. Induction of millimolar amounts of 5-phosphoribosyl-1-pyrophosphate in human erythrocytes by incubation in inosine-pyruvate-phosphate medium. A 31P-NMR study.

Quistorff, Bjørn; Petersen, Aqqaluk

Published in:
Biomed. Biochim. Acta

Publication date:
1990

Document version
Publisher's PDF, also known as Version of record

Biomed. Biochim. Acta 49 (1990) 2/3, S 111 - S 116

Induction of millimolar amounts of 5-phosphoribosyl-1-pyrophosphate in human erythrocytes by incubation in inosine-pyruvate-phosphate medium. A ^{31}P-NMR study.

Aqqaluk Petersen and [1]Bjørn Quistorff.

Dept. of Clinical Chemistry, Rigshospitalet and [1]The NMR-Center, The Panum Institute, University of Copenhagen, Denmark.

Summary.

Incubation of human erythrocytes in inosine (10 mM), pyruvate (10 mM), phosphate (50 mM) and NaCl (75 mM) (IPP-medium)[1] at pH 6.6 leads to a ca. 1000 fold increase in the concentration of 5-phosphoribosyl-1-pyrophosphate (PRPP) as identified and measured by ^{31}P-NMR spectroscopy. PRPP accumulation is pH-dependent with a maximum at extracellular pH 6.60 and the maximum value of 1.3-1.6 mmol/l RBC is reached within one hour at 37 °C. PRPP accumulated despite high concentrations of 2,3-diphosphoglycerate (2,3-DPG), an inhibitor of PRPP-synthetase. The concentration of PRPP attained correlated with the intracellular concentration of inorganic phosphate (P_i). Substitution of either adenosine or adenosine plus inosine for inosine in the medium (APP or A+IPP media) did not lead to NMR detectable accumulation of PRPP, despite the same high concentrations of intracellular P_i. The lack of accumulation of PRPP in adenosine containing media may be related to the higher concentrations of nucleoside diphosphates induced in these media. These results show that neither 2,3-DPG nor PRPP itself inhibit the synthesis of PRPP in the human erythrocyte under these circumstances.

Introduction.

5-phosphoribosyl-1-pyrophosphate (PRPP) is a central metabolite in the salvage pathway of purines (1). It has earlier been shown that the synthesis of PRPP in human erythrocytes can be stimulated by inorganic phosphate (P_i) (2). Here we present the novel

---

[1]Abbreviations used: APP-medium, adenosine-pyruvate-phosphate; 2,3-DPG, 2,3-diphosphoglycerate; IPP-medium, inosine-pyruvate-phosphate; PRPP, 5-phosphoribosyl-1-pyrophosphate.

observation, that fresh human erythrocytes accumulate millimolar levels of PRPP by incubating the cells in inosine (10 mM), pyruvate (10 mM) and P_i (50 mM). Furthermore we show that adenosine in the medium prevents the accumulation of PRPP.

Materials and methods.

Erythrocytes were isolated from human venous blood and washed 3 times in an Hepes (25 mM) buffered saline solution at pH=7.40 (3). After the third wash the erythrocytes were resuspended in a medium containing 75 mM NaCl, 10 mM pyruvate, 10 mM inosine and 50 mM P_i (4). pH varied within the range 6.2-7.6 and the kinetic experiments were performed at optimum pH=6.60. In some experiments either adenosine (10 mM) or adenosine (10 mM) and inosine (10 mM) were substituted for inosine (10 mM). The erythrocytes were incubated in a shaking water bath at 37 °C at a hematocrit of 5-10%. After incubation the erythrocytes were washed in the Hepes-buffered saline solution before the ^{31}P-NMR measurements in a Bruker AM 250 instrument at 101.3 MHz. 125 mM methylenediphosphonic acid (MDP) at pH=9.00 in 50 mM Tris-HCl buffer served as external reference (16.90 ppm relative to 85% H_3PO_4).

Results.

Incubation of human erythrocytes in inosine-pyruvate-phosphate-medium leads to a pH-dependent accumulation of PRPP, Fig. 1B, while Fig. 1A is a control spectrum of erythrocytes suspended in normal saline solution. That the peak at -11 ppm together with the peaks at -5.4 ppm and at 4.3 ppm was due to PRPP was verified by pH-titrations on water hemolyzed extracts both before and after addition of genuine PRPP, Fig 2A+C. The assignment of the α-phosphourus peak of PRPP was verified by the doublet split pattern of the peak at -11 ppm in proton coupled spectra, Fig. 2B. With a constant incubation time of 1 h the extracellular pH maximum found was 6.60 corresponding to an intracellular pH of 6.50, Fig. 3A. At pH=6.60 PRPP accumulated quickly in the erythrocyte and reached a maximum level of 1.65 mmol/l erythrocytes within 1 h, 500-1000 times its normal value, decreasing slowly during the following hours, Fig. 3B. Varying pH between 6.2-7.6, the accumulation of PRPP correlated with the intracellular levels of the activator of PRPP synthetase, P_i, Fig. 3A+3B.

