Activities of Key Gluconeogenic Enzymes and Glycogen Synthase in Rat and Human Livers, Hepatomas, and Hepatoma Cell Cultures

Kathryn D. Hammond² and Doris Balinsky³

Enzyme Research Unit, The South African Institute for Medical Research, Johannesburg, South Africa

ABSTRACT

The activities of pyruvate carboxylase (PC), phosphoenolpyruvate carboxykinase (PEPCK), glucose-6-phosphatase (G6Pase), and glycogen synthetase (GS) were determined in the cancerous and in the apparently uninvolved (host) regions of livers from primary hepatoma patients as well as in normal adult human livers and human fetal livers. The activities of these enzymes were also assayed in a fairly fast-growing, 3′-methyl-4-dimethylaminoazobenzene-induced transplantable rat hepatoma and in hepatoma cell lines derived from both rat and human tumors.

In the human hepatoma, as in the rat hepatoma, the activities of PC, PEPCK, and G6Pase were considerably reduced, compared to those in the host liver. The activities of both the a (glucose 6-phosphate-independent) and b (glucose 6-phosphate-dependent) forms of GS were also lower in human and rat hepatomas than in the respective host livers. Activities of PC, PEPCK, and G6Pase in the human hepatomas were often comparable with those of fetal livers. In rat and human hepatoma cells, the activities of PC, PEPCK, and G6Pase were similar to or lower than the activities in the respective hepatomas; the activities of GS a were also similar to those in the hepatoma, whereas the activities of GS b were somewhat higher.

INTRODUCTION

Alterations in carbohydrate metabolism in rat hepatomas have been studied extensively, particularly since the production by Morris (14) of a series of chemically induced transplantable tumors of varying growth rate and differentiation. It has been shown that the activities of the key glycolytic enzymes increase in parallel with increasing growth rate and loss of differentiation of the tumor, whereas the activities of key gluconeogenic enzymes decrease with increasing growth rate (22). These changes in enzyme activity correlate closely with overall increased rates of glycolysis and reduced rates of gluconeogenesis (22). Decreases in the activities of GS a and glycogen phosphorylase have also been observed in rat hepatomas; it appears that glycogen metabolism in fast-growing tumors differs from that in normal liver to a greater extent than it does in slow-growing tumors (11, 19).

In human primary hepatomas obtained from patients from various geographical areas, changes in the activities of several enzymes of carbohydrate metabolism resembled those occurring in rat hepatomas (8). The activities of key gluconeogenic enzymes were reduced in hepatomas from Chinese patients (26).

Human primary hepatoma, apparently of an etiology distinct from that of the subjects of Boxer and Shonk (8) and Yeung et al. (26), occurs with high incidence in southern Africa, particularly in Mozambique, among the Bantu-speaking Negro population (16). An earlier study from this laboratory showed that the activities of several enzymes of carbohydrate metabolism were changed in the human hepatoma, compared to those of normal liver or apparently uninvolved liver from hepatoma patients (4). In the work presented here, the investigation was extended to include the key gluconeogenic enzymes, PC, PEPCK, and G6Pase, and a key enzyme of glycogen metabolism, GS. Since fetal liver, unlike adult liver, is a growing, dividing tissue, comparison of its enzyme activities with those of hepatoma was considered of interest. In addition, the availability of a chemically induced, transplantable rat hepatoma and cell lines derived from human and rat hepatomas permitted comparison with these systems.

MATERIALS AND METHODS

Substrates and Cofactors

Monosodium phosphoenolpyruvate, disodium ATP, disodium NADH+, and malate dehydrogenase were obtained from Boehringer Mannheim, Mannheim, Germany. Disodium IDP, trilithium S-acetyl-CoA, sodium uridine diphosphoglucose, and glycogen (Type III from rabbit liver) were from Sigma Chemical Co., St. Louis, Mo. Potassium pyruvate was from Calbiochem, San Diego, Calif., and glucose 6-phosphate was from British Drug Houses, Poole, England. Sodium [14C]bicarbonate and UDP-D-[6-3H]glucose (ammonium salt) were obtained from the Radiochemical Centre, Amersham, England.

