Glycoproteins in cystic fibrosis: a lectin binding study

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SUMMARY Ten lectins have been used to detect glycoproteins, after SDS polyacrylamide gel electrophoresis and gel isoelectric focusing, in fibroblasts, red cell membranes, urine, and plasma of patients and obligate heterozygotes with cystic fibrosis. No disease specific changes were detected but considerable individual variation was observed, some of which was attributed to known genetic polymorphisms unrelated to cystic fibrosis.

There is abundant evidence to suggest that abnormalities of glycoproteins occur in patients with cystic fibrosis.¹ Some of these features may be secondary effects, for example the changed density and solubility of glycoproteins in cystic fibrosis are probably attributable to increased Ca^{2+} concentration.^{2,3} However, differences in the sugar content might reflect a constitutional defect in glycosylation. Decreased fucose or increased sialic acid or both have been reported in duodenal fluid, pulmonary secretions, and rectal mucus, on the serum proteins which bind heparin, mucus glycopeptides from meconium, and decreased sialic acid and altered binding to lectins have been reported for serum α_2 -macroglobulin.^{1,4-6} Although there have been conflicting reports, these observations suggest a generalised defect in soluble glycoproteins possibly involving their processing. The possible role of the cell membrane in the pathogenesis of cystic fibrosis has led to a number of studies on the cell membrane glycoproteins and has provided some evidence for alterations of these components in fibroblasts,⁷⁻⁹ although several other studies failed to show differences.¹⁰⁻¹⁴ The purpose of this study was to investigate the glycan structure of soluble and membrane glycoproteins in cystic fibrosis patients, heterozygotes, and controls, using a wide variety of lectins to detect the glycoproteins after electrophoresis, employing the general technique originally described by Burridge¹⁵ and extending the study of Harris and Bramwell¹⁴ in which this technique was used to analyse fibroblast membrane glycopro-

teins. Using this approach several tissues could be investigated, deficient or additional components could be identified, and abnormal glycan structures could, in principle, be pinpointed to particular molecules without previous purification. The material examined was plasma, red cell membranes, urine, and fibroblasts. These tissues were chosen because of their accessibility and also because considerable background information had already been acquired in this laboratory about the normal glycoprotein profiles in healthy subjects. Particular attention was paid to the newly described peanut lectin reactive urinary mucin (PUM) which shows genetically determined variation and occurs in urine, kidney, and lung.¹⁶ In selecting other lectins, attention was paid to the possibility of changes in fucose and sialic acid content. Thus the fucose binding lectin from *Ulex europaeus* (UEA-I) was included, and comparisons were made between the wheat germ agglutinin (WGA) binding profile (sialic acid and *N*-acetylglucosamine) and that of succinylated wheat germ agglutinin (sucWGA) which binds *N*-acetylglucosamine alone. Material treated with bacterial sialidase was also compared with untreated material.

A more limited survey of glycoproteins was also done using the same detection technique following isoelectric focusing in polyacrylamide gel with the purpose of detecting glycoproteins with altered charge characteristics.

Materials and methods

SDS POLYACRYLAMIDE ELECTROPHORESIS
Vertical sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis (PAGE) was carried out as described previously.¹⁶

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ISOELECTRIC FOCUSING

Isoelectric focusing (IF) of plasma samples was done using a pH gradient of 3.5 to 10. Urine samples (concentrated approximately 20 times and made 5% in mercaptoethanol and 9.5 mol/l in urea) were focused on gels containing 6 mol/l urea. The gels (0.2×11×25 cm) were run for 2 hours adjusting the power pack to 25 W maximum with unlimited current and voltage, with cooling by water circulating at 8 to 10°C.

LECTIN STAINING

Table 1 shows a list of the lectins used in the present study and their principal specificities. Lectin staining was carried out as described previously.¹⁶

SAMPLES

The 10 to 20 control samples tested from each type of tissue were from healthy young adults and children. On the IF gels fewer controls were tested but equal numbers of control and patient samples

were tested on each gel, matched as far as possible for age of sample and age of patient. Samples from cystic fibrosis patients (a total of 29 different patients, age range 2 to 22) and four obligate heterozygotes were obtained from Sydenham Children's Hospital, University College Hospital, and the Blood Bank, Reykjavik. The six patients whose fibroblasts were examined were six of the 17 studied previously by Harris and Bramwell.¹⁴

Urine samples were concentrated approximately 20 times using dialysis tubing and Aquacide (Calbiochem-Boehringer). Plasma samples from heparinised blood were diluted 10 times before analysis with SDS-PAGE but were diluted 1/3 for analysis with isoelectric focusing. Red cell membranes were made using the technique of Fairbanks *et al*¹⁷ and the membranes stored up to 6 weeks at -70°C. Fibroblasts were grown in MEM (Flow Laboratories) containing 10% fetal calf serum. The cells were harvested using a rubber policeman and disrupted by freezing and thawing twice. The preparation was centrifuged at 100 000 g to obtain a membrane-rich pellet.

SIALIDASE TREATMENT

Sialidase treatment was done by using *Clostridium perfringens* type VI (Sigma) sialidase in citrate phosphate buffer (pH 5.0) at a concentration of 0.1 unit/ml. Two volumes of sample and one volume of the sialidase solution were incubated for one hour at 37°C.

Results

PROTEIN PROFILES

SDS-PAGE was used to analyse urine from 10 cystic fibrosis patients, red cell membranes from three,

TABLE 1 Lectins used in the present study, their abbreviations, and principal specificity.