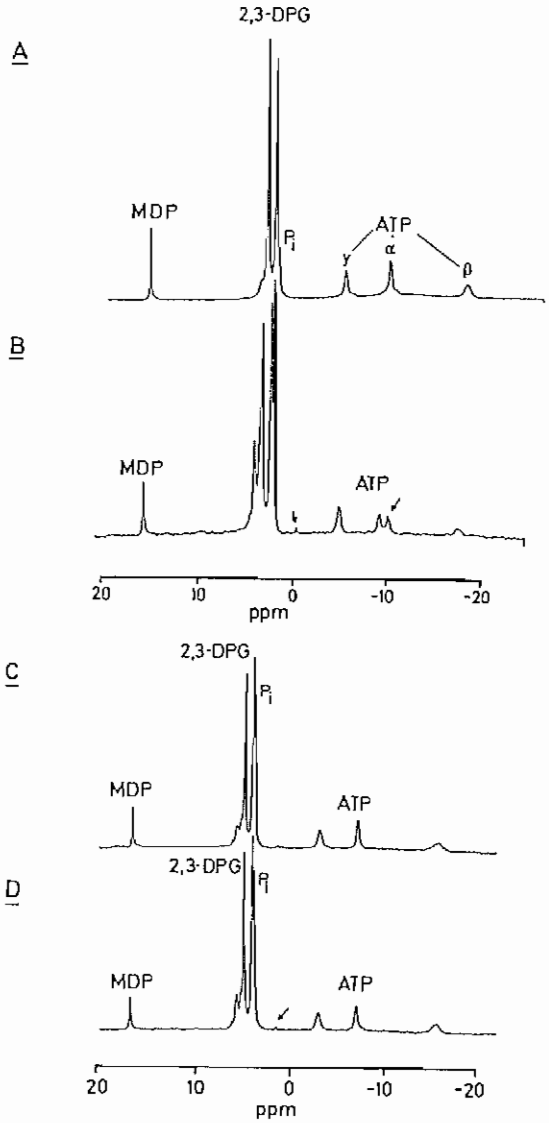

Fig. 1.

^{31}P-NMR spectra at 101.3 MHz of human erythrocytes suspended in normal saline (A), preincubated in inosine-pyruvate-phosphate medium at pH=6.6 (B), preincubated in adenosine-pyruvate-phosphate medium (C), and preincubated in the combined adenosine-inosine-pyruvate-phosphate medium (D).

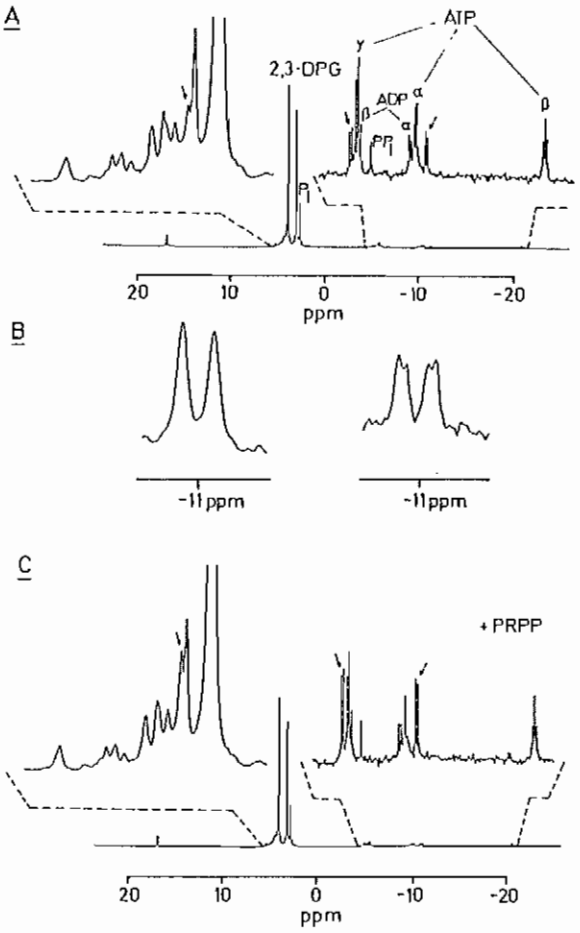

Fig. 2.

^{31}P-NMR spectra of extracts of erythrocytes preincubated in inosine-pyruvate-phosphate medium without (A) and with added PRPP (C), while (B) shows the doublet at -11 ppm in a proton-coupled spectrum.

Fig 3A.

