Purine and Pyrimidine Enzymic Programs and Nucleotide Pattern in Sarcoma

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ABSTRACT

The purpose of this study was to elucidate the enzymic program and nucleotide pattern of a chemically induced, transplantable sarcoma and to compare the biochemical makeup with that of normal and differentiating skeletal muscle in the rat.

The activities of 28 key enzymes of pyrimidine, purine, and carbohydrate metabolism were determined in the 100,000 x g supernatant fluid or in purified extracts. The concentrations of the adenosine, guanosine, uridine, and cytidine mono-, di-, and triphosphates were measured by high-pressure liquid chromatography of samples prepared by the freeze-clamp method.

The results of enzymic and metabolite assays were given in nmol/hr/mg protein and nmol/g, respectively, and for comparability they were also expressed as percentages of the muscle of adult rat as the reference normal tissue in this study. These percentages denote that the activities in sarcoma or in differentiating muscle were higher or lower than those in the muscle of adult rats.

In pyrimidine metabolism, the specific activities of cytidine diphosphate reductase, cytidine triphosphate synthetase, and thymidine kinase increased in the sarcoma 60-, 78-, and 80-fold over those of the muscle. The activities of the key glycolytic enzymes, hexokinase and phosphofructokinase, increased 7- and 3-fold, whereas that of pyruvate kinase decreased to 35%.

The activities of glucose-6-phosphatase and fructose-1,6-diphosphatase decreased to 42 and 48%, respectively. The activities of enzymes involved in pentose phosphate production and utilization increased, with that of the glucose-6-phosphate dehydrogenase being elevated 288-fold. The activity of galactose kinase was unchanged, whereas that of uridine diphosphoglucose pyrophosphorylase decreased to 22%.

In purine metabolism, the activities of the first three enzymes of guanosine triphosphate biosynthesis, inosine monophosphate dehydrogenase, guanosine monophosphate synthetase, and guanosine monophosphate kinase, increased 22-, 2-, and 5-fold, respectively.

In contrast, the activity of adenosine deaminase and kinase was unchanged, whereas that of uridine diphosphoglucose dehydrogenase being decreased 10-fold. The activity of galactokinase was unchanged, whereas that of uridine diphosphoglucose dehydrogenase was decreased 22%.

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INTRODUCTION

Previous work in this laboratory demonstrated, in a spectrum of chemically induced, transplantable hepatocellular carcinomas of the liver, a transformation- and progression-linked imbalance in the activities of key enzymes of carbohydrate, purine, and pyrimidine metabolism and in the pattern of ribonucleotides and deoxyribonucleoside triphosphates (3, 9, 10). Subsequent studies indicated the presence of the metabolic imbalance in a series of carcinomas of kidney and colon in the rat and mouse and, recently, in human renal and colon carcinomas (1, 2, 12). The purpose of the present investigation was to answer the following questions. Is the biochemical imbalance discovered in carcinomas applicable to sarcoma? Are the enzymic indications of an imbalance in the cellular metabolic programs verifiable by the pattern of nucleotides in sarcoma? Is the biochemical pattern of the sarcoma different from that of differentiating muscle? Is it possible to identify a shared pattern of biochemical imbalance in carcinomas and sarcoma? Are there any sarcoma-specific biochemical alterations?

MATERIALS AND METHODS

For these studies, a methylcholanthrene-induced sarcoma line carried in male Fischer (F344) rats (Charles River Laboratories, Portage,
Mich.) and skeletal muscles from control, normal rats of the same sex, strain, age, and weight were used. The tumor was a rhabdomyosarcoma; the induction, maintenance, histology, and biological behavior are described in detail elsewhere. This is a fairly rapidly growing neoplasm which resembles the macroscopic appearance and growth rate of hepatoma 3924A and reaches a diameter of 1.5 cm in about 14 days, with death occurring 30 to 34 days after inoculation. The 12- to 18-day-old tumor has no necrosis, and it is well suited for the metabolic studies that were conducted.

