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# DECREASE IN PHOSPHOFRUCTOKINASE ACTIVITY DURING BLOOD PRESERVATION AND THE EFFECT OF INTRACELLULAR ATP

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SUMMARY

The activities of 15 enzymes, including all the glycolytic enzymes, were observed during the preservation of human red cells at 4°C. After 8 weeks, phosphofructokinase (PFK) activity had decreased most. The decrease was prevented by the addition of adenine and inosine to the preservation medium, but not at all by inosine alone and only slightly by adenine alone. This decrease paralleled that of the decrease of intracellular ATP. PFK in the hemolysate was inactivated rapidly, but the inactivation was effectively prevented by ATP. The decrease in glycolytic activity during preservation was concluded to be a result of the loss of PFK activity, and this in turn was due to the decrease of ATP.

During the preservation of human red cells *in vitro*, their glycolytic activity decreases gradually, as does the content of ATP. The addition of both adenine and inosine was effective in maintaining the glycolytic activity and the ATP level (1-5) as well as the red cell shape (3-6), osmotic fragility (7,8) and posttransfusional viability (7,9,10). However, the molecular mechanism of the decrease in glycolytic activity is still unclear. We determined all the glycolytic enzyme activities during preservation and found that phosphofructokinase (PFK) activity was the most significantly decreased. The decrease in PFK was prevented by the addition of adenine and inosine, which produce intracellular ATP.

Human ACD blood (NIH Formula A) was supplied by the Japan Red Cross Blood Centre in Tokyo. Thirty ml of

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packed cells (Hct about 80%) was mixed with 0.5 ml of 100 mM $Na_2HPO_4$, 0.5 ml of 100 mM $MgCl_2$, 7 ml of ACD solution and 15 ml of physiological saline containing 4 mM adenine plus 40 mM inosine, 40 mM inosine alone, 4 mM adenine alone or neither compound, and the suspension was preserved at 4°C. Enzyme activities were determined according to the method of Beutler (11) as slightly modified by Kitamura (12). ATP was determined by high performance liquid chromatography on LiChrosorb $NH_2$ using $Na_2$ ATP (Kyowa Co. in Tokyo) as a standard. Hemoglobin was determined according to Van Kampen (13) and red cell fragility was assayed according to Parpart (14). As indicated in Table 1, only PFK activity was decreased very much in the control (no addition). The PFK activity in the control decreased gradually over several weeks, then fell abruptly as the pH fell.

The addition of adenine and inosine to the preservation medium was very effective in maintaining the PFK activity. Adenine alone was slightly effective, and inosine alone was almost completely ineffective. Activities of other enzymes were within the normal range, though with slight decreases of TPI, AK and GSSGR (for abbreviations, see the legend to Table 1); these changes were not prevented by the addition of adenine and inosine, inosine alone or adenine alone. This protection of PFK by the addition of adenine and inosine is primarily related to the ATP level in the cells, because adenine alone or inosine alone was not effective. Adenine and inosine together rapidly form ATP in the cell, probably by way of a positive feedback mechanism, as one of the

Table 1

Enzyme Activities in Red Cells Stored for 8 weeks

| | No additive | Plus adenine | Plus inosine | Plus adenine & inosine | Normal range IU/g hemoglobin |
|---|---|---|---|---|---|
| HK | 0.8 | 0.7 | 0,7 | 0.7 | 0.2 - 1.3 |
| GPI | 27.8 | 27.8 | 28.4 | 27.7 | 28.0 -45.0 |
| PFK | 0.3 | 1.2 | 0.5 | 3.0 | 3.0 -11.5 |
| ALD | 2.1 | 2.1 | 2.0 | 2.0 | 0.8 - 4.4 |
| TPI | 864.0 | 887.0 | 890.0 | 900.0 | 1,069-2,253 |
| GA3PD | 48.2 | 51.7 | 52.1 | 55.3 | 49.0 -79.0 |
| PGK | 53.6 | 54.0 | 57.6 | 59.9 | 52.0 -86.0 |
| PGM | 28.8 | 31.2 | 31.7 | 32.4 | 23.9 -44.0 |
| Enolase | 2.2 | 2.2 | 2.0 | 2.2 | 2.4 -14.8 |
| PK | 16.0 | 16.9 | 19.8 | 19.9 | 9.1 -23.5 |
| LDH | 105.7 | 107.6 | 108.5 | 112.8 | 102.0 -193.0 |
| AK | 131.0 | 139.0 | 135.0 | 142.0 | 158.0 -234.0 |
| G6PD | 5.4 | 6.4 | 5.8 | 7.2 | 4.6 - 9.1 |
| 6PGD | 4.8 | 4.8 | 4.9 | 5.2 | 3.9 -15.2 |
| GSSGR | 3.9 | 3.7 | 3.6 | 3.4 | 4.7 - 9.1 |

ACD blood (packed cells) was divided to 4 bottles, which contained no additive, adenine 3.5 mM, inosine 17.3 mM, and adenine plus inosine as described in the text, and the suspensions were preserved at 4°C. The enzyme activities were assayed according to Beutler (11).
Abbreviations: HK, hexokinase; GPI, glucosephosphate isomerase; PFK, 6-phosphofructokinase; ALD, fructose-bisphosphate aldolase; TPI, triosephosphate isomerase; GA3PD, glyceraldehydephosphate dehydrogenase; PGK, phosphoglycerate kinase; PGM, phosphoglyceromutase; PK, pyruvate kinase; LDH, lactate dehydrogenase; AK, adenylate kinase; G6PD, glucose 6-phosphate dehydrogenase; 6PGD, 6-phosphogluconate dehydrogenase.

authors and his collaborators reported many years ago (4). The PFK activity in the hemolysate was unstable to dilution, high ionic strength and low pH. The stability of PFK in the hemolysate at 4°C and the reactivation of PFK at 37°C were also studied. Addition of ATP, ADP, GTP or UTP protected the PFK activity, as shown in Table 2.

Table 2

Effects of Various Compounds on PFK in the Hemolysate

| Buffer (mM) / Addition | Bis-tris* 50 | $KPO_4$ 10 |
|---|---|---|
| None | 19% | 16% |
| Hypoxanthine | 18 | 5 |
| Adenine | 11 | 3 |
| Inosine | 22 | 10 |
| AMP | 13 | 13 |
| ADP | 46 | 96 |
| ATP | 91 | 100 |
| Fructose 1,6-biphosphate | 59 | 61 |
| Fructose 6-phosphate | 16 | 14 |
| Glucose 6-phosphate | 12 | 10 |
| Glucose 1-phosphate | 18 | 15 |
| 2,3-diphosphoglycerate | 15 | 34 |
| Cyclic AMP | 11 | 18 |
| UTP | 23 | 82 |
| ITP | 38 | 100 |
| GTP | 77 | 100 |
| GDP | 31 | 50 |

Hemolysate of fresh erythrocytes was diluted with 100 volumes of bis-(2 hydroxyethyl)-imino-tris-(hydroxymethyl) methane (Bis tris), pH 6.0, or potassium phosphate buffer, pH 6.0 ($KPO_4$), containing 0.1 mM or 1 mM concentration, respectively, of a test compound as indicated in the table. After incubation at 0°C for one hour, PFK activity was measured. The activity before dilution was taken as 100%.

Thus, the decrease in glycolytic activity during aging in vitro at 4°C was mainly due to the decrease of PFK induced by the decrease in the ATP level inside the cell. This is why addition of adenine and inosine together was superior to the addition of adenine or inosine alone for the protection of PFK in the cell. The low PFK level may in turn induce a further decrease of ATP level in the cell.

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