Impaired Erythrocyte Phosphoribosylpyrophosphate Formation in Hemolytic Anemia due to Pyruvate Kinase Deficiency


RBCs from patients with hemolytic anemia due to pyruvate kinase (PK) deficiency are characterized by a decreased total adenine and pyridine nucleotide content. Because phosphoribosylpyrophosphate (PRPP) is a precursor of both adenine and pyridine nucleotides, we investigated the ability of intact PK-deficient RBCs to accumulate PRPP. The rate of PRPP formation in normal RBCs (n = 11) was 2.89 ± 0.80 nmol/min · mL RBCs. In contrast, the rate of PRPP formation in PK-deficient RBCs (n = 4) was markedly impaired at 1.03 ± 0.39 nmol/min · mL RBCs. Impaired PRPP formation in these cells was not due to the higher proportion of reticulocytes. To study the mechanism of impaired PRPP formation, PK deficiency was simulated by incubating normal RBCs with fluoride. In normal RBCs, fluoride inhibited PRPP formation, caused adenosine triphosphate (ATP) depletion, prevented 2,3-diphosphoglycerate (DPG) depletion, and inhibited pentose phosphate shunt (PPS) activity. These results together with other data suggest that impaired PRPP formation is mediated by changes in ATP and DPG concentration, which lead to decreased PPS and perhaps decreased hexokinase and PRPP synthetase activities. Impaired PRPP formation may be a mechanism for the decreased adenine and pyridine nucleotide content in PK-deficient RBCs.

The concentrations of several intermediates are known to be abnormal in RBCs from patients with nonspherocytic hemolytic anemia due to pyruvate kinase (PK) deficiency. RBCs from PK-deficient patients have increased 2,3-diphosphoglycerate (DPG) and often have decreased adenosine triphosphate (ATP) concentrations. PK-deficient RBCs are also characterized by decreased total adenine nucleotide (adenosine monophosphate, adenosine diphosphate, and ATP) and total nicotinamide adenine dinucleotide (NAD* and NADH) concentrations. Because these decreases in total nucleotide content are not caused by the PK deficiency directly and because they probably play a role in mediating hemolysis, we have recently studied the mechanism responsible for these decreases. Since 5-phosphoribosyl-1-pyrophosphate (PRPP) is a common precursor of both adenine and pyridine nucleotides, previous studies from this laboratory have included the examination of PRPP synthetase, the enzyme responsible for PRPP formation. We have shown that PK-deficient RBCs have decreased PRPP synthetase subunit aggregation and therefore decreased PRPP synthetase activity in vivo. We have suggested that this plays a role in mediating the decreases in total adenine nucleotide and total NAD content. Furthermore, we found that NAD synthesis was impaired in intact PK-deficient RBCs and that this was the primary mechanism for the decrease in total NAD content in these cells. The decrease in the rate of NAD synthesis in PK-deficient RBCs was a result of the impaired ability of these cells to regenerate ATP due to the enzyme deficiency.

We have previously suggested that decreased PRPP synthetase subunit aggregation should result in impaired PRPP formation in intact PK-deficient RBCs in vivo. However, because the rate of PRPP formation in intact cells is also dependent on factors other than PRPP synthetase subunit aggregation (eg, the availability of ATP or ribose-5-phosphate [R5P], the substrates of PRPP synthetase) and because PRPP formation can be readily demonstrated in intact RBCs in the presence of glucose and inorganic phosphate, the current studies were undertaken to determine whether the rate of PRPP formation is decreased in intact PK-deficient RBCs. The data presented in this report indicate that PRPP formation is impaired markedly in RBCs from all of the PK-deficient patients studied. Impaired PRPP formation appears to be caused by changes in ATP and DPG content, which lead to decreases in pentose phosphate shunt (PPS) and perhaps hexokinase and PRPP synthetase activities.

MATERIALS AND METHODS

Materials

Sephadex G-200 was purchased from Pharmacia, Inc, Piscataway, NJ. All other reagents were purchased from Sigma Chemical Co, St Louis. The potassium salt of fluoride was used throughout this study.

Methods

Isolation of erythrocytes and preparation of hemolysates. With informed consent, blood was obtained from PK-deficient patients by routine venipuncture and collected in heparin-coated tubes (15 U heparin/mL whole blood). Blood samples from normal subjects and patients with pyrimidine-5'-nucleotidase (PSN) deficiency or hemolytic anemia of unknown etiology were used as controls where appropriate. A red cell–enriched fraction was prepared by passing whole blood through a column of α-cellulose and microcrystalline cellulose to deplete white cells and platelets as described by Beutler. The washing of RBCs and the preparation of hemolysates have been described previously.

