Differential binding of serum glycoproteins to lectins during hepatic regeneration in hepatocellular carcinoma and fulminant hepatic failure

M.-Q. DU, W. L. HUTCHINSON, P. J. JOHNSON AND ROGER WILLIAMS
Liver Unit, King's College Hospital and School of Medicine and Dentistry, London

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SUMMARY
1. The concentrations of four serum glycoproteins, thyroxine-binding globulin, α1-macroglobulin, α1-antitrypsin and transferrin, as well as their reactivities with concanavalin A and lentil-lectin, were measured in patients with hepatocellular carcinoma or fulminant hepatic failure and in normal subjects.

2. Serum concentrations of thyroxine-binding globulin and α1-antitrypsin were significantly greater in patients with hepatocellular carcinoma than in normal subjects, and the percentage lentil-lectin reactivity of these two proteins was markedly increased.

3. With the exception of transferrin, which did not bind to lentil-lectin, an enhancement of lentil-lectin reactivity was observed for the glycoproteins in serum from patients with fulminant hepatic failure. No difference in concanavalin A binding was found between the groups for any of the glycoproteins.

4. Altered fucosylation, as indicated by increased lentil-lectin binding, occurs in several glycoproteins arising in malignant and non-malignant conditions associated with abnormal hepatic regeneration.

Key words: fucosylation, fulminant hepatic failure, glycoprotein, hepatic regeneration, hepatocellular carcinoma, lectin.

Abbreviations: AFP, α-fetoprotein; α1-AT, α1-antitrypsin; Con A, concanavalin A; FHF, fulminant hepatic failure; HCC, hepatocellular carcinoma; α2-MG, α2-macroglobulin; TBG, thyroxine-binding globulin.

INTRODUCTION
α-Fetoprotein (AFP) is a transitory foetal protein produced by the yolk-sac and the liver [1]. It is undetectable, or presents only in very low concentrations, in normal adult sera [2], but may be synthesized and secreted by hepatocytes which have undergone malignant change [3]. Serum levels of AFP are also increased in conditions associated with abnormal hepatic regeneration such as hepatic cirrhosis, chronic active hepatitis and fulminant hepatic failure (FHF) [4–6]. We have previously reported that concanavalin A (Con A)- and lentil-lectin-reactive AFP are significantly increased in patients with hepatocellular carcinoma (HCC) relative to patients with non-malignant liver disease and normal pregnant subjects [7]. It is not clear, however, if this increase, which implies abnormal glycosylation, is specific to AFP, a characteristic of all glycoproteins synthesized by malignant hepatocytes, or a feature of any type of hepatic regeneration.

In this study the differential Con A- and lentil-lectin-binding characteristics of four additional serum glycoproteins were investigated in sera from a series of patients with HCC. We also studied sera from patients with FHF who have raised AFP levels, since elevations of AFP have been considered indicative of active regeneration in this condition [8].

Patients
Sera for this study were obtained from 15 patients (aged 20–75 years) with HCC, in all of whom the diagnosis was confirmed by histological examination of liver biopsy specimens (serum AFP range = 390–9000,000 ng/ml). With the exception of one female patient, all had hepatic cirrhosis. Fifteen patients with FHF and elevated serum AFP levels (42–422 ng/ml) were also studied, together with 10 normal subjects.

Lectin-affinity column chromatography
Affinity chromatography on lentil-lectin–Sepharose 4B was carried out at 4°C. Serum samples were firstly
dialysed against Tris–HCl (50 mmol/l, pH 7.2) containing 150 mmol/l NaCl, 1 mmol/l CaCl₂ and 1 mmol/l MgCl₂. A volume between 0.3 and 0.5 ml from each dialyzed serum sample was applied to the column (5.0 ml bed vol.) of lentil-lectin-Sepharose 4B which had previously been equilibrated with the dialysis buffer. Each column was washed with 50 ml of the same buffer (10 column vol./20 ml/h) and then eluted with 0.5 mol/l α-methyl-D-mannoside dissolved in the buffer. A total of 20–26 ml of eluate containing the binding glycoproteins was collected for analysis along with the unbound material. The column was finally eluted with the same buffer for regeneration.

