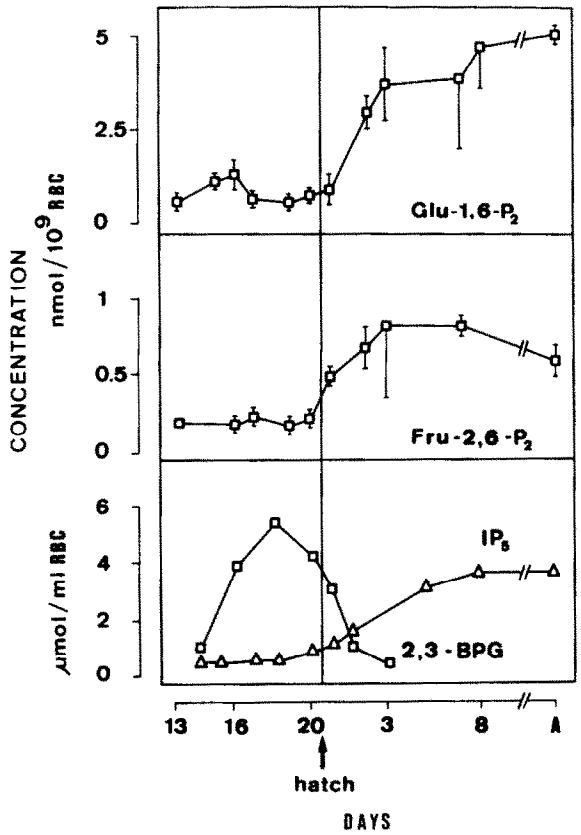

Fig.1. Levels of phosphorylated metabolites in developing chick erythrocytes. Values of Glu-1,6-P_2 and Fru-2,6-P_2 are means ± SE of 3–8 animals. IP_5 and 2,3-BPG values are taken from [18]. A, adult animal (6 weeks old); RBC, red blood cells.

In mammalian tissues four enzymatic reactions for the synthesis of Glu-1,6-P_2 have been detected [11], but in the erythrocytes only the Glu-1,6-P_2 synthase reaction catalyzed by phosphoglucomutase (glucose monophosphate + glycerate 1,3-P_2 → Glu-1,6-P_2 + glycerate 3-P) was found [13]. Similar results are obtained in chick embryo and chicken erythrocytes, in which the Glu-1,6-P_2 synthase activity is about 30-fold higher than the PFK-2 activity (Fru-6-P + ATP → Fru-2,6-P_2 + ADP) involved in the synthesis of Fru-2,6-P_2. Neither the Glu-1,6-P_2 synthase activity nor the PFK-2 activity in chick erythrocytes varies significantly during development. Hexokinase (HK), PFK-1 and pyruvate kinase (PK) activities, which could indirectly influence the concentration of bisphosphorylated hexoses, remain also essentially constant along embryo and chick development (table 1).

Two different populations of erythrocytes are present in the blood of chick embryo, as a consequence of the embryonic (primitive) erythrocytes formed in the yolk sac being replaced by definitive erythrocytes of medullar origin. It has been postulated that both red cell populations, which possess different types of hemoglobin, also differ in the concentration of 2,3-BPG and IP_5 as a consequence of their different capability of synthesiz-

Table 1

Glu-1,6-P_2 synthase, PFK-2, HK, PFK-1 and PK activities in erythrocytes during chick development

Age (days)	Glu-1,6-P_2 synthase	PFK-2	HK	PFK-1	PK
14–15	5.2 ± 0.2 (4)	0.10 ± 0.02 (4)	155 ± 94 (2)	30 ± 2.5 (4)	1333 ± 33 (3)
17–18	4.8 ± 0.1 (4)	0.28 ± 0.07 (8)	109 ± 75 (3)	43 ± 7.0 (5)	1686 ± 370 (3)
19–20	6.0 ± 1.2 (4)	0.22 ± 0.04 (7)	81 ± 11 (4)	42 ± 6.5 (6)	1200 ± 231 (3)
Hatching	6.1 ± 2.0 (4)	0.21 ± 0.06 (4)	225 ± 49 (2)	31 ± 1.3 (5)	1225 ± 25 (2)
7–8	3.6 ± 0.9 (3)	0.27 ± 0.08 (6)	153 ± 26 (3)	31 ± 3.2 (6)	1450 ± 49 (2)
Adult	3.5 ± 0.4 (6)	0.12 ± 0.02 (4)	199 ± 60 (3)	25 ± 2.3 (3)	1943 ± 133 (3)

Activities are expressed as mU/10^9 cells and are means ± SE of the number of animals in parentheses. Adult chicken blood contained 2.5 × 10^{12} cells/l. The hematocrit value was 30%

ing those metabolites [14,15]. The results herein suggest that the definitive erythrocytes possess higher levels of Fru-2,6-P_2 and of Glu-1,6-P_2 than the embryonic erythrocytes. The progressive substitution of the primitive population by the definitive population of erythrocytes would produce an increase in the content of Fru-2,6-P_2 and Glu-1,6-P_2 in circulating blood cells. Since the levels of PFK-2 and Glu-1,6-P_2 synthase activities remain constant during chick development, the different concentration of Glu-1,6-P_2 and Fru-2,6-P_2 in primitive and in definitive erythrocytes could involve differences in their Glu-1,6-bisphosphatase and Fru-2,6-bisphosphatase content. However, it could also result from differences either in the kinetic properties of the enzymes or in the levels of substrates and enzyme effectors.

It has been shown that in spite of possessing the whole set of glycolytic enzymes, chicken erythrocytes consume no measurable amounts of glucose and do not produce lactate [16]. Further biochemical characterization of the two populations of chick erythrocytes is needed for understanding of their energetic metabolism. The study of the effect of Fru-2,6-P_2 and Glu-1,6-P_2 on the key enzymes of carbohydrate metabolism is in progress.

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