Vol. 93, No. 1, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
March 13, 1980 Pages 93-99

ENZYME ACTIVITIES RELATED TO 2,3-P_2-GLYCERATE METABOLISM IN EMBRYONIC AND FETAL RED CELLS

Wolfgang Jelkmann and Christian Bauer

Physiologisches Institut, Universität Regensburg, 8400 Regensburg, West-Germany

Received December 10, 1979

SUMMARY

The intraerythrocytic concentration of 2,3-P_2-glycerate decreases conspicuously during the intrauterine development of rabbits. In order to obtain informations on possible causes for these changes, we have measured in embryonic and fetal red cells the activities of those glycolytic enzymes which are thought to be most important in the regulation of the steady-state level of 2,3-P_2-glycerate, namely hexokinase, phosphofructokinase, bisphosphoglyceromutase and pyruvate kinase. Hexokinase and bisphosphoglyceromutase activities are significantly higher in embryonic red cells, when compared to fetal or adult erythrocytes, whilst the activity of phosphofructokinase is not significantly different at either developmental stage. Most important are the changes found in the activity of pyruvate kinase which is low in embryonic and early fetal erythrocytes and increases drastically thereafter. The activity of this enzyme is inversely related to the concentration of 2,3-P2-glycerate, which is highest in embryonic red cells and very low in the late fetal period. These results support the conclusion that the pyruvate kinase activity is of great importance for the adjustment of the concentration of 2,3-P_2-glycerate and therefore of the blood oxygen affinity during rabbit ontogeny.

INTRODUCTION

Red cells are known to change their morphological and functional properties in the course of mammalian ontogeny (1-4). Thus, during the embryonic period, nucleated red cells deriving from yolk sac stem cells are produced, which contain specific embryonic hemoglobins. Later, in the fetal stage anucleated red cells mostly of liver origin appear in the circulation which contain either fetal or adult hemoglobins depending on the species (4). In general, the oxygen binding properties of red cells are not only determined by the respective type of hemoglobin but are modified by the interaction of the hemoglobin with intraerythrocytic phosphates, mostly 2,3-P_2-glycerate. Mammalian fetuses without a specific fetal hemoglobin attain a high oxygen affinity of their blood by low concentrations of 2,3-P_2-glycerate in

0006-291X/80/050093-07$01.00/0

their red cells, which at least in one species, the rabbit, is secondary to a very high activity of pyruvate kinase (5).

However, the primitive nucleated erythrocytes of rabbit embryos contain high concentrations of 2,3-P_2-glycerate and have a low oxygen affinity which may aid the oxygen flow from the blood to the very actively metabolizing tissues (3). In order to obtain informations on the relationship between the concentration of 2,3-P_2-glycerate and red cell enzymes we have measured in embryonic red cells the activity of those glycolytic enzymes which are considered to be the most relevant ones for the regulation of 2,3-P_2-glycerate metabolism, namely hexokinase [EC 2.7.1.1.] , phosphofructokinase [EC 2.7.1.11.], bisphosphoglyceromutase [EC 2.7.5.4.] , and pyruvate kinase [EC 2.7.1.40]. Such measurements were also performed at various gestational ages during the fetal period which allows us to link enzyme activity with 2,3-P_2-glycerate concentration and therefore oxygen affinity throughout a large part of intrauterine development.

METHODS

White New Zealand rabbits were mated at a local animal breeding farm (Dr. Ivanovas, Kisslegg). The gestation period of rabbits is 31 days. Red cell suspensions or blood, respectively, were sampled from embryos 14 days after conception (14 dpc), from fetuses 19, 22, and 26 dpc, from newborns 2 days after birth (2 dpp), and from adult rabbits as described earlier (3). Washed red cells were depleted of white cells and platelets by chromatography on mixed α-cellulose and microcrystalline cellulose (Sigma) columns prior to hemolysis in water to which 1 mmol/l EDTA had been added (6).
Enzyme activities in hemolysates were determined at 37 °C and pH 8.0 as described by Beutler (6) but without the addition of mercaptoethanol. Enzymes and substrates were obtained from Boehringer, Mannheim. Enzyme activities were expressed as µmoles substrate converted per min (IU) and related to the concentration of hemoglobin in the sample rather than to red cell volume because only minute amounts of embryonic red cells were available and because the exact solute space is uncertain in nucleated cells. Statistical significance of the data was determined using Dunnett's test for multiple comparisons. P was considered significant at a level of 1 %.