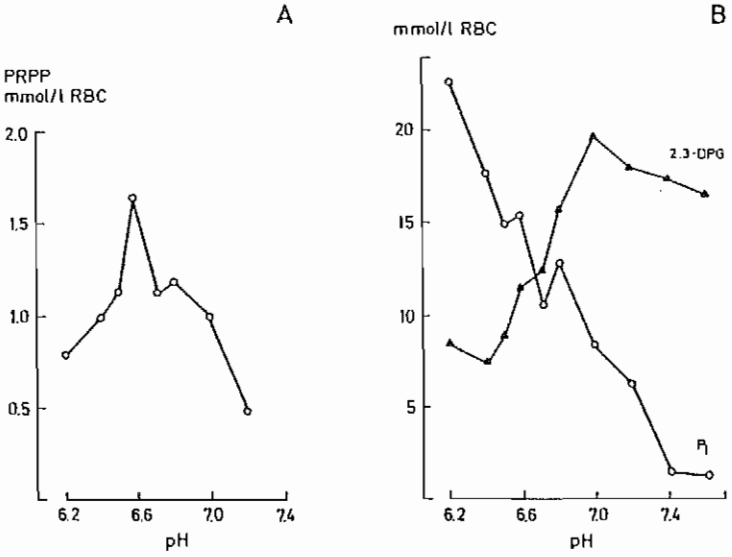

Fig 3B.

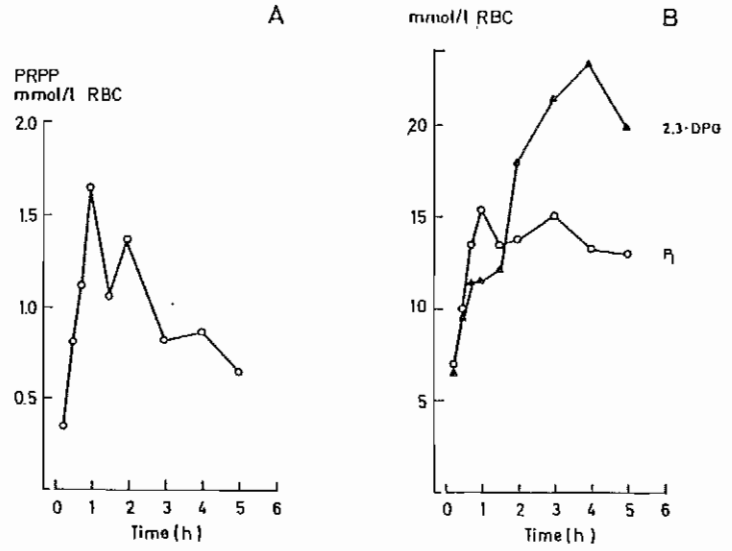

Fig. 3A.

The concentration of PRPP (left panel) and 2,3-DPG and P_i (right panel) in human erythrocytes as a function of extracellular pH in the inosine-pyruvate-phosphate medium.

Fig. 3B.

The time dependence of the concentration of PRPP (left panel) and 2,3-DPG and P_i (right panel) in human erythrocytes incubated in inosine-pyruvate-phosphate medium at pH=6.6.

Despite high levels (10 mmol/l RBC) of the physiological inhibitor of PRPP-synthetase, 2,3-diphosphoglycerate (2,3-DPG), PRPP still accumulated in the erythrocytes, Fig. 3A+3B. PRPP did not accumulate when adenosine or adenosine plus inosine were substituted for inosine, Fig. 1C+1D. With both adenosine and inosine in the medium the synthesized nucleoside triphosphate was shown to be ATP and not ITP. ADP concentration was 0.39±0.10 (SE) mmol/l, n=9 in erythrocytes preincubated in the IPP-medium while the adenosine containing media induced an ADP concentration of 0.79±0.09 (SE) mmol/l, n=5. Another finding in this study was the time-and pH dependent accumulation of phosphoenolpyruvate (PEP), Fig. 1B+1D in both adenosine and inosine containing media.

Discussion.

Here we show by ^{31}P-NMR spectroscopy that erythrocytes incubated at low pH in IPP-medium (4) accumulate high levels of PRPP and that the accumulation is correlated to the level of intracellular P_i, an activator of PRPP synthetase, and despite high levels of the physiological inhibitor of PRPP synthetase, 2,3-DPG (1). Thus the highest concentration of PRPP attained was 1.65 mmol/l RBC after 1 h of incubation of erythrocytes in IPP medium at pH=6.60. Despite several attempts, substitution of inosine for either adenosine or adenosine plus inosine in the medium did not lead to ^{31}P-NMR detectable accumulation of PRPP. The human erythrocyte lacks the capacity of phosphorylytic cleavage of adenosine, which is instead either deaminated to inosine or phosphorylated directly to AMP. Our results indicate that adenosine inhibits the inosine induced accumulation of PRPP in the human erythrocyte, despite the same high levels of the PRPP synthetase activator P_i as in the IPP medium. The effect of adenosine may be related to the higher concentrations of ADP induced in adenosine containing media. Furthermore the inhibition of PRPP synthetase by 2,3-DPG seems to be negligible.

References:

1) BECKER, M.A., K.O. RAIVO and J.E. SEEGMILLER: Adv. Enzymol 29, 281-306 (1979).
2) HERSHKO, A., A. RAZIN and J. MAGER: Biochim. Biophys. Acta 184, 64-76 (1969).
3) PETERSEN, A., J.P. JACOBSEN and M. HØRDER: Magn. Res. Med. 4, 341-350 (1987).
4) DEUTICKE, B., J. DUHM and R. DIERKESMANN: Pflügers Arch. 326, 15-34 (1971).