Affinity chromatography on Con A-Sepharose 4B (5 ml) was similarly performed in Tris–HCl (50 mmol/l, pH 7.5) containing 1 mol/l NaCl, 1 mmol/l CaCl₂ and 1 mmol/l MgCl₂, plus 0.2 ml of Tween 20 (polyoxyethylene sorbiton monolaurate wetting agent)/l. α-Methyl-D-mannoside (0.2 mol/l) dissolved in the buffer was used for elution.

Glycoprotein, albumin and total protein determinations

Thyroxine-binding globulin (TBG) in sera and column eluates was determined by a commercial radioimmunoassay method (Immophase; Corning). Albumin, α₁-antitrypsin (α₁-AT), α₂-macroglobulin (α₂-MG) and transferrin in sera and column eluates were measured by single radial immunodiffusion according to the manufacturer’s procedure (LC Partigen; Behring). Total protein in sera and in the column eluates was estimated using the bicinchoninic acid protein assay reagent (Pierce) as described by Smith et al. [9]. The individual lectin-reactive glycoproteins were expressed as a percentage of each individual applied protein. Total lectin-reactive glycoprotein was assessed as that which was eluted by α-methyl-D-mannoside. This ligand-eluted protein was expressed as a percentage of the total protein applied to the column. The differences in Con A- and lentil-lectin-binding characteristics between the different groups were assessed by Student’s t-test.

RESULTS

Serum TBG and α₁-AT concentrations were significantly increased in patients with HCC (P<0.05 and P<0.02, respectively, Fig. 1), whereas serum α₂-MG and transferrin were unaltered (Table 1). In FHF there were significant decreases in transferrin (P<0.01) and α₂-MG (P<0.0005). Serum albumin was significantly lower in both HCC and FHF groups than in normal control subjects (P<0.005 and P<0.0005, respectively). Serum total protein concentration was significantly greater in the HCC group but was reduced in the FHF group (P<0.05 in each case) compared with control subjects.

Lectin-affinity column chromatography

Albumin did not bind to either the Con A or lentil-lectin column in HCC patients, FHF patients or control subjects. With the exception of transferrin, all glycoproteins were detected in material eluted by α-methyl-D-mannoside from the lentil-lectin column. This result with transferrin was due to non-binding rather than degradation or loss on the column, as shown by total recoveries of 90–110% in each case.

Lentil-lectin-reactive TBG and α₁-AT were significantly elevated in patients with HCC (P<0.005 and P<0.01, respectively, Fig. 2 and Table 2), whereas α₂-MG showed identical results compared with normal control subjects. In FHF there was a significant enhancement in the binding to lentil-lectin of TBG (P<0.0005), α₁-AT (P<0.0005) and α₂-MG (P<0.05). There were also significant differences between the binding to lentil-lectin of TBG (P<0.005), α₁-AT (P<0.005) and α₂-MG (P<0.025) from patients with HCC and FHF, due largely to more enhanced binding in the latter condition. No significant differences in Con A-binding activities were found.

Table 1. Glycoprotein, albumin and total protein concentrations in sera from HCC patients, FHF patients and normal subjects

<table>
<thead>
<tr>
<th></th>
<th>TBG (μg/ml)</th>
<th>α₁-AT (mg/ml)</th>
<th>α₂-MG (mg/ml)</th>
<th>Transferrin (mg/ml)</th>
<th>Albumin (mg/ml)</th>
<th>Total protein (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCC (n=15)</td>
<td>29.8±13.3</td>
<td>3.7±1.8</td>
<td>3.8±1.1</td>
<td>32±1.0</td>
<td>35.0±13.6</td>
<td>83.1±14.3</td>
</tr>
<tr>
<td>FHF (n=15)</td>
<td>15.2±7.7</td>
<td>1.9±0.8</td>
<td>2.4±0.7</td>
<td>2.3±0.7</td>
<td>30.2±11.3</td>
<td>57.2±16.6</td>
</tr>
<tr>
<td>Normal (n=10)</td>
<td>18.7±4.2</td>
<td>2.1±0.6</td>
<td>3.6±0.6</td>
<td>3.1±0.7</td>
<td>52.2±6.4</td>
<td>70.0±12.5</td>
</tr>
</tbody>
</table>

