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A Comparative Study of Glucose-6-phosphate Dehydrogenase in Ehrlich Ascites Carcinoma and Host's Liver

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Glucose-6-phosphate dehydrogenase was studied in Ehrlich ascites carcinoma and compared with that of the host's liver. Distinct differences were observed in thermal stability, *p*H optima and Km for Glucose-6-phosphate. However, no difference in the electrophoretic mobilities on polyacrylamide gel was observed.

GLUCOSE-6-phosphate dehydrogenase (G-6-PD) attracted the attention of several workers in the field of cancer-research[1,2,3]. It was reported to be increased in variety of experimentally induced and spontaneous tumours[4,5,6]. Recently, it has been observed in our laboratory that liver G-6-PD increases proportionally with the growth of Ehrlich ascites carcinoma (EAC) and induced skin carcinoma[7]. It was, therefore, decided to compare the behaviour of this enzyme in Ehrlich ascites carcinoma with that of the host's liver.

Experimental

EAC was maintained in Swiss male mice by intraperitoneal inoculation of ascitic fluid.

Ascites cells were taken out from the peritoneal cavity and washed thrice in cold 0.1*M* Na-K-phosphate buffer. *p*H 7.2 containing 5 mM $MgCl_2$ and finally resuspended in such a way, so that the suspension contains 4 mg dry wt/ml in this buffer. While vigorously agitated on a Vortex mixer, 0.01 vol of toluene : ethanol (1 : 9 v/v) was added to the cell-suspension and the agitation continued for a further period of 10 mins. The toluene treated cells[8] was then kept in ice.

10% liver homogenate from normal and tumour-bearing mice was prepared in cold 0.25*M* sucrose. Both the toluene-treated ascites cell-suspension and the liver homogenates were centrifuged at 100,000 g for 1 hr and the supernatants were used for enzyme assay. G-6-PD was assayed spectrophotometrically[9] by following optical density change at 340 nm. Specific activity was expressed as micromoles of NADPH produced/milligram of protein/minute.

Michaelis constant of G-6-P at 0.15 mM NADP concentration was determined at *p*H 7.5, using concentration of G-6-P ranging from 0.1 to 1.5 mM for liver enzyme and from 0.01 to 0.5 mM for tumour enzyme. The Km values of NADP at 1.0 mM and 0.1 mM concentration of G-6-P for liver and tumour enzymes respectively, were also determined at *p*H 7.5 with concentrations of NADP ranging from 0.015 to 0.21 mM.

Electrophoresis of G-6-PD of samples was carried out with soluble supernatant on 5.5% polyacrylamide gel at *p*H 7.5 and stained according to the method of Dietz *et al.*[10].

Results

pH optima : G-6-PD activities of ascites cells as well as host's liver at different *p*H are shown in Fig 1. Enzyme preparation from normal and host's liver shows peak at *p*H 8, whereas, the *p*H optima of ascites cell preparation is *p*H 7.5. It is also evident from the figure that the specific activity of the enzyme is much higher in tumour cells than that of the host's liver.

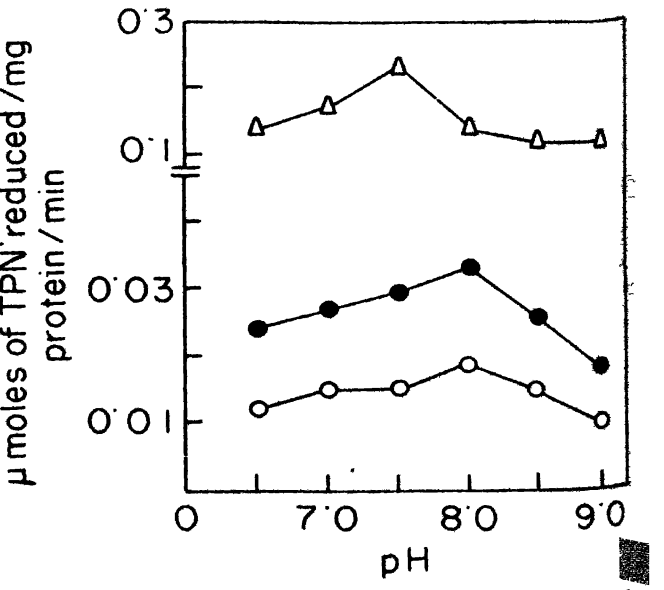

Fig. 1. Specific activity of G-6-PD with respect to *p*H, normal liver (○—○) ; host's liver (●—●) ; EAC (▲—▲)

Thermal stability : Heat inactivation studies of enzyme preparations were carried out at 45° and 55° according to Messina *et al*[12]. Enzymes in all preparations were inactivated at 55°. Whereas, it can be observed from Fig 2 that at 45°, the host's liver enzyme retains about 60% of the initial activity at the end of 10 minutes. The tumour cell enzyme seems to be more heat-labile which is evident from the figure.