The animals were inoculated s.c. by injection of $10^6$ viable tumor cells, and 1 week later the tumor-bearing and control rats were shipped by air from the National Cancer Institute to Indiana University. All rats were housed in individual cages in air-conditioned rooms, which were illuminated daily from 6 a.m. to 7 p.m. Purina laboratory chow and water were available ad libitum. Rats were always killed between 9 and 10 a.m.

**Biochemical Methods.** Animals were stunned, decapitated, and bled; tumors were excised, and 10% homogenates were prepared in the appropriate media as reported earlier (1, 2, 13). For the freeze-clamp studies, the animals received light ether anesthesia, and tumors and muscles were removed as described elsewhere (17).

Preparation of the extracts and methods for measurement of enzymes were described in detail elsewhere (1, 2, 5, 13). Preliminary kinetic studies were carried out to ensure that the enzyme assays were conducted under linear kinetic conditions, at optimum substrate and activator concentrations, and that the activities measured were proportional with the amount of enzyme added and reaction time elapsed. Preparation of the tissue material for determination of concentrations of ribonucleotides and deoxynucleoside triphosphates by high-pressure liquid chromatography and enzymatic methods (3) was cited elsewhere. Protein content was determined by a standard procedure (7).

**Expression and Evaluation of Results.** Enzymic activities were calculated as nmol product formed per hr, per g (wet weight) of tissue, per average cell or per mg protein, as specific activity. The concentrations of nucleotides were calculated in nmol per g (wet weight) of tissue.

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

**RESULTS AND DISCUSSION**

**Enzymic Programs of Muscle and Sarcoma**

The activities of key enzymes of carbohydrate, purine, and pyrimidine metabolism are summarized in Table 1, which provides the means and standard errors of activities observed in normal muscle and the results in the sarcoma as percentages of the values in muscle. In pyrimidine metabolism, the activities of the synthetic enzymes were low as compared to those observed in rat liver (16). In the muscle, the rate-limiting enzyme of CTP biosynthesis, CTP synthetase, had an activity of uridine kinase was 5 times higher than that in the muscle. The activity of uridine kinase was 5 times higher than that of the enzymes of the de novo synthetic pathway, orotidine 5'-monophosphate decarboxylase and orotate phosphoribosyltransferase.

In the sarcoma, the activities of all synthetic enzymes examined were very markedly increased as compared to muscle, ranging from a rise of 78- to 80-fold for thymidine kinase and CTP synthetase to increases of 26- and 14-fold for uridine kinase and uracil phosphoribosyltransferase. These elevations were much more marked (about 10-fold higher) than were those observed in the rapidly growing liver tumors compared to the resting liver (16).

In carbohydrate metabolism in the muscle, the activities of the key glycolytic enzymes, hexokinase, phosphofructokinase, and pyruvate kinase, were higher than those in rat liver (11). This is expected in view of the well-known glycolytic capacity of skeletal muscle. In the sarcoma, the activities of phosphofructokinase and hexokinase increased 3- to 7-fold, which is in line with observations made in hepatic carcinomas of similar rapid growth rates (11). In contrast to results in hepatomas, the activity of pyruvate kinase, which is orders of magnitude higher than any other enzyme that we have studied, was decreased to one-third of that observed in the control muscle.

In muscle, the gluconeogenic enzymes, glucose-6-phosphatase and fructose-1,6-diphosphatase, were present only in low activities, which were further decreased to 42 to 48% in the sarcoma. These results are in line with the decline in activities of gluconeogenic enzymes first reported in hepatomas (11).

In pentose phosphate metabolism in the muscle, the activity of glucose-6-phosphate dehydrogenase proved to be rate limiting, being 10 times lower than that of the subsequent enzyme, 6-phosphogluconate dehydrogenase. The activity of glucose-6-phosphate dehydrogenase was the lowest among the enzymes of carbohydrate metabolism examined in this study. Therefore, it is interesting that in the sarcoma the activity of this dehydrogenase was increased the most markedly of all the enzymes assayed, i.e., 288-fold over the value of normal control muscle. The 6-phosphogluconate dehydrogenase activity was elevated 12-fold. Transaldolase, which channels fructose 6-phosphate into ribose 5-phosphate biosynthesis, in the muscle had an activity twice as high as that of 6-phosphogluconate dehydrogenase, and the activity increased 7.7-fold in the sarcoma. The enzyme that utilized ribose 5-phosphate for phosphoribosylpyrophosphate biosynthesis, phosphoribosyl pyrophosphate synthetase, also had low activity in the muscle, but it increased only 1.5-fold in the sarcoma.

