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ON THE MOLECULAR BASIS OF PYRUVATE KINASE DEFICIENCY

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Summary

Oxidized glutathione was found to be in the normal range in pyruvate kinase (ATP· pyruvate 2-*O*-phosphotransferase, EC 2.7.1.40) deficient erythrocytes and in erythrocytes from obligate heterozygotes with this inborn error of metabolism. Physiological concentrations of oxidized glutathione failed to affect the kinetics and stability of pyruvate kinase From the data reported in this paper it seems to be unlikely that pyruvate kinase deficiency is the consequence of an increased oxidized glutathione concentration in the red blood cell

In the twelve years following the first description [1] of hemolytic anemia due to inherited deficiency of red blood cell pyruvate kinase (ATP pyruvate 2 *O*-phosphotransferase, EC 2 7 1.40) about 200 additional cases with this inborn error of metabolism have been observed. The molecular heterogeneity of the deficient enzyme protein has been demonstrated by electrophoretic, kinetic, and immunological methods [2—5] There is also a considerable variability in the expression of the clinical disorder and no correlation between residual enzyme activity and severity of hemolytic disease has been found

In two recent papers [6,7] it was suggested, on the basis of in vitro experiments, that pyruvate kinase deficiency is a secondary defect which could be the consequence of an increased oxidized glutathione concentration in the red blood cell and that the change in the -SH groups of the enzyme might also be responsible for the increased rate of hemolysis. However, the concentration of

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oxidized glutathione was not determined in normal erythrocytes and in the patients' erythrocytes. Moreover, the concentration of oxidized glutathione used in the in vitro experiments (2.5 mM) [6] was higher by about three orders of magnitude than has been observed earlier in vivo by using a reliable method for determination (3 6 ± 1 4 μmol/l erythrocytes) [8]

Conflicting results concerning the ratio of reduced oxidized glutathione in pyruvate kinase-deficient red blood cells have been reported [9] oxidized glutathione was markedly increased in one patient but was found to be normal in two other patients However, the method which had been used for the determination of oxidized glutathione [10] did not allow the accurate measurement of the oxidized form of this tripeptide.

To elucidate the relation between pyruvate kinase deficiency and oxidized glutathione we performed biochemical studies in six pyruvate kinase patients, five obligate heterozygotes for pyruvate kinase deficiency and ten healthy controls. For all determinations we followed earlier-described methods pyruvate kinase [11], oxidized glutathione [8], reduced glutathione [12], glutathione reductase [13], and hexokinase [14] Pyruvate kinase was purified up to Step 4 of our previously published method [15], gaining a 2460-fold purification with a yield of 53 % Mercaptoethanol was omitted from all buffers. Prior to kinetic analysis, hemolyzate and enzyme preparation were dialyzed against 250 mM Tris—HCl buffer pH 8 0 The conditions for kinetic studies and for heat-stability tests were identical to those reported earlier [11,15] All biochemicals used in this study were purchased from C F Boehringer, Mannheim (Germany), except for *N*-ethylmaleimide which was obtained from Sigma Co , St Louis, U S.A

TABLE I

ACTIVITY OF PYRUVATE KINASE, HEXOKINASE AND GLUTATHIONE REDUCTASE WITH AND WITHOUT FLAVIN ADENINE DINUCLEOTIDE, AND CONCENTRATION OF REDUCED AND OXIDIZED GLUTATHIONE IN ERYTHROCYTES FROM 10 NORMAL SAMPLES, 5 OBLIGATE HETEROZYGOTES FOR PYRUVATE KINASE DEFICIENCY AND 6 PYRUVATE KINASE-DEFICIENT PATIENTS

	Normal Samples (n = 10)	Obligate Heterozygotes (n = 5)	Pyruvate Kinase deficient patients (n = 6)
Pyruvate kinase (units/g hemoglobin)	8 61 ± 1 47	5 31 ± 1 31	2 67 ± 1 47
Hexokinase (units/g hemoglobin)	0 64 ± 0 23	0 61 ± 0 19	1 24 ± 0 32
Glutathione reductase without FAD (units/g hemoglobin)	3 67 ± 0 78	3 86 ± 0 99	3 86 ± 0 96
Glutathione reductase with FAD (units/g hemoglobin)	5 56 ± 1 13	4 92 ± 0 89	5 34 ± 0 91
Reduced glutathione (μmol/l erythrocytes)	2190 ± 205	2202 ± 207	2158 ± 184
Oxidized glutathione (μmol/l erythrocytes)	5 29 ± 0 93	5 77 ± 2 47	5 19 ± 1 70

The results of red cell-enzyme determinations and estimations of glutathione are summarized in Table I. The pyruvate kinase-deficient patients have markedly reduced pyruvate kinase activity whereas this enzyme shows intermediate values in heterozygotes, as it has been observed earlier [1] Hexokinase, which has been determined as a marker for red cell age, is in the same range for normal samples and in erythrocytes from heterozygotes, and is increased in pyruvate kinase deficiency, reflecting the high content of young red cells Glutathione reductase, which is not a cell age-dependent enzyme, shows no essential difference between the three groups The effect of flavin adenine dinucleotide on glutathione reductase is also similar in the three groups There is no difference in content and ratio of reduced or oxidized glutathione between normal samples, heterozygotes and pyruvate kinase deficiency Especially oxidized glutathione is not increased in pyruvate kinase deficiency

The kinetic data in Table II show that there is no remarkable effect of physiological concentrations of oxidized glutathione on pyruvate kinase The thermostability of the enzyme is not significantly affected by 5 μM oxidized glutathione

From our data it seems unlikely that an increased oxidized glutathione concentration in the red blood cell is the causative factor for pyruvate kinase deficiency, as has been discussed recently [6,7]

TABLE II

KINETIC DATA AND HEAT STABILITY OF PYRUVATE KINASE IN A HEMOLYSATE AND A PYRUVATE KINASE PREPARATION WITH AND WITHOUT PREINCUBATION WITH 5 μM OXIDIZED GLUTATHIONE

	Hemolyzate without oxidized glutathione	Hemolyzate with oxidized glutathione	Preparation without oxidized glutathione	Preparation with oxidized glutathione
$K_{1/2}$ Phosphoenol-pyruvate (mM)	0 37	0 31	0 51	0 47
K_m Adenosine diphosphate (mM)	0 29	0 24	0 28	0 24
$K_{1/2}$ Fructose 1,6-diphosphate (μM)	0 06	0 06	0 14	0 19
Thermostability (Loss of activity after 60 min at 53 °C)	19	26	23	18

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