Other Chemicals

Dithiothreitol was obtained from Calbiochem. Glutathione (reduced form) was from Sigma. Folin Ciocalteu's reagent was from E. Merck, Darmstadt, Germany. Bovine plasma albumin was from Armour Pharmaceutical Co., Eastbourne, England. Instagel scintillation fluid was from Packard Instrument Co., Downers Grove, Ill. Other standard
chemicals were obtained from British Drug Houses, E. Merck, and Hopkin and Williams (Chadwell Heath, England) and were of analytical grade.

Source of Material

Albino Wistar rats bearing in their hind legs transplantable hepatomas induced originally by the carcinogen 3'-methyl-4-dimethylaminoazobenzene (2) were provided by Dr. C. F. Albrecht and Dr. L. D. Nourse. Normal livers were also obtained from albino Wistar rats. The rats were killed by cervical dislocation, their livers were removed, hepatomas were dissected, and any necrotic tissue was discarded. Tissues were kept on ice and assayed immediately.

Normal human tissues, removed at autopsy from accident victims, were provided by Dr. M. G. G. Maier. Fetal tissues from spontaneously or therapeutically aborted fetuses of Caucasian origin were made available by Dr. L. G. R. van Dongen and staff. Livers and hepatomas, obtained at autopsy from patients in whom primary hepatocellular carcinoma had been confirmed histologically, were provided by Dr. E. W. Geddes, Professor I. Webster, and staff. The patients were Bantu-speaking Negro males originating from Mozambique. Gross liver weight varied from 3 to 6 kg. Evidence of cirrhosis was apparent in a few cases. The cancerous regions of the livers were dissected from the regions that appeared to be morphologically normal ("host liver"). Only hepatoma tissue in which there was <15% necrosis (as determined histologically by the Histopathology Department, The South African Institute for Medical Research) was assayed. In 1 instance, host liver and hepatoma, obtained during surgery from a Bantu female of South African origin, was provided by Dr. J. A. Hunt. Autopsy material was assayed within a maximum of 6 hr after death, and fetal material was assayed as soon as possible after abortion, usually within 2 to 6 hr. After removal from the body, tissues were kept on ice and assayed immediately. In a few cases, G6Pase, which was stable to freezing, was assayed in tissue that had been stored at -20°.

A rat hepatoma cell line, derived from the previously mentioned 3'-methyl-4-dimethylaminoazobenzene-induced hepatoma and grown under the conditions described by Albrecht et al. (1), was provided by Dr. L. D. Nourse. Rat hepatoma cells, derived from the line provided by Dr. Nourse, and a human hepatoma cell line, established from the tumor of a patient in whom primary liver cancer had been confirmed histologically (17), were supplied by Professor O. W. Prozesky. The cells were obtained in serum-free medium after trypsinization, centrifuged at 50 × g for 2 min at room temperature, and washed twice with 0.9% NaCl solution. Extracts were prepared immediately.

Preparation of Extracts and Assay Procedures

All assays were carried out at 30°. Reaction rates were linear with time and enzyme concentration, under the conditions used.

PC and PEPCK. Tissues were homogenized at 4° in 10 mM Tris-HCl buffer (pH 7.4) containing 1 mM dithiothreitol, with an Ultra-Turrax homogenizer. The homogenates were centrifuged at 36,000 × g for 30 min at 4°, and the clear supernatants were used for enzyme assays. Homogenization and centrifugation at room temperature did not increase the activity of either enzyme. Cell extracts were prepared in 50 mM Tris-HCl buffer (pH 7.4) containing 0.25 mM sucrose, 1 mM EDTA, and 5 mM mercaptoethanol, by freezing and thawing 3 times in acetone-dry ice. The extracts were centrifuged at 36,000 × g for 1 hr at 4°, and the clear supernatants were used for enzyme assays. For PC the reaction mixture was based on that described by Ballard and Hanson (5) and contained 125 mM Tris-HCl buffer (pH 7.4), 6.6 mM potassium pyruvate, 8.3 mM MgSO4, 2.2 mM ATP, 0.75 mM acetyl-CoA, 50 mM NaH14CO3 (0.013 μCi/μmol), and enzyme in a total volume of 0.3 ml (final pH, 7.2). For PEPCK the reaction mixture was the same as that described by Ballard and Hanson (5), except that NaH14CO3 (0.04 μCi/μmol) was used instead of KH14CO3. In both assays, reactions were stopped by addition of 0.5 ml 10% trichloroacetic acid. Precipitated protein was removed by centrifugation at 50 × g for 5 min at room temperature, and nitrogen was bubbled through the supernatant to remove any remaining 14CO2. Aliquots of the supernatant were transferred to scintillation vials containing 10 ml Instagel, and the radioactivity was determined with a Packard Model 3390 liquid scintillation spectrometer.

