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Imbalance of Purine Metabolism in Hepatomas of Different Growth Rates as Expressed in Behavior of Glutamine-Phosphoribosylpyrophosphate Amidotransferase (Amidophosphoribosyltransferase, EC 2.4.2.14)

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SUMMARY

The behavior of glutamine-phosphoribosylpyrophosphate amidotransferase (amidophosphoribosyltransferase, EC 2.4.2.14) was determined in normal, differentiating, and regenerating liver and in a spectrum of hepatomas of widely different growth rates. The liver and tumor enzymes were measured in 100,000 × *g* supernatants prepared from 20% tissue homogenates containing 0.25 M sucrose and 1 mM $MgCl_2$.

Kinetic studies were carried out on the amidotransferase in the crude supernatant from liver and rapidly growing hepatoma 3924A so that under optimum standard assay conditions only the enzyme amount would be the limiting factor. The kinetic results showed that certain properties of the amidotransferase from liver and hepatoma were similar. The liver and hepatoma enzyme exhibited apparent K_m's for: glutamine, 1.7 and 2.3 mM; $MgCl_2$, 0.7 and 1.1 mM, and phosphoribosylpyrophosphate, $S_{0.5}$ for 0.9 and 0.4 mM, respectively. The liver amidotransferase showed a sigmoid curve for phosphoribosylpyrophosphate, but under the same conditions the behavior of the hepatoma amidotransferase was similar to Michaelis-Menten kinetics. The liver and hepatoma enzymes exhibited a pH optimum at approximately 6.5 to 7.2. However, the plateau for liver was broad, but for the tumor it was sharp. Neither enzyme had activity under pH 4.8. At and above pH 9.0 the hepatoma exhibited no amidotransferase activity; however, the liver enzyme retained approximately 50% of its maximal activity. A standard assay was developed for liver and hepatoma in which good proportionality was achieved over a 120-min incubation period and with various amounts of enzyme added.

In a spectrum of hepatomas, where the growth rate of the various tumor lines was between 12.4 and 0.5 months, the amidotransferase activity in the average cell was significantly increased in all neoplasms to approximately 175 to 256% of the values observed in the normal liver of control rats of the same strain, sex, age, and weight.

In the regenerating liver the amidotransferase activity in the average cell was significantly increased at 24 (142%), 48 (181%), and 72 (124%) hr after partial hepatectomy above the values observed in the sham-operated control liver. The activity returned to normal 96 hr after operation. The enzyme activity did not change in the sham-operated control livers. In postnatal differentiation the amidotransferase activity in the average liver cell at 1, 6, 18, and 25 days of age was 45, 46, 50, and 59%, respectively, of the activity observed in the liver of the normal adult rat.

Since the tumor amidotransferase was increased in activity, it exhibited more favorable kinetics for phosphoribosylpyrophosphate and was less inhibited by the physiological feedback inhibitor, adenosine 5'-monophosphate; these changes in the activity and molecular properties of this key purine-synthesizing enzyme should provide an increase in the capacity of the purine-synthetic pathway. These alterations in gene expression, which are manifested in the behavior of amidotransferase in the hepatoma, should confer selective advantages to the neoplastic cells.

INTRODUCTION

Previous studies carried out in this laboratory and other centers demonstrated that in the spectrum of hepatomas of different growth rates there exists a profound imbalance in the activities of certain key enzymes involved in carbohydrate, pentose phosphate, pyrimidine, DNA, ornithine, and membrane cyclic adenosine 3':5'-monophosphate metabolism (4, 5, 16, 18, 20, 22, 23, 26). A number of alterations indicating reprogramming of gene expression correlated with the growth rate of the neoplasms (for reviews, see Refs. 19 and 20). The molecular correlation concept that is the conceptual and experimental approach in this laboratory

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grouped these alterations in Class 1, discriminants that appear to be linked with the degrees in the expression of malignant properties in the hepatoma spectrum (19, 20). In addition to these cancer-linked discriminants, there are alterations in gene expression that appear to be linked with the malignant transformation *per se*, since they occur in all hepatomas, even in the slowest growing, most differentiated neoplasms (19, 20). These transformation-linked discriminants (Class 2) include increased activity of key enzymes involved in channeling hexoses into pentose phosphate biosynthesis through the oxidative pathway (glucose-6-P[5] dehydrogenase, EC 1.1.1.49) and the nonoxidative pathway [transaldolase, EC 2.2.1.2] (22). These alterations are in line with observations that indicate an increased potential for pentose phosphate formation in the hepatomas (17). Ribose-5-P is channeled into purine biosynthesis by PRPP synthetase (EC 2.7.6.1), the activity of which is increased in the rapidly growing liver tumors (8).

