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GUANOSINE-5′-PHOSPHATE SYNTHETASE AND GUANOSINE-5′-PHOSPHATE KINASE IN RAT HEPATOMAS AND KIDNEY TUMORS

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Summary

The behavior of the activities of GMP synthetase (xanthosine-5′-phosphate: L-glutamine amido-ligase(AMP-forming),EC 6.3.5.2) and GMP kinase (ATP: (d)GMP phosphotransferase,EC 2.7.4.8) was elucidated in cytosol preparations of rat tissues, including fetal, neonatal and regenerating liver, in a transplantable kidney tumor, and in a spectrum of 11 hepatomas of different growth rates. GMP kinase activity was 60-fold or more higher than GMP synthetase activity in all of the examined tissues. GMP synthetase activity was increased in all hepatomas and in the kidney tumor, compared to control tissues, reaching 5.5-fold the normal liver values in the most rapidly growing hepatoma. This increase correlated with the tumor growth rates. GMP kinase activity showed no consistent pattern of alteration in the tumors. In both fetal and neonatal rat liver the activity of GMP synthetase was 2.5-times higher than in livers of adult rats, but GMP kinase activity did not change markedly during liver development. After partial hepatectomy GMP synthetase activity was elevated, reaching a peak of 155% of the sham-operated control values by 36 h after the operation. GMP kinase activity was not affected by partial hepatectomy. After 3 days starvation hepatic GMP kinase activity decreased slightly faster than total cytosol protein, while GMP synthetase activity was preferentially maintained. These results indicate that GMP synthetase activity was linked with cellular proliferation in differentiating, regenerating and neoplastic tissues.

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Introduction

In normal rat liver most of the IMP produced by the de novo and salvage pathways of purine nucleotide biosynthesis appears to be utilized for biosynthesis of adenine nucleotides, which are required as nucleic acid precursors and as cofactors for energy metabolism. In ischemia the adenine nucleotides may be recycled to IMP, to maintain a high adenylate charge [1—4]. IMP is also the metabolic precursor for guanine nucleotides, and in rapid cell proliferation, such as embryonic development, liver regeneration after partial hepatectomy or neoplastic transformation, the activity of IMP dehydrogenase (IMP: NAD^+ oxidoreductase,EC 1.2.1.14), the rate-limiting enzyme of the guanine nucleotide biosynthetic pathway, is increased [5,6], perhaps reflecting a need for approx. equal amounts of guanine nucleotides and adenine nucleotides when the rate of nucleic acid biosynthesis is greatly accelerated. There are important differences between the enzyme pattern observed in neoplasia, and in the non-malignant conditions of development and regeneration. Activities of the enzymes of the purine ribonucleotide cycle, adenylosuccinate synthetase (IMP: L-aspartate ligase (GDP-forming), EC 6.3.4.4), adenylosuccinate lyase (adenylosuccinate AMP-lyase,EC 4.3.2.2), and adenylate deaminase (AMP aminohydrolase,EC 3.5.4.6) showed no change during neonatal development or hepatic regeneration, but were increased in neoplasia [7—9]. IMP dehydrogenase activity was markedly increased in even the most slowly growing hepatomas, and correlated positively with tumor growth rate [5,6]. The final enzyme leading to GTP, GDP kinase (an activity of nucleoside diphosphokinase, EC 2.7.4.6) also had elevated activity in hepatomas [10]. The relationships of these enzymes are summarized in Fig. 1.

Clearly, for a complete understanding of the changes in control of guanine nucleotide biosynthesis in malignant tissue, it was necessary to clarify the behavior of GMP synthetase (xanthosine-5′-phosphate:L-glutamine amido-

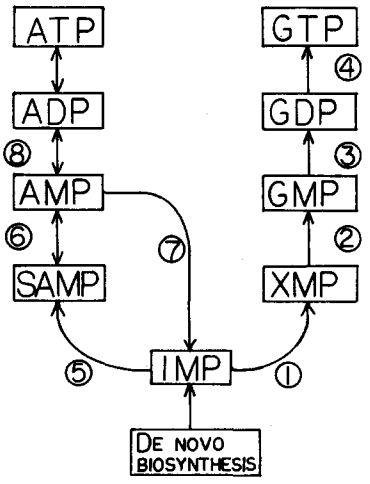

Fig. 1. Purine ribonucleotide interconversions. The enzymes are: (1) IMP dehydrogenase; (2) GMP synthetase; (3) GMP kinase; (4) nucleoside diphosphate kinase; (5) adenylosuccinate synthetase; (6) adenylosuccinase; (7) AMP deaminase and (8) AMP kinase. Abbreviation: SAMP, adenylosuccinate.

lyase (AMP-forming), EC 6.3.5.2) and GMP kinase (ATP:(d)GMP phosphotransferase, EC 2.7.4.8) in malignant tissues. GMP synthetase catalyzes the reaction:

XMP + ATP + glutamine → GMP + AMP + PP_i + glutamate

Some properties of GMP synthetase from Ehrlich ascites cells have been described [11,12].

