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A pyruvate kinase variant in different mouse transplanted tumors

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Summary. Mouse transplanted tumors, in contrast to normal tissues, contain a pyruvate kinase (PK) variant sensitive to the inhibitory action of L-cysteine and less sensitive to saturated fatty acids than the normal enzyme. In selected normal and tumor materials two fractions of PK were separated. Fraction A (20–30% $(NH_4)_2SO_4$ saturation) dominated in normal liver, and fraction B (50–60% $(NH_4)_2SO_4$ saturation) in skeletal muscles and Ehrlich ascites tumor. Only this fraction B from tumor material was sensitive to L-cysteine, and seems to contain a tumor-specific PK variant which might be considered as a marker of neoplastic transformation in a broad spectrum of mouse experimental tumors.
Key words. Pyruvate kinase; mouse transplanted tumors; tumor marker; L-cysteine; stearic acid.

Pyruvate kinase is one of the key regulatory enzymes of the glycolytic pathway, which is directly involved in cytoplasmatic energy formation [1,2]. Its activity increases significantly during 'spontaneous' or viral cell transformation in vitro [3,4]. In Ehrlich ascites tumor cells this enzyme, in addition to increased activity, acquires a new sensitivity to the inhibitory action of L-cysteine [5–7] and shows a decreased sensitivity to several normal effectors and signal molecules, including saturated fatty acids [8] and ATP [9]. These effects, observed previously both in metabolic studies and in direct enzymatic determinations [5–7] suggested that in tumor cells a PK variant might be present.

The aim of the study now reported was to see whether a PK variant, sensitive to the inhibitory action of L-cysteine and with decreased sensitivity to fatty acids, is specific for a broad spectrum of mouse transplanted tumors, and thus to evaluate its role as a marker of neoplastic transformation.

Material and methods. Cytosolic fractions obtained from various mouse solid and ascites tumors were studied.

Mouse solid tumors (in brackets mouse strains used for transplantations): 1) Mammary adenocarcinoma 16C (C_3H), 2) Madison lung carcinoma (BALB/C), 3) Lewis lung carcinoma (C57BL/6), 4) Colon carcinoma C-28 (C57BL/6), 5) Colon carcinoma C-26 (BALB/C), 6) Melanoma B-16 (C57BL/6), 7) Leukemia L-1210 (DBA-2), 8) Polyoma-induced tumor (DBA-2).

Mouse ascites tumors (in brackets mouse strains used for transplantations): 1) Ehrlich ascites tumor (Swiss), 2) Leukemia L-1210 (DBA 2), 3) Leukemia P-388 (AKR), 4) Leukemia AKSL-4 (AKR).

All solid tumors were transplanted s.c. into syngenic recipients. Ascites neoplastic cells were transplanted i.p. As a rule 10^5–10^6 cells were inoculated by both routes. For comparison cytosolic fractions of normal mouse tissues (liver, skeletal muscle, spleen), and mouse embryo were used.

Tumors and normal tissues obtained from animals sacrificed by cervical dislocation were homogenized with 20 mmol/l Tris-HCl buffer (pH = 7.4), containing 115 mmol/l KCl, 10 mmol/l $MgCl_2$, 1 mmol/l EDTA, in a Potter Elvehjem glass homogenizer, and centrifuged at 100,000 × g at 4 °C for 15 min in a Spinco preparative ultracentrifuge. The cytosolic fractions were used directly for enzymatic studies. In the case of Ehrlich ascites tumor, and for comparison of normal muscle and liver, the cytosol enzyme activity was determined also after ammonium sulphate fractionation between 20 and 70% of saturation, in the range of 10%.

PK activity was determined spectrophotometrically [10] in conditions of pseudozero-order kinetics in the absence or presence of 0.05 mmol/l L-cysteine or 0.05 mmol/l stearic acid in liposomes from 1 mmol/l phosphatidylcholine. The results were expressed in mIU per mg of protein.

Protein concentration was measured by the method of Lowry et al. [11]. The results were analyzed statistically using Student's t-test.

Results and discussion. The activity of PK in cytosolic fractions of various mouse tumors as well as of mouse embryo and normal skeletal muscle was much higher (about 3000–10,000 mIU/mg protein) than in corresponding fractions of mouse spleen or mouse liver (about 250–1000 mIU/mg protein) (table 1).

Stearic acid (0.05 mmol/l) in liposomes decreased cytosolic PK activity in solid tumors (88.8–98.6%) less than in normal tissues studied (59.8–78.9%) when compared with corresponding control activity.

