Biosynthesis of abnormally glycosylated hepatoma secretory proteins in cell cultures

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We studied, by electrophoretic techniques, the physicochemical properties of 4 glycoproteins, α_1-antitrypsin, α_1-antichymotrypsin, α_1-acid glycoprotein and transferrin synthesized by three different human hepatoma cell lines. A common feature was the export of glycoproteins with retarded electrophoretic mobility, indicating incomplete sialylation, and a predominance of atypical, highly branched carbohydrate chains. The abnormal glycosylation pattern may be specific for malignant transformation of hepatocytes and possibly related to the intracellular accumulation of some of these proteins in malignant cells.

Glycoprotein *Abnormal glycosylation* *Crossed affinoimmunoelectrophoresis* *Hepatoma cell line*

1. INTRODUCTION

Several human hepatocellular carcinoma cell lines have biosynthetic capabilities similar to those of normal liver parenchymal cells. The lines Hep G2 and Hep 3B synthesize and secrete most major plasma proteins including albumin and α-fetoprotein [1]. Another previously described line (PLC/PRF/5) [2] has a narrower range of synthetic ability but expresses HB_sAg a property in common with Hep 3B. The availability of these cell lines in continuous culture provide a major tool for studies of biosynthetic patterns and mechanisms modulating the rate of biosynthesis of individual proteins. Nearly all plasma proteins are glycoproteins the majority of which are formed by *N*-glycosylation. The intracellular transfer is assumed to be receptor mediated with characteristic rates for the different proteins [3]. Before export from the hepatocyte the high-mannose core oligosaccharide is trimmed resulting in the final complex glycoprotein having sialic acid, the only electrically charged sugar residue, at the terminal position. The mobility on agarose electrophoresis reflects the number of sialic acid residues. In the process of trimming and complex glycosylation variations in the branching of the oligosaccharide occur. Chains exist as bi-, tri-, tetraantennary or hybrid forms. Circulating glycoproteins exhibit a large degree of microheterogeneity which can be studied by crossed affinoimmunoelectrophoresis with lectins particularly concanavalin A (Con A). Only glycans corresponding to the bi-antennary structure, which is the least modified in the glycosylation process, bind to Con A [4].

We have previously used PLC/PRF/5 to delineate the biosynthetic steps for human α_1-antitrypsin (AAT) and found that this process is similar to that found for rat AAT [5]. However, it was demonstrated that export AAT from these hepatocytes, although having a normal molecular size, had a retarded electrophoretic mobility consistent with incomplete terminal sialylation of the glycoprotein. In addition Con A electrophoresis displayed reduced affinity indicating an excess of highly branched but incompletely sialylated carbohydrate chains. We extended these studies to in-

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Abbreviations: PEG, polyethylene glycol; SDS-PAGE, SDS-polyacrylamide gel electrophoresis

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clude α_1-antichymotrypsin (ACT), another closely related antiprotease and acute phase reactant; α_1-acid glycoprotein (AGP), a non-antiprotease acute phase reactant with high carbohydrate content; and transferrin (TRF), a glycoprotein whose export does not increase in acute phase situations.

2. MATERIALS AND METHODS

PLC/PRF/5 cells were maintained as described [5]. The cell lines Hep G2 and 3B were provided by Dr Darlington, Department of Pathology, Baylor College of Medicine, Houston, TX, with the kind permission of Dr B. Knowles, Wistar Institute, Philadelphia. They were maintained as described [1,5]. To avoid high concentrations of fetal calf serum standard medium was replaced by Williams medium E supplemented as described [5] in some experiments.

Quantitation of proteins in cell culture media was performed by electroimmunoassay [6] using dilutions of a standard normal serum (Seronorm® Protein, Nyegaard & Co., Oslo, Norway) as reference. Monospecific antisera against AAT, TRF, and AGP were gifts from the Department of Clinical Chemistry, Malmö, antiserum against ACT was purchased from Dakopatts, Copenhagen, Denmark. Crossed immunoelectrophoresis was performed according to Ganrot [7] and crossed affinoimmunoelectrophoresis with free Con A in the first dimension according to Hansen et al. [8]. [^{35}S]Methionine labelling of export proteins as described [5]. SDS-PAGE was carried out as previously described with 20 × 30 cm slab gels containing a 10–15% polyacrylamide gradient with a 2 cm spacing gel [5]. Sialidase activity in cell cultures was analyzed by adding 0.5 ml normal human serum to 5 ml medium with cells close to confluence in a culture flask. Incubation was then continued for 24 h at 37°C and electrophoretic mobility of the glycoproteins in the medium was studied by crossed immunoelectrophoresis.