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**PRPP formation in intact erythrocytes.** PRPP formation was measured by using a slight modification of the method of Hershko et al.9 Because PRPP formation decreased upon storage, washed RBCs were used immediately after isolation. The incubation to measure PRPP formation was begun within 60 to 90 minutes of phlebotomy. The incubation mixture contained 60 μmol potassium phosphate, pH 7.4, 300 μmol NaCl, 20 μmol b-glucose, and intact RBCs (final hematocrit of 35% to 40%) in a total volume of 2.00 mL. The mixture was maintained at 37°C. At the indicated times, 0.25-mL aliquots were removed and immediately mixed with 5.0 mL ice-cold 0.15 mol/L NaCl. RBCs were collected by centrifuging at 1,000 g for one minute. The supernatant was discarded, and 1.25 mL 1.0 mmol/L EDTA (pH 7) was added to the RBC pellet. The resulting hemolysate was mixed and immediately placed in a boiling water bath for two minutes. After chilling to 0°C, 0.15 mL 10% (wt/vol) activated charcoal was added to the boiled extract. Coagulated protein and charcoal were removed by centrifugation (1,000 g for one minute). An aliquot of this supernatant was immediately assayed for PRPP by using a modification of the spectrophotometric method of Kornberg et al.10 The assay mixture contained 20 μmol Tris-HCl, pH 8.0, 2.0 μmol MgCl2, 0.20 μmol orotic acid, 0.92 mL of the aforementioned supernatant, 0.40 unit (1 unit = 1 μmol/h) of orotidine-5'-monophosphate pyrophosphorylase (OMPase), and 0.40 unit of orotidine-5'-monophosphate dehydrogenase (ODCase) in a total volume of 1.00 mL. The assay mixture was preincubated for five minutes at 37°C, and the reaction was started by adding a mixture of OMPase and ODCase. Absorbance was monitored at 295 nm until no further decreases were apparent. PRPP concentration was calculated by using a millimolar extinction coefficient of 3.95 for orotic acid.11 The rate of PRPP formation was determined from a plot of PRPP concentration vs. time with at least five data points and linear regression analysis.

PRPP was also quantitated by using a modification of the radioactive method of Hershko et al.8,10,12 Partially purified human RBC hypoxanthine-guanine phosphoribosyl transferase (HGPRT) was used instead of the charcoal-treated hemolysate previously used as a source of HGPRT.12 HGPRT purification was performed by using the heating method of Tax and Veerkamp13 followed by precipitation using 60% saturated ammonium sulfate. The resulting HGPRT preparation was purified 30-fold with respect to crude hemolysate. Results of PRPP quantitation with this assay were virtually identical to those obtained with the spectrophotometric assay described earlier.

**PRPP synthetase subunit aggregation.** Freshly isolated RBCs were incubated at 37°C for 1.5 hours in the PRPP formation mixture described earlier. RBCs were quickly washed with excess ice-cold 0.15 mmol/L NaCl and collected by centrifugation (1,000 g for two minutes). Two volumes of water were then added to the packed RBCs, and the mixture was frozen in an acetone-dry ice bath and thawed to ensure complete hemolysis. Hemolysate was centrifuged at 40,000 g for ten minutes to remove stroma, and the proportion of aggregated PRPP synthetase was determined by gel permeation chromatography as described previously.14 Briefly, 0.60 mL of the stroma-free hemolysate was applied to a Sephadex G-200 column (1.6 × 34 cm) that had been equilibrated with a buffer containing 0.18 mol/L KCl, 1.0 mmol/L 2-mercaptoethanol, 20 mmol/L potassium phosphate (pH 7.4), 1.5% (wt/vol) glycerol, and 2.0 mmol/L MgCl2. The column was developed by using the same buffer.