G6Pase. Tissues were homogenized manually in ice-cold 0.25 mM sucrose by using 5 to 6 strokes of a Potter-Elvehjem homogenizer. Cell extracts were prepared as described previously. The incubation medium was the same as that described by Nordlie and Snoke (15) for determination of phosphohydrolase activity at pH 6.5. After the reactions had been stopped by addition of 10% trichloroacetic acid and after the precipitated protein had been removed by centrifugation at 50 × g for 5 min at room temperature, the inorganic phosphate present in the supernatant was determined in the same way as described by Lindberg and Ernster (12).

GS. Tissues were homogenized manually in 10 mM Tris-HCl buffer (pH 7.4) containing 125 mM NaF (to inhibit conversion of GS a to GS b) and 5 mM diithiothreitol by using 5 to 6 strokes of a Potter-Elvehjem homogenizer. Cell extracts were prepared in the same buffer by freezing and thawing 3 times in an acetone-dry ice mixture. Since GS is bound to glycogen associated with mitochondria as well as to microsomal and particulate glycogen, the homogenates and extracts were centrifuged at 5,000 × g for 10 min at 4°, and the supernatants were used for enzyme assays. The assay mixture was the same as that described by Sato et al. (19), except that UDP glucose labeled with 3H (0.03 μCi/μmol) instead of with 14C was used. Reactions were stopped by addition of 30% KOH solution containing 2 mg glycogen per ml. Glycogen was recovered by the method of Steiner et al. (21) and dissolved in water. An aliquot of the solution was transferred to a scintillation vial containing 10 ml Instagel, and the radioactivity was determined with a Packard Model 3390 liquid scintillation spectrometer. Activity was determined in the presence and absence of glucose 6-phosphate, and the total (a + b) activity (with glucose 6-phosphate) minus GS a activity (without glucose 6-phosphate) was taken as the GS b activity.

A unit of enzyme activity is defined as the amount of enzyme catalyzing the conversion of 1 μmol substrate to
Enzyme Levels in Livers, Hepatomas, and Hepatoma Cells

product per min at 30°, under the conditions specified previously.

Protein Determinations

Proteins were determined by the method of Lowry et al. (13).

RESULTS AND DISCUSSION

Enzyme Activities in Rat and Human Tissues. The activities of PC, PEPCK, G6Pase, and GS in livers, hepatomas, and hepatoma cells are shown in Charts 1 and 2. The activities of some of the enzymes of carbohydrate metabolism are known to be affected by diet. Many of the patients in this study were emaciated, partly as a result of inability to eat adequately in the late stages of the disease, due to gastric discomfort. Normal liver from well-nourished, non-tumor-bearing individuals thus may not be suitable control material for assessing enzyme changes associated with cancer. Thus, for reducing the effects of dietary differences between individuals, the activity in a particular host liver and hepatoma were compared. In this way, liver and hepatoma that are subject to the same dietary carbohydrate depletion and exposed to the same levels of circulating hormones and blood sugar can be compared. However, host liver cannot be equated to normal liver since it has been exposed to the same carcinogenic stimuli that gave rise to the cancer; moreover, its metabolism could have been affected by the presence of the adjacent hepatoma.