The present work reports that amidotransferase, the 1st enzyme committed to *de novo* purine biosynthesis, is increased in all hepatomas.

MATERIALS AND METHODS

Adult male or pregnant female rats were housed in individual cages in air-conditioned rooms that were illuminated daily from 6 a.m. to 7 p.m. Treatment of the litters is given below. Purina laboratory chow and water were available *ad libitum*. All rats were killed between 9 and 10 a.m. Examination of stomach contents at autopsy confirmed that rats were well fed during the night before death.

Tumor-bearing and Control Animals

The hepatomas of different growth rates were carried in the inbred strains of Buffalo or ACI/N rats. The liver tumors were transplanted s.c. bilaterally. Normal rats of the same strain, sex, age, and weight were killed along with the tumor-bearing rats under the same experimental circumstances. We examined a number of tumor lines, including the very slowly growing, medium-growing, and very rapidly growing hepatomas. The neoplasms were harvested when they reached a diameter of about 1.5 cm. The biological and growth properties (15), the biochemical techniques for quantitating growth rate (20), and the conceptual and experimental approaches of the molecular correlation concept (19, 20) that were used in this study were outlined elsewhere.

[5] The abbreviations used are: glucose-6-P, glucose 6-phosphate; ribose-5-P, ribose 5-phosphate; amidotransferase, glutamine PRPP amidotransferase (amidophosphoribosyltransferase, EC 2.4.2.14); PRPP, phosphoribosylpyrophosphate.

Investigations on Regenerating Liver

For investigation on the effects of partial hepatectomy and sham operation, ACI/N rats were purchased from Laboratory Supply Co., Indianapolis, Ind. The rats, weighing 180 to 200 g, were partially hepatectomized under light ether anesthesia by removal of 66% of the liver according to the standard procedure of Higgins and Anderson (9). Sham-operated animals were used as controls. The animals were killed 12, 24, 48, 72, and 96 hr after operation.

Studies on Differentiating Liver

To investigate the expression of the genetic program during differentiation pregnant Wistar rats were purchased from Harlan Industries, Cumberland, Ind. The litters were allowed to stay in the same cage with the mothers for 18 days after birth; then they were placed in individual cages.

Experimental Procedures, Biochemical Assays, and Cell Counts

The rats were stunned, decapitated, and exsanguinated. Tumors and livers were quickly excised and placed in beakers imbedded in crushed ice. The neoplasms were dissected free of hemorrhagic, necrotic, and nontumorous material. The careful selection of only the viable tumor tissue parts is important in ensuring the comparability of tumors of different age groups and different tumor lines.

Assay of Amidotransferase Activity. For measuring this enzyme 20% homogenates were prepared from normal liver and hepatoma tissues in 0.25 M sucrose containing 1 mM $MgCl_2$ (13). The homogenate was centrifuged at 100,000 × *g* for 30 min at 3° in a Beckman Model L5-50 preparative centrifuge. The resulting clear supernatant was used for the assays.

For determination of amidotransferase activity, a standard enzyme assay system was worked out which was an adaptation of the technique of Katunuma *et al.* (12) to the kinetic conditions of the rat liver and hepatoma systems. The 1-ml reaction mixture contained in final concentrations: PRPP, 5 mM; glutamine, 20 mM; $MgCl_2$, 15 mM; Tris-HCl buffer, 50 mM; pH, 7.2; KF, 1.0 mM; and 8 mg liver or 2.4 mg hepatoma protein. The reactions were carried out at 37° and they were stopped at 0, 30, and 45 min of incubation by boiling for 5 min. The tubes were centrifuged and in the clear supernatant the concentration of glutamic acid, which had formed in the 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 Model 2400-S recording spectrophotometer. The blanks contained the identical reaction mixture without PRPP, and the activity was recorded simultaneously. The enzyme activity was provided by the difference between the rate observed in the full reaction mixture and that of the blank which contained no added PRPP. Under these experimental conditions no ammonia was liberated from

glutamine, indicating that glutaminase activity did not interfere with this assay. Through careful studies the various kinetic constants were established for the crude enzyme in liver and hepatomas.

