Vol. 125, No. 1, 1984 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 30, 1984 Pages 14-17

RELATIONSHIP BETWEEN THE RATE OF ERYTHROCYTE HEXOSE MONOPHOSPHATE PATHWAY AND THE GLUCOSE 6-PHOSPHATE CONCENTRATION

Mauro Magnani, Vilberto Stocchi, Antonio Fazi, Marina Dachà and Giorgio Fornaini

Istituto di Chimica Biologica, Università degli Studi, Urbino, Italy

Received September 27, 1984

SUMMARY: Erythrocytes of individuals with increased (+ 50%) or reduced (-35%) hexokinase activity contain respectively 70 and 17 nmole/ml RBC of glucose-6-phosphate (normal concentration 30 ± 5nmole/ml RBC) and show comparable rates of the HMP (60±5nmole/hr/ml RBC). Similarly, in RBC of different ages, obtained by density gradient ultracentrifugation, the glucose-6-phosphate concentration range from 57 (young cells) to 18 (old cells) nmole/ml RBC but the rate at which glucose is utilized in the HMP is unchanged. These data exclude a regulatory role of glucose 6-phosphate in the HMP even if its concentration is under that required for maximal G6PD activity.

The control of the HMP in RBC has been extensively investigated. It is generally agreed that G6PD catalyzes the rate limiting step in this pathway and that regulation occurs by control of G6PD activity. In fact, in resting cells the enzyme is markedly inhibited by NADPH (1-3) and both substrates, glucose 6-phosphate and NADP, are well below saturating levels explaining the very low HMP activity (4). These considerations come from studies of G6PD and have been confirmed, at least in part (5), by studies on intact erythrocytes where the NADP/NADPH ratio can be easily altered by oxidative agents (6). In contrast, no information is avaible on the consequences of possible physiological changes in the concentration of glucose-6-phosphate.

In the present study we have evaluated the role of glucose 6-phosphate as a modulator of the HMP. RBC with increased (trisomy 10p) or reduced (enzyme deficiency) hexokinase activity, and in turn glucose 6-phosphate levels, provide a useful model to investigate this problem. Furthermore

ABBREVIATIONS: RBC, Red blood cells; HMP, hexose monophosphate pathway; G6PD, glucose 6-phosphate dehydrogenase, Hb, hemoglobin.

0006-291X/84 $1.50

similar results have also been obtained on RBC of different ages prepared from normal individuals. The main conclusion that can be drawn from this study is that no modulation of HMP is observed changing the concentration of glucose 6-phosphate from 17 to 70 nmole/ml RBC and that probably only the NADP/NADPH ratio is responsible for HMP activity in the erythrocytes of normal individuals.

MATERIALS AND METHODS

Enzymes, substrates and coenzymes were obtained from Sigma Chem. Co. D-[1-^{14}C]-glucose (58 mCi/mmol) was obtained from the Radiochemical Centre, Amersham, U.K., Ficoll was from Pharmacia, Uppsala, Sweden, and Triosil from Nyegaard, Oslo, Norway. All other reagents were obtained from Merck, F.R.G.

Human blood samples were obtained in heparin from a patient with trisomy 10p whose detailed cytogenetic and biochemical data have been previously reported (7) and from two subjects, heterozygous for hexokinase deficiency, that will be described in detail elsewhere (Magnani et al. Blood, submitted for publication). As control blood was also obtained from ten normal subjects.

Enzymes and glucose 6-phosphate were determined by the Beutler methods (8) and glucose utilization in the HMP by measurement of $^{14}C\ O_2$ production using [1-^{14}C]-glucose as previously described (7).

Red blood cells were separated into fractions of different mean age by ultracentrifugation through a density gradient of Ficoll-Triosil layers as previously reported (9). After centrifugation the red cells were separated into six fractions representing erythrocytes of increasing density (age).