In purine metabolism among the synthetic enzymes, the activity of IMP dehydrogenase was the lowest, resembling that in liver (5). However, the muscle activity was much lower than that in the rat liver. In the sarcoma, the dehydrogenase activity increased 22-fold, the highest rise among all the purine enzymes examined. The activities of the enzymes involved in GTP biosynthesis subsequent to the action of IMP dehydrogenase, GMP synthetase and GMP kinase, were orders of magnitude higher than that of the rate-limiting enzyme, the dehydrogenase. These enzymic activities increased in the sarcoma to 2- to 5-fold the values of the muscle. It is interesting that the activities of the enzymes of the purine cycle, adenylosuccinase and AMP deaminase, were decreased in the sarcoma, indicating that this cycle which may be a characteristic liver and muscle function was decreased in the sarcoma. The activity of AMP deaminase was the highest among the purine enzymes in the muscle. The activities of enzymes involved in adenosine metabolism, adenosine deaminase and kinase, were also high, but these activities further increased 1.8- and 3.5-fold. This
behavior is in contrast with that in hepatic carcinoma where in the rapidly growing tumors the activity of adenosine deaminase was increased 3- to 4.5-fold and that of adenosine kinase decreased to less than 20% of that observed in the control liver (4).

The activity of the first committed enzyme of de novo purine biosynthesis, amidophosphoribosyltransferase (amidotransferase), in the sarcoma was markedly increased (13-fold), whereas that of the rate-limiting catabolic enzyme, xanthine oxidase, was decreased to 54%. As a result, the ratio of amidotransferase to xanthine oxidase was markedly elevated.

**Enzymic Program of Differentiating Muscle**

The enzymic program of differentiating muscle of the rat (Table 1) shows some similarities to and also sharp differences from that of the sarcoma. The activities of the pyrimidine-synthetic enzymes were increased in comparison with normal muscle, but to a smaller extent than those of the sarcoma. However, uridine phosphorylase activity was decreased. The activity of hexokinase was decreased to 47%, whereas in the sarcoma it was increased to 686%. The pyruvate kinase activity was 18%, whereas in the sarcoma it was 35%, of the normal muscle. Minor increases were observed in the activities of glucose-6-phosphate dehydrogenase and transaldolase. However, the activity of 6-phosphogluconate dehydrogenase was decreased, whereas in the sarcoma it was elevated 12-fold. In purine metabolism, the GMP kinase activity was as high as in the sarcoma. However, the activities of AMP deaminase and adenosine deaminase in the developing muscle were the same as in normal muscle, whereas in sarcoma the first one decreased and the latter increased. In the 6-day-old muscle, the amidotransferase activity was 2-fold higher whereas in the sarcoma it was 13-fold higher than in the normal muscle. In the sarcoma, the xanthine oxidase activity was decreased, whereas it was high in the differentiating muscle. Thus, the enzymic program of the sarcoma can be readily separated from that of the differentiating muscle through the quantitatively more pronounced alterations in the sarcoma and by the different behavior of uridine phosphorylase, hexokinase, 6-phos-
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phosphogluconate dehydrogenase, AMP deaminase, adenosine deaminase, and xanthine oxidase.