Cell Counts and Protein Determinations. The cell counts from liver and hepatomas were prepared as cited previously (20). Protein concentration was assayed by biuret reaction (7), with a standard curve using bovine serum albumin.

Expression and Evaluation of Results. Amidotransferase activity was calculated in μmoles of glutamate formed per hr; per g, wet weight, of tissue; per average cell; or per mg protein (specific activity). The cell counts were expressed as cellularity calculated in millions of nuclei per g, wet weight, of tissue. The activities per cell or per mg protein are to be multiplied by the exponentials given to arrive at the actual values.

The results were subjected to statistical evaluation by means of the *t* test for small samples. Differences between means giving a probability of less than 5% were considered to be significant.

RESULTS

Comparison of Kinetic Conditions of Amidotransferase in Hepatoma and in Liver

To establish that linear kinetics operate in the crude system used in the amidotransferase determination and that under the assay conditions the enzyme activity measured reflected the enzyme concentration, the properties of the amidotransferase were compared in extracts from normal rat liver (ACI/N strain) and the rapidly growing hepatoma 3924A (carried in ACI/N rats).

Effect of Glutamine Concentration. Chart 1 shows the effect of glutamine concentration on amidotransferase activity in normal rat liver and rapidly growing hepatoma 3924A. The enzyme activity in both tissues was saturated at a glutamine concentration of approximately 20 mM, and it was not inhibited by excess levels of glutamine up to 40 mM. The affinity of amidotransferase activity to glutamine for liver and hepatoma 3924A yielded an apparent K_m of 1.7 and 2.3 mM, respectively.

Effect of Magnesium Concentration. The amidotransferase activity of both normal and neoplastic liver was saturated at a $MgCl_2$ concentration of about 10 mM and was not inhibited by excess levels of $MgCl_2$ up to concentrations of 20 mM. The affinity of amidotransferase activity to $MgCl_2$ for liver and hepatoma yielded apparent K_m's of 0.7 and 1.1 mM, respectively.

Effect of PRPP Concentration. The effect of PRPP on liver and hepatoma amidotransferase activity is compared in Chart 2. The enzymatic activity of both normal and neoplastic liver was saturated at 2 mM PRPP concentration. The substrate curve for the liver appeared to yield sigmoid kinetics, whereas the one for the hepatoma seemed to follow Michaelis-Menten kinetics. These observations were made at optimum concentration of all other reactants, varying only the PRPP levels and stopping the enzyme assay at 0,

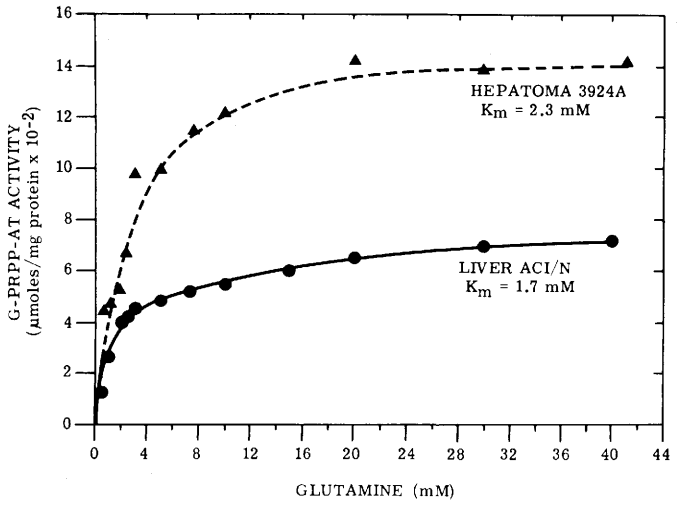

Chart 1. Comparison of the effect of glutamine concentration on activity of amidotransferase in liver and hepatoma 3924A. The standard assay described in "Materials and Methods" was used for both tissues, varying only the glutamine concentration. For the normal liver and hepatoma, the assay was carried out on 20% supernatant fluid (0.4 and 0.2 ml, respectively). The standard assay concentration of glutamine is 20 mM. Enzyme activity was calculated as μmoles/hr/mg protein $\times 10^{-2}$. The activities are to be multiplied by the exponential given to arrive at the actual values. *G-PRPP-AT*, amidotransferase.