**PPS activity in intact erythrocytes.** PPS activity was determined by using a modification of the method of Davidson and Tanaka.15,16 Incubation mixtures were identical to those used for PRPP formation (see method above) except that they were supplemented with 2.5 μCi [1-14C]glucose (final specific radioactivity, 0.125 μCi/μmol). The assay was started by adding packed RBCs, and 14CO2 generation was continuously monitored by using a vibrating reed electrometer and ionization chamber in a closed system as described by Lachant et al.17,18 Other methods. Hexokinase, glucose-6-phosphate dehydrogenase (G6PD), 6-phosphofructokinase (PFK), and 6-phosphogluconate dehydrogenase (6PGD), ATP, and DPG were assayed spectrophotometrically as described by Beutler.19 PK was assayed as described by Tanaka,20 and PRPP synthetase was assayed as described by Valentine and Kurschner.21

**RESULTS**

We measured the rate of PRPP formation in intact RBCs from normal and PK-deficient individuals. Although previous investigators used a radioactive assay to quantitate PRPP formation in intact RBCs,8,9 we noted that the quantity of PRPP formed by intact RBCs incubated with glucose and inorganic phosphate was high enough to allow the use of a faster and more convenient spectrophotometric assay. Results obtained with this spectrophotometric assay were identical to those obtained with the radioactive assay (data not shown). Normal RBCs were found to accumulate PRPP in the presence of glucose and inorganic phosphate in a time-dependent and linear manner (Fig 1). No PRPP accumulation when either glucose or phosphate was omitted from the incubation mixture (Fig 1). In contrast, the rate of PRPP accumulation was severalfold higher in the presence of new methylene blue (NMB) (Fig 1), which stimulates the PPS pathway and increases the availability of R5P, the immediate precursor of PRPP.4 These results were similar to those of Hershko et al.8 and suggest that the spectrophotometric assay used in this report is functioning properly.

When using this assay, we found that RBCs from 11 normal subjects accumulated PRPP at a rate of 1.85 to 4.10 nmol/min · mL RBC (Table 1). In contrast, RBCs from...
four PK-deficient patients accumulated PRPP at a rate of 0.46 to 1.31 nmol/min · mL RBC (Table 1). The degree of impairment of PRPP formation in PK-deficient RBCs did not appear to correlate with the severity of the PK deficiency. P5N-deficient RBCs which have decreased PRPP synthetase activity, had impaired PRPP formation (Table 1). In contrast, RBCs from three patients with hemolytic anemia of unknown etiology, which had a higher proportion of reticulocytes and a higher activity of the cell age-dependent enzymes hexokinase and PK, had elevated rates of PRPP formation in PK-deficient RBCs.

To determine the mechanism responsible for impaired PRPP formation in PK-deficient RBCs, we investigated the effects of fluoride, an inhibitor of enolase (and therefore glycolysis), on PRPP formation in intact normal RBCs. We found that fluoride is a potent inhibitor of PRPP formation in normal RBCs, with 0.30 mmol/L fluoride causing 50% inhibition of PRPP accumulation and 0.50 mmol/L fluoride causing nearly complete inhibition of PRPP accumulation (Fig 2). Fluoride, even at concentrations as high as 5.00 mmol/L, has been shown previously not to affect PRPP synthetase activity.”}

### Table 1. Rate of PRPP Formation, Enzyme Activities, and Intermediate Concentrations in Erythrocytes From Normal Subjects and in Patients With PK Deficiency, P5N Deficiency, and Hemolytic Anemia of Unknown Etiology

