Glucosamine 6-Phosphate Synthase of Regenerating Rat Liver

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ABSTRACT

When rats were subjected to partial hepatectomy, glucosamine 6-phosphate synthase (EC 5.3.1.19) of the remaining liver underwent alterations both in activity and in molecular form. To study the molecular alterations, glucosamine 6-phosphate synthase was purified from regenerating as well as control liver and was analyzed by isoelectric focusing. Although control liver exhibited only one form of glucosamine 6-phosphate synthase with a pI of 5.0, sequential and transient appearance of three other forms, with pI values of 4.3, 4.8, and 4.5, respectively, was observed for regenerating liver within 72 hr following partial hepatectomy. Laparotomy, on the other hand, induced in the liver only the pI 4.8 form, and injection of a mixture containing triiodothyronine, amino acids, glucagon, and heparin induced only the pI 4.3 and 4.5 forms. It therefore appears that the pI 4.3 and 4.5, but not the pI 4.8, form, are associated with hepatic DNA synthesis. The pI 4.8 form is induced in the liver in response to surgical stress.

INTRODUCTION

GlcN-6-P synthase [glucosaminephosphate isomerase (glutamine-forming), EC 5.3.1.19; formerly L-glutamine:D-fructose 6-phosphate amidotransferase (EC 2.6.1.16)] transfers the amide group of glutamine to fructose 6-phosphate and is the first and rate-limiting enzyme in the formation of UDP-N-acetylglucosamine (6, 8). Since the major fate of UDP-N-acetylglycosamine is to supply N-acetylamino sugars as components for glycoproteins, mucopolysaccharides, and glycolipids, GlcN-6-P synthase plays an important role in regulating the biosynthesis of these macromolecules.

We have reported previously that hepatocarcinogenesis profoundly affects GlcN-6-P synthase of rat liver both quantitatively (6) and qualitatively (10, 11). While these alterations are readily explained in terms of dedifferentiation (7, 11), they may also be associated with the autonomous growth characteristic of neoplasia, since certain glycoproteins and glycolipids on the cell surface have been implicated in the control of cell replication (4, 9, 17).

As one approach to this problem, the effect of partial hepatectomy on the molecular states of hepatic GlcN-6-P synthase has now been studied by means of isoelectric focusing. Previous studies in other laboratories showed that the level of hepatic GlcN-6-P synthase rose after partial hepatectomy (12). Preliminary accounts of this work have been presented (15).

MATERIALS AND METHODS

Treatments of Rats. Male Donryu rats weighing 150 to 200 g and fed ad libitum were used. Partial hepatectomy (the removal of 70% of the liver) was performed under ether anesthesia using the procedure of Higgins and Anderson (5). Laparotomy was carried out in a similar manner without the ligation and removal of the liver lobes. TAGH was prepared according to the procedure of Short et al. (14) and injected s.c. into rats.

Purification of GlcN-6-P Synthase. The procedure described in the preceding paper (11) was used to purify GlcN-6-P synthase from regenerating and control liver. The data reported in Table 1 show that, using this procedure, GlcN-6-P synthase from 47-hr regenerating liver could be purified almost 70-fold with a recovery of 50%. Partial hepatectomy itself did not affect significantly the purification and recovery of GlcN-6-P synthase.

Isoelectric Focusing and Assay of GlcN-6-P Synthase. These were conducted under the same conditions as described in the preceding paper (11). One unit of the enzyme was defined as the amount which catalyzed the formation of 1 μmol of GlcN-6-P per min.

Other Assays. In vivo DNA synthesis was measured by the incorporation of [3H]thymidine into liver DNA. DNA was extracted by a modification of the method of Schmidt and Thannhauser (13) and determined by the method of Burton (2). The radioactivity was counted with a Beckman liquid scintillation counter.

Chemicals. Triiodothyronine was purchased from Sigma Chemical Co., Saint Louis, Mo.; glucagon was from Lilly Research Laboratories, Indianapolis, Ind.; and heparin was from The Upjohn Company, Kalamazoo, Mich. [3H]Thymidine was obtained from Daiichi Kagaku Company, Tokyo, Japan. The source of other chemicals was described elsewhere (11).