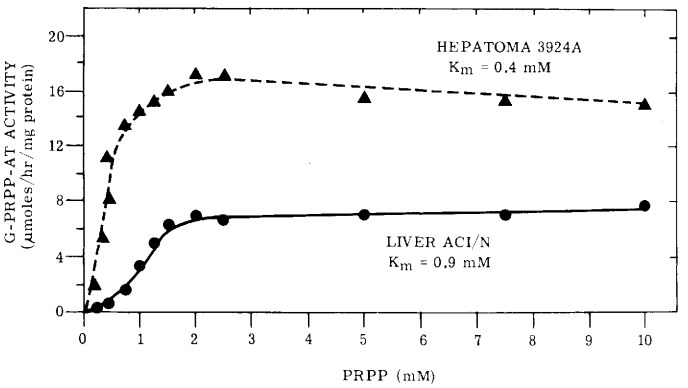

Chart 2. Comparison of the effect of PRPP concentration on activity of amidotransferase in liver and hepatoma 3924A. The assay conditions were as described in "Materials and Methods," varying only the PRPP concentration. Enzyme concentration levels used were as in Chart 1. The standard assay concentration of PRPP is 5 mM. Enzyme activity was calculated as μmoles/hr/mg protein $\times 10^{-2}$. The activities are to be multiplied by the exponential given to arrive at the actual values.

30, and 45 min of incubation. To ascertain the behavior of the saturation curves at initial enzyme velocity concentrations, experiments were conducted in assay systems where the reaction was stopped at 15 min incubation, this being the shortest incubation period when the absorbance differences between blank and experimental systems were large enough to be reliable and easily repeatable. Under such initial velocity recording conditions, the pattern of affinity curves was similar to the one given in Chart 2. The affinity of amidotransferase activity to PRPP for liver and hepatoma yielded $S_{0.5}$ of 0.9 and 0.4 mM, respectively.

Effect of pH. Both liver and hepatoma amidotransferase

exhibited a pH optimum at approximately 6.5 to 7.2 (Chart 3). The plateau for the liver was broad and for the hepatoma it was a sharp one, indicating a pH optimum for the hepatoma around 7.0. Neither enzyme had any activity under pH 4.8. The hepatoma exhibited no activity at and above pH 9.0, but the liver enzyme still retained approximately 50% of its maximal activity.

Through systematic kinetic studies a standard assay was established for determination of liver and hepatoma amidotransferase activities. In the standard assay, good proportionality was achieved with length of reaction time over a 120-min incubation period (not shown) and with amount of enzyme added (Chart 4). The standard assays of liver and hepatomas were carried out at pH 7.2 at 37° with the optimum reaction mixture conditions given in "Materials and Methods."

Amidotransferase Activity in Normal Liver and in Hepatomas of Different Growth Rates (Table 1). For examination of the behavior of this enzyme in tumors with as wide a range of growth rate as possible, the activity was studied in the spectrum of liver neoplasms, which varied from 12.4 to 0.5 month in reaching 1.5 cm in diameter. The protein concentration in the 100,000 × *g* supernatant in normal liver varied between 92.4 and 114.0 mg/g, wet weight, of tissue. In hepatomas the protein content was decreased significantly, with the exception of 1 tumor. The amidotransferase activity in the normal liver varied from 5.1 to 7.1 μmoles/hr/g, wet weight, but the activity was significantly increased in all liver neoplasms irrespective of their growth rate. The activity in hepatomas was increased on a per g basis to 168 to 248% and on a per cell basis to 175 to 256% of the values observed in the normal liver of control rats. The tumor amidotransferase activity per mg protein (specific activity) was also increased to 218 to 309% of the values in normal rats (not shown).