<table>
<thead>
<tr>
<th>Subject</th>
<th>Reticulocytes (%)</th>
<th>Hexokinase (IU/mL RBC)</th>
<th>PK (IU/mL RBC)</th>
<th>PRPP Synthetase (µmol/h · mL RBC)</th>
<th>ATP (µmol/mL RBC)</th>
<th>DPG (µmol/mL RBC)</th>
<th>Rate of PRPP Formation (nmol/min · mL RBC)</th>
</tr>
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<tbody>
<tr>
<td>Normal (n = 12)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Mean ± SD</td>
<td>1.2 ± 0.6</td>
<td>0.41 ± 0.07</td>
<td>5.27 ± 1.02</td>
<td>48.2 ± 8.8</td>
<td>1.24 ± 0.16</td>
<td>4.51 ± 0.64</td>
<td>2.89 ± 0.80</td>
</tr>
<tr>
<td>Range</td>
<td>0.5-2.0</td>
<td>0.31-0.55</td>
<td>3.70-6.97</td>
<td>35.7-62.1</td>
<td>0.96-1.48</td>
<td>3.66-5.25</td>
<td>1.85-4.10</td>
</tr>
<tr>
<td>Patient 1</td>
<td>2.7</td>
<td>0.69</td>
<td>—</td>
<td>61.9</td>
<td>1.48</td>
<td>5.39</td>
<td>3.54</td>
</tr>
<tr>
<td>Patient 2</td>
<td>17</td>
<td>1.44</td>
<td>9.29</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>4.83</td>
</tr>
<tr>
<td>Patient 3</td>
<td>45</td>
<td>1.46</td>
<td>12.4</td>
<td>82.5</td>
<td>1.90</td>
<td>5.98</td>
<td>6.00</td>
</tr>
<tr>
<td>Patient 1</td>
<td>4.2</td>
<td>0.62</td>
<td>1.08</td>
<td>40.7</td>
<td>0.88</td>
<td>8.63</td>
<td>1.31</td>
</tr>
<tr>
<td>Patient 2</td>
<td>29</td>
<td>0.86</td>
<td>0.57</td>
<td>82.2</td>
<td>1.34</td>
<td>6.05</td>
<td>1.28</td>
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<tr>
<td>Patient 3</td>
<td>24</td>
<td>0.93</td>
<td>2.61</td>
<td>76.9</td>
<td>1.71</td>
<td>7.63</td>
<td>1.08</td>
</tr>
<tr>
<td>Patient 4</td>
<td>3.9</td>
<td>0.63</td>
<td>0.59</td>
<td>31.4</td>
<td>0.71</td>
<td>7.63</td>
<td>0.46</td>
</tr>
<tr>
<td>Patient 1</td>
<td>16</td>
<td>0.91</td>
<td>7.74</td>
<td>23.5</td>
<td>1.48</td>
<td>4.36</td>
<td>0.61</td>
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For ATP and DPG determinations, perchloric acid extracts of whole blood were prepared immediately after phlebotomy. From another aliquot of whole blood, erythrocytes were isolated and used immediately to measure the rate of PRPP formation as described (see Materials and Methods). The activities of hexokinase, PK, and PRPP synthetase were determined on the same day that the blood sample was obtained.

Abbreviation: HA, hemolytic anemia of unknown etiology.

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**Table 1.** Rate of PRPP Formation, Enzyme Activities, and Intermediate Concentrations in Erythrocytes From Normal Subjects and in Patients With PK Deficiency, P5N Deficiency, and Hemolytic Anemia of Unknown Etiology

**Fig 2.** The effect of fluoride on PRPP formation in intact normal erythrocytes. Freshly obtained RBCs were incubated as described in Fig 1 except that the indicated reaction mixtures were supplemented with fluoride. The data shown represent the mean of two determinations. Symbols used: ●, without fluoride; ○, with 0.30 mmol/L fluoride; ▲, with 0.50 mmol/L fluoride.
to measure PRPP formation (Fig 4A). Because the proportion of aggregated PRPP synthetase in freshly obtained RBCs is approximately 38%, it is noteworthy that incubating RBCs in the PRPP formation mixture caused a marked (66%) decrease in aggregation. After 1.5 hours of incubation in the presence of 0.50 mmol/L fluoride, the proportion of aggregated PRPP synthetase remained essentially unchanged at 14% (Fig 4B). There were no significant changes in PRPP synthetase subunit aggregation even when normal RBCs were incubated with 2.0 mmol/L fluoride for two hours (data not shown).

The impairment of PRPP formation in normal RBCs in the presence of fluoride may be due to decreased generation of R5P as a result of inhibition of the activities of hexokinase, G6PD, and 6PGD by DPG. We tested this hypothesis by determining the effect of fluoride on PPS activity in intact normal RBCs by measuring 14CO2 generation from [1-14C]glucose. The rate of 14CO2 generation increased steadily in normal unsupplemented RBCs (Fig 5). However, when 0.50 mmol/L fluoride was added to the incubation mixture (as indicated by the arrow in Fig 5), there was a significant and reproducible suppression of 14CO2 generation. It is noteworthy that, although 0.50 mmol/L fluoride caused almost complete impairment of PRPP formation, only partial inhibition of 14CO2 generation was apparent at this fluoride concentration. This suggests that inhibition of R5P generation is only a partial cause of impaired PRPP formation and that other factors are also responsible.

To determine the contribution of the PPS pathway in causing impaired PRPP formation, we examined the effect of NMB on PRPP formation in PK-deficient RBCs. NMB caused a 1.5- to twofold stimulation of PRPP formation in PK-deficient RBC. However, the rate of PRPP formation in PK-deficient RBCs in the presence of NMB was considerably lower than the corresponding rate for NMB-incubated normal RBCs (Fig 1). Given that PPS activity in NMB-
stimulated RBCs is approximately the same in normal and PK-deficient RBCs, this suggests that other factors in addition to decreased PPS activity are also responsible for impaired PRPP formation in PK-deficient RBC.

