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Fructose 2,6-bisphosphate and glucose 1,6-bisphosphate in rabbit erythroid cells during differentiation

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Fructose 2,6-bisphosphate concentration and 6-phosphofructo-2-kinase activity markedly decrease during differentiation of rabbit erythroid cells, being higher in erythroblasts (654±97 pmol/10^9 cells; 238±81 $U\mu$/10^9 cells) than in reticulocytes (40±15 pmol/10^9 cells; 11±3 $U\mu$/10^9 cells) and much higher than in mature erythrocytes (10±0.8 pmol/10^9 cells; 2±1 $U\mu$/10^9 cells). The enzymatic activities involved in glucose 1,6-bisphosphate metabolism also decrease, but the levels of aldohexose 1,6-bisphosphates remain essentially constant during differentiation of erythroid cells.

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

1. INTRODUCTION

Differentiation and maturation of the erythroid cells involve significant changes in energy metabolism which are not fully understood. The control of carbohydrate metabolism has been extensively studied in mature erythrocytes, but it is not so well known in erythroid cells [1]. Fructose 2,6-bisphosphate (Fru-2,6-P_2) and glucose 1,6-bisphosphate (Glu-1,6-P_2), which have been implicated in the regulation of glucose metabolism [2–4], could also play a relevant role in erythroid cells. Both bisphosphorylated hexoses are present in chicken erythrocytes at concentrations which vary during development [5]. Mammalian erythrocytes are rich in Glu-1,6-P_2 [6], and it has been recently reported that they also contain Fru-2,6-P_2, although its concentration is negligible in freshly isolated cells [7].

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The aim of this work was to study the concentrations of Fru-2,6-P_2 and Glu-1,6-P_2, and the levels of the enzymatic activities involved in the metabolism of these compounds in rabbit erythroblasts, reticulocytes and erythrocytes. For comparison, the enzymes implicated in the metabolism of 2,3-bisphosphoglycerate (2,3-BPG), known to be a modulator of haemoglobin affinity for oxygen and of other erythrocyte functions [4], have also been determined.

2. MATERIALS AND METHODS

Enzymes and biochemical reagents were obtained from either Boehringer or Sigma. New Zealand white male rabbits (3–4 kg body wt) were used. Blood was collected from the lateral ear vein and drawn into 1 vol. of 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. Anaemia was induced by daily bleeding for 3 days followed by an interval of 2 days, and on the 6th day reticulocytes were prepared from

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peripheral blood (40% reticulocytosis) by density fractionation [8]. The preparations contained more than 80% reticulocytes. Bone marrow was scraped from the femur and humerus of anaemic rabbits, and erythroblasts were separated by centrifugation through a Percoll gradient [9]. The preparations contained 65–80% erythroblasts.

Cell extracts were prepared as in [5]. Haemoglobin was determined as in [10]. Fru-2,6-P_2 and 2,3-BPG were measured, and enzymatic activities were assayed as reported [5]. Prior to Fru-2,6-P_2 determination, haemoglobin was separated from the erythrocyte extracts and 6-phosphofructo-2-kinase (PFK-2) incubation mixtures by centrifugation at 2000 × g with Amicon-CF 25 membranes. It has been shown that both the potato enzyme (pyrophosphate: fructose-6-phosphate 1-phosphotransferase, PPi:PFK) used for measurement of Fru-2,6-P_2 and PFK-2 are inhibited by 2,3-BPG [7]. However, under the assay conditions used, the 2,3-BPG present in the incubation mixtures (4–40 μM) would produce less than 20% inhibition. Glu-1,6-P_2 and mannose 1,6-P_2 are present in mammalian erythrocytes at similar concentrations [11]. Both compounds have been measured as cofactors of the phosphoglucomutase reaction which does not differentiate the two aldohexose 1,6-bisphosphates [11].

3. RESULTS AND DISCUSSION

As shown in table 1, the levels of Fru-2,6-P_2 markedly decrease during differentiation of rabbit erythroid cells. Fru-2,6-P_2 levels in erythroblasts are about 15-times higher than those found in reticulocytes, and 60-times higher than those present in mature erythrocytes. In contrast, the levels of aldohexose 1,6-bisphosphates are of the same order of magnitude in the three types of erythroid cells, although they appear to be slightly higher in reticulocytes. As reported by others [12], the levels of 2,3-BPG increase during differentiation of erythroid cells, concurrently with haemoglobin concentration. In mature rabbit erythrocytes, the levels of Fru-2,6-P_2 are three orders of magnitude lower than those of aldohexoses-1,6-P_2 and five orders of magnitude lower than those of 2,3-BPG. The fact that Fru-2,6-P_2 had not been previously detected in mammalian erythrocytes [13] could be due to interference from 2,3-BPG [7] and haemoglobin in the assay. Fru-2,6-P_2 was barely detectable in rabbit erythrocyte extracts containing haemoglobin. Control experiments showed that haemoglobin markedly interferes with the stimulation of PPi:PFK by Fru-2,6-P_2.

Table 2 summarizes the changes in enzymatic activities during differentiation of rabbit erythroid cells. As shown, a marked decline in PFK-2 activity, which is involved in the synthesis of Fru-2,6-P_2, is produced. This decrease, as well as the possible inhibition of PFK-2 produced by the increasing levels of 2,3-BPG [7], could explain the decline in Fru-2,6-P_2 concentration during differentiation of erythroid cells.