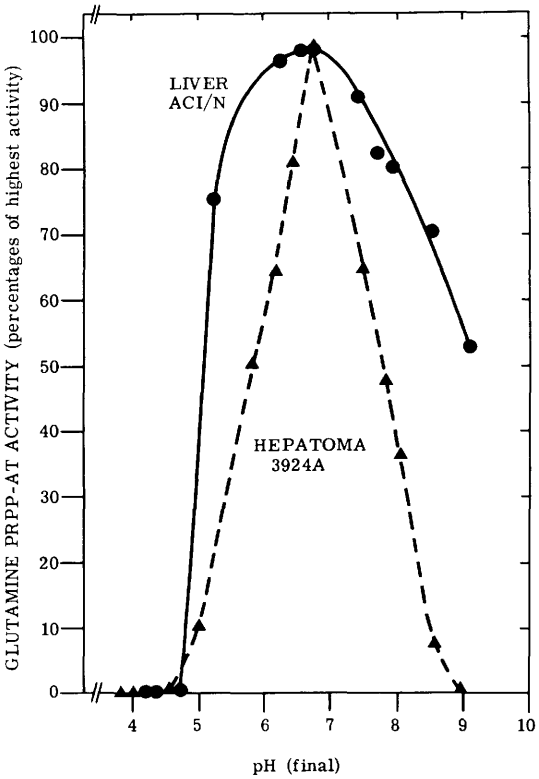

Chart 3. Comparison of the effect of pH on amidotransferase activity in liver and hepatoma 3924A. The standard assay was performed as described in Chart 1, varying only the pH of the reaction mixture. Acetate buffer was used for pH 4.0 through 6.0; above this value Tris buffer was used. The final pH of the reaction mixture is given.

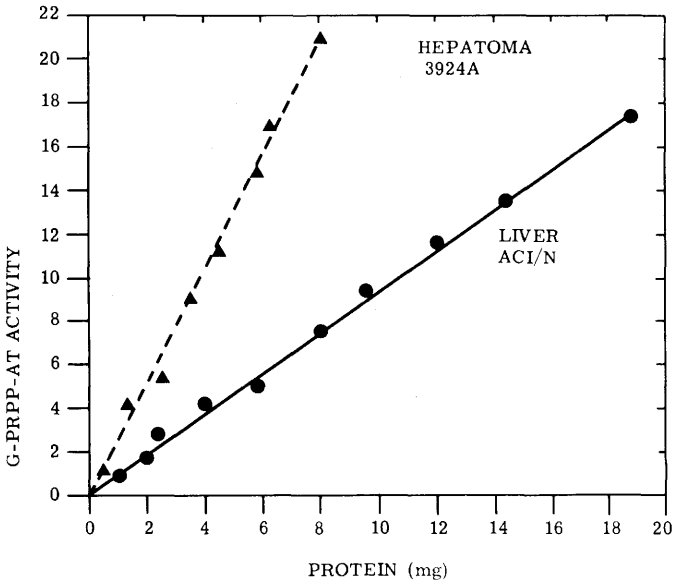

Chart 4. Proportionality of amidotransferase activity with amount of liver and hepatoma 3924A added. The standard assay described in "Materials and Methods" was used, varying only the amount of supernatant. The activity given in the *ordinate* is to be multiplied by 5×10^{-2} to express enzyme activity in μmoles/hr/mg protein. The standard enzyme assays had 0.4 and 0.2 ml supernatant fluid, containing 8.0 mg liver or 2.4 mg hepatoma protein in the final 1-ml reaction mixture.

Amidotransferase Activity in Regenerating Liver (Table 2). Sham operation had no effect on enzyme activity or protein concentration, but amidotransferase activity in the average cell was markedly increased in the regenerating liver. The activity increased little at 12 hr after operation; however, at 24 hr it was 142%, at 48 hr it was 181%, and at 72 hr it was 124% of the activity of the sham-operated control livers. After having reached a peak at 48 hr the activity returned to normal by 96 hr. The results were similar when enzyme activity was expressed on a protein basis (not shown).

Amidotransferase Activity in Differentiating Liver. Since the amidotransferase activity was markedly increased in all hepatomas and in the rapidly growing regenerating liver, it was of interest to examine the enzyme activity in the rapidly growing differentiating liver. In postnatal differentiation of the rat, the liver amidotransferase activity on a wet weight basis was high; however, when the liver cellularity was taken into consideration, the activity per cell in the liver of the newborn rat was 45% and in the 6-, 18-, and 25-day-old animals activities were 46, 50, and 59%, respectively, of that observed in the liver of normal adult rat.

Table 1

Amidotransferase activity in hepatomas of different growth rates

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 activities per cell are to be multiplied by the exponential given to arrive at the actual values. Assay conditions are described in "Materials and Methods." Growth rate is expressed as the mean transplantation time given in months between inoculation and growth to a size of approximately 1.5 cm diameter.