**DISCUSSION**

There are several possible mechanisms that could account for impaired PRPP formation in PK-deficient RBCs. These mechanisms are (a) decreased availability of ATP, a substrate of PRPP synthetase, through impaired glycolysis; (b) decreased availability of R5P, a cosubstrate of PRPP synthetase, via impaired hexokinase and/or impaired PPS activity; and (c) decreased PRPP synthetase activity in vivo through either kinetic inhibition by the higher concentration of DPG, decreased subunit aggregation due to the higher DPG and lower ATP concentrations, or other causes including a decrease in the quantity of PRPP synthetase as manifested by a decrease in measured enzyme activity in hemolysate.

To determine whether ATP availability can be a cause of impaired PRPP formation in PK-deficient RBCs we considered the kinetic properties of PRPP synthetase. The $K_a$ for ATP of PRPP synthetase is 14 $\mu$mol/L. Because this is approximately 1% of the ATP concentration in normal or PK-deficient RBCs, decreased ATP formation in PK-deficient RBCs cannot be a direct cause of impaired PRPP formation in these cells. Similarly, the R5P kinetic properties of PRPP synthetase were considered to determine whether R5P availability could be a cause of impaired PRPP formation. The $K_a$ for R5P of PRPP synthetase is 33 $\mu$mol/L and the physiological concentration of R5P is approximately 6 $\mu$mol/L. This indicates that R5P availability can be a cause of impaired PRPP formation.

To test the possibility that decreased PRPP synthetase activity can cause impaired PRPP formation, we measured the rate of PRPP formation in PSN-deficient RBCs that have previously been shown to have decreased PRPP synthetase activity as an epiphenomenon. The rate of PRPP formation in PSN-deficient RBCs that had half the PRPP synthetase activity of normal RBCs was markedly decreased even though ATP and DPG levels in these cells were normal (Table 1). In addition, although we previously found that PPS activity in PSN-deficient RBCs is decreased compared with high reticulocyte controls, PPS activity is slightly increased compared with normal RBCs. This indicates that PSN-deficient RBCs have an R5P generation rate that is comparable to normal RBCs. These data strongly suggest that decreased PRPP formation in PSN-deficient RBC is caused by their decreased PRPP synthetase activity. Thus, PRPP synthetase activity appears to be a limiting factor in the rate of PRPP formation. The limiting nature of PRPP synthetase activity is also supported by the observation that PK-deficient patient 4 RBCs, which have decreased PRPP synthetase activity, have less than half the rate of PRPP formation as the other PK-deficient RBCs (Table 1). These data suggest that a decreased activity of PRPP synthetase per se can cause impaired PRPP formation.

To determine which of the remaining possible causes may also be responsible for impaired PRPP formation in PK-deficient RBC, we studied the effect of fluoride on PRPP formation in normal RBCs. Fluoride has previously been used to inhibit glycolysis and simulate some of the characteristics of PK deficiency. Relative low concentrations (0.30 to 0.50 mmol/L) of fluoride inhibited PRPP formation, whereas relatively high concentrations (10 mmol/L) of fluoride did not inhibit hexokinase, G6PD, 6PGD, or PRPP synthetase activity. This suggests that decreased PRPP formation in normal RBCs in the presence of fluoride is not due to direct inhibition of the latter enzymes by fluoride. The same concentrations of fluoride that decreased the rate of PRPP formation were found to cause ATP depletion and prevent DPG depletion during incubation (Fig 3). These results suggest that impairment of PRPP formation in normal RBCs by fluoride is mediated indirectly through changes in ATP and DPG concentrations.

Previous studies have demonstrated that DPG is an inhibitor of human RBC PRPP synthetase. Thus, a possible contributing factor to impairment of PRPP formation is the kinetic inhibition of PRPP synthetase activity by DPG. Because the DPG concentration is increased in PK-deficient RBCs and because fluoride prevents the depletion of DPG in normal RBCs under our incubation conditions, a functional decrease in PRPP synthetase activity in vivo, due to increased DPG concentration, is a possible contributing factor to impaired PRPP formation. Our observation that PRPP synthetase activity can be a rate-limiting factor of PRPP formation and the failure of NMB to stimulate PRPP formation in PK-deficient RBCs to the same extent as normal RBCs support this hypothesis.