GMP is phosphorylated to GDP by GMP kinase:

GMP + ATP → GDP + ADP

This enzyme is specific for monophosphates of guanosine, deoxyguanosine and some closely related synthetic analogues. It has been studied in pig brain [13], rat liver [14] and human erythrocytes [15]. The present report describes the distribution of these two enzymes in normal rat tissues, and presents the first study of their activities in regenerating and developing rat liver, and in a series of transplantable rat hepatomas of widely different growth rates. Results of measurements of GMP synthetase and GMP kinase are also given for rat renal cortex and a transplantable rat renal cell carcinoma.

Experimental Procedures

Animals and tissues. Male Wistar rats were used for the tissue distribution and embryonic liver studies. Male ACI/N rats were used for hepatic regeneration experiments, and for the hepatomas 3924A and 3683. Other hepatomas were carried in male Buffalo (BUF) rats, as were the MK-3 kidney tumors. Maintenance of the tumors and preparation of tissues were as previously described [16,19].

Reagents. Coenzymes and substrates were obtained from Sigma Chemical Company, St. Louis, MO. [^{3}H]GMP was purchased from the Amersham Corp., Arlington Heights, IL. [^{3}H]XMP was prepared from [^{3}H]GMP by diazotization [17]; the purity of the product was >99% as assessed by liquid chromatography [4]. Sephacryl S-200 was a product of Pharmacia Inc., Piscataway, NJ, and polyethyleneimine cellulose (PEI) thin layer plates were from Brinkman, Inc., Westbury, NY.

Enzyme assays. GMP synthetase was assayed as follows: a cocktail was prepared containing 1 vol. ATP (40 mM); 1 vol. L-glutamine (40 mM); 2 vol. 1.5 M Tris-chloride buffer (pH 7.6)/200 mM magnesium sulfate; 2 vol. phospho*enol*pyruvate (30 mM); 2 vol. pyruvate kinase from rabbit muscle (100 units/ml) and 6 vol. 8-[^{3}H]XMP (0.5 mM; 0.2 Ci/mmol). Homogenates (25%) were prepared in buffered 0.25 M sucrose, and centrifuged at 105 000 × *g* for 60 min. From this enzyme preparation 15 µl were added to 25 µl of assay cocktail in a microanalytical tube, the contents were mixed by a 10 s centrifugation in a Beckman Microfuge B, then incubated at 37°C for 10 min. Reaction was stopped by boiling for 3 min, then after centrifugation of the tubes, 10 µl supernatant fraction was applied to a thin layer plate of polyethyleneimine cellulose, which had previously been washed in 2 M formic acid and deionized water. Plates were developed for 5 h in 2 M formic acid.

Under these conditions GDP and GTP remained at the origin, and XMP and GMP were cleanly resolved. Spots were located under an ultraviolet lamp, and the XMP, GMP and origin spots were cut out and counted in a liquid scintillation spectrometer. Activity of GMP synthetase was calculated from the amount of radioactivity recovered in GMP, GDP and GTP.

GMP kinase was also assayed isotopically. An assay cocktail was prepared from equal volumes of 8-[^{3}H]GMP (1.2 mM; 4 ci/mmol); potassium chloride (1 M); Tris-acetate buffer, pH 7.4 (1 M); magnesium chloride (0.1 M); ATP (43 mM). 25 μl of the same enzyme preparation as was used for the GMP synthetase assay were added to microanalytical tubes, together with 0.2 ml of assay cocktail and 0.2 ml of distilled water; blank tubes contained buffer instead of enzyme. After a 3 min incubation at 37° C, reaction was stopped by boiling for 3 min. Tubes were centrifuged, and 25-μl aliquots of supernatant fraction were applied to 1-cm squares of PEI-cellulose thin layer plate. These squares were given three successive 5 min washes in 50 ml 0.5 M formic acid/sodium formate, pH 3.4. Squares were dried, and counted in scintillation counters. Counting efficiency was measured by applying known amounts of activity to PEI-cellulose squares and omitting the washing procedure, and under these conditions it averaged 9%.