3. RESULTS AND DISCUSSION

The approximate concentrations of the different proteins reached in culture media at confluence is given in table 1. All individual glycoproteins studied show a more or less pronounced retarded mobility towards the anode. The patterns with broad, heterogenous and retarded peaks of ACT, TRF and AGP on crossed immunoelectrophoresis (fig.1) is uniform and resembles that described for AAT [5]. After [^{35}S]methionine labelling export ACT was studied and appeared as a broad, heterogenous immunoprecipitate. Tunicamycin, known to totally prevent addition of asparagine-linked carbohydrate chains, resulted in delayed export of a smaller molecule with sharper demarcation of the immunoprecipitate (fig.2). The experiment demonstrates that ACT heterogeneity resides in the carbohydrate chains rather than in the polypeptide. Absence of sialidase activity in the media (not shown) suggests incomplete de novo sialylation rather than postexport alterations. On crossed affinoimmunoelectrophoresis a low lectin

Table 1

Electroimmunoassay of 4 glycoproteins in hepatoma supernatants

Protein	Carbohydrate content (%)	Cell line		
		PLC/PRF/5	Hep G2	Hep 3B
AAT	12	0.95	0.65	0.15
ACT	25	0[a]	0.45	0[a]
AGP	45	<0.01	0.05	0.02
TRF	6	0.04	0.50	0.18

Concentrations are expressed as per cent of normal plasma level (= 100). [a] Not detectable

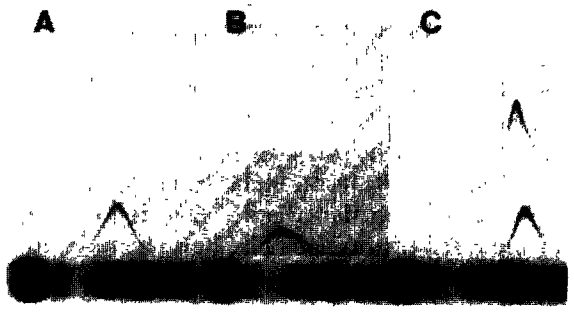

Fig.1. Crossed immunoelectrophoresis of a normal plasma pool (above) and medium from Hep G2 (below). ACT (A), AGP (B) and TRF (C). The electrophoretic pattern of normal plasma is given for reference. Anode to the left. Medium was collected after 3 days culture and concentrated 20 × before analysis.

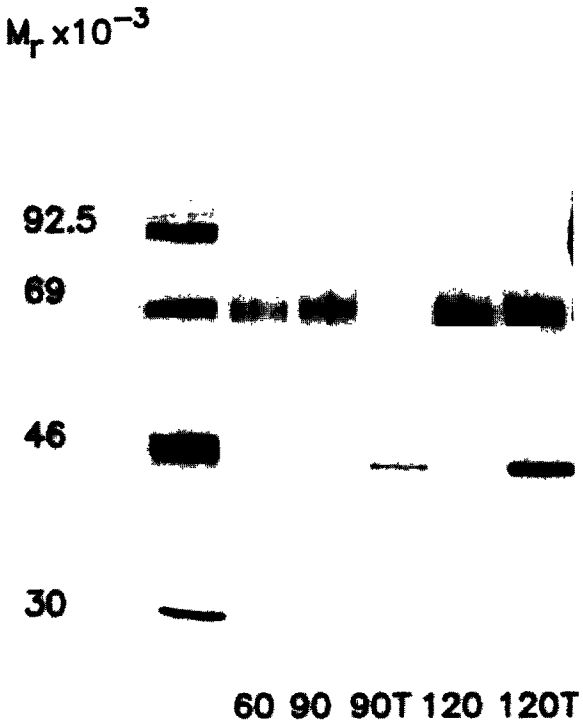

Fig.2. SDS-PAGE of immunoprecipitated export ACT from a pulse chase experiment. Hep G2 cells were incubated for 75 min in a methionine deficient medium with tunicamycin 3 μg/ml in lanes marked T. Pulse time was 15 min with 100 μCi [^{35}S]methionine per culture. SDS-PAGE in a 10–15% gradient slab gel with fluorographic enhancement is shown at chase time 60, 90 and 120 min.