In Charts 1 and 2 a line is drawn between the enzyme activity in a tumor and that in the corresponding host liver from the same patient. Thus, for example, although human host liver and hepatoma activities of PC overlapped, in every case the activity in the hepatoma was lower than that in the host liver from the same patient. The mean activity in the human hepatomas, compared to that in the host livers, was 12.3 ± 3.0% (S.E.). The host liver in the human differs from that of the rat, in that the human hepatoma is primary, with the host liver (which was normal by gross morphological and histological studies) adjacent to the tumor, whereas the rat host liver is remote from the hepatoma, which had been transplanted into the animal’s hind leg.

Interestingly, the activity of PEPCK in human host and normal liver was much higher than that in rat host liver, whereas rat host liver PC had a higher activity than that of either human host liver or normal rat liver. Chang and Morris (9) found that the activities in rat host livers were no different or were lower than the PC activities in normal rat livers. The activities of the other enzymes studied were similar in livers from normal and tumor-bearing rats, as found by others (19, 22), and similar to those in human host livers. Since only a few human normal livers were available, no such comparison could satisfactorily be made. The human host liver values varied over a wide range, possibly as a result of dietary differences between the individuals studied; the values obtained for the normal livers were within the ranges of the host liver values.

As can be clearly seen from Chart 1, the activities of PC, PEPCK, and G6Pase in hepatomas were considerably reduced in every case (except for the G6Pase of 1 tumor sample) in both rat and human hepatoma, compared to those of the host livers. Similarly, Chart 2 shows that the activities of both the a and b forms of GS were lower in all the hepatomas. Moreover, the proportions of GS a and b were similar in normal and host livers and hepatomas.

Decreases in the activities of key gluconeogenic enzymes in fast-growing rat hepatomas had previously been shown, whereas slow-growing tumors had activities comparable with those of normal livers (22, 23). However, a more recent report showed that, although PC activities were reduced in many hepatomas, there was no correlation of activity with growth rate (9). A report published while the present study was underway (26) showed reduced activities of G6Pase, PC, and PEPCK in hepatomas of patients in Hong Kong. Reductions of GS and glycogen levels in most experimental rat hepatomas have been observed (11, 19). In a few fast-growing rat hepatomas, the liver enzyme was replaced by a nonhepatic form, possibly that of muscle (18). The

Chart 1. Activities of gluconeogenic enzymes PC, PEPCK, and G6Pase in rat and human livers. N, normal liver; HL, host liver; H, hepatomas; HC, hepatoma cell cultures; FL, fetal liver; Δ, surgical tissue. A line joins the activities in host livers to those of the corresponding hepatomas.
Chart 2. GS activities in rat and human livers. N, normal liver; HL, host liver; H, hepatomas; HC, hepatoma cell cultures; FL, fetal liver; Δ, surgical tissue. A line joins the activities in host livers to those of the corresponding hepatomas. The difference between total GS activity \((a+b)\), measured in the presence of glucose 6-phosphate, and GS a activity, measured in the absence of glucose 6-phosphate, was taken as GS b activity. The activity ratio, \(\frac{a}{a+b}\), is also shown.

Present results give no indication of this.

Mean values ± S.E. have been calculated, both in milli-units/mg protein and units/g wet weight, for comparison with most published values. These figures are available from the authors on request. All the differences between the means for enzyme activities in host liver and hepatoma were highly significant.

Apart from the data for G6Pase, the differences in activity between host liver and hepatoma are only 2- to 3-fold greater for the rapidly growing rat hepatoma than for the human hepatoma.

Our previous findings (4) had also shown that human hepatomas have some enzyme differences resembling rapidly growing rat hepatomas, whereas others are more analogous to those found in rat hepatomas of slow or intermediate growth rate. Thus, tumor fructose-1,6-bisphosphatase and phosphoglucomutase activities were very much reduced, and pyruvate kinase activity was increased approximately 2-fold, whereas hexokinase and glucose-6-phosphate dehydrogenase activities were not significantly altered.

On 2 occasions human tissue was obtained during sur-
gery, and the data are included in Charts 1 and 2. The activities of PC, PEPCK, and G6Pase in the surgical tissues were comparable with those of the autopsy tissues. This suggests that there was little loss of activity of these enzymes in autopsy material during the time that elapsed between death and performance of the assays. The activity of GS in the surgical tissues was higher than it was in the autopsy tissues. This could be due to the dietary state of the individuals from whom tissues were obtained or, possibly, the enzyme may have lost activity. In the only instance in which both surgical host liver and hepatoma were available for assay, the activities of PC, PEPCK, and GS (both forms) were lower in the hepatoma than they were in the host liver, as in autopsy tissues. For G6Pase a higher value was again obtained for 1 of 2 samples of the same tissue.

