GLUTAMINE PHOSPHORIBOSYLPYROPHOSPHATE AMIDOTRANSFERASE: INCREASED ACTIVITY IN HEPATOMAS

Nobuhiko KATUNUMA and George WEBER*

Laboratory for Experimental Oncology and Department of Pharmacology, Indiana University School of Medicine, Indianapolis, Indiana 46202, USA

Received 9 October 1974

1. Introduction

Glutamine phosphoribosylpyrophosphate (PRPP) amidotransferase (amidophosphoribosyltransferase, EC 2.4.2.14) was markedly increased in all hepatomas examined and the sensitivity of the hepatoma enzyme to feedback inhibition by AMP was an order of magnitude less than that of the liver enzyme. These observations are of special relevance in the elucidation of the reprogramming of gene expression in cancer because amidotransferase is the first enzyme committed to channeling PRPP into de novo purine biosynthesis and it is subject to stringent feedback regulation [1].

2. Materials and methods

2.1. *Animals and tissues*

Inbred strain male Buffalo and ACI/N rats carrying bilateral subcutaneous transplants of hepatoma lines of different growth rates were maintained in separate cages with water and Purina Chow available ad libitum. Control normal rats of the same strains were sacrificed along with tumor-bearing rats. The handling of the tumor-bearing animals, the preparation of the regenerating liver, the investigation in developing rats, the killing of animals and excision of livers and tumors were conducted as outlined previously [2]. The relevant biological aspects [3] and biochemical properties [2] of the different transplantable tumor lines in the spectrum of hepatomas of different growth rates were reported elsewhere.

* To whom correspondence should be sent.

2.2. *Preparation of extracts for enzyme assay*

20% Homogenates (w/v) were prepared from normal liver and tumor tissues in 0.25 M sucrose containing 1 mM $MgCl_2$ [4]. The homogenate was centrifuged at 100 000 *g* for 30 min at 0°C in Spinco ultracentrifuge and the clear supernate was used for assays. Careful kinetic studies were carried out to insure proportionality with elapsed time and amount of enzyme added.

2.3. *The glutamine PRPP amidotransferase assay system*

As a result of such investigations a standard assay system was worked out which was an adaptation of that of Katunuma et al. [4] to the kinetic conditions of the rat liver and hepatoma systems. The reaction mixture contained in final concentrations: PRPP 10 mM, glutamine 20 mM, $MgCl_2$ 15 mM, Tris–HCl buffer (50 mM), pH 7.2, and KF 1.0 mM. The reactions were carried out at 37°C and they were stopped at 0, 30, and 45 min of incubation by boiling for exactly 5 min. The tubes were centrifuged and in the clear supernate the concentration of glutamic acid, that had formed in presence of PRPP, was determined. This was carried out by coupling to the reduction of NAD in presence of added excess glutamate dehydrogenase in a Gilford 2000 recording spectrophotometer. Under these experimental conditions no PRPP dependent ammonia was liberated from glutamine, indicating that glutaminase activity did not interfere with this assay. The results were calculated in μmoles/hr/g wet weight of tissue and were expressed as specific activities in μmoles of substrate metabolized per hr per mg protein.

3. Results and discussion

3.1. *Comparison of glutamine PRPP amidotransferase activity in normal and regenerating rat liver and in hepatomas of different growth rates*

Table 1 indicates that in normal liver the amidotransferase specific activity varied from 4.4 to 8.2 μmoles substrate metabolized per hr per mg protein $\times 10^{-2}$. Taking these values as 100%, the activities in the slow growing hepatomas 66, 20, 8999, 47C, and 44 were 170, 209, 317, 251, and 259%, respectively, of the activities of corresponding normal rat livers. In the rapidly growing hepatomas 7777, 3924A, 3683, and $9618A_2$ the specific activities were 303, 195, 278, and 309%, respectively, of the normal liver values.

