INCREASED PRPP SYNTHETASE ACTIVITY IN RAPIDLY GROWING HEPATOMAS

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1. Introduction

Previous work demonstrated that in a spectrum of hepatomas of different growth rates the key enzymes of glycolysis increased and the key enzymes of gluconeogenesis decreased in parallel with tumor growth rate [1]. It was also shown that the two enzymes of the pentose phosphate pathway, glucose-6-phosphate dehydrogenase [2] and transaldolase [3], that are involved in the channeling of glycolytic intermediates into pentose phosphate biosynthesis were increased in all hepatomas. The metabolic consequences of these alterations in the reprogramming of gene expression in hepatoma cells should be an increase in glycolysis and ribose-5-phosphate production. Isotope studies demonstrated that in rapidly growing liver tumors there was an increase in the glycolytic and in the direct oxidative pathway [4] and in the incorporation of precursors into the de novo synthesis of purine ribonucleotides [5].

A stepping up of ribose-5-phosphate synthesis and utilization in the rapidly growing neoplasms might also entail an increase in certain enzymes involved in channeling this metabolite into purine biosynthesis. In order to test this prediction the first enzyme committed to routing ribose-5-phosphate into de novo purine biosynthesis, phosphoribosylpyrophosphate (PRPP) synthetase was studied. The action of PRPP synthetase (ribosephosphate pyrophosphokinase, EC 2.7.6.1) converts ribose-5-phosphate, in presence of ATP, into PRPP and AMP. In turn, PRPP is a key precursor in purine, RNA and DNA biosynthesis.

In this communication we report that PRPP synthetase activity was markedly increased in rapidly growing hepatomas; in contrast, the activity was low in the differentiating liver and was in normal range in the regenerating liver. The kinetic properties of PRPP synthetase were compared in tissue extracts of liver the hepatomas and the activity of the enzyme of various organs was contrasted with that observed in liver.

2. Materials and methods

2.1. *Animals and tissues*

Male Buffalo and ACI/N rats were maintained in separate cages with water and Purina chow available ad libitum. The handling of the tumorbearing rats, preparation of the regenerating liver, studies on developing animals, killing of the rats and excision of livers and tumors were carried out as reported previously [1,2].

2.2. *Preparation of extracts for enzyme assay*

Ten percent homogenates (w/v) were prepared from the liver and tumor tissues in 0.1 M potassium phosphate buffer, pH 7.4, containing 1 mM dithioerythritol. To 6 ml of clear 100 000 *g* supernatant fluid 1.4 g solid ammonium sulfate was added (40% saturation).

After standing at 0°C for 15 min it was centrifuged at 100 000 *g* for 10 min. The precipitate was dissolved in 0.3 ml of 0.1 M potassium phosphate buffer and 1 mM dithioerythritol, pH 7.4, recentrifuged at 12 000 *g* for 10 min and used for the PRPP synthetase assay.

2.3. *The PRPP synthetase assay system*

For the enzyme assay the methods of Kornberg et al. [6] and Flaks [7] were adapted to the conditions of the liver and the hepatoma systems. A standard assay, the kinetic details of which will be published elsewhere [8], was established for determination of liver and hepatoma enzyme activities. In this assay proportionality with the amount of enzyme added and with length of reaction time over a 15-min period was achieved.

In the two-step assay procedure PRPP is accumulated in step I and is quantitatively converted to orotidylic and uridylic acid in step II by addition of ancillary enzymes. The disappearance of orotate in step II was followed in a Gilford Model 2000 spectrophotometer at 295 nm at 25°C.

Step I.

The incubation mixture contained in a final volume of 1 ml 2 mM ATP, 1 mM ribose-5-phosphate, 5 mM $MgCl_2$ (added after ATP and phosphate), 50 mM potassium phosphate in 50 mM Tris–HCl buffer, pH 8.9. The reaction was started by addition of liver (0.60 mg protein) or tumor (0.48 mg protein) extracts and incubated at 37°C for 10 min; the reaction was terminated by boiling for 60 sec at 100°C. (During this treatment less than 2% of PRPP was decomposed and this was taken into consideration in calculating the PRPP synthetase activity.) The tubes were immediately cooled in ice water and centrifuged.

Step II.

The reaction mixture contained in a final volume of 1 ml in a quartz cuvette; 0.2 mM orotate, 2 mM $MgCl_2$, 20 mM Tris–HCl buffer, pH 7.6, 0.2 ml of orotidine-5′-phosphate pyrophosphorylase (EC 2.4.2.10) and orotidine-5′-phosphate decarboxylase (EC 4.1.1.23), and 0.3 ml of the step I incubation mixture. The pH of the reaction mixture in step II after addition of 0.3 ml step I was 7.6. The amount of orotidine-5′-phosphate pyrophosphorylase-decarboxylase used was chosen so that 25 nmoles of PRPP caused the disappearance of orotate within 30 min at room temperature (25°C). The disappearance of 1 μmole of orotate corresponded to a decrease in absorbance of 3.95 at 295 nm [6].

Highly purified preparations of PRPP synthetase (3.5 μmoles/min/mg protein) were obtained from rat liver by the following procedure. Tissue was homogenized in 0.1 M potassium phosphate and 1 mM dithioerythritol, centrifuged at 100 000 *g* for 30 min, precipitated with ammonium sulfate (40% saturation), brought to 65°C for 5 min, precipitated at pH 4.8 and subjected to Sepharose 4B-200 gel filtration [8].