RESULTS and DISCUSSION

We have recently reported important differences in red cell 2,3-P_2-glycerate levels during ontogenetic development of rabbits. Thus, the mean concentrations of 2,3-P_2-glycerate were 21.6 µmol/g

hemoglobin (Hb) in the embryo, 0,6 µmol/g Hb in the fetus near term, and 29.0 µmol/g Hb in the adult animal (3). These changes in red cell 2,3-P_2-glycerate concentrations were found to be correlated with changes in the oxygen affinity of red cells (3). In the present study, we were interested in the accompanying changes in the activity of certain red cell enzymes in order to explain these marked differences in the concentration of 2,3-P_2-glycerate.

Energy production in mature erythrocytes occurs predominantly via anaerobic glycolysis. The rate-controlling steps in the glycolytic pathway are most likely the initial kinase reactions (hexokinase and phosphofructokinase) and under conditions of stimulated glycolysis, the pyruvate kinase reaction (7,8). These reactions have been demonstrated to be far from thermodynamic equilibrium (9). In looking at the interrelation between red cells 2,3-P_2-glycerate concentration and glycolytic enzyme activities special attention has been paid to the following enzymes: hexokinase, phosphofructokinase, pyruvate kinase, and bisphosphoglyceromutase. We have hereby taken evidence into consideration, which suggests that the synthesis of 2,3-P_2-glycerate depends mainly on the availability of the substrates 1,3-P_2-glycerate, 2-P-glycerate and 3-P-glycerate (7,10,11) and on 2,3-P_2-glyceromutase activity (12-15).

The hexokinase activity was found to be highest in the embryonic red cells and lowest in the erythrocytes from adult animals with a steady, significant decrease during the fetal period until the end of gestation (Fig. 1). No significant differences were found in red cell phosphofructokinase activities at either developmental stage.

In Fig. 2 are shown the variations of bisphosphoglyceromutase and of pyruvate kinase activities as well as of the concentration of 2,3-P_2-glycerate during the prenatal development. The activity of bisphosphoglyceromutase is significantly higher in red cells from embryos and young fetuses and decreases from day 19 after conception onwards. The enzymatic activity of bisphosphoglyceromutase has been shown to be closely associated with the appearance and disappearance of 2,3-P_2-glycerate in the red blood cells of chicks before and after hatching although its precise role in

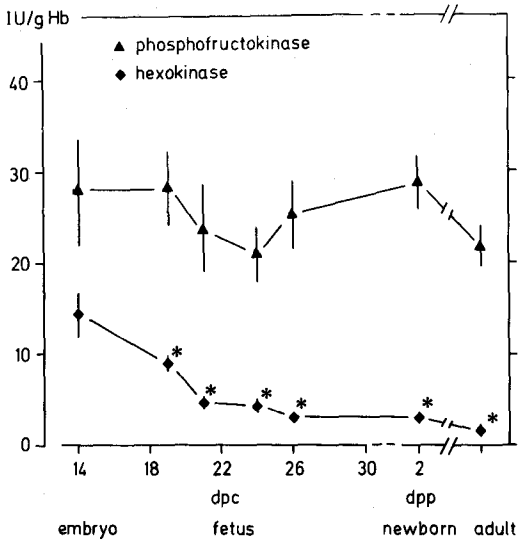

Fig. 1 Phosphofructokinase and hexokinase activities of red cells during rabbit ontogeny. Asteriks indicate significant differences when compared to embryonic red cells ($P < .01$, mean $\pm$ SD, $n = 4 - 7$).

the regulation of the synthesis and breakdown of the phosphate ester in the nucleated red cells of fetal chicks remains to be investigated (12).

Those mammals which have only trace amounts of 2,3-P_2-glycerate in their red cells also possess very low activities of bisphosphoglyceromutase (13,14). Among the mammals with high intraerythrocytic concentrations of 2,3-P_2-glycerate on the other hand, the relationship between the bisphosphoglyceromutase activity and the concentration of 2,3-P_2-glycerate is rather variable (13) which is also borne out by the present experiments (Fig. 2). It is obviously the almost complete absence of bisphosphoglyceromutase activity that leads to extremely low levels of 2,3-P_2-glycerate as for example in ruminants and adult chickens (12-14) as well as in bisphosphoglyceromutase deficient human red cells (15) whilst in species with significant activities of bisphosphoglyceromutase other control points of red cell glycolysis seem to be more important.