Table 1
Increased glutamine PRPP amidotransferase activity in hepatomas

Tissues	Growth rate (months)	Specific activity (μmoles/hr/mg protein $\times 10^{-2}$)	
Normal liver (Buffalo)			
Control for 20		4.4 ± 0.4	(100)
Control for 66		6.8 ± 0.4	(100)
Control for 47C		6.8 ± 0.5	(100)
Control for 8999		6.9 ± 0.1	(100)
Control for 44		8.2 ± 1.1	(100)
Control for 7777		6.7 ± 0.8	(100)
Control for 9618A2		5.1 ± 0.4	(100)
Normal liver (ACI/N)			
Control for 3924A		7.5 ± 0.5	(100)
Control for 3683		5.4 ± 0.4	(100)
Sham-operated (Wistar)		7.0 ± 0.4	(100)
Regenerating liver, 24-hr (Wistar)		7.9 ± 0.6	(114)
Hepatomas			
20	11.5	9.3 ± 0.6	(209)*
66	10.2	11.5 ± 0.6	(170)*
47C	7.0	17.1 ± 0.7	(251)*
8999	6.5	22.0 ± 0.5	(317)*
44	5.4	21.1 ± 0.2	(259)*
7777	1.0	20.3 ± 1.6	(303)*
3924A	0.9	14.7 ± 0.5	(195)*
3683	0.6	15.1 ± 0.9	(278)*
9618A2	0.5	15.6 ± 1.5	(309)*

The data are given as means ± S.E. of 3 to 10 rats in the various groups with percentages of corresponding control liver values in parentheses. The specific activities are to be multiplied by the exponential given to arrive at the actual values. Growth rate is expressed as the mean transplantation time in months between inoculation and growth to 1.5 cm diameter.

* Values statistically significantly different from the respective controls ($p < 0.05$).

The amidotransferase activity in the 24-hr regenerating liver was not different from that of the liver of sham-operated control rats. Investigation in rats at 6 days and at 4 weeks of age showed that the amidotransferase activities were less than one-third of those in the adult rat liver. As the increased amidotransferase activity was present only in the liver tumors, and it did not occur in rapidly growing differentiating or regenerating liver, the increased activity of this key purine synthesizing enzyme appears to be specific to the neoplastic transformation.

Since in the assays the enzyme activity was proportionate with the enzyme amount, it is assumed that the increased enzyme activity in the hepatomas represents increased enzyme concentration in the neoplasms. The rigorous verification of this assumption by a method independent from the assay of enzyme activity, such as by immunological technics, should be available, since the purification and immunotitration of liver and hepatoma enzymes are in progress in this laboratory.

3.2. *Alteration of feedback inhibition by AMP on glutamine PRPP amidotransferase in hepatoma 3924A*

In examining the feedback inhibition exerted by AMP, it was observed during in vitro studies that the amidotransferase in rapidly growing hepatoma 3924A was more than 10 times less sensitive to the inhibitory action of AMP (K_i = 1.5 mM) than the enzyme from normal liver (K_i = 0.1 mM). The inhibition by AMP was relieved in both liver and hepatoma by high concentrations (10 mM) of PRPP. The kinetics of this inhibition is in agreement with reports on chicken and rat liver enzyme [4].

The observation that amidotransferase activity was increased 2- to 3-fold in all hepatomas, irrespective of the degree of malignancy, differentiation, or growth rate, is important, since it suggests that the reprogramming of gene expression that occurs in malignant transformation is linked with an increase in the expression of this key purine-synthesizing enzyme activity. In our laboratories to date we discovered 4 such malignant transformation-linked increases

in enzyme activities, and they all relate to an increased potential in the channeling of precursors to key biosynthetic processes. Thus, the increase in the activities of glucose 6-phosphate dehydrogenase and transaldolase provides an increased potential for routing glycolytic intermediates into pentose phosphate biosynthesis [5]. In turn, the presently reported increase in amidotransferase activity should provide an increased potential for purine biosynthesis. The fourth alteration observed in all hepatomas was an increase in UDP kinase activity (J. C. Williams, G. Weber, in preparation) which also provides an increased potential for nuclei acid biosynthesis.

3.3. *Glutamine PRPP amidotransferase activity in rat kidney tumors*

The increased amidotransferase activity is not restricted to liver tumors, since we also observed in two series of transplantable rat kidney tumors of different growth rates that this enzyme activity was elevated 2- to 3-fold over that of normal kidney. Increased amidotransferase activity was also reported in the spleen of Friend leukemia-bearing mice [6]. In addition to the presence of an increased enzyme concentration in the hepatomas, we provide evidence that the increased enzyme activities at PRPP concentrations that may approach the physiological levels are much less sensitive to normal feedback inhibition exerted by AMP. Since the level of AMP is in the range of 0.3 to 0.5 mM in these hepatomas (7), it is likely that the hepatoma enzyme under in vivo circumstances is deinhibited from the influence of AMP. Work is in progress to elucidate whether the decreased sensitivity to feedback inhibition by AMP was due to an alteration in the hepatoma amidotransferase enzyme protein or the hepatoma enzyme is an isozyme different from that contained in normal liver.