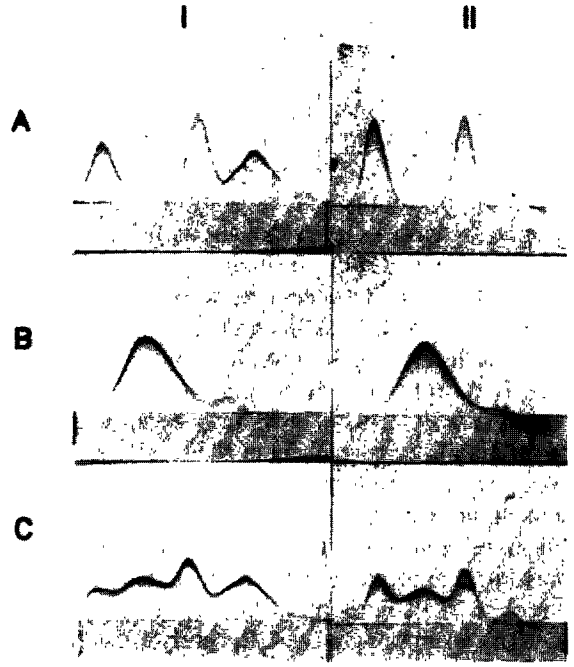

Fig.3. Crossed Con A affinoimmunoelectrophoresis of ACT (I) and AGP (II). A, normal plasma pool; B, Hep G2 medium concentrated 20 × and C, mixture of equal volumes of A and B. Anode to the left. Second dimension: 0.7% anti-human ACT and 1% anti-human AGP, 4% PEG 6000 respectively and 50 mM methyl-α-D-mannopyranoside.

affinity was noted. The patterns are similar to that previously described for AAT (fig.3) and indicate, in addition to hyposialylation, a relative predominance of highly branched carbohydrate chains.

The data presented thus indicate the presence of a similar glycosylation defect in 4 different glycoproteins secreted from 3 different cell lines and therefore suggest a common underlying mechanism. At present it is uncertain whether these alterations are due to cell culture conditions or to inherent characteristics of the malignant cells per se. If the putative mechanism is operating in vivo one would anticipate the biosynthesis and export of incompletely sialylated glycoproteins from hepatoma cells with a very rapid clearance from the circulation due to binding by the hepatic galactose-binding protein [9]. The presence of small amounts of asialoglycoproteins in the circulation has been demonstrated by indirect techniques in a variety of liver diseases [10] and do not seem to be a specific feature of malignantly transformed hepatocytes although one remarkable case has been reported [11]. The presence of an excess of triantennary carbohydrate chains has been described for AAT in acute phase situations [12]. Presently, however, one may speculate that the carbohydrate chains of the proteins studied here are not only highly branched but in addition atypical and similar to the unusual biantennary, complex and asialylated sugar chains that have been shown to be present in choriongonadotropin produced by a chorioncarcinoma [13]. The Con A affinoimmunoelectrophoresis is a relatively crude technique, incapable of providing subtle structural details. When interpreting results it is also impor-

tant to carefully consider the proportions between total amount of glycoprotein applied to the gels and their Con A content [8]. We therefore studied the influence of increasing Con A concentrations (1–10.5 mg/ml) on the pattern shown in fig.3. Higher concentrations resulted in slower mobility and slightly improved resolution but did not change the basic pattern (not shown). 3 mg/ml was found to be the optimal concentration to demonstrate differences between normal and hepatoma proteins in this system.

Intracellular AAT revealed by periodic-acid Schiff staining or by immunoperoxidase techniques in PLC/PRF/5 cells may be an expression of an inability of hepatoma cells to glycosylate export proteins completely thus promoting intracellular accumulation [5]. There is increasing evidence that AAT accumulation may occur not only in hepatoma tissue but also in many other tumours [14] suggesting a broader significance of AAT as a tumour marker. Recently ACT has also been shown, by the immunoperoxidase technique, to accumulate in hepatoma cells and to be even more sensitive than AAT as a tumour marker [15]. The appearance of abnormally glycosylated export proteins in the circulation and/or in malignant hepatocytes may thus have a potential diagnostic value.

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