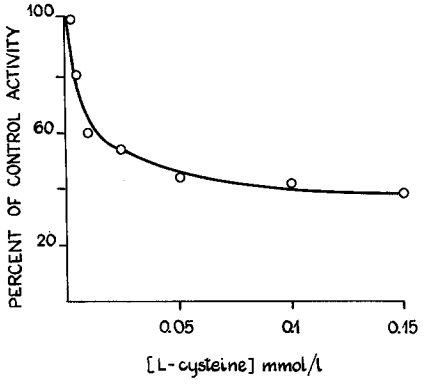

Figure 1. The effect of different concentrations of L-cysteine on PK activity in cytosole of Ehrlich ascites tumor cells. The activity is expressed as a percentage of control value.

Table 1. The effect of L-cysteine and stearic acid in liposomes on the pyruvate kinase activity in cytosolic fractions of different tumors and normal tissues. L-cysteine (0.05 mmol/l final conc.) or stearic acid (0.05 mmol/l final conc.) in liposomes were added to the reaction mixture. Pyruvate kinase activities were measured spectrophotometrically at 340 nm for 3 min at 1-min intervals in 25 °C. The results calculated in mIU per mg of protein were expressed as a percentage of the control values (n = number of animals).

Material	Control (mIU/mg protein) n = 6			+ L-cysteine (% of control) n = 4			+ Stearic acid (% of control) n = 4		
	$\bar{x}$	±	SD	$\bar{x}$	±	SD	$\bar{x}$	±	SD
Mice solid tumors									
Mammary adenocarcinoma	5652		868	75.4		19.7	96.3		3.8
Madison lung carcinoma	10418		1209	60.6		8.0***	92.1		9.0
Lewis lung carcinoma	3543		133	74.4		18.8*	93.1		4.5*
Colon carcinoma C-38	9561		2605	71.4		24.2	93.7		4.7
Colon carcinoma C-26	3882		1100	76.3		19.7	94.2		6.2
Melanoma B-16	7854		2438	94.2		20.2	98.6		5.3
Polyoma	2171		826	70.9		20.9	88.8		2.3
Leukemia L-1210 (n = 2)	8499		2977	76.2			68.9		
Mice ascites tumors									
Ehrlich ascites tumor	3943		970	50.8		9.5***	84.0		7.8***
Leukemia L-1210	3324		753	74.5		3.8*	87.4		6.6
Leukemia P-388	4337		1038	61.7		7.1**	83.0		7.6*
Leukemia AKSL-4 (n = 2)	6903		2473	83.1			81.7		
Normal mice tissues									
Skeletal muscle	4552		1833	99.6		10.2	76.9		10.1
Liver	435		155	108.2		16.6	78.5		8.6
Spleen	1060		250	105.7		8.6***	59.8		10.3
Mouse embryo	4793		149	100.0		0.0	53.7		12.0

*** $0.001 < p < 0.005$; ** $0.01 < p < 0.05$; * $0.05 < p < 0.1$.

L-cysteine 0.05 mmol/l decreased PK activity in all the solid tumors studied, with the exception of skin melanoma, to about 60.6–76.3% of the initial control values. The effects were slightly greater in ascitic tumors (50.8–74.5%) and statistically significant. In contrast to tumor materials, L-cysteine had no effect on PK from cytosolic fractions of normal tissues (99.6–108.2%), irrespective of whether the initial enzyme activities were low (liver and spleen) or high (skeletal muscle and embryo).

With increasing L-cysteine concentrations, inhibition of cytosolic PK activity from Ehrlich ascites tumor was stabilized at about 50% of the control activity (fig. 1). This indicated that tumor cytosol contains a PK fraction insensitive to L-cysteine, in addition to the sensitive fraction.

Ammonium sulphate fractionation revealed the presence of two PK variants: 'A' and 'B' in all materials studied (fig. 2).

Table 2. The effect of L-cysteine (0.05 mmol/l) on the pyruvate kinase activity in fractions A and B obtained by ammonium sulphate precipitation from cytosol of Ehrlich ascites tumor cells, mouse liver and skeletal muscle.

	mIU/mg protein (control)	Effect of L-cysteine (% of control)
EAT		
Fraction A	750	100
Fraction B	4250	33
Muscle		
Fraction A	1200	100
Fraction B	5400	100
Liver		
Fraction A	2750	100
Fraction B	550	100