4. Conclusions

Glutamine phosphoribosylpyrophosphate (PRPP) amidotransferase (EC 2.4.2.14) was increased 2- to 3-fold in all examined hepatomas irrespective to the tumor growth rate and the degrees of histological differentiation. Amidotransferase in rapidly growing hepatomas was much less sensitive to inhibitory action of AMP (K_i = 1.5 mM) than the enzyme from normal liver (K_i = 0.1 mM). Since reprogramming of gene expression, as manifested in the increased activity of this key purine-synthesizing enzyme, was present only in hepatomas and not in rapidly growing differentiating or regenerating liver, this increased enzyme activity appears to be specific to the neoplastic transformation.

Much of the previous work in the hepatoma spectrum emphasized the important alterations that are linked with the increase in tumor growth rate. These malignant progression-linked alterations in gene expression include the increase in the activities of key glycolytic, pyrimidine and DNA synthesizing enzymes [8–12]. Concurrently, there was a progressive decrease in the activities of key enzymes of gluconeogenesis, pyrimidine catabolism and the urea cycle [13–15]. In addition to this progression-linked metabolic imbalance we now provide new observations indicating that the reprogramming of gene expression in the cancer cells entails transformation-linked alterations such as we report for the marked increase in activity of the key purine-synthesizing enzyme, glutamine PRPP amidotransferase. Since the tumors contain more amidotransferase activity and the enzyme is less sensitive to physiological regulation, this reprogramming of gene expression should confer a selective biological advantage to the cancer cells.

Acknowledgement

The research work outlined in this paper was supported by grants from the United States Public Health Service, National Cancer Institute, Grant Nos. CA-05034 and CA-13526 and from the Damon Runyon-Walter Winchell Cancer Fund.

We thank Miss Coleen Higgings and Miss Maureen Gordon for excellent technical assistance.

References

[1] Caskey, C. T., Ashton, D. M. and Wyngaarden, J. B. (1964) J. Biol. Chem. 239, 2570–2579.
[2] Weber, G. (1974) in: The Molecular Biology of Cancer (Busch, H., ed.) pp. 487–521, Academic Press, New York.
[3] Morris, H. P. and Wagner, B. P. (1968) in: Methods in Cancer Research (Busch, H., ed.) Vol. 4, pp. 125–152, Academic Press, New York.

[4] Katunuma, N., Katsunuma, T., Towatari, T. and Tomino, I. (1973) in: The Enzymes of Glutamine Metabolism (Prusiner, S. and Stadtman, E. R., eds.) pp. 227–275, Academic Press, New York.
[5] Weber, G., Trevisani, A. and Heinrich, P. C. (1974) Adv. Enzyme Regul. 12, 11–41.
[6] Friend, C. and Reem, G. H. (1967) Science 157, 1203–1205.
[7] Weber, G., Stubbs, M. and Morris, H. P. (1971) Cancer Res. 31, 2177–2183.
[8] Weber, G., Queener, S. F. and Ferdinandus, J. A. (1971) Adv. Enzyme Regul. 9, 63–95.
[9] Lo, C., Cristofalo, V. J., Morris, H. P. and Weinhouse, S. (1968) Cancer Res. 28, 1–10.
[10] Sweeney, M. J., Parton, J. W. and Hoffman, D. H. (1974) Adv. Enzyme Regul. 12, 385–396.
[11] Elford, H. L., Freese, M., Passamani, E. and Morris, H. P. (1970) J. Biol. Chem. 245, 5228–5233.
[12] Ove, P., Laszlo, J., Jenkins, M. D. and Morris, H. P. (1969) Cancer Res. 29, 1557–1561.
[13] Weber, G. and Lea, M. A. (1967) in: Methods in Cancer Research (Busch, H., ed.) Vol. 2, pp. 523–578, Academic Press, New York.
[14] Queener, S. F., Morris, H. P. and Weber, G. (1971) Cancer Res. 31, 1004–1009.
[15] Weber, G., Queener, S. F. and Morris, H. P. (1971) Cancer Res. 31, 1933–1940.