For kinetic studies a 50-fold purified preparation of GMP kinase was used, obtained by $(NH_4)_2SO_4$ precipitation and calcium phosphate absorption as described by Miech and Parks [14]. GMP synthetase was purified 20-fold from liver cytosol by chromatography on DEAE-Sephadex A-50, followed by $(NH_4)_2SO_4$ precipitation, using the conditions described by Spector et al. [12].

Results

Preliminary tests of the GMP synthetase assay procedure indicated that measured activity was proportionate with the amount of tissue extract used, up to 9-times the standard assay level, and proportionate with time for 20 min. The routine concentrations of the three substrates, XMP, ATP and glutamine, were saturating, and no indication of inhibition by excess substrate was observed. For a 20-fold purified preparation of GMP synthetase from rat liver, which was free of interfering XMP and GMP phosphatase activities, K_m values were 0.11 ± 0.07 mM for $MgATP^{2+}$, 0.21 ± 0.06 mM for glutamine and 4.4 ± 1.2 μM for XMP. The pH optimum was between 7.6 and 7.8. The molecular weight as estimated by gel filtration on Sephacryl S-200 was 83 000 ± 6000. These properties were similar to those previously described for the GMP synthetase from Ehrlich ascites cells [11]. We also purified GMP synthetase 20-fold from the rapidly growing hepatoma 3683. The hepatoma enzyme closely resembled the normal liver enzyme with respect to kinetic parameters, pH optimum and molecular weight.

For GMP kinase, the activity in the routine assay procedure was proportionate with enzyme amount up to 4-times the normal level, and with time for up to 11 min. The standard concentrations of GMP and ATP were in excess of the saturating values. Calculated K_m values for a 50-fold purified preparation of liver GMP kinase were 0.20 ± 0.03 mM for ATP and 11.2 ± 1.8 μM for GMP.

Organ distribution. Table I shows the distribution of the two enzymes in

TABLE I

GMP SYNTHETASE AND GMP KINASE IN RAT TISSUES AND ORGANS

Specific activities are expressed as percentages of the liver values. Means of three or more determinations are shown, followed by S.E.

Tissues	Cytosol protein mg/g wet weight	GMP synthetase	GMP kinase
Testis	36	497 ± 11 *	890 ± 30 *
Brain	26	240 ± 8 *	550 ± 35 *
Bone marrow	20	239 ± 10 *	278 ± 26 *
Intestinal mucosa	46	225 ± 48 *	147 ± 39
Thymus	55	178 ± 7 *	320 ± 37 *
Heart	46	161 ± 7 *	152 ± 25
Liver	87	(100 ± 4)	(100 ± 6)
Spleen	76	94 ± 3	331 ± 35 *
Lung	70	92 ± 3	218 ± 27 *
Skeletal muscle	44	76 ± 2	83 ± 11
Kidney	62	62 ± 5 *	64 ± 22
Adipose tissue	9	6 ± 2 *	267 ± 32 *
Leucocytes	31	5 ± 3 *	36 ± 12 *
Erythrocytes	174	1 ± 1 *	256 ± 24 *
Plasma	50	0	0

* Statistically significant difference from liver activity ($P < 0.05$ in Student's 2-tail t-test).

organs and tissues of normal adult male Wistar rats. The highest activity of both enzymes was in testis and the next richest source of both enzymes was brain. GMP synthetase levels were generally high in such organs of cell renewal as bone marrow, intestinal mucosa and thymus, and low in skeletal muscle, adipose tissue and blood cells. In normal rat liver cytosol preparations the mean specific activity of GMP synthetase was 30.4 ± 1.2 nmol/h per mg protein and for GMP kinase it was 6300 ± 390 nmol/h per mg protein. Activity of GMP

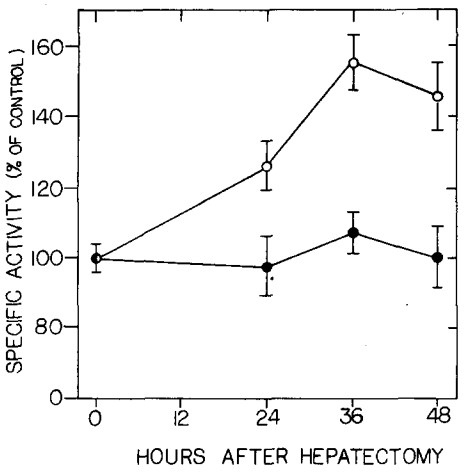

Fig. 2. GMP synthetase (○———○) and GMP kinase (●———●) activities in regenerating rat liver. Partial hepatectomy was performed by the standard method [18]. Points are means of quadruplicate determinations and vertical bars show S.E. GMP synthetase activities in the regenerating liver samples were significantly higher than sham-operated controls at all three time points.