Tissues	Transplant generations	Growth rates (mos.)	Protein concentration (mg/g)	Amidotransferase activity (μmoles/hr)	
				Per g	Per cell × 10^{-8}
Normal livers (Buffalo)					
Control for 9618A			100.2 ± 5.3	5.2 ± 1.0	2.4 ± 0.3
Control for 20			98.9 ± 10.0	5.9 ± 0.3	2.8 ± 0.2
Control for 47C			114.0 ± 2.3	7.1 ± 0.4	3.2 ± 0.1
Control for 8999			99.7 ± 5.8	6.4 ± 0.4	3.0 ± 0.1
Control for 44			93.9 ± 7.6	7.0 ± 0.3	3.2 ± 0.1
Control for 7777			98.7 ± 3.3	6.1 ± 0.5	3.1 ± 0.2
Normal livers (ACI/N)					
Control for 3924A			92.4 ± 2.7	6.5 ± 0.3	2.9 ± 0.1
Control for 9618A2			108.0 ± 5.4	5.1 ± 0.2	2.6 ± 0.1
Control for 3683F			105.0 ± 2.7	5.5 ± 0.4	2.8 ± 0.2
Hepatomas					
9618A	8 (3)	12.4	74.0 ± 0.9 (74)[a]	8.8 ± 1.0 (168)[a]	4.2 ± 0.3 (175)[a]
20	6	11.6	84.1 ± 5.1 (73)[a]	10.9 ± 0.2 (185)[a]	5.5 ± 0.1 (196)[a]
47C	11	7.0	75.8 ± 3.5 (66)[a]	12.8 ± 0.4 (181)[a]	6.6 ± 0.1 (206)[a]
8999	17	6.3	76.4 ± 2.4 (76)[a]	15.9 ± 0.9 (248)[a]	7.2 ± 0.2 (240)[a]
44	17	5.4	84.4 ± 8.5 (89)	16.9 ± 1.8 (242)[a]	8.5 ± 0.3 (256)[a]
7777	111	1.0	76.6 ± 3.3 (78)[a]	14.6 ± 0.5 (239)[a]	7.3 ± 0.2 (239)[a]
3924A	319 (2)	0.9	57.9 ± 1.4 (63)[a]	11.2 ± 0.6 (180)[a]	6.2 ± 0.2 (221)[a]
9618A2	71	0.6	71.8 ± 2.4 (66)[a]	10.5 ± 0.7 (208)[a]	6.4 ± 0.3 (247)[a]
3683F	524	0.5	74.8 ± 1.7 (71)[a]	11.0 ± 0.8 (202)[a]	5.8 ± 0.3 (212)[a]

[a] Values are statistically significantly different from the respective controls ($p < 0.05$).

Table 2

Behavior of amidotransferase activity in regenerating liver

Means ± S.E. of experiments from 4 or more rats are given in the various groups with percentages of values of corresponding control sham-operated rat liver in parentheses. The activities per cell are to be multiplied by the exponential given to arrive at the actual values. Assay conditions are described in "Materials and Methods."

Time after operation (hr)	Sham-operated liver			Regenerating liver		
	Protein concentration	Amidotransferase activity (μmoles/hr)		Protein concentration	Amidotransferase activity (μmoles/hr)	
	mg/g	Per g	Per cell × 10^{-8}	mg/g	Per g	Per cell × 10^{-8}
12	90.8 ± 2.2 (100)	5.3 ± 0.2 (100)	2.1 ± 0.1 (100)	85.7 ± 2.5 (94)	5.7 ± 0.4 (107)	2.5 ± 0.2 (119)
24	100.2 ± 3.4 (100)	5.8 ± 0.1 (100)	2.4 ± 0.1 (100)	95.2 ± 2.9 (95)	8.3 ± 0.1 (143)[a]	3.4 ± 0.1 (142)[a]
48	89.5 ± 2.2 (100)	5.1 ± 0.2 (100)	2.2 ± 0.1 (100)	80.0 ± 2.5 (89)[a]	7.7 ± 0.2 (150)[a]	4.0 ± 0.1 (181)[a]
72	97.3 ± 2.1 (100)	5.5 ± 0.2 (100)	2.5 ± 0.1 (100)	92.5 ± 2.9 (99)	7.6 ± 0.4 (138)[a]	3.1 ± 0.2 (124)[a]
96	82.0 ± 1.7 (100)	6.2 ± 0.2 (100)	2.5 ± 0.0 (100)	82.9 ± 2.9 (101)	6.0 ± 0.2 (97)	2.5 ± 0.1 (100)

[a] Values are statistically significantly different from the respective controls ($p < 0.05$).

