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Pyruvate kinase in cultivated amniotic fluid cells

After glucose-6-phosphate dehydrogenase deficiency L-type pyruvate kinase deficiency is the most common erythrocyte metabolic error. Although the clinical pattern is reported to be quite variable, a rather high rate of mortality in early life has been found[1]. Recently, pyruvate kinase was purified from human erythrocytes[2] and it was found that the enzyme exhibits allosteric properties. These properties were compared with the partially purified enzyme from pyruvate kinase-deficient patients.

In three of four unrelated patients the mutant enzyme did not show allosteric properties, while in all patients an increased thermolability was found[3].

The properties of red blood cell pyruvate kinase is very much like the (liver) L-type pyruvate kinase. Besides the L-type the liver also contains the (muscle) M-type pyruvate kinase which in deficient patients remains unchanged. It has been shown that in pyruvate kinase deficiency there is also a decrease of total liver pyruvate kinase activity[4]. However, to perform antenatal diagnosis of this deficiency it is necessary to establish which type (L- or M-type) of pyruvate kinase is present in human cultured fibroblasts and amniotic fluid cells.

Pyruvate kinase activity was measured according to the method of Bücher and Pfleiderer[5]. Starch gel electrophoresis was performed with the method of Bigley *et al.*[6], the pyruvate kinase activity was detected with the fluorescent technique[7].

Fig. 1 shows the saturation curve for phosphoenolpyruvate (PEP) of fibroblast pyruvate kinase with [ADP] = 2 mM. The curve is hyperbolic and fructose-1,6-diphosphate (Fru-1,6-P_2) does not stimulate the enzymatic reaction. The same result was obtained with cultivated amniotic fluid cells. It is known that the L-type pyruvate kinase of liver and erythrocyte exhibits an S-shaped saturation curve, while the M-type does not. Furthermore, Fru-1,6-P_2 stimulates the L-type and transforms the sigmoidal curve into a hyperbolic one. The enzymatic activity of the M-type is not influenced by Fru-1,6-P_2.

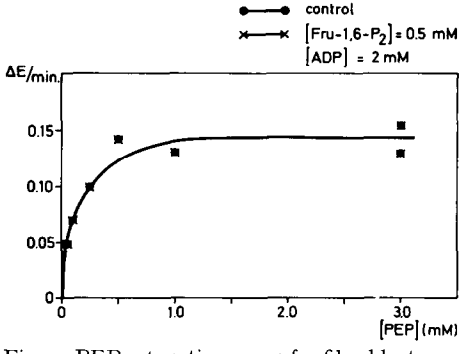

Fig. 1. PEP saturation curve for fibroblast pyruvate kinase in the absence and presence of Fru-1,6-P_2. Buffer 0.1 M Tris–HCl (pH 7.6), [ADP] = 2 mM, reaction medium contains 0.08 mg protein. ●–●, control; x–x, in the presence of Fru-1,6-P_2 (0.5 mM).

Table I summarizes the apparent K_m values for PEP at fixed ADP concentration. It shows that the apparent K_m values obtained for pyruvate kinase in fibroblasts

and cultivated amniotic fluid cells are in the same order as those for the M-type from various origins.

TABLE I

THE APPARENT K_m VALUES FOR THE SUBSTRATE PHOSPHOENOLPYRUVATE AT [ADP] = 2 mM FOR RAT LIVER (L- AND M-TYPE), HUMAN ERYTHROCYTE, HUMAN FIBROBLAST, HUMAN AMNIOTIC FLUID CELLS, AND HUMAN LEUCOCYTES PYRUVATE KINASE

	Type	K_m *(mM)*
Liver (rat)*	L	0.80
	M	0.07[5]
Erythrocyte (human)	L	0.45
Muscle (human)	M	0.02
Leucocytes (human)	M	0.05
Cultured amniotic fluid cells (human)	M	0.10
Cultured fibroblasts (human)	M	0.10

* Obtained from Tanaka *et al.*, *J. Biochem. (Tokyo)*, 62 (1967) 71.

Furthermore, electrophoresis showed that the mobility of fibroblast pyruvate kinase is the same as that of the M-type, but quite different from the erythrocyte L-type pyruvate kinase.

From these data, it can be concluded that cultivated fibroblasts and amniotic fluid cells contain the M-type pyruvate kinase. As the diagnosis pyruvate kinase deficiency is based on a decreased activity of the L-type, fibroblasts nor amniotic fluid cell cultures can be used in diagnosis of this disease. Therefore, antenatal diagnosis of this disease by using cultured amniotic fluid cells is impossible.

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