RESULTS

In agreement with previous workers (1, 12), the level of GlcN-6-P synthase in the liver rose after partial hepatectomy (Chart 1). While DNA synthesis began to increase at about 20 hr after partial hepatectomy and reached a maximum at 26 hr, the level of GlcN-6-P synthase did not reach a peak until 48 hr, after which time the level slowly declined to normal. It is noteworthy that the rise was more pronounced when the (NH4)2SO4 precipitate fraction was assayed. The level of hepatic GlcN-6-P synthase also rose after laparotomy (Chart 2), but here the level was maximal at 24 hr and returned to normal within 48 hr; the rise was not particularly pronounced after (NH4)2SO4 precipitation.

To investigate the mechanism by which the level of GlcN-6-
Three rats were sacrificed at 47 hr after 70% partial hepatectomy. The livers, amounting to 12 g, were homogenized, and GlcN-6-P synthase was purified from the homogenate by the procedure described elsewhere (11).

<table>
<thead>
<tr>
<th>Steps</th>
<th>Total protein (mg)</th>
<th>Total activity (units x 10^3)</th>
<th>Specific activity (units/mg x 10^3)</th>
<th>Purification (fold)</th>
<th>Recovery (%)</th>
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<tbody>
<tr>
<td>105,000 x g supernatant</td>
<td>511</td>
<td>939</td>
<td>1.84</td>
<td>(1)</td>
<td>(100)</td>
</tr>
<tr>
<td>(NH₄)₂SO₄ precipitate</td>
<td>172</td>
<td>874</td>
<td>5.06</td>
<td>2.8</td>
<td>93</td>
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<tr>
<td>DEAE-Sephadex eluate</td>
<td>27.3</td>
<td>542</td>
<td>19.8</td>
<td>11</td>
<td>58</td>
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<tr>
<td>Hydroxylapatite eluate</td>
<td>3.52</td>
<td>433</td>
<td>123</td>
<td>67</td>
<td>46</td>
</tr>
</tbody>
</table>

P synthase in the liver rises after partial hepatectomy, the enzyme from 47-hr regenerating liver was chromatographed on a hydroxylapatite column. As shown in Chart 3, the enzyme from control liver emerged as a single peak, but the regenerating liver enzyme exhibited more than one peak, thereby suggesting that the rise in GlcN-6-P synthase might involve the induction of new enzyme form(s).

To investigate this point further, partially hepatectomized rats were sacrificed at various time intervals, and GlcN-6-P synthase purified from the liver was subjected to isoelectric focusing, which had been a valuable technique to resolve and characterize isozymic forms of GlcN-6-P synthase (11). Confirming the previous observation (11), the enzyme from unoperated rats focused at a pH of 5.0 (see Chart 4, 0 hr), but the regenerating livers contained additional peaks, with pl values of 4.3, 4.5, and 4.8, respectively (see Chart 4, 7 to 47 hr).

The results of a typical time study are shown in Chart 4. While the pl 4.3 form is already prominent at 7 hr after partial hepatectomy, the pl 4.8 form reaches a maximum only after 24 hr. The pl 4.5 form then appears and becomes a major peak at 47 hr. At 72 hr, however, all of these forms have disappeared, and the pattern has almost reverted to that seen before partial hepatectomy. Although variation was frequently seen among experiments, the general qualitative pattern was always similar.

In order to obtain further insights into the nature of these molecular alterations, rats were laparotomized or given injections of TAGH, and their hepatic GlcN-6-P synthase was purified and subjected to isoelectric focusing. In confirmation of the data of Short et al. (14), the injection of TAGH was followed by hepatic DNA synthesis. The time curve was the same as after partial hepatectomy, although the extent was somewhat smaller.