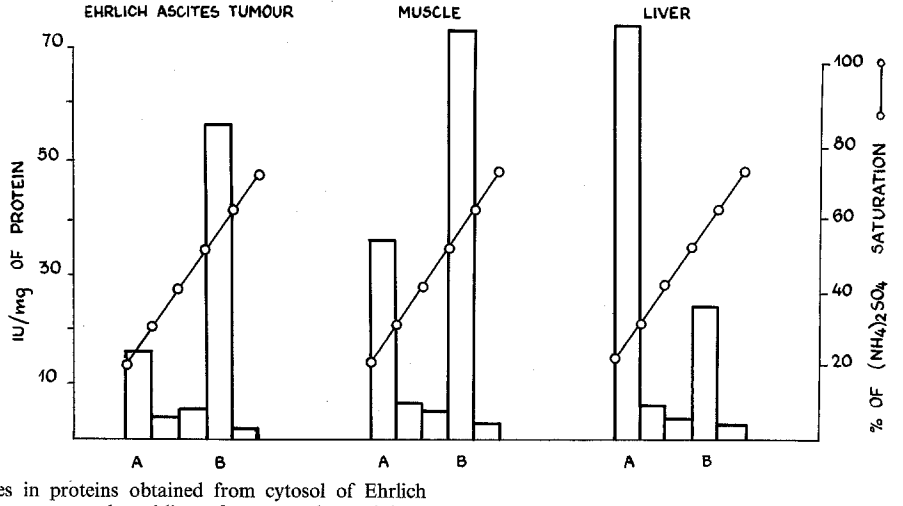

Figure 2. PK activities in proteins obtained from cytosol of Ehrlich ascites tumor, normal mouse muscle and liver after ammonium sulphate fractionation.

Fraction 'A' (20–30% of ammonium sulphate saturation) dominates in mouse liver, fraction 'B' (50–60% of ammonium sulphate saturation) dominates in Ehrlich ascites tumor and the muscle cytosolic fractions.
All fractions 'A' were insensitive to L-cysteine. Only fraction 'B' from Ehrlich ascites tumor was markedly inhibited by L-cysteine, but not completely, which also may point to its heterogeneity (table 2).
Among many PK variants two molecular forms were described; the L type from liver and the M type from muscles[12–14]. Neoplastic PK belongs to the type M. It appears, however, that it differs in its sensitivity to L-cysteine. This inhibitory effect does not seem to be caused by the thiol groups, since D-cysteine, or other compounds with free thiol groups such as reduced glutathione or homocysteine, do not influence neoplastic glucose catabolism[7]. It seems thus that this stereospecific inhibitory effect of L-cysteine might be an allosteric effect, characteristic only for tumor PK in a broad spectrum of mouse experimental tumors. Further studies to characterize this PK variant in more detail have been undertaken.

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Recent observations on the structure and the properties of yeast NMN adenylyltransferase

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Summary. A homogeneous preparation of yeast NMN adenylyltransferase (EC 2.7.7.1) showed microheterogeneity, which was revealed by FPLC (Fast Protein Liquid Chromatography) ion exchange chromatography. The resolved components have been characterized with respect to electrophoretic behavior and adenine content. The results led to a hypothesis about a possible role of poly(ADP-ribosylation) in modulating the enzyme activity.
Key words. NMN adenylyltransferase; yeast (*Saccharomyces cerevisiae*); enzyme purification.

Both in the de novo and the salvage synthesis of nicotinamide nucleotides, a central role is played by the nuclear enzyme nicotinic acid/nicotinamide-mononucleotide adenylyltransferase, catalyzing the reaction NaMN (NMN) + ATP → NAD^+ + PPi. The nuclear localization of NAD biosynthesis seems to be associated with the regulation of cellular activities exerted by the nucleus[1]. Indeed, not only NAD biosynthesis, but also NAD degradation is unique to the nuclear compartment, so that the stability of the NAD level in the whole cell appears to be controlled by nuclear events[2]. NAD consumption takes place in the reaction of poly(ADP-ribosylation) of nuclear proteins catalyzed by the nuclear enzyme poly(ADP-ribose) polymerase[3].
The biological significance of this latter reaction is not well understood, as yet; however, data are accumulating suggesting a close relationship between poly(ADP-ribosylation) and many biological events, such as DNA repair, cell cycle, cell differentiation and oncogenesis[4]. The whole of the cell's activity, then, appears to respond to the concomitant fluctuation of NAD levels.
In order to investigate the mechanism of one such phenomenon, we have carried out the purification of the enzyme NMN adenylyltransferase from yeast. Chemical analysis showed the enzyme to be a glycoprotein containing 2% carbohydrates, and two moles of alkali-labile phosphate as well as one mole of adenine derivative per mols of protein (200,000 mol. wt)[5]. Carbohydrate content could be referred mainly to pentoses on the basis of the absorption maximum of the colored product formed, according to the procedure of Dubois et al.[6]. These findings suggested a possible poly(ADP-ribosylation) of the enzyme, that, on the other hand, seems to be plausible in view of the nuclear compartmentation of both NMN adenylyltransferase and poly(ADP-ribose) polymerase. In addition, a modification of this kind seems very likely to possess a regulatory value, both enzymes being involved in the NAD turnover.
However, the data seem only to be consistent with no more than a single ADP-ribose unit per enzyme molecule. The apparent discrepancy might be due to one of the following: a) only a limited fraction of the enzyme molecules is poly-modified; b) the native poly-modified enzymes undergoes extensive demodification (hydrolysis of n-1 ADP-ribose units) at some stage during the purification procedure.
As a result some molecular microheterogeneity would be displayed by the purified enzyme preparation, which might be demonstrated by a sufficiently selective separation proce-