PHOSPHOFRUCTOKINASE ACTIVITY AND RATE OF GLYCOLYSIS IN THE RAT LIVER DURING CARCINOGENESIS

B. L. Rubenchik and A. S. Petrun'

UDC 616-006.6-092.9-07 :616.36-008.931-074

The rate of glycolysis and the phosphofructokinase activity in the liver are increased in rats during carcinogenesis induced by administration of p-dimethylaminoazobenzene with the diet. The changes are accompanied by lowering of the sensitivity of this enzyme to allosteric inhibitors, ATP, and citrate.

Phosphofructokinase (PFK) (ATP:D-fructose-6-phosphate-1-phosphotransferase; 2.7.1.11) limits the rate of glycolysis in certain tissues [6-9] and tumors [10, 11] and participates in regulation of the Pasteur effect. The system of aerobic glycolysis in formed tumors is known to be disturbed. Yet during the induction of malignant changes in the liver by chemical carcinogens, these changes develop gradually, and long before the formation of hepatomas [2, 5].

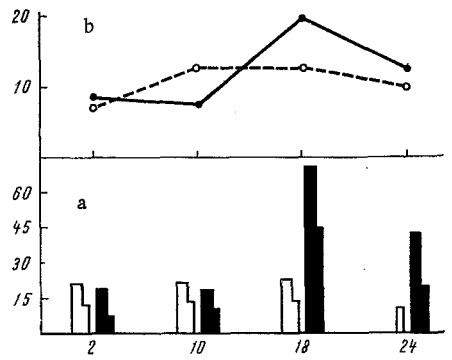

Fig. 1. Glycolysis and PFK activity in hyaloplasm of rat liver in control group and during carcinogenesis. Abscissa: Duration of experiments (in weeks); ordinate: a) level of glycolysis (in mg lactic acid/g protein at 37°C); b) PFK activity (in μmoles NAD $\cdot H_2$/min/g protein at 25°C); unshaded column and broken line represent control; black column and continuous line represent carcinogenesis; lower part of column indicates deficiency, upper part excess of coenzymes of glycolysis in medium.

The object of this investigation was to study the PFK activity and the rate of glycolysis in the rat liver during chemical carcinogenesis.

EXPERIMENTAL METHOD

The tests were carried out at different stages of carcinogenesis induced in rats by administration of p-dimethylaminoazobenzene (DAB) along with the diet [2]. By differential centrifugation [3] the nuclei and mitochondria were successively isolated from the liver, and in this way the hyaloplasm was obtained. Activity of aerobic glycolysis was estimated from the increase in lactic acid in the presence of glucose and of an excess or deficiency of the coenzymes of glycolysis [1].

The method adopted for isolation and partial purification of PFK [9] was followed by treatment to the enzyme with potassium phosphate gel, after which its specific activity was increased by 25 times. The activity of the enzyme was determined spectrophotometrically from the rate of dehydrogenation of NAD $\cdot H_2$ in a coupled system in the presence of enzymes of glycolysis [9].

Laboratory of Carcinogenic Substances, Kiev Institute of Food Hygiene, Ministry of Health of the Ukrainian SSR. (Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 71, No. 1, pp. 68-70, January, 1971. Original article submitted March 23, 1970.

TABLE 1. Action of ATP, Citrate, AMP, and Metallic Ions on Partly Purified PFK from Rat Liver (in % of optimal activity)

Composition of samples		Control	Carcinogenesis (DAB)
ATP (in mmoles)	other components		
0.2	5 mmoles $MgCl_2$ + 20 mmoles KCl	100.0	100.0
	20 mmoles KCl	34.6 ± 4.9	20 ± 3.5
	5 mmoles $MgCl_2$	83.3 ± 4.4	101 ± 5.0
3	5 mmoles $MgCl_2$ + 20 mmoles KCl	53.6 ± 7.9	105.4 ± 7.7
4	The same	46.7 ± 5.5	105.9 ± 11.0
6	" "	28.1 ± 6.0	97.6 ± 11.7
4	The same + 5 mmoles citrate	15.3 ± 0.7	64 ± 1.4
6	The same + 0.1 mmoles AMP	39.9 ± 7.3	100.5 ± 6.4
Activity of PFK (in μmoles NAD · H_2/min/g protein at 25°C)		77.7	126.9