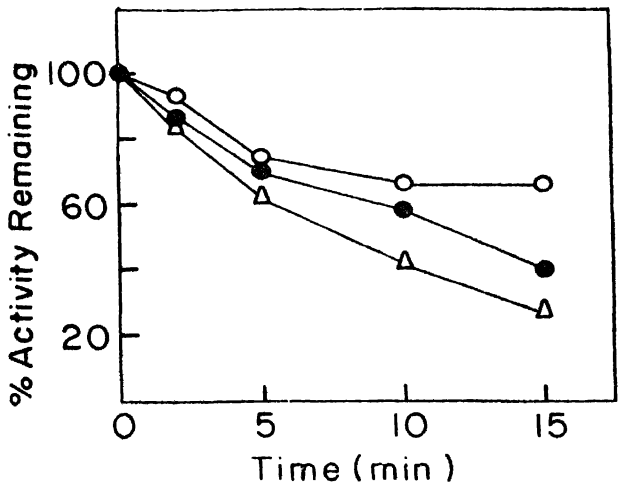

Fig. 2. Thermal stability studies of G-6-PD, normal liver (O—O) ; host's liver (●—●) ; EAC (Δ—Δ).

Electtophoretic mobility :

Enzyme preparation from both tumour cells and host's liver demonstrated one band on activity staining with same mobility on 5.5% polyacrylamide gel at *p*H 8.3. The band is more distinct in host's liver preparation in comparison to that of the toluene-treated ascites cells.

Discussions

It is well known that the tumour tissues have a high rate of aerobic glycolysis. Hexose monophosphate shunt being an oxidative pathway is also higher in tumours. The activity of G-6-PD, the first enzyme of the shunt pathway, has been found to be increased in tumour cells[4,5] and also in host's liver[7]. From the results presented in this communication it shows that the specific activity of G-6-PD is much higher in toluene-treated ascites cells in comparison to that of the host's liver. It may also be mentioned here that the enzyme from EAC although has a much higher affinity for G-6-P than the liver enzymes, it is very much susceptible to G-6-P. This enzyme is significantly inhibited by G-6-P at a concentration higher than 0.6 mM whereas the liver enzymes (both normal and host's) are not inhibited even at a concentration of 1.5 mM (data not shown). This means that the EAC enzyme shows substrate inhibition at high concentration whereas the liver enzymes are not. An attempt to compare the behaviour of the enzyme in liver and ascites cells reveals a marked difference in thermal stability and *p*H

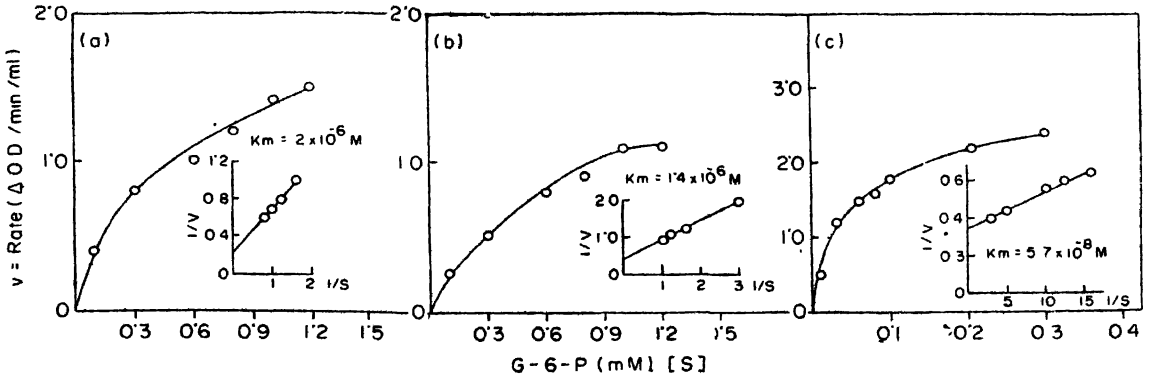

Fig. 3. Substrate concentration curves of G-6-PD,
(a) Normal liver,
(b) host's liver,
(c) Ehrlich ascites cells.
Insets represent double reciprocal plot from where Kms were calculated.

Michaelis-Menten constants : In case of host's liver, the Km for G-6-P ($1.4 \times 10^{-6} M$) is similar in magnitude with that of normal liver (Fig 3), whereas in ascites cells, the Km is significantly lower and is $5.7 \times 10^{-8} M$ (Fig 3). There is not much of difference in Km values for NADP in host's liver and ascites cell ($7.7 \times 10^{-7} M$ and $1.1 \times 10^{-7} M$ respectively) enzymes. In normal liver, the Km value for NADP has been found to be $1 \times 10^{-6} M$.

optima. It can thus be concluded that host's liver G-6-PD, the activity of which increases with the growth of the ascites tumour, behaves like the normal liver enzyme and thus differs significantly from the G-6-PD of toluene-treated ascites cells.

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