Glutamine-Phosphoribosylpyrophosphate Amidotransferase (Amidophosphoribosyltransferase, EC 2.4.2.14) Activity in Normal, Differentiating, and Neoplastic Kidney

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ABSTRACT

The behavior of glutamine-phosphoribosylpyrophosphate amidotransferase (amidophosphoribosyltransferase, EC 2.4.2.14), the first enzyme committed to de novo purine biosynthesis, was elucidated in normal, differentiating, and neoplastic rat kidneys. Enzymic activities were measured spectrophotometrically in the 100,000 x g supernatants prepared from 20% tissue homogenates containing 0.25 M sucrose and 1 mM MgCl₂.

Kinetic studies were carried out on the amidotransferase in the crude supernatant from rat kidney cortex and renal cell carcinoma (MK-3) so that under optimum standard assay conditions only the enzyme amount would be the limiting factor. The kinetic results indicated that certain properties of the amidotransferase in kidney and in the renal tubular cell tumor were similar. In normal kidney cortex and in the kidney tumor (MK-3), the pH optimum was 7.2 to 8.5; the Kₘ's for glutamine were 2.0 and 1.7 mM, respectively, and for MgCl₂ the Kₘ was 1 mM. For phosphoribosylpyrophosphate of the kidney and tumor enzyme, the S₀ₕ's were 0.9 and 0.5 mM, respectively. The amidotransferase in the kidney had sigmoid kinetics for phosphoribosylpyrophosphate, but in the renal tumor the enzyme exhibited a hyperbolic curve. The 50% feedback inhibition by adenosine 5'-monophosphate of the amidotransferase was 2.2- to 2.7-fold over that of the kidney and tumor enzyme.

The amidotransferase specific activities in rat thymus, testes, bone marrow, gut, kidney cortex, spleen, lung, brain, adipose tissue, heart, and skeletal muscle were 271, 259, 173, 167, 128, 96, 70, 64, 49, 24, and <1.0%, respectively, of that of the liver.

In three lines of chemically induced, transplantable renal tumors (MK-1, MK-2, and MK-3), the amidotransferase specific activities were increased 2.2- to 2.7-fold over that of the kidney cortex of normal control rats. During development, the enzyme activities in the average kidney cell of 1-, 7-, 30-, and 40-day-old rats were 57, 71, 79, and 114% of adult activity.

These studies indicate the applicability of the pattern of enzymic imbalance in purine metabolism first discovered in transplanted hepatomas to kidney tumors and further confirm the conclusion that the behavior of amidotransferase activity is a transformation-linked alteration. The elevation in the amidotransferase activity should increase the capacity of the de novo purine-biosynthetic pathway, and this enzymic imbalance should confer selective advantages to the neoplastic cells.

INTRODUCTION

Amidotransferase⁴ is the rate-limiting enzyme and the first enzyme committed in the de novo purine-biosynthetic pathway. This enzyme catalyzes an irreversible reaction between PRPP and glutamine to form phosphoribosylamine, L-glutamate, and glutamine-phosphoribosylpyrophosphate (amidophosphoribosyltransferase, EC 2.4.2.14): PRPP, phosphoribosylpyrophosphate.

Previously, we have demonstrated that the activity of amidotransferase increased 2- to 4-fold in all the chemically induced, transplantable rat hepatomas examined and that the behavior of this enzyme was transformation linked and characteristic of neoplasia (13, 17). The purpose of this investigation was to determine the applicability to renal neoplasia of the pattern of reprogramming of gene expression in purine metabolism, as expressed in the behavior of the amidotransferase activity.

Previous studies in this laboratory and in other centers led to the formulation of the molecular correlation concept, which resulted in the identification of an ordered imbalance in the activities of the key enzymes involved in purine, pyrimidine, carbohydrate, ornithine, and membrane cyclic adenosine 3': 5'-monophosphate metabolism in a spectrum of rat hepatomas of different growth rates (2, 3, 16, 20, 22-24, 29, 30, 34, 35). In analyzing this reprogramming of gene expression, the molecular correlation concept that has been the conceptual and experimental guide in this laboratory, grouped in Class I those alterations in which the discriminants were linked with the degrees in the expression of malignancy in the hepatoma spectrum (22-24). In addition to these progression-linked discriminants, there are alterations in gene expression that are linked with the malignant transformation per se, since they occurred in all liver tumors, even in the slowest growing, most differentiated hepatomas (22-24). These transformation-linked alterations (Class 2) include increased activity of key enzymes that channel hexoses into pentose phosphate biosynthesis through the oxidative pathway, glucose-6-phosphate dehydrogenase (EC 1.1.1.49), and the nonoxidative pathway, transaldolase (EC 2.2.1.2) (32). These enzymic indications are in line with isotopic evidence for an increased potential for pentose phosphate formation in the hepatomas (19). Ribose 5-phos-
from normal kidney cortex and tumor tissues in 0.25 M sucrose. The transformation-linked increase in the activity of amidotransferase in all rat hepatomas examined was an important observation because it suggested the operation of an increased capacity for purine biosynthesis in the presence of a decrease in the activity of the rate-limiting enzyme of purine catabolism, xanthine oxidase (18).