**Ribonucleotide Pattern in Muscle and Sarcoma**

The overall pattern of purine and pyrimidine ribonucleotide concentrations in muscle (Table 2) resembled that of liver in that the concentrations of adenylates were 2 orders of magnitude higher than those of the other nucleotides and the concentrations of cytidylylates were the lowest (3). Particularly low CTP concentration was found in the muscle, the other nucleotides (CDP, IMP, and XMP) being too low to measure with accuracy by current methods. The strikingly high concentrations of ATP and ADP were in line with expectations for these nucleotides because of their vital role in muscle function. Comparison of the purine and pyrimidine ribonucleotide concentrations shows a characteristic pattern in the sarcoma. The concentrations of ATP, ADP, and AMP and total adenylates in the sarcoma decreased to 24, 49, 76, and 32%, respectively, of those observed in normal muscle. The decrease in concentration of adenylates is similar to that in renal cell carcinomas (18) and hepatomas (3, 17) in the rat. By contrast, the concentrations of GTP, GDP, GMP, and total guanylates were very markedly increased over those in muscle. The 9-fold increase in concentration of GTP and the elevation in that of total guanylates (14-fold) were in marked contrast with values in rat liver carcinomas which were in the normal range of the control liver. The concentrations of UTP, UDP, the UDP sugars, and total uridylylates were markedly increased in the sarcoma but were not significantly altered in the rapidly growing hepatoma. The 17-fold increase in CTP concentration is the most marked among the ribonucleotides in the sarcoma; a 4- to 5-fold elevation was observed in the rapidly growing hepatoma 3924A.

The decreased concentration of adenylates is in line with the decrease in the activities of enzymes of de novo ATP production in the sarcoma (Table 2). The increased concentrations of GTP, GDP, and GMP reflect the elevated activities of IMP dehydrogenase, GMP synthetase, and GMP kinase (Table 1). The elevated concentrations of UTP, UDP, and UMP are in good agreement with the marked increase in the activities of enzymes involved in the de novo and salvage pathways of uridylate biosynthesis. The increased concentration of CTP reflects the elevation in the activity of the rate-limiting enzyme of biosynthesis, CTP synthetase (Table 1).

**Identification of Shared Programs in Neoplasms and Tumor-specific Enzymic and Metabolic Pattern in Sarcoma**

**Shared Programs in Different Types of Neoplastic Cells.** In elucidating the biochemical strategy of gene logic, it was reported from this laboratory that there are shared programs in the enzymic and metabolic imbalance that are displayed in various types of neoplasms (9, 10, 14). Evidence was presented that important segments of alterations in enzymology of carbohydrate, purine, pyrimidine, and other metabolic areas occurred in chemically induced and virus-derived, transplantable rat, mouse, and avian tumors and in primary human liver and kidney and colon neoplasms (1, 6, 8, 15). Three major aspects of gene logic were identified in the various tumors (14).

Reciprocal regulation plays a vital role in control of the rate and direction of opposing metabolic pathways by determining the amounts of antagonistic key enzymes (14). This metabolic imbalance amplifies the metabolic capabilities of the tumor cells. Such a pattern was observed in the present investigation in the sarcoma in the elevation in activity of the synthetic enzyme, amidotransferase, and the decline in that of the opposing catabolic enzyme, xanthine oxidase. This reciprocal regulation resulted in a 31-fold increase in the ratio of the activities of the opposing enzymes of purine metabolism which is more marked than the rise observed for this enzyme ratio in other tumors.

A second principle that we identified in purine and pyrimidine metabolism was the relationship of the extent of rise in activity of an enzyme in a tumor compared with the absolute activity observed in the homologous normal tissue of origin. An example of this relationship is in human colon tumor where the activities of thymidine kinase and CTP synthetase which were the lowest in normal colon mucosa were increased the most markedly in the neoplasms (1). A similar relationship holds in the sarcoma where thymidine kinase, CTP synthetase, and CDP reductase had the lowest activities in the muscle, but these activities were increased to the greatest extent among the pyrimidine-synthetic enzymes in the sarcoma. In purine metabolism in the muscle, the activity of IMP dehydrogenase was the lowest; in the sarcoma, it was increased the most markedly among the enzymes of purine biosynthesis.