Fig. 1. Comparison of serum concentrations of TBG (a) and α₁-AT (b) in HCC patients, FHF patients and normal subjects. Bars indicate means ± SD.
among any of these glycoproteins between the HCC, FHF and normal control groups (Table 2).

Additionally, the percentage lentil-lectin binding among the total ligand-eluted glycoproteins was significantly greater in both patients with HCC and patients with FHF \((P<0.02\) and \(P<0.001\), respectively) compared with normal control subjects (Table 3). No differences were found in Con A-binding activities of total glycoprotein among the three groups. There was no correlation between lentil-lectin binding of individual glycoproteins and AFP levels in HCC or control subjects. Similarly, no correlations were observed between reactivities of total glycoproteins and AFP levels.

**DISCUSSION**

It has previously been demonstrated that the percentage of lentil-lectin-reactive AFP (i.e. fucosylated) is significantly greater in patients with HCC than in patients with chronic liver disease or in cord serum [10-13]. In our previous work we examined material from serum which had been obtained from HCC patients, FHF patients and pregnant subjects and applied it to lentil and Con A lectins [7]. The quantities of lentil-lectin-reactive AFP and Con A-reactive AFP, as judged by the material specifically eluted with \(\alpha\)-methyl-D-mannoside, were significantly greater in patients with HCC than those found in serum from patients with FHF and normal pregnant subjects. By extending the study to additional serum glycoproteins, we now demonstrate significant differences in binding activities between other serum glycoproteins in HCC and FHF, namely TBG, \(\alpha_1\)-AT and \(\alpha_2\)-MG.

Somewhat surprisingly, the mean percentage binding of these glycoproteins was significantly greater in patients with FHF than in patients with HCC. So far as we are aware, the glycosylation of these serum proteins has not been investigated previously in patients with FHF, and our results suggest a greater degree of apparent fucosylation in this group than among those with HCC. The overlap in binding seen between these two groups and between either group and the normal control group contrasts with the situation seen for AFP [7]. No overlap was observed for the lentil-lectin binding of this protein in HCC and FHF. A possible explanation for such a difference may be the contribution made by non-tumour tissue in HCC and FHF of a large proportion of these glycoproteins which are normally present in serum. With AFP on the other hand, the major proportion derives from tumour tissue, particularly at higher serum levels. Nevertheless, it is clearly shown that carbohydrate structural alterations in HCC are not specific to AFP.

Differences in lentil-lectin binding between glycoproteins and the non-glycosylated albumin are not altogether surprising. Similarly, differing serum patterns might be expected between glycosylated and non-glycosylated components in conditions such as HCC which affect glycosylation states, in view of the profound effect of sugar moieties on glycoprotein secretion [14, 15]. Some of

**Table 2. Binding of serum glycoproteins (a) to lentil-lectin and (b) to Con A**

Results are means ± sd.

