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A new variant of red blood cell pyruvate kinase deficiency

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SUMMARY

A new mutant pyruvate kinase (ATP: pyruvate phosphotransferase, EC 2.7.1.40) from human erythrocytes is described. The mutant enzyme shows an increased thermolability, a decreased K_m value for the substrate phosphoenolpyruvate and a loss of allosteric properties during the lifespan of the erythrocytes. By comparing the previous obtained data from other patients it seems that there can be distinguished at least three types of pyruvate kinase deficiencies.

Erythrocyte pyruvate kinase (ATP:pyruvate phosphotransferase, EC 2.7.1.40) is an important key enzyme in glycolysis. Reduced activity of pyruvate kinase causes a defect in the energy supply of the erythrocytes. This defect may cause haemolysis and anaemia. Pyruvate kinase of normal human erythrocytes exhibits allosteric properties[1]. Recently it was found[2] that the mutant enzyme of three non-related patients showed no allosteric properties and an increased thermolability. Another patient, however, showed increased thermolability but normal allosteric properties. The mutant enzyme of all patients had the same K_m value for phosphoenolpyruvate (PEP) and ADP as the normal enzyme. This paper deals with a new variant of pyruvate kinase deficiencies, of which the clinical data was presented elsewhere[3]. The enzyme activity was measured according to the method of Bücher and Pfleiderer[4]. Separation of the erythrocytes into an "old" and "young" fraction was obtained according to Ten Brinke and de Regt[5]. The enzyme was partially purified to Stage 4, according to the method of Staal *et al.*[1] and finally dialysed overnight against 0.01 M Tris-maleate (pH 8.0) containing 50% (v/v) glycerol.

Fig. 1 shows the $1/v$–$1/[PEP]$ plots of the partially purified mutant enzyme of the "old" (B) and "young" (A) erythrocytes at infinite [ADP]. From Fig. 1 a K_m value for the substrate PEP of 0.16 mM ("young" erythrocytes) and of 0.25 mM ("old"

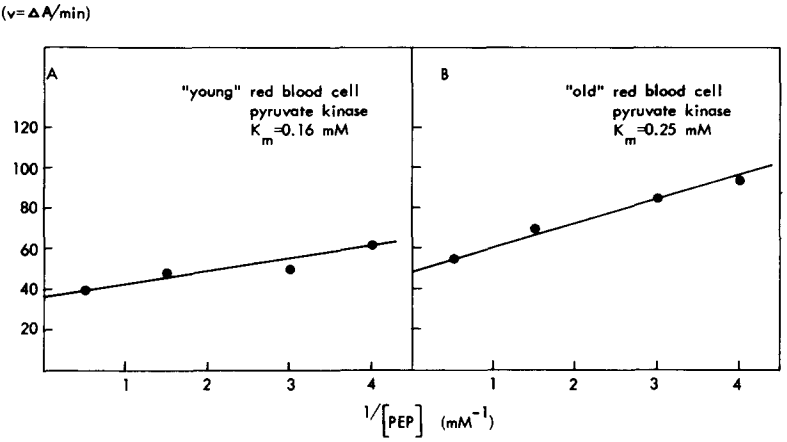

Fig. 1. The $1/v$ *versus* $1/[PEP]$ of the red blood cell pyruvate kinase from "young" and "old" erythrocytes at [ADP] = ∞. A. Pyruvate kinase from "young" cells. B. Pyruvate kinase from "old" cells.

erythrocytes) was calculated. The K_m value for ADP at [PEP] = ∞ was in both cases 0.5 mM. For the normal enzyme the K_m values for PEP and ADP are 0.6 mM (see ref. 1). From these data it can be concluded that the K_m value for the substrate PEP is decreased.

The Hill plot at [ADP] = ∞ shows for the "young" erythrocytes positive cooperativity (n=1.2) with respect to PEP, although considerably less than the normal enzyme (n=1.6). However, for the "old" erythrocytes no cooperativity is found (n=0.9) at all. Therefore, it can be concluded that the allosteric properties of the mutant enzyme are diminished.

The heat stability of the mutant enzyme is considerably less than that of the normal enzyme. Heating (performed as described in ref. 1) causes a loss of activity of 75%, while the normal enzyme remains fully active. This was also found with the mutant enzymes of the other patients described in ref. 2, although with those mutant enzymes

TABLE I

THE KINETIC PARAMETERS OF THE MUTANT ENZYMES OF THE VARIOUS NON-RELATED PATIENTS

Type	*Patient*	K_m^{PEP} *([ADP] = ∞)*	K_m^{ADP} *([PEP] = ∞)*	*Allosteric properties*	*Heat stability*	*Ref.*
I	J. v/d K. N.S. S.S.	Normal	Normal	Lost (n=0.9)	Decreased	2
II	B.H.	Normal	Normal	Present (n=1.6)	Decreased	2
III	M.V.	Decreased	Normal	Lost (n=0.9)	Decreased	This study

the thermolability was less (50%). Further evidence for an increased instability of the enzyme was found by measuring the ratio of pyruvate kinase activity in the "young" and "old" fraction compared with the ratio for the enzyme glucose-6-phosphate dehydrogenase. For pyruvate kinase a ratio of 2 was found, while the ratio for glucose-6-phosphate dehydrogenase was 1.1. This means that during the lifespan of the erythrocyte pyruvate kinase of the patient is inactivated.

From the data obtained from the various non-related patients, we can now distinguish at least three different types of pyruvate kinase deficiency, as shown in Table I. To prove the validity of the conclusions more patients should be studied. However, it should be kept in mind that there is no relationship between the enzymatic activity and the degree of haemolysis. Therefore, the existence of various types of this deficiency is possible.

1 G.E.J. Staal, J.F. Koster, H. Kamp, L. v. Milligen-Boersma and C. Veeger, *Biochim. Biophys. Acta*, 227 (1971) 86.
2 G.E.J. Staal, J.F. Koster and L. v. Milligen-Boersma, *Biochim. Biophys. Acta*, 220 (1970) 613.
3 J.G. Nijessen, G.E.J. Staal and M.C. Verloop, *Abstr. 1st Meeting Eur. Div. Int. Soc. Haematol.*, 1971, p. 109.
4 T. Bücher and G. Pfleiderer, in S.P. Colowick and N.O. Kaplan, *Methods in Enzymology*, Vol. I, Academic Press, New York, 1965, p. 435.
5 M. Ten Brinke and J. de Regt, *Scand. J. Haematol.*, 7 (1970) 336.