The determination of the protein content of the extracts [9] and the cell counts were made as cited previously [2].

All chemicals were of the highest purity grade available and the various compounds and ancillary enzymes were purchased from Sigma Chemical Co., St. Louis, Mo. From the stock solution of orotidine-5′-phosphate pyrophosphorylase-decarboxylase (2.8 mg/ml of 60 mM Tris–HCl, pH 7.5) appropriate dilutions were prepared immediately before the assays.

3. Results and discussion

3.1. *Comparison of properties of PRPP synthetase from rat liver and hepatoma 3924A*

Both liver and hepatoma enzymes have an absolute requirement for inorganic phosphate, as have the enzymes isolated from Salmonella typhimurium [10] and human red blood cells [11,12]. The pH optimum for the liver enzyme was 8.8 (shoulder at 6.9) and for the hepatoma 8.6 (shoulder at 7.2). For Mg-ATP, without excess Mg^{2+}, a sigmoidal curve was observed. Additional Mg^{2+} converts the sigmoidal curves into hyperbolic ones. The apparent K_m values for Mg-ATP, in the presence of 3 mM Mg^{2+} and 20 mM phosphate, were 0.3 and 0.6 mM for liver and hepatoma, respectively. Mg^{2+}-ATP in concentrations higher than 3.0 mM inhibited the enzyme. The Mg^{2+} saturation curves were sigmoidal and the half maximal activities at 20 mM phosphate for liver and hepatoma were 0.5 and 0.6 mM, respectively. For ribose-5-phosphate the apparent K_m was 0.1 mM for both tissues and the substrate inhibited PRPP synthetase activity at concentrations higher than 1.0 mM. Both liver and hepatoma PRPP synthetase were inactivated by *p*-chloromercuriphenyl sulfonic acid, signalling the importance of sulfhydryl groups for the catalytic activity. These ob-

Table 1
PRPP synthetase activity in neoplastic, regenerating, and differentiating rat liver

Tissues	Growth rate (weeks)	PRPP synthetase activity (μmoles per hr)	
		per g wet weight	per cell × 10^{-8}
Normal liver (Buffalo)			
Control for 7777		9.4 ± 0.2	4.0 ± 0.1
Control for 9618A2		10.8 ± 0.5	7.2 ± 0.3
Normal liver (ACI/N)			
Control for 3924A		10.4 ± 0.2	5.7 ± 0.1
Sham operated (Wistar)		8.8 ± 0.1	4.7 ± 0.1
24 hr regenerating liver (Wistar)		10.5 ± 0.5 (120)	5.6 ± 0.2 (118)
5-day-old liver		9.3 ± 0.3 (89)	1.5 ± 0.1 (32)*
Hepatomas 7777	4.1	14.0 ± 0.4 (149)*	5.8 ± 0.2 (147)*
3924A	3.8	19.5 ± 0.4 (187)*	11.5 ± 0.2 (202)*
9618A2	2.3	18.5 ± 1.1 (172)*	11.9 ± 0.7 (166)*

The data are means ± S.E. of 4 experiments with percentages of corresponding control liver values in parentheses. The activities per cell are to be multiplied with the exponential given to arrive at the actual numbers.
*Values statistically significantly different from the respective controls ($p < 0.05$).

servations indicated that the PRPP synthetase was kinetically similar in the liver and in the rapidly growing hepatoma 3924A.

The enzyme preparations catalyzed the formation of ATP and ribose-5-phosphate from PRPP and AMP, indicating reversibility of the reaction. Determination of the equilibrium constant will be reported elsewhere [8].

3.2. *Activity of PRPP synthetase in extracts of different rat tissues*

The specific activities of PRPP synthetase as percentages of normal liver were the following: brain 308, thymus 220, heart 147, spleen 125, liver 100, intestinal mucosa 75, lung 60, kidney 50, and muscle 28. The brain and thymus which have high RNA and DNA synthesis have the highest, whereas skeletal muscle has the lowest, enzyme activity.

3.3. *PRPP synthetase activity in normal, differentiating and regenerating liver and in rapidly growing hepatomas*

In table 1 PRPP synthetase activity is expressed per gram wet weight and per average cell. In the average cell of the rapidly growing hepatoma, 7777, 3924A and 9618A2, the PRPP synthetase activity was significantly increased 1.5 to 2.0-fold over that of the control normal liver. In contrast, the enzyme activity in the regenerating liver was in normal range and in the differentiating liver it was 40% of the activity of the adult rat. The results were similar when the activity was expressed on a per gram wet weight of tissue basis (table 1).

4. Conclusions

The reprogramming of gene expression that occurs in the neoplastic transformation of the liver entails in addition to the increase in the activities of the key enzymes of glycolysis and pentose phosphate synthesis also a rise in the activity of PRPP synthetase which is the first enzyme committed to the channeling of ribose-5-phosphate into purine biosynthesis. This alteration in the expression of gene program should provide selective advantages for the cancer cell.

The increase in PRPP synthetase activity in the rapidly growing hepatomas appears to be specific to neoplastic rapid growth rate; since no similar alteration is observed in the rapidly growing regenerating or differentiating liver. In view of these considerations PRPP synthetase might be an important target in the design of selective chemotherapy. The molecular and regulatory properties of this enzyme are under investigation.

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