DISCUSSION

Comparison of Kinetic Parameters of Liver and Hepatoma Amidotransferase Activity

The kinetic studies showed the applicability of the liver enzyme assay to hepatomas under the circumstances of the present investigation. The apparent K_m's were similar in liver of control normal rats and in the rapidly growing liver tumors. Current studies indicate that the affinity constants of the highly purified liver and hepatoma 3924A enzymes are similar to those observed in the crude assay system.[6] For glutamine both enzymes are half-saturated at approximately 1.7 to 2.3 mM. This is in line with observations on purified rat liver and human lymphoblast amidotransferases (2, 27). Since the glutamine content of liver is 4.68 mM (1), the amidotransferase in both liver and hepatoma may well be half-saturated by the substrate.

[6] M. Tsuda, N. Katunuma, and G. Weber, to be published.

The sigmoid kinetics for PRPP of the normal and regenerating liver observed in the standard enzyme assay system (45-min incubation) were also found when initial rates were measured at 15 min. The S-shaped curve for PRPP of the liver enzyme is in agreement with other reports (10, 27). The kinetics of the crude liver enzyme differed from that of the hepatoma enzyme which exhibited behavior more in line with Michaelis-Menten kinetics. Thus, under the same assay conditions the hepatoma enzyme is rapidly saturated with low concentrations of PRPP (Chart 2). Since PRPP concentrations in rodent liver are very low (14), this might mean that at physiological PRPP concentrations the liver amidotransferase would be largely inactive, whereas at the same PRPP levels the hepatoma amidotransferase would be active. Present work in rapidly growing hepatoma 3683F confirmed observations in rapidly growing hepatoma 3924A (13) that the amidotransferase was much less sensitive to inhibitory action of AMP (K_i 2.6 mM) than the normal liver enzyme (K_i = 0.3 mM) and the inhibition was relieved by high levels of PRPP. The kinetic results are to be interpreted with caution as they pertain to those crude systems that are being compared carefully under identical conditions in this study. To elucidate the possible interplay of various factors in crude systems, all kinetic parameters are under current investigation in highly purified liver and hepatoma enzymes.

Selective Biological Advantage That the Increased Amidotransferase Activity Might Confer to the Cancer Cell

Table 1 indicates that the amidotransferase activity was markedly increased in all liver tumors irrespective of rate of growth of the neoplasms. Since in the standard assay amidotransferase activity was proportionate with the enzyme amount, it is assumed that the increased enzyme activity in the hepatomas represents increased enzyme concentration. Current work on immunotitration of the liver and hepatoma enzymes provided an independent line of evidence for demonstrating the presence of an increased amidotransferase concentration in the hepatomas.[6] The increase in the concentration of this key purine-synthesizing enzyme should provide an increased potential for purine biosynthesis. This enzymatic indication is in good agreement with isotopic studies of the behavior of purine metabolism (24).

Another factor important in the assessment of the role of amidotransferase in purine biosynthesis in the cancer cells is the observation that the amidotransferase in rapidly growing hepatomas 3924A and 3683F was less sensitive to the physiological inhibitory action of AMP than the normal liver enzyme and that the inhibition by AMP was relieved in liver and hepatomas by high concentrations of PRPP. The kinetics of this inhibition by AMP was in agreement with reports on avian and rat liver amidotransferase (2, 11, 12, 27). Since the concentration of AMP in liver and hepatomas is 0.3 to 0.4 ml (21), the normal liver amidotransferase at low PRPP concentrations would be in part or completely inhibited. In contrast, the hepatoma amidotransferase under similar *in vivo* circumstances would be released from the influence of AMP because of its decreased sensitivity to this feedback signal.

Since in hepatomas there is more amidotransferase activity, the enzyme has a better affinity to PRPP and it is less sensitive to inhibition by AMP; these alterations in gene expression should increase the purine-synthetic potential and might confer selective advantages to the cancer cells.