TABLE II

GMP SYNTHETASE AND GMP KINASE ACTIVITY IN FETAL AND NEONATAL RAT LIVER

Values are mean + S.E. for quadruplicate samples. Numbers in parentheses give results as percent of control.

Liver	GMP synthetase	GMP kinase
	(nmol/h per mg protein)	(nmol/h per mg protein)
Adult control	22.0 ± 0.8	5310 ± 270
16 day fetal	56.6 ± 0.4 (258)	4140 ± 205 (78)
6 day neonatal	56.2 ± 1.7 (256)	6640 ± 740 (125)

kinase exceeded the GMP synthetase activity by at least a factor of 60 in all the tissues examined.

Regenerating liver. The effects of partial hepatectomy on the activities of the two enzymes are shown in Fig. 2. GMP kinase activity was not significantly changed, but GMP synthetase activity was significantly increased after 24 h, reaching a peak of 155% of the normal value by 36 h after operation, thereafter declining. GMP synthetase activity was not increased following sham operation.

Differentiating liver. Table II summarizes the specific activities of GMP

TABLE III

GMP SYNTHETASE AND GMP KINASE ACTIVITIES IN RAT HEPATOMAS

Activities are means for quadruplicate samples, followed by S.E. Values in parentheses give hepatoma activity as percentage of the appropriate liver value.

Tissue	Transplant interval (months)	GMP synthetase	GMP kinase
		(nmol/h per mg protein)	(nmol/h per mg protein)
Liver (BUF)			
control for 9618A		26.1 ± 1.7	6850 ± 290
control for 20 and 9633		14.7 ± 0.7	4720 ± 270
control for 47C and 9618A$_2$		28.4 ± 1.1	6360 ± 160
control for 44 and 7794A		33.2 ± 3.3	6020 ± 180
control for 5123D		32.1 ± 1.4	5990 ± 250
control for 7777		16.0 ± 1.6	6720 ± 300
Liver (ACI/N)			
control for 3924A		32.6 ± 1.8	6090 ± 320
control for 3683		10.1 ± 1.5	6670 ± 140
Hepatomas			
9618A	12.0	31.2 ± 1.7 (120) *	6580 ± 610 (96)
20	8.0	27.3 ± 0.2 (186) *	3780 ± 390 (80)
9633	6.5	20.2 ± 2.1 (137) *	5620 ± 560 (118)
47C	6.0	42.8 ± 2.7 (151) *	5530 ± 320 (87)
44	5.6	40.5 ± 2.4 (122)	7530 ± 300 (125) *
7794A	3.5	52.2 ± 2.9 (157) *	5540 ± 250 (92)
5123D	1.8	49.9 ± 0.6 (155) *	6290 ± 490 (105)
7777	1.0	29.4 ± 1.2 (184) *	7860 ± 500 (117)
3924A	0.9	121.0 ± 2.6 (371) *	7060 ± 460 (116)
9618A$_2$	0.8	110.7 ± 3.6 (390) *	9350 ± 600 (147) *
3683	0.6	55.2 ± 4.5 (548) *	8070 ± 570 (121) *

* Statistically significant difference from control ($P < 0.05$ in Student's t-test).

TABLE IV

GMP SYNTHETASE AND GMP KINASE ACTIVITY IN RAT KIDNEY CARCINOMA

Values are means with S.E. from quadruplicate samples.

Tissues	Specific activity (nmole/h per mg protein)	
	GMP synthetase	GMP kinase
Normal kidney cortex	14.2 ± 2.0	6040 ± 220
Renal carcinoma MK-3	42.3 ± 4.3 *	5070 ± 90
MK-3 as % normal kidney	297	84

* Significant difference from control ($P < 0.05$).

synthetase and GMP kinase in fetal and neonatal rat liver. 6-day-old rats were chosen for the neonatal measurements because by this time the liver is clear of hemopoietic cells. In both the fetal and neonatal samples the GMP synthetase activity was over 2.5-fold the adult liver value. By contrast, the activity of GMP kinase in the same samples did not differ markedly from the adult level.

Hepatomas of different growth rates. Specific activities of the enzymes in cytosol preparations of 11 transplanted hepatomas are shown in Table III. The activity of GMP kinase showed no consistent alteration in the tumors, and was between 80 and 147% of the control liver activity in all cases. The activity of GMP synthetase was increased in all the hepatomas. In the tumors of slow or medium growth rate the increases were small, activities ranging from 120 to 186% of control. GMP synthetase was considerably more active in the tumors of rapid growth rate, and in the most rapidly growing tumor the activity reached 548% of control. GMP synthetase correlated positively with growth rate in the hepatoma spectrum, giving a Spearman rank correlation coefficient of 0.736, a correlation significant at the 1% level.