The present work reports that, in a spectrum of chemically induced, transplantable rat kidney tumors, the amidotransferase activity increased 2- to 3-fold. This investigation indicates the applicability of the enzymic imbalance observed in purine metabolism in hepatomas to renal tumors.

MATERIALS AND METHODS

All rats were housed in individual cages in air-conditioned rooms that were illuminated daily from 6 a.m. to 7 p.m. Purina laboratory chow and water were available ad libitum. Rats were killed between 9 and 10 a.m. Examination of stomach contents at autopsy confirmed that all rats were well fed during the night before death.

Tumor-bearing and Control Animals. Renal cell tumors (MK-1, MK-2, and MK-3) were implanted bilaterally in a s.c. position in male Buffalo rats. These rat kidney tumors were induced in Buffalo rats that were fed a diet containing 4'-fluoro-4-biphenylacetamide. Histologically, they were well-differentiated adenocarcinomas composed of generally uniform cells resembling kidney tubular cells, and they appeared to deviate only slightly from normal kidney tissue. The induction, transplantation, and some biological and histological characteristics of these tumors were reported previously (9, 15). All three are classified as some biological and histological characteristics of these tumors were reported previously (9, 15). All three are classified as slow-growing tumors, and they would relate to the slow-growing part of the spectrum of hepatomas of different growth rates (14, 15). Tumors MK-1 and MK-2 had a very slow growth rate (10 to 11 months old when assayed); Tumor MK-3 was somewhat faster (4 to 5 months old). Kidney cortex from normal animals of the same strain, sex, age, and weight was compared with the tumors.

Amidotransferase Activity in Different Rat Organs. For investigation of organ distribution of the amidotransferase activity, male ACI/N rats (180 to 220 g; Harlan Industries, Cumberland, Ind.) were used.

Differentiating Kidney. Pregnant Wistar rats were purchased from Harlan Industries, and the litters were allowed to remain in the same cage with the mother for 18 days after birth; then each rat was placed in an individual cage.

Preparation of Kidney and Tumor Extracts. The rats were stunned, decapitated, and exsanguinated. Tumors and kidney cortex 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 the viable tumor tissue parts is important in ensuring the comparability of neoplasms of different age groups and tumor lines. For the enzyme assay, 20% homogenates were prepared from normal kidney cortex and tumor tissues in 0.25 M sucrose containing 1 mM MgCl₂ (17). The homogenate was centrifuged at 100,000 × g for 30 min at 2⁰C in a Beckman Model L3-50 preparative centrifuge. The resulting clear supernatant was used for the assays.

Assay of Amidotransferase Activity. A standard enzyme assay system was worked out which was an adaptation of the technique of Prajda et al. (17) to the kinetic conditions of the rat kidney cortex and renal tumor systems. The reaction mixture contained, in final concentrations: PRPP (5 mM); glutamine (20 mM); MgCl₂ (15 mM); Tris-HCl buffer (50 mM, pH 7.2); and KF (1.0 mM). The determinations were carried out at 37⁰C and were stopped at 0, 30, and 45 min of incubation by boiling for exactly 5 min. The tubes were centrifuged, and in the clear supernatant the concentration of glutamic acid that had formed in the presence of PRPP was determined. This was carried out by coupling to the reduction of NAD⁺ in the presence of added excess glutamate dehydrogenase in a Gilford 2400 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 PRPP-independent 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 kidney cortex and renal tumors, and a standard assay was designed where the enzyme activity was proportional with the amount of tissue equivalent added and the time elapsed. The relevant kinetic studies will be described below. The PRPP was purchased as sodium salt, approximately 88% pure, from Sigma Chemical Co., St. Louis, Mo. The glutamine and glutamate dehydrogenase were also obtained from Sigma.

Protein concentration was assayed by the biuret reaction (5) with a standard curve, using crystalline bovine serum albumin.