Specificity to Neoplasia of the Increase in Amidotransferase Activity. The increased amidotransferase activity was present in all the hepatomas. An increase in this enzyme activity also occurred in regenerating liver, which has a growth rate similar to that of the rapidly growing hepatomas (5). Since amidotransferase activity was increased even in very slowly growing tumors, and in the rapidly growing hepatomas it was more highly increased than in the regenerating liver, the elevated activity of this enzyme in the hepatomas does not reflect the increase in growth rate alone, but it appears to be characteristic to the neoplastic proliferation. The increased amidotransferase activity in the hepatomas might be used as a quantitative discriminant of neoplasia (19). In this it resembles the amplified increase observed in the rapidly growing hepatomas as compared to the elevations in the regenerating liver for ribonucleotide reductase, DNA polymerase, thymidine kinase, dTMP kinase, dTMP synthase, dCMP deaminase, and the increase in the incorporation of thymidine to DNA (for review, see Ref. 20). The other 3 enzymes that are increased in all the hepatomas (glucose-6-P dehydrogenase, transaldolase, and UDP kinase) were not altered in the regenerating liver. The amidotransferase activity in rapidly growing differentiating liver was lower than in the liver of adult normal rats.

General Applicability of Reprogramming of Gene Expression as Manifested in Increased Amidotransferase Activity. The amidotransferase activity was increased in 9 different lines of transplantable rodent hepatomas (Table 1). We also observed increased amidotransferase activity in 2 cases of human primary hepatomas and in 2 different types of kidney tumors in the rat.[7] Increased amidotransferase activity was reported in the spleen of mice bearing Friend leukemia (6). Further work is required to ascertain whether an increased amidotransferase activity is an integral part of neoplastic transformation in all types of tumors.

Integrated Pattern of Imbalance in Purine Metabolism in Hepatomas. There is enzymatic and isotopic evidence for the operation of an imbalance in the opposing pathways of purine synthesis and degradation. Studies in this laboratory, entailing determination in the hepatoma spectrum of the key enzymes of the oxidative and nonoxidative pathways of ribose-5-P production [glucose-6-P dehydrogenase, transaldolase (22)] and the isotope exploration of these pathways (17), demonstrated that there was also an increased potential for the production of ribose-5-P. With the discovery of increased PRPP synthetase activity (8) it became of interest to elucidate the behavior of the enzymes that utilize PRPP for *de novo* purine biosynthesis (amidotransferase) and

[7] N. Prajda, H. P. Morris, and G. Weber, to be published.

through the salvage or recycling pathway. The present results demonstrated that amidotransferase activity was increased in all hepatomas. The importance to neoplasia of the *de novo* purine-synthetic pathway is further underlined by the antineoplastic action of the glutamine analog, azaserine.

On the basis of the behavior of the enzymes involved in purine catabolism, the potential of the catabolic pathway should be markedly increased in the rapidly growing liver tumors (3, 24). The 5′-nucleotidase was markedly decreased in the rapidly growing hepatoma 3924A.[8] Current work showed that in 9 Morris hepatomas of different growth rates xanthine oxidase activity was markedly decreased.[7] This enzyme was also very low in the rapidly growing Novikoff hepatoma (3). Isotope results (24) are in good agreement with these enzymatic indications. Thus, the integrated features of purine metabolism indicate a reprogramming of gene expression resulting in a metabolic imbalance where the synthetic pathway of purine biosynthesis should predominate over the degradative pathway.

Biological Significance of Malignant Transformation-linked Alterations in Gene Expression. Investigations carried out with the molecular correlation concept as a conceptual and experimental approach resulted in gaining a degree of insight into the biochemical strategy of the cancer cell (19, 20). Our demonstration of high amidotransferase activity present in all hepatomas, irrespective of the degree of cancer, suggests that the reprogramming of gene expression in the malignant transformation is linked with an increase in the expression of the activity of this key purine-synthesizing enzyme. In this laboratory recently there were discovered 4 transformation-linked increases in enzyme activities, and they all heightened the potential for the channeling of precursors to strategic biosynthetic processes (22, 25). Thus, the increased glucose-6-P dehydrogenase and transaldolase activities enhance the potential for routing glycolytic metabolites into pentose phosphate biosynthesis (22). The increased UDP kinase (EC 2.7.4.6) activity (25) should yield an enhanced potential for UTP and consequently for RNA and DNA biosynthesis. The presently reported increased amidotransferase activity should provide an increased potential for purine biosynthesis. These transformation-linked increases in the activities of the key enzymes of ribose-5-P biosynthesis, purine production, and UTP biosynthesis indicate a reprogramming of gene expression that should confer selective biological advantages to the neoplastic cells.

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