Because in vitro PRPP synthetase activity is determined in diluted hemolysate, inhibitors of PRPP synthetase such as DPG are diluted out and may become ineffective. Consequently, a normal or increased in vitro PRPP synthetase activity does not necessarily indicate a normal or increased in vivo activity. This may explain why the rate of RBC PRPP formation in PK-deficient patients 1, 2, and 3 is impaired despite normal or, in the case of patients 2 and 3, elevated in vitro PRPP synthetase activity.

Because both ATP and DPG affect the activity and the state of PRPP synthetase subunit aggregation, we investigated the effect of fluoride on PRPP synthetase subunit aggregation. We found that fluoride did not affect the proportion of aggregated PRPP synthetase recovered from normal RBCs. However, the incubation of RBCs in the PRPP formation mixture without fluoride caused a marked decrease in the recovered proportion of aggregated PRPP synthetase (13%) (Fig 4A) relative to freshly obtained RBCs (38%). As a result, it may not have been possible to detect further decreases in aggregation. Thus, the role that decreased PRPP synthetase subunit aggregation plays in mediating impaired PRPP formation in PK-deficient RBCs remains unclear.

The conclusion that decreased R5P availability can be a rate-limiting factor in PRPP formation prompted the investigation of PPS pathway activity. R5P is synthesized from glucose-6-phosphate (G6P) via the reactions catalyzed by G6PD, G6P lactonase, 6PGD, and ribosephosphate isomerase (henceforth referred to as the G6P-PPS pathway). Our results indicate that fluoride inhibits G6P-PPS activity in intact RBCs under the same conditions used to measure the
rate of PRPP formation (Fig 5). This suggests that impaired G6P-PPS activity is a contributing factor to impaired PRPP formation in normal fluoride-incubated RBCs. In addition, because previous studies in our laboratory have shown that there is a relative impairment of G6P-PPS activity in PK-deficient RBCs, a3 impaired G6P-PPS activity appears to be a contributing factor to impaired PRPP formation in PK-deficient RBCs.

Because hexokinase is inhibited by DPG22,23 impaired G6P-PPS activity in both PK-deficient and normal fluoride-incubated RBCs could be due to inhibition of hexokinase by DPG. In addition, the K_m for MgATP of hexokinase has been shown to be 0.59 mmol/L, a value that is approximately half the physiological concentration of ATP in RBCs. Hence, impaired G6P-PPS activity in PK-deficient and fluoride-incubated normal RBCs could also be caused by decreased hexokinase activity in vivo as a result of the decrease in ATP concentration. Since both the increase in DPG content and the decrease in ATP content cause a decrease in availability of R5P through the G6P-PPS pathway, our data suggest that impaired PPS activity is mediated by decreased hexokinase activity in vivo as a result of changes in ATP and DPG content.

The partial inhibition of G6P-PPS activity at a fluoride concentration that caused nearly complete inhibition of PRPP formation suggests that inhibition of R5P generation from G6P is only a partial mechanism for impaired PRPP formation and that other factors including the kinetic inhibition of PRPP synthetase by DPG are also responsible. Because the reactions catalyzed by transketolase, transaldolase, ribulosephosphate epimerase, and ribosephosphate isomerase are reversible, R5P can also be synthesized from fructose-6-phosphate (F6P) and glyceraldehyde-3-phosphate (G3P) (henceforth referred to as the F6P/G3P-PPS pathway). Dische and Igalis have shown that DPG is a potent inhibitor of both transaldolase and transketolase in RBCs.36 Thus, inhibition of these enzymes by DPG can decrease R5P generation from F6P and G3P and may also contribute to causing impaired PRPP formation. Our results are consistent with the hypothesis that impaired PRPP formation is caused by (a) inhibition of PRPP synthetase by DPG and (b) a decrease in the rate of R5P generation due to one or more of the following: (a) decreased hexokinase activity, (b) decreased G6P-PPS activity, and (c) perhaps decreased F6P/G3P-PPS activity. Furthermore, our data suggest that impaired PRPP formation in PK-deficient RBCs is a mechanism for their decreased concentrations of total adenine and pyridine nucleotides. Because PRPP is a precursor of other nucleotides, it is possible that PK-deficient RBCs have decreased concentrations of other nucleotides that may play an important role in hemolysis. This possibility is currently under investigation in our laboratory.

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REFERENCES

Impaired erythrocyte phosphoribosylpyrophosphate formation in hemolytic anemia due to pyruvate kinase deficiency

CR Zerez, MD Wong, NA Lachant and KR Tanaka