Expression and Evaluation of Results. Amidotransferase activity was calculated in µmol of glutamate formed per hr per mg of protein (specific activity), per g (wet weight) of tissue, or per cell. The cell counts were made as described previously and were expressed as cellularity calculated in millions of nuclei per g, wet weight, of tissue (25).

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 Normal Kidney Cortex and Renal Tumor. In order to establish that linear kinetics operates in the crude system used in the amidotransferase measurements and that under the assay conditions the enzyme activity measured reflected the enzyme concentration, the properties and behavior of the amidotransferase were compared in extracts from normal rat kidney cortex (Buffalo rats) and Renal Tumor MK-3 (carried in Buffalo rats).

Effect of Glutamine Concentration. Chart 1 shows the effect of glutamine concentration on amidotransferase activity in normal rat kidney and in Renal Tumor MK-3. The enzyme activity in both normal and neoplastic tissue was saturated at a glutamine concentration of approximately 10 mM, and it was not inhibited by excess levels of glutamine up to concentrations of 44 mM. The affinity of amidotransferase to glutamine for kidney and Tumor MK-3 yielded apparent Kₘ's of 2.0 and 1.7 mM, respectively.

Effect of Magnesium Concentration. The enzyme activity of
Amidotransferase Activity in Rat Kidney

both normal and neoplastic tissue was saturated at a MgCl₂ concentration of about 10 mM. The enzyme activity in normal kidney and in the tumor was not inhibited by excess levels of MgCl₂ up to concentrations of 20 mM. The affinity of amidotransferase to MgCl₂ for both tissues yielded a Kᵣ of 1.0 mM.

Effect of PRPP Concentration. The effects of PRPP on kidney and tumor amidotransferase activities are compared in Chart 2. The enzymic activity of both normal control and neoplastic tissue was saturated at a PRPP concentration of approximately 2 mM. The substrate curve for the kidney yielded sigmoid kinetics, whereas the one in the tumor exhibited only slight, if any, sigmoidicity. These observations were made at optimum concentrations of all other reactants with variations only of the PRPP levels and of stopping the enzyme assay at 0, 30, or 45 min of incubation. In order to ascertain the behavior of the saturation curves at initial enzyme velocity concentrations, these experiments were also carried out in enzyme assay systems in which 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 readily repeatable. The results indicated that under such initial velocity-recording conditions the pattern of affinity curves was similar to the one given in Chart 2. The affinity of amidotransferase to PRPP for kidney and tumor yielded S₀.₅’s of 0.9 and 0.5 mM, respectively.

Effect of pH. Both kidney and tumor amidotransferase exhibited a pH optimum at approximately 7.2 to 8.5 (Chart 3). The shape of the pH curve was similar in the 2 tissues.

Proportionality of Amidotransferase Activity with Incubation Time and Enzyme Amount. Through systematic kinetic studies, a standard assay was established for determination of kidney cortex and renal tumor amidotransferase activities. In the standard assay, good proportionality was achieved with length of reaction time over a 120-min incubation period and with amount of enzyme added (Chart 4). The standard assays of kidney and tumor were carried out at pH 7.2 at 37° with the optimum reaction mixture conditions given in "Materials and Methods." Further information is provided in the legends for Charts 1 to 4.

Comparison of Activities of Amidotransferase in Different

Chart 1. Comparison of the effect of glutamine concentration on activity of amidotransferase in kidney cortex and Renal Tumor MK-3. The standard assay described in "Materials and Methods" was used for both tissues, varying only the glutamine concentration. For the normal kidney and tumor, the assay was carried out on supernatant fluid of 20% homogenate (0.4 and 0.2 ml, respectively). The standard assay concentration of glutamine is 20 mM.

Chart 2. Comparison of the effect of PRPP concentration on activity of amidotransferase in kidney cortex and Renal Tumor MK-3. The assay conditions were as described in "Materials and Methods," varying only the PRPP concentration. Enzyme concentrations used were as in Chart 1. The standard assay concentration of PRPP is 5 mM.
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P pH (Final)

Chart 3. Comparison of the effect of pH on amidotransferase activity in kidney cortex and renal tumor. The standard assay was performed as described in Chart 1, varying only the pH of the reaction mixture. Acetate buffer was used for pH in liver. In contrast, the amidotransferase activities in the adipose tissue, heart, and skeletal muscle were much lower than those observed in the liver.

Feedback Inhibition by AMP of Amidotransferase Activity in Normal Kidney Cortex and Neoplastic Tissue. The amidotransferase activity in Renal Tumor MK-3 and normal kidney was similarly sensitive to the inhibitory action of AMP. In kidney cortex, the 50% inhibition was 9.5 mm, and in kidney tumor tissue it was 9.3 mm.