RESULTS

HMP in erythrocytes with different hexokinase levels

Erythrocytes of individuals carryng a trisomy 10p contain an increased amount of hexokinase being its gene locus on chromosomes 10 in the 10 p 11.2 region (10). As a consequence the glucose 6-phosphate concentration in these cells is strongly increased (Table I). By contrast, in cases of hexokinase deficiency low erythrocyte levels of glucose 6-phosphate are found. These cells provide a useful model to investigate the role of glucose 6-phosphate in the control of HMP rate. The results we have obtained are reported in table I. RBC with reduced hexokinase activity were obtained from individuals heterozygous for the defect and not from homozygous patients since these are severely anemic and with high reticulocyte counts.

Table I - HMP in human erythrocytes with different levels of glucose 6-phosphate

	Hexokinase (U/gHb)	G6PD (U/gHb)	Glucose 6-phosphate (nmole/ml RBC)	HMP (nmole/hr/ml RBC)
Trisomy 10p	1.50	9.0	70	60
Hexokinase deficiency	0.67	8.38	16.7	69
Normal controls	0.98±0.16	8.5±2.5	28±7	60±7

HMP in erythrocytes of different ages

RBC from normal subjects fractionated by ultracentrifugation into six fractions of different mean age and with different levels of glucose-6-phosphate show the same HMP rate (Table II). G6PD activity in these cells ranges from 18 U/g Hb in fraction 1 to 6 U/gHb in fraction 6.

DISCUSSION

G6PD catalyzes the rate-limiting reaction in the HMP. The Km for glucose 6-phosphate of this enzyme is in the 50-70 μM range• (4) so that physiologic concentrations of the substrate (about 30 μM) are below the level required to saturate the enzyme. Furthermore, ATP, at physiologic concentrations, is a competitive inhibitor of G6PD with

Table II - HMP in erythrocytes of different ages

Fraction n°	Glucose 6-phosphate (nmol/ml RBC)	HMP (nmol/hr/ml RBC)
1	57 ± 5	62 ± 4
2	25 ± 3	60 ± 4
3	23 ± 2	62 ± 3
4	22 ± 3	60 ± 3
5	18 ± 1	61 ± 5

Fractions from 1 to 5 represent RBC of increasing density (age) obtained by ultracentrifugation on discontinuous Ficoll-Triosil gradients. There were too few cells in fraction six (not shows) to be utilized for biochemical determinations.

respect to glucose 6-phosphate (11). On the basis of these facts we must expect an increase of G6PD activity (and consequently of the HMP) increasing the cellular concentration of glucose 6-phosphate. The data we report in this paper show that this is not the case. RBC with glucose 6-phosphate in the range 17 to 70 μM metabolize glucose through the HMP at similar rates indicating that factor(s) other than glucose 6-phosphate are important in the regulation of HMP under resting conditions.

REFERENCES

1. Soldin, S.J., and Balinsky, D. (1968) Biochemistry 7: 1077-1080.
2. Kirkman, H.M. (1962) J. Biol. Chem. 237: 2364-2370.
3. Afolayan, A., and Luzzatto, L. (1971) Biochemistry 10: 415-419.
4. Yoshida, A. and Lin, M. (1973) Blood 41: 877-891.
5. Gaetani, G.D., Parker, J.C., and Kirkman, H.M. (1974) Proc. Nat. Acad. Sci. USA 71: 3584-3587.
6. Beutler, E. (1983) The metabolic basis inherited disease, pp. 1629-1653 Mc Graw-Hill Book Company, New York.
7. Magnani, M., Stocchi, V., Piatti, E., Dachà, M., Dallapiccola, B., and Fornaini, G. (1983) Blood 61: 915-919.
8. Beutler, E. (1975) Red Cell Metabolism. A Manual of Biochemical Methods (ed. 2). Grune & Stratton, New York.
9. Magnani, M., Piatti, E., Serafini, N., Palma, F., Dachà, M., and Fornaini, G. (1983) Mech. Ageing Dev. 22: 295-308.
10. Dallapiccola, B., Novelli, G., Micara, G., Delaroche, I. Moris Petrovic, S., and Magnani, M. (1984) Hum. Hered. 34: 156-160.
11. Avigad, G., (1966) Proc. Nat. Acad. Sci. USA 56: 1543-1547.