Observations in an array of 14 different rat, mouse, avian, and human tumors indicated the operation of an integrated program of enzymic and metabolic imbalance that conferred selective advantages to the cancer cells (6, 8, 9, 10, 14). In the sarcoma, we confirmed the presence of a coordinated pattern of alterations in the activities of key enzymes yielding a highly meaningful picture (Tables 1 and 2).

**Tumor-specific Differences.** In parallel with striking similarities in the overall biochemical strategy of different cancer cells, we also expect organ- and cell-specific alterations in neoplasms of different cellular origin, and we reported such differences for the various tumor systems (1, 14). In the sar-
coma, a striking contrast with other neoplasms was the decrease in activity of pyruvate kinase which was ubiquitously increased in all other tumors examined thus far. In sarcoma, galactokinase activity did not increase, but it was elevated 4-fold in human colon carcinoma. In sarcoma, the activities of AMP deaminase and adenylosuccinase decreased, but they increased in liver and kidney tumors. The activity of adenosine kinase that was low in rapidly growing hepatomas showed a 3.5-fold increase in the sarcoma. These alterations provide a pattern of sarcoma-specific markers that distinguishes the enzymic features of this tumor from those of any others that we have examined thus far. There are also differences in the nucleotide pattern where the marked increases in concentrations of guanylates and uridylates contrast with the normal liver values observed in hepatocellular carcinomas. In sarcoma, the 17-fold increase of CTP concentration was quantitatively far beyond the rise in hepatoma 3924A (4- to 5-fold). These differences assist in characterizing the metabolic individuality of the sarcoma and in the biochemical differential diagnosis of this tumor.

Selective Advantages Conferred to Sarcoma Cells by the Enzymic and Metabolic Imbalance. The biological malignancy and rapid growth rate of the sarcoma are reflected in the pronounced elevations in the activities of key enzymes of pyrimidine and purine biosynthesis and in the decrease in catabolic capacity (9, 10). The increased activities of key enzymes of glycolysis and pentose phosphate production and utilization should provide an increased capability for the production of ribose 5-phosphate and phosphoribosyl pyrophosphate.

The markedly enlarged pools of guanylates, uridylates, and cytidylates should provide a stepped-up capability for nucleic acid production, in particular, the provision of substrate for ribonucleotide reductase for the de novo biosynthesis of deoxyribonucleoside triphosphates. The markedly increased capacities of the salvage pathways, as shown by elevated activities of thymidine kinase (80-fold) and uridine kinase (26-fold), would contribute strongly to the increased potential for nucleic acid biosynthesis. The enzymic and metabolic imbalance discovered in the sarcoma should confer selective advantages to these cancer cells. The pronounced enzymic and metabolic imbalance and particularly the increase in both de novo and salvage enzymic activities explain, in part at least, the difficulties encountered in the chemotherapeutic control of this malignant neoplasm.

Relevance of Enzymology of Sarcoma to the Design of Anticancer Drug Treatment. The difficulties in achieving lasting remissions in experimental or human sarcomas by inhibitors of the de novo pathways of pyrimidine metabolism may be explained, in part at least, by the operation and increased activities of the powerful salvage enzymes, uridine kinase, uracil phosphoribosyltransferase, and thymidine kinase, which were markedly elevated in the sarcoma. The stepped-up activity of uridine-cytidine kinase is particularly important because this enzyme provides the uridylates, the pool of which is profoundly enlarged in the sarcoma. This enzyme also activates cytidine and yields CMP. Thus, through the elevated activity of this enzyme, an inhibition by drugs of the final common pathway of CTP biosynthesis, CTP synthetase (e.g., by glutamine antagonists or competitive inhibitors), would be circumvented. The marked elevation in thymidine kinase activity provides a heightened capacity for production of thymidylates which are also crucial for DNA biosynthesis. In the design of combination chemotherapy of sarcoma, there is a need for agents that in addition to inhibition of the de novo pathways would block the activities of the salvage synthetic processes. The present studies, while revealing the formidable biochemical capacities displayed by sarcoma cells, also point out possible approaches in the strategy of anticancer drug treatment of this neoplasm.

REFERENCES

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