In mammalian tissues four enzymatic reactions for the synthesis of Glu-1,6-P_2 have been detected [4], but in erythrocytes only the Glu-1,6-P_2 synthase reaction catalyzed by phosphoglucomutase has been found [14]. As shown in table 2, this enzymatic activity is reduced during differentiation of rabbit erythroid cells, although its decrease (about 50%) is much lower than that corresponding to PFK-2 (about 99%). No Glu-1,6-P_2 phosphatase activity is detectable in rabbit erythroid cells. Attempts to assess the existence of Glu-1,6-P_2 phosphatase activity in mature human erythrocytes have also been unsuccessful, and it has been suggested that Glu-1,6-P_2 breakdown could result from the collateral activities of phosphoglucomutase [14]. This enzyme markedly decreases during differentiation.

In agreement with the results of others [12], both the 2,3-BPG synthase and glycolate 2-P-stimulated 2,3-BPG phosphatase activities have been found to increase markedly during differentiation of rabbit erythroid cells. Narita et al. [15] concluded that the enhanced enzymatic activities resulted primarily from an increase in the amount of the multifunctional enzyme 2,3-BPG synthase-phosphatase responsible for the synthesis and breakdown of 2,3-BPG in most mammalian erythrocytes [4]. As shown in table 2, the 2,3-BPG phosphatase activity not stimulated by glycolate 2-P remains essentially constant during differentiation of erythroid cells. Both the enzymes 2,3-BPG synthase-phosphatase and phosphoglycerate mutase contribute to this activity [4]. Whereas the former enzyme increases, phosphoglycerate mutase decreases during erythroid cell differentiation (table 2). In addition to the increase in 2,3-BPG synthase activity, pro-

Table 1

Levels of phosphorylated metabolites and haemoglobin in erythroid cells

	Erythroblasts	Reticulocytes	Erythrocytes
Fructose 2,6-P_2 (pmol/10^9 cells)	654 ± 97	40 ± 15	10 ± 0.8
Aldohexose 1,6-bisphosphates (nmol/10^9 cells)	18 ± 6	74 ± 3	21 ± 4
2,3-Bisphosphoglycerate (μmol/10^9 cells)	0.08 ± 0.05	0.46 ± 0.02	0.8 ± 0.2
Haemoglobin (mg/10^9 cells)	8.2 ± 3.2	12 ± 5	22 ± 10

Values represent the means ± SD of five animals. The erythrocyte mean corpuscular value was 74 ± 0.9, and the mean corpuscular haemoglobin concentration 32 ± 0.4

gressive enhancement of the ratio phosphofructokinase/pyruvate kinase activity occurred during differentiation of erythroid cells could contribute to the increase in 2,3-BPG concentration by increasing 1,3-bisphosphoglycerate levels [16].

Although nucleated erythroid cells as well as reticulocytes derive most of their energy from respiration and possess a highly active citrate cycle, they also exhibit much higher glycolytic capacity than mature erythrocytes [1]. This could be a consequence of the progressive decline of the key glycolytic enzymes produced during differentiation of the erythroid cells (see table 2 and [1]), although the present results suggest that the decrease in concentration of Fru-2,6-P_2 could also be implicated. This bisphosphorylated sugar activates erythrocyte

Table 2

Enzymatic activities in erythroid cells

	Erythroblasts	Reticulocytes	Erythrocytes
Phosphofructo 2-kinase (mU/10^9 cells)	0.24 ± 0.08	0.01 ± 0.003	0.002 ± 0.001
Glu-1,6-P_2 synthase (mU/10^9 cells)	1.9 ± 0.5	1.3 ± 0.6	1.04 ± 0.2
2,3-BPG synthase (mU/10^9 cells)	7.1 ± 3	90 ± 4	120 ± 60
2,3-BPG phosphatase (mU/10^9 cells)	2.0 ± 1	1.7 ± 0.5	1.5 ± 0.4
2,3-BPG phosphatase[a] (mU/10^9 cells)	5.8 ± 4	43 ± 20	75 ± 30
Phosphoglucomutase (U/10^9 cells)	0.9 ± 0.2	0.018 ± 0.003	0.015 ± 0.009
Phosphoglycerate mutase (U/10^9 cells)	2.9 ± 0.6	1.6 ± 0.4	0.6 ± 0.2
Hexokinase (U/10^9 cells)	2.4 ± 0.4	0.3 ± 0.06	0.02 ± 0.009
Phosphofructokinase (U/10^9 cells)	0.3 ± 0.08	0.25 ± 0.05	0.17 ± 0.06
Pyruvate kinase (U/10^9 cells)	4.3 ± 2.5	0.6 ± 0.3	0.15 ± 0.07
Lactic dehydrogenase (U/10^9 cells)	24 ± 9	4.6 ± 1.5	2.2 ± 0.3

[a] Stimulated by glycolate-2-P

Values represent the means ± SD of five animals

phosphofructokinase similarly to the enzyme from other tissues [2,13]. Glu-1,6-P_2 is also an activator of phosphofructokinase [3] and one of the main inhibitors of hexokinases [17]. Stimulation of the adenylate cyclase activity of rabbit erythroblasts by erythropoietin has been demonstrated [18], and activation and desensitization of glycolysis by stimulation of adenylate cyclase in rat reticulocytes have been recently reported [19]. The decrease in glycolytic flux during desensitization was accompanied by a decline in glucose 6-P and Glu-1,6-P_2 levels [19]. This supports the possible involvement of bisphosphorylated sugars in the control of carbohydrate metabolism in erythroid cells.

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