Of special relevance in this respect is the pyruvate kinase activity which apparently regulates the distribution between the main glycolytic pathway and the 2,3-P_2-glycerate bypass (7).

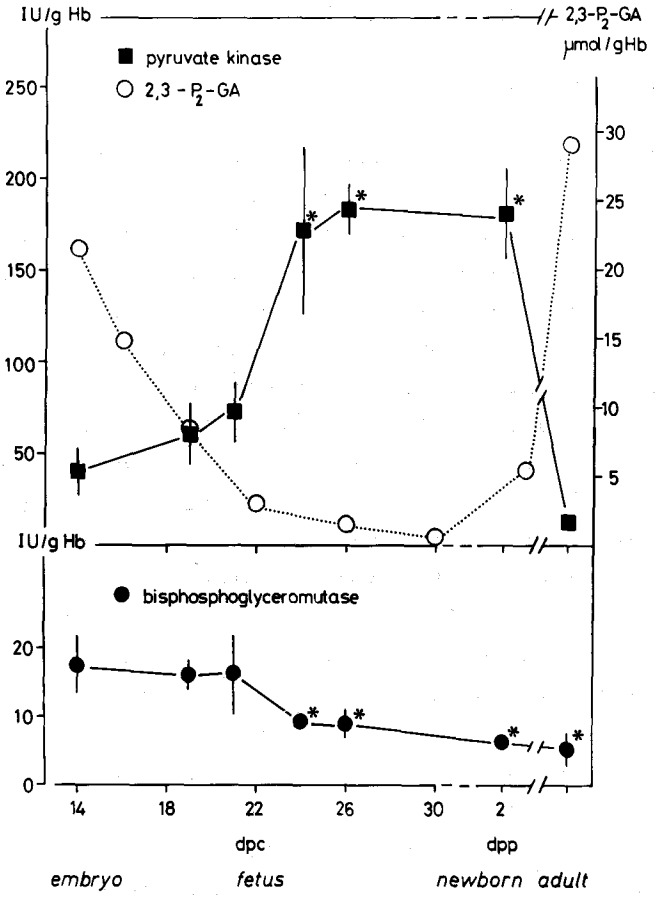

Fig. 2 Pyruvate kinase and bisphosphoglyceromutase activities of red cells during rabbit ontogeny. Asteriks indicate significant differences when compared to embryonic red cells ($P < .01$, mean $\pm$ SD, $n = 5 - 9$). 2,3-P_2-glycerate (2,3-P_2-GA) levels were taken from reference (3).

Jacobasch et al. (7) have drawn attention to the fact that whenever there is a preponderance of the initiating steps of glycolysis, particularly of the phosphofructokinase reaction over the pyruvate kinase reaction, 2,3-P_2-glycerate will accumulate. This interpretation is in full accord with the present results concerning the change of pyruvate kinase activity in red cells of the developing rabbit.

It can be seen from Fig. 2 that the pyruvate kinase activity is relatively low in embryonic red cells but increases as gestation continues and is significantly higher in the erythrocytes from animals between day 22 of gestation and 2 days after birth when

compared to embryonic red cells. The pyruvate kinase activity measured in red cells from adults is not significantly different from that of the embryo.

Since the activity of phosphofructokinase is not significantly different at either developmental stage (Fig. 1) it follows that the ratio of phosphofructokinase activity over pyruvate kinase activity is high in embryonic red cells, then declines during the fetal stage and rises again in the course of the postnatal development. These changes are probably associated with a prenatal decrease and postnatal increase of those intermediates which are relevant for the steady-state concentration of 2,3-P_2-glycerate, namely 1,3-P_2-glycerate, 3-P-glycerate and 2-P-glycerate (7,10,11). In accord with this interpretation is the prenatal decrease of the concentration of 2,3-P_2-glycerate (Fig. 2) and its rise after birth which is mirrored by the changes in pyruvate kinase activity (5). Our results support the hypothesis that the physiologically significant alterations of the concentration of 2,3-P_2-glycerate are secondary to changes in the pyruvate kinase activity. Note, however, that these conclusions are based on measurements under conditions of maximal reaction velocity, which may not exactly reflect the catalytic functioning of the enzyme in the circulating red cell. The in vivo reaction velocities are,of course, influenced by the intracellular conditions such as substrate concentrations and pH-value. In addition, the reaction kinetics may be altered in relation to enzyme multiplicity, when pyruvate kinase isozymes appear and disappear in the various populations of red cells (16,17).

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