TABLE 2. Rate of Formation of F-1,6-DP in Rat Liver in Control and during Carcinogenesis (in μmoles F-1,6-DP/min/g protein at 37°C)

Group of animals	Duration of experiments (in weeks)			
	2	10	18	24
Experimental (DAB)	11.5 ± 1.2	21.6 ± 2.2	21.4 ± 5.1	28.3 ± 4.5
Control	10.14 ± 1.1	7.8 ± 0.97	11.2 ± 1.8	11.2 ± 1.8

EXPERIMENTAL RESULTS

In the presence of a deficiency or an excess of coenzymes of glycolysis (Fig. 1), the velocity of the reaction increased sharply in the hyaloplasm in the last stages of carcinogenesis, especially after the 18th week, before macroscopically visible tumors were found in the rats' liver. A parallel was observed between the change in glycolytic and PFK activity.

PFK was then isolated preparatively from the liver of the control and experimental rats at the stage of carcinogenesis (18 weeks) when the increase in activity of this enzyme and of glycolysis in the hyaloplasm reached its maximum. Some of the kinetic properties of PFK after partial purification are given in Table 1.

Optimal PFK activity (taken as 100%) was obtained in both experimental and control groups when the ATP content in the sample was 0.2 mmole in the presence of 5 mmoles $MgCl_2$ and 20 mmoles KCl. As Table 1 shows, the enzyme requires magnesium and potassium ions in order to exhibit activity, and it is inhibited by an excess of ATP and citrate, characteristic features of PFK from liver and other tissues [6-9]. However, during carcinogenesis PFK was much less sensitive than in the control to the inhibitory action of ATP. Even when the sample contained 6 mmoles ATP, when the enzyme activity in the control was inhibited by 72%, no decrease in its activity was observed during carcinogenesis. Citrate together with ATP inhibited activity in the control by 85%, but only by 36% during carcinogenesis.

Further investigations showed that the decrease in the sensitivity of PFK to ATP starts to appear in the preceding stage of carcinogenesis, before any increase has occurred in PFK activity or in the glycolytic activity coupled with it. The writers' investigations and those of other workers [8, 9] have shown that PFK is activated by a reaction product, fructose-1,6-diphosphate (F-1,6-DP), which sharply lowers the sensitivity of the enzyme to inhibitors.

The results given in Table 2 demonstrate a sharp increase in the rate of formation of F-1,6-DP in the rat liver during carcinogenesis, coinciding in time with the beginning of the decrease in sensitivity of PFK to ATP. At the same time, the writers' previous experiments [2] showed that the activity of enzymes removing the excess of F-1,6-DP, namely aldolase (4.1.2.13) and hexose diphosphatase (3.1.3.11), in the liver remains substantially unchanged during carcinogenesis.

The hypothetical model of the structure of PFK at the present time envisages the existence of two active centers for substrates, at least three for inhibitors, and four for activators [6]. It has been shown,

in this connection, that substitution of even one of the centers by an activator sharply lowers the sensitivity of the enzyme to inhibitors. It can accordingly be postulated that DAB or its metabolic products, by virtue of their specific ability to bind themselves to liver proteins [4], act on the enzyme and lower its sensitivity to allosteric regulators. This results in an increase in the activity of PFK and of the glycolysis coupled with it.

LITERATURE CITED

1. S. A. Neifakh et al., Biochim. Biophys. Acta, 100, 329 (1965).
2. B. L. Rubenchik and A. S. Petrun', Ukr. Biokhim. Zh., No. 3, 319 (1968).
3. G. Hogeboom and W. Schneider, in: The Nucleic Acids [Russian translation], Moscow (1957), p. 102.
4. I. A. Khodosova, Tsitologiya, No. 6, 661 (1968).
5. H. F. Druckrey, F. Bresciani, and H. Schneider, Z. Naturforsch., 13b, 516 (1958).
6. O. H. Lowry and J. V. Passonneau, J. Biol. Chem., 241, 2268 (1966).
7. T. E. Mansour, in: Control of Energy Metabolism, New York (1965), p. 81.
8. J. V. Passonneau and O. H. Lowry, Biochem. Biophys. Res. Commun., 7, 10 (1962).
9. A. H. Underwood and E. A. Newscholme, Biochem. J., 95, 868 (1965).
10. R. Wu, Biochem. Biophys. Res. Commun., 14, 89 (1964).
11. R. Wu, J. Biol. Chem., 241, 4680 (1966).