Since fetal liver, unlike normal differentiated adult liver, is rapidly growing tissue, activities in these tissues were also assayed for comparison. The human fetal livers examined were obtained from fetuses ranging from 13 to 28 weeks in gestational age. The activities of PC, PEPCK, and G6Pase were in most cases lower than those in adult livers and comparable with those in hepatomas. No definite correlation of the activities of these enzymes with fetal age was observed, which is in agreement with the findings of others (3, 10, 18, 20). The 2 high values for PEPCK were for a set of twins. The activities of GS in fetal livers varied over a wide range and included values higher than those of adult livers. The activities of both forms of the enzyme appeared to increase up to 24 weeks; GS b activity then decreased, whereas GS a activity was not noticeably affected, consistent with the fact that glycogen has been shown to be present in human fetal livers from as early as the second quarter of pregnancy and to increase during development (10).

Enzyme Activities in Cell Cultures. The enzyme activities in the 2 rat hepatoma cell lines fell within a similar range and are included together in Charts 1 and 2.

In the rat hepatoma cells, the activities of PC were significantly lower than those in hepatoma tissue; the activities of PEPCK and G6Pase were similar to those of the hepatoma. The activities of PC, PEPCK, and G6Pase in the human hepatoma cells were significantly lower than those in autopsy hepatomas. Similarly, activities of G6Pase in cell lines or suspensions established from spontaneous mouse mammary carcinomas and uterine sarcomas and from thioacetamide-induced mouse hepatomas have been shown to be similar to or lower than those in the corresponding tumor tissues (7, 25). Wicks et al. (24), however, showed that the activities of PEPCK in Reuber H35 and M, H, C, rat hepatoma cells were similar to those of normal liver, whereas, in HTC rat hepatoma cells and RLC rat liver cells, the activities were lower than those in normal liver.

In both rat and human hepatoma cells, GS b activities were significantly higher than those in the respective tumors; GS a activities were similar in the cells and hepatomas. The increased activities of GS b can probably be correlated with the presence of glycogen; glycogen particles have in fact been detected in the rat hepatoma cells (1). Bernaert et al. (6) have shown that the formation of glycogen particles in cultures of isolated rat hepatocytes is associated with conversion of GS b to GS a, initially, and then with reconversion of GS a to GS b as glycogen accumulates.

The activities of enzymes in cell lines are not necessarily similar to those of the tissue of origin, presumably as a result of adaptation to culture conditions. The low activities of PC, PEPCK, and G6Pase and the increases of GS activity in the rat and human hepatoma cells are what might be expected in cells growing in a medium containing an adequate supply of glucose.

CONCLUSION

In human hepatoma, as in rat hepatoma, the activities of PC, PEPCK, G6Pase, and GS were lower than those in host tissue, indicating the probability of reduced gluconeogenesis and glycogenesis in the tumor. These findings are consistent with our earlier observations (4) that the activities of fructose-1,6-bisphosphatase and phosphoglucomutase are reduced in the human hepatoma. Increased activities of pyruvate kinase, indicative of increased glycolytic activity in the tumor, were also observed previously (4). The activities of PC, PEPCK, and G6Pase in human hepatoma were in general comparable with those of fetal livers, indicating perhaps similar rates of gluconeogenesis in these tissues. Activities of GS and, hence, probably the rates of glycogen synthesis were, however, lower in hepatoma than in fetal liver. The low activities of PC, PEPCK, and G6Pase and the increases of GS activity in the cells are possibly related to the glucose content of the culture medium.

REFERENCES

K. D. Hammond and D. Balinsky


Activities of Key Gluconeogenic Enzymes and Glycogen Synthase in Rat and Human Livers, Hepatomas, and Hepatoma Cell Cultures

Kathryn D. Hammond and Doris Balinsky


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/38/5/1317

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.