Comparison of Amidotransferase Activity in Normal Kidney Cortex and in Tumors MK-1, MK-2, and MK-3. In order to examine the behavior of this enzyme in kidney neoplasia, the activity was studied in 3 renal tumors of different growth rates (Table 2). Tumor MK-3 has a faster growth rate (4 to 5 months) than do Tumors MK-1 and MK-2 (10 to 11 months). The protein concentration in the 100,000 x g supernatant in the normal kidney cortex of Buffalo rats was 71.3 ± 1.3 (S.E.) mg protein per g, wet weight, of tissue. In the tumors, protein content was slightly increased. The amidotransferase activity in normal kidney was 8.9 ± 0.4 μmol/hr/mg protein x 10^-2, and the specific activity was significantly increased in all 3 tumors, irrespective of the growth rate of the neoplasms. The amidotransferase activities were significantly elevated in all 3 kidney tumors to 2.2- to 2.7-fold over the values observed in the normal kidney of the control rats of corresponding strain, sex, age, and weight. The enzyme activities per average cell were

Organs of the Adult Rat. Table 1 shows that the enzyme specific activity was highest in organs of high cell renewal, the thymus, testes, and bone. The activities in kidney cortex, spleen, lung, and brain were in the same range as those of the

<table>
<thead>
<tr>
<th>Organ</th>
<th>Amidotransferase activity (μmol/hr/mg protein x 10^-2)</th>
<th>% of liver</th>
<th>Protein concentration (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymus</td>
<td>18.2</td>
<td>271</td>
<td>57.7</td>
</tr>
<tr>
<td>Testes</td>
<td>17.4</td>
<td>259</td>
<td>41.8</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>11.8</td>
<td>173</td>
<td>51.0</td>
</tr>
<tr>
<td>Gut</td>
<td>11.2</td>
<td>167</td>
<td>32.9</td>
</tr>
<tr>
<td>Liver</td>
<td>6.7</td>
<td>100</td>
<td>101.6</td>
</tr>
<tr>
<td>Kidney</td>
<td>8.6</td>
<td>128</td>
<td>78.0</td>
</tr>
<tr>
<td>Spleen</td>
<td>6.4</td>
<td>96</td>
<td>77.0</td>
</tr>
<tr>
<td>Lung</td>
<td>4.7</td>
<td>70</td>
<td>79.0</td>
</tr>
<tr>
<td>Brain</td>
<td>4.3</td>
<td>64</td>
<td>33.6</td>
</tr>
<tr>
<td>Adipose tissue</td>
<td>3.3</td>
<td>49</td>
<td>27.2</td>
</tr>
<tr>
<td>Heart</td>
<td>1.6</td>
<td>24</td>
<td>54.9</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>46.7</td>
</tr>
</tbody>
</table>

Table 2

Comparison of amidotransferase activity in rat kidney cortex and renal tumors

The data are means ± S.E. of 5 rats in each group with percentages of corresponding control kidney values. The activities per mg protein are to be multiplied by the exponential given to arrive at the absolute values and are also expressed as percentages of those observed in liver.

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Protein content (mg/g)</th>
<th>Amidotransferase activity (μmol/hr/mg protein x 10^-2)</th>
<th>% of liver</th>
<th>Protein concentration (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>71.3 ± 1.3</td>
<td>100</td>
<td>8.9 ± 0.4</td>
<td>100</td>
</tr>
<tr>
<td>Tumor MK-1</td>
<td>80.0 ± 1.2</td>
<td>112</td>
<td>24.3 ± 0.8</td>
<td>273^</td>
</tr>
<tr>
<td>Tumor MK-2</td>
<td>78.5 ± 1.9</td>
<td>100</td>
<td>24.3 ± 0.8</td>
<td>273^</td>
</tr>
<tr>
<td>Tumor MK-3</td>
<td>85.1 ± 2.5</td>
<td>119</td>
<td>19.6 ± 1.0</td>
<td>220^</td>
</tr>
</tbody>
</table>

Table 2

Comparison of amidotransferase activity in rat kidney cortex and renal tumors

The data are means ± S.E. of 5 rats in each group with percentages of corresponding control kidney values. The activities per mg protein are to be multiplied by the exponential given to arrive at the actual values. Assay conditions are described in "Materials and Methods."