<table>
<thead>
<tr>
<th></th>
<th>Lentil-lectin binding (%)</th>
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<th>Con A binding (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TBG</td>
<td>(\alpha_1)-AT</td>
<td>(\alpha_2)-MG</td>
<td>Transferrin</td>
</tr>
<tr>
<td>HCC (n=15)</td>
<td>14.7 ± 3.6</td>
<td>17.7 ± 8.2</td>
<td>52.4 ± 10.7</td>
<td></td>
</tr>
<tr>
<td>FHF (n=15)</td>
<td>21.0 ± 7.1</td>
<td>28.4 ± 8.8</td>
<td>63.3 ± 13.7</td>
<td></td>
</tr>
<tr>
<td>Normal (n=10)</td>
<td>9.1 ± 4.8</td>
<td>8.8 ± 3.2</td>
<td>50.3 ± 8.4</td>
<td></td>
</tr>
<tr>
<td>HCC (n=15)</td>
<td>78.9 ± 8.3</td>
<td>81.9 ± 10.2</td>
<td>70.1 ± 7.6</td>
<td>66.6 ± 8.1</td>
</tr>
<tr>
<td>FHF (n=15)</td>
<td>78.3 ± 8.9</td>
<td>79.6 ± 9.6</td>
<td>71.6 ± 8.0</td>
<td>65.2 ± 10.5</td>
</tr>
<tr>
<td>Normal (n=10)</td>
<td>82.8 ± 5.1</td>
<td>83.6 ± 7.4</td>
<td>76.2 ± 12.9</td>
<td>63.9 ± 4.7</td>
</tr>
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Table 3. Binding of total glycoproteins to lentil-lectin and Con A

<table>
<thead>
<tr>
<th></th>
<th>Lentil-lectin-binding glycoproteins (%)</th>
<th>Con A-binding glycoproteins (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCC (n = 15)</td>
<td>21.5 ± 5.8</td>
<td>21.9 ± 3.2</td>
</tr>
<tr>
<td>FHF (n = 15)</td>
<td>24.7 ± 6.4</td>
<td>21.9 ± 4.4</td>
</tr>
<tr>
<td>Normal (n = 10)</td>
<td>16.1 ± 3.1</td>
<td>20.4 ± 1.8</td>
</tr>
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</table>

the glycoproteins (e.g. α1-AT, α2-MG) are acute-phase reactants [16] and may be further expected to have certain synthetic and secretory features in common. In this context, our observation of differences in lectin-binding behaviour and serum levels in HCC among these glycoproteins was initially surprising (serum TBG and α1-AT increased in HCC, α2-MG and transferrin did not). It has been observed previously, however, that in various cancer states (ovarian, breast, gastrointestinal), serum protein-bound fucose is often increased and correlates positively with the levels of those glycoproteins found to increase in acute-phase reactions (e.g. haptoglobin, α1-acid glycoprotein, α1-AT) [17]. It is possible, therefore, that increased fucosylation may affect directly the secretion of certain glycoproteins and may explain our observation of increased lentil-lectin binding only among those proteins manifesting elevated serum levels in HCC. It is unlikely that such a mechanism would apply in a similar manner in FHF where increased lentil-lectin binding was also observed for TBG, α1-AT and α2-MG, for which elevated serum levels were not observed.

Unlike previous studies which have demonstrated increased binding of AFP from HCC patients to Con A, relative to that seen for AFP from FHF patients or normal pregnant women, no differences were found in Con A-binding characteristics among serum glycoproteins involved in this study between HCC, FHF and normal control groups. These results do not necessarily imply the absence of structural modifications affecting glucose and mannose residues. Con A lectin has been shown to bind glycoproteins containing these sugars when they form an accessible part of a carbohydrate chain of the biantennary type [18]. Glycoproteins containing chains of the multiantennary type (> 2 antennae) appear to be unretarded by this lectin. It is possible therefore that modifications involving these sugars may well occur in these proteins during HCC development, but remain undetected by Con A reactivity if they affect the multiantennary chains only. Certainly, forms of AFP and TBG containing multiantennary carbohydrate chains are well known [18, 19].

The results indicate that altered fucosylation is a property of proteins which arises during abnormal regeneration in both malignant and non-malignant conditions. The suggestion of altered fucosylation in these states is further supported by the significant increases in total protein eluted by α-methyl-D-mannoside in both conditions from the lentil-lectin column. Such increases cannot be accounted for by the specific proteins investigated in the present work and it raises the possibility of structural modifications in pre-existing proteins or the generation of new disease-related components. The enzymatic basis of these changes has not been elucidated, although increased fucosyltransferase activities have been reported in malignant liver tissues and in the serum from patients with cancer [20, 21].

ACKNOWLEDGMENTS

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REFERENCES