Of the 3 GlcN-6-P synthase forms that were detected in regenerating liver, only the pl 4.8 form could be induced by laparotomy alone (Chart 5A). As was the case with partial hepatectomy, this form was manifest at 22 hr and absent at 46 hr (Chart 5B). This observation when coupled with the failure of TAGH injection to induce the pl 4.8 form (Chart 6) suggests that the pl 4.8 form is induced in the liver in response to hepatic DNA synthesis.
Regenerating Liver GlcN-6-P Synthase

Chart 5. Isoelectric focusing of GlcN-6-P synthase purified from the liver of laparotomized or adrenalectomized rats. A, 22 hr after laparotomy; B, 46 hr after laparotomy; C, 22 hr after bilateral adrenalectomy; ●, GlcN-6-P synthase activity; x, pH.

Chart 4. Isoelectric focusing of GlcN-6-P synthase purified from the liver of partially hepatectomized rats. The rats were sacrificed at 0, 7, 24, 47, and 72 hr following the operation. ●, GlcN-6-P synthase activity; x, pH.

Chart 6. Isoelectric focusing of GlcN-6-P synthase purified from the liver of the rats given injections of TAGH. 24 and 46 hr, time after injection. ●, GlcN-6-P synthase activity; x, pH.

surgical stress. The response may be adrenal dependent, since adrenalectomy, although it is a surgical operation, does not induce the pl 4.8 form in the liver (Chart 5C).

These considerations leave the sequential appearance and disappearance of the pl 4.3 and 4.5 enzymes as a possible candidate for the GlcN-6-P synthase alteration associated with the proliferative cycle of the liver. Further support for this contention was provided by the data reported in Chart 6. When rats were treated with TAGH, the early appearance of the pl 4.3 form in the liver (24 hr) was followed by its disappearance and the appearance of the pl 4.5 form (46 hr).

DISCUSSION

In the present work, isoelectric focusing was used to resolve and characterize the multimolecular forms of GlcN-6-P synthase in regenerating liver. The hypothesis that the conditions of isoelectric focusing experiments in some way converted the enzyme into multiple forms is unlikely since, during the purification, the regenerating liver enzyme was as stable as the control liver enzyme (see "Materials and Methods"), which gave a single peak upon isoelectric focusing. Also important in this respect is the finding that the regenerating liver enzyme, but not the control liver enzyme, was multiple upon hydroxylapatite chromatography.

Although these forms also emerge after laparotomy or TAGH injection, their patterns are distinct from the pattern for regenerating liver and from each other. This indicates that each form is inducible individually and independently. Moreover, one of these forms (the pl 4.3 form) is even the major GlcN-6-P synthase species of Morris hepatoma 5123D and 14-day whole fetus (data not shown). These observations further substantiate the view that the GlcN-6-P synthase forms of regenerating liver do not result from artificial modifications of the native enzyme.

We thus come to a conclusion that these GlcN-6-P synthase forms, when found in regenerating liver, are certainly associated with partial hepatectomy. The conclusion is also in har-
mony with the early observation that the rise in GlcN-6-P synthase after partial hepatectomy can be prevented by either actinomycin D or cycloheximide (1).

Comparison of these GlcN-6-P synthase alterations with those induced by laparotomy or TAGH injection has enabled postulation of the sequential appearance of the pl 4.3 and 4.5 forms, which is associated with the proliferative cycle of hepatocytes. The pl 4.8 form, on the other hand, emerges in the liver in response to surgical stress and may thus be associated with the enhanced seromucoid synthesis observed under these conditions (3).

It remains to be learned how the pl 4.3 and 4.5 forms of GlcN-6-P synthase are functionally related to hepatic DNA synthesis. The demonstration of the fetal isozyme of pyruvate kinase in regenerating and preneoplastic liver has led Walker and Potter (16) to suggest that the fully mature hepatocytes cannot undergo division without some degree of dedifferentiation. The sequential and transient appearance of the pl 4.3 and 4.5 forms of GlcN-6-P synthase may also be a reflection of the rapid transition of hepatocytes between a functioning and a dividing state. Although these forms are absent from ascites hepatomas (11), they may be present in less rapidly growing hepatomas; work currently in progress has indicated the presence of the pl 4.3 form in Morris hepatoma 5123D.

REFERENCES

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