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Protein content (mg/g)</th>
<th>Amidotransferase activity (μmol/hr/mg protein x 10^-2)</th>
<th>% of liver</th>
<th>Protein concentration (mg/g)</th>
</tr>
</thead>
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<td>100</td>
<td>8.9 ± 0.4</td>
<td>100</td>
</tr>
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<td>80.0 ± 1.2</td>
<td>112</td>
<td>24.3 ± 0.8</td>
<td>273^</td>
</tr>
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<td>220^</td>
</tr>
</tbody>
</table>

^ Significantly different from values of kidney cortex of normal rats (p < 0.05).
in this laboratory that the highly purified liver amidotransferase (1, 8, 17, 37). On the other hand, it has recently been shown in other reports in the literature on crude enzyme preparations that the rates were measured (15-min incubation). The S-shaped affinity for glutamine and Mg²⁺ was also present when initial enzyme assay (45-min incubation) was also present when initial.

The data are means ± S.E. of 4 or more rats in the various groups with percentages of corresponding control adult kidney values in parentheses. The activities are to be multiplied by the exponential given to arrive at the actual value. Assay conditions are described in “Materials and Methods.”

## Table 3

<table>
<thead>
<tr>
<th>Postnatal age (days)</th>
<th>Cellularity/g x 10⁶</th>
<th>mg/mg x 10⁻²</th>
<th>mg/cell x 10⁻⁶</th>
<th>Protein concentration</th>
<th>Amidotransferase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10⁻²</td>
<td>μmol/hr/mg protein x 10⁻⁶</td>
</tr>
<tr>
<td>1</td>
<td>630 ± 4.0 (172)</td>
<td>46.8 ± 2.1 (65)</td>
<td>7.4 ± 0.3 (38)</td>
<td>10.9 ± 1.0 (143)</td>
<td>0.8 ± 0.1 (57)</td>
</tr>
<tr>
<td>7</td>
<td>590 ± 4.0 (160)</td>
<td>50.9 ± 0.0 (68)</td>
<td>8.6 ± 0.0 (42)</td>
<td>11.8 ± 0.3 (155)</td>
<td>1.0 ± 0.0 (71)</td>
</tr>
<tr>
<td>30</td>
<td>594 ± 3.0 (162)</td>
<td>70.0 ± 3.4 (93)</td>
<td>11.8 ± 0.9 (58)</td>
<td>9.0 ± 0.4 (118)</td>
<td>1.1 ± 0.1 (79)</td>
</tr>
<tr>
<td>40</td>
<td>408 ± 2.4 (111)</td>
<td>75.2 ± 0.0 (100)</td>
<td>18.4 ± 0.3 (90)</td>
<td>8.6 ± 0.3 (113)</td>
<td>1.6 ± 0.1 (114)</td>
</tr>
<tr>
<td>60 (adult control)</td>
<td>367 ± 1.5 (100)</td>
<td>75.0 ± 3.1 (100)</td>
<td>20.4 ± 0.8 (100)</td>
<td>7.6 ± 0.3 (100)</td>
<td>1.4 ± 0.0 (100)</td>
</tr>
</tbody>
</table>

*Significantly different from the respective control adult kidney values (p < 0.05).
served in the newborn kidney, but only on a protein basis; when the high cellularity of the postnatal kidney was taken into consideration, it was apparent that the activity of this enzyme in the average cell was less than 60% of that in the adult kidney. In contrast, the amidotransferase activity in the kidney tumors increased 2- to 3-fold above the normal values on a per cell basis. Thus, the marked increase of amidotransferase activity appears to be characteristic of neoplastic transformation.

Biochemical studies have revealed that in kidney tumors a neoplastic program is displayed that is similar to that observed in liver tumors. Thus, as in hepatomas, in the renal tumors examined in this work the activities of the key gluconeogenic enzymes decreased, whereas those of the glycolytic enzymes increased (26). The incorporation of thymidine into DNA was increased (14) and the level of adenine nucleotides and the concentrations of glycolytic intermediates were altered in the same direction in both kidney and liver tumors (31, 36). Studies in this laboratory have indicated that the activities of CTP synthetase (34), IMP dehydrogenase (10, 11), adenylosuccinate synthetase, adenylosuccinase, and adenylate deaminase (10, 12) increased in both hepatic and renal neoplasms in the rat. Recent work has shown that xanthine oxidase, the rate-limiting enzyme of purine degradation, was low in all examined transplantable hepatomas (18) and that it also decreased in the kidney tumors. While there may be differences in organ-specific characteristics of the metabolic imbalance in the various types of tumors, there appears to be present a shared program of biochemical alterations in various tumors that characterizes the enzymic and metabolic phenotype that is stringently linked with the malignant transformation.

REFERENCES

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