Kidney and renal cell carcinoma. In Table IV results are shown of measurements of GMP synthetase and GMP kinase in BUF rat kidney and in the transplantable kidney carcinoma MK-3. In the kidney tumor GMP kinase activity was not significantly changed, but GMP synthetase activity was elevated to 297% of the activity in the normal renal cortex control.

Effect of starvation and refeeding. During starvation, when most enzyme levels decrease, except those involved in gluconeogenesis, IMP dehydrogenase,

TABLE V

GMP SYNTHETASE AND GMP KINASE ACTIVITY IN FASTED AND RE-FED RATS

Values are mean ± S.E. for quadruplicate samples. Numbers in parentheses express results as percent of control.

Group	GMP synthetase (nmol/h per mg protein)	GMP kinase (nmol/h per mg protein)	Cytosol protein (mg/g wet weight)
Control, fed	26.6 ± 0.8	5730 ± 480	86.7 ± 3.5
3 day starved	32.6 ± 2.5 (122)	4410 ± 650 (77)	97.4 ± 4.1 (112)
3 day starved then 1 day re-fed	38.9 ± 2.6 (146)	4350 ± 260 (76)	69.0 ± 2.9 (80)

the rate-limiting enzyme of guanine nucleotide biosynthesis, has increased activity [6]. Table V shows corresponding data for GMP synthetase and GMP kinase. Activity of GMP kinase declined more rapidly than the total cytosol protein, but GMP synthetase was preferentially retained, its specific activity increasing to 122% of the value in fed controls and further increased for 1 day after refeeding. This higher enzymatic activity may be related to the elevated requirement for GTP in liver for gluconeogenesis during starvation.

Discussion

The GMP synthetase activity of all tissues examined in the present study was about an order of magnitude greater than the IMP dehydrogenase activity [5], which together with the very low intracellular concentration of XMP [4] provides support for earlier suggestions that IMP dehydrogenase may be the rate-limiting enzyme of de novo guanine nucleotide biosynthesis. Nevertheless, it appears that in conditions of cell proliferation, including normal development, regeneration, and neoplastic transformation, increased GMP synthetase activity is required. The increase is small in tumors of slow growth rates and over 5-fold in the most rapidly growing hepatoma. This correlation with growth rate resembles the pattern seen with IMP dehydrogenase and AMP deaminase (an enzyme that may be involved in deamination of adenine nucleotides into IMP for subsequent conversion to guanine nucleotides), but differs from the behavior of the enzymes of adenine nucleotide biosynthesis (adenylosuccinate synthetase and adenylosuccinate lyase) which were increased in all hepatomas but showed no correlation with growth rate [9]. By contrast with the synthetase, GMP kinase activity was in large excess in the normal tissues, and no consistent increase was seen in the tumors. These observations are in line with the molecular correlation concept which suggested that rate-limiting and key enzymes are most stringently linked with neoplasia and thus, their activities correlate with transformation and/or progression; enzymes present in great excess, such as GMP kinase, do not correlate [19,20]. GMP synthetase, although probably not rate-limiting for guanylate biosynthesis in normal liver, could become so in the hepatomas if its activity remained at the untransformed level, because the IMP dehydrogenase activity in some hepatomas rises to a higher level than the normal liver GMP synthetase activity [5]. The increases in GMP synthetase activity in hepatomas are less extensive than the increases in IMP dehydrogenase activity, but sufficient to keep IMP dehydrogenase rate-limiting in the pathway. The high GMP kinase activity of normal liver is consistent with the relatively low steady-state pool size of GMP observed in rat liver [4]. In the rapidly growing hepatomas, the combination of increased GMP synthetase with essentially unchanged GMP kinase might account for the increased pool of GMP measured in the rapid growth-rate hepatoma 3924A [4]. However, the steady-state pool of GTP was not increased in this tumor, perhaps because GTP biosynthesis and utilization were increased to a similar extent.

The increased GMP synthetase activity in hepatomas may be an adaptation to conditions of enhanced nucleic acid biosynthesis and may provide some selective advantage to these neoplastically transformed cells. The fact that a similar increase in GMP synthetase was observed in a transplantable kidney

tumor and in rat sarcoma (unpublished observation) indicates that the effect is not limited to hepatomas, but also occurs in other classes of tumors.

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