Lectin	Abbreviation	Principal specificity
<i>Ulex europaeus</i> agglutinin I	UEA-I	α-L-Fuc
<i>Helix pomatia</i> agglutinin	HPA	α-D-GalNAc
Soybean agglutinin	SBA	α-D-GalNAc
<i>Ricinus communis</i> agglutinin I	RCA-I	β-D-Gal
Peanut agglutinin	PNA	β-D-Gal(1→3)D-GalNAc
<i>Phaseolus vulgaris</i> erythroglutinin	PHA-E	β-D-Gal(1→4)D-GlcNAc
Wheat germ agglutinin	WGA	β-D-GlcNAc, sialic acid
Succinylated wheat germ agglutinin	sucWGA	β-D-GlcNAc
Concanavalin A	CON A	α-D-Man
<i>Lens culinaris</i> agglutinin	LCA	α-D-Man

TABLE 2 Summary of material tested and results obtained by lectin analysis after electrophoresis.

Material studied	Sialidase treatment	No of patients	No of heterozygotes	Electrophoretic method	PHA-E	CONA	WGA	LCA	RCA-I	sucWGA	PNA	SBA	HPA	UEA-I
Urine	-	10	15	SDS-PAGE	nt	+	+	+	+	+	+	(+)	nt	(+)
	+/-	5		SDS-PAGE	+	+	+	nt	+	nt	+	nt	+	nt
	-	6		IF	nt	+	+	+	+	(+)	(+)	(+)	+	nt
Red cell membranes	+/-	3	4(+1 sib)	SDS-PAGE	+	+	+	nt	+	+	+	(+)	+	+
Plasma	+/-	2	4(+1 sib)	SDS-PAGE	+	+	+	nt	+	(+)	+	(+)	(+)	(+)
	-	1		SDS-PAGE	nt	+	+	nt	nt	nt	nt	nt	nt	nt
	+/-	3		SDS-PAGE	+	nt	+	nt	+	nt	+	nt	nt	nt
	+/-	3		IF	+	+	+	nt	+	(+)	(+)	(+)	(+)	(+)
	+/-	3		IF	nt	nt	nt	nt	+	nt	nt	nt	nt	nt
Fibroblast membranes	-	3	6	SDS-PAGE	+	+	+	nt	+	(+)	(+)	(+)	(+)	(+)
	+	3		SDS-PAGE	nt	nt	nt	nt	nt	(+)	+	(+)	(+)	(+)
	+/-	3		SDS-PAGE	nt	nt	+	nt	+	nt	nt	nt	nt	nt

SDS-PAGE=sodium dodecyl sulphate polyacrylamide gel electrophoresis.

IF=gel isoelectric focusing.

+

indicates glycoproteins detected.

(+) indicates little or no glycoprotein detected in either patients or controls.

nt=not tested.

FIG 1 SDS gel stained with WGA showing urine specimens from six patients (P1-6) and three healthy controls (C1-3). For other abbreviations see text.

plasma from two, and fibroblasts from six. Both sialidase treated and untreated red cell membrane preparations, plasma, and fibroblasts were examined. The gels were stained with Coomassie Brilliant Blue (CBB) and the protein profiles compared with those of control samples. We failed to detect any differences between cystic fibrosis patients and controls although several differences were observed within each group.

GLYCOPROTEIN PROFILES

The tissues examined and the lectins used are summarised in table 2. In most cases material treated with bacterial sialidase was also examined as indicated in table 2.

GLYCOPROTEINS IN URINE

Urine was analysed from 15 cystic fibrosis patients using SDS electrophoresis and from six patients using isoelectric focusing gels followed by staining with several lectins (table 2). There was considerable individual variation in the concentration of all or particular glycoproteins but these variations are not related to the disease. The PUM components¹⁶ were detected by PNA staining after SDS-PAGE of samples from 20 patients and they displayed a

FIG 2 IF gel stained with RCA-I showing plasma samples (both sialidase treated and untreated) from three patients (P1-3) and three healthy controls (C1-3).

random selection of the phenotypes detected in healthy subjects. With WGA this family of components was also detected (fig 1) together with many

other components. The variation in the region designated T-H is probably an artefact of the preparation method, since the centrifugation used to

FIG 3 Pieces of SDS gels stained with various lectins (for abbreviations see table 1) showing red cell membrane preparations (with and/or without sialidase treatment) from one patient (P), one heterozygote (HZ), and two healthy controls (C1, C2). Both the patient and heterozygote are blood group B, C1 is A₁, and C2 is O.

clarify the samples removes variable amounts of the Tamm-Horsfall glycoprotein (T-H).¹⁸

GLYCOPROTEINS IN PLASMA

Plasma samples from six cystic fibrosis patients and four heterozygotes were analysed on SDS-PAGE and from six cystic fibrosis patients on isoelectric focusing gels (table 2). No reproducible differences between the patient and control samples were seen using either sialidase treated or untreated material, although in one IF experiment (fig 2) a component (indicated with an arrow) was detected with RCA-I in sialidase treated samples from three patients but not in the controls. However, a similar component was weakly visible in the control material after prolonged exposure (3 days) of the autoradiograph and, furthermore, was not visible on subsequent examination of the same material from the patients and controls or in several other cases.

RED CELL MEMBRANE GLYCOPROTEINS

There were no differences in glycoprotein profiles between the cystic fibrosis patients and the controls. However, some differences in staining were seen with the blood group reactive lectins, UEA-I and HPA, which appeared to be related to the different blood groups of the subjects analysed. HPA showed strong binding to the glycoproteins of A₁ subjects and UEA-I to the glycoproteins of type O subjects. These observations are compatible with those of Finne.¹⁹ Quite surprisingly sucWGA and WGA after sialidase treatment also distinguished the red cell membrane glycoproteins from persons of different blood groups. They bind strongly to those of blood group A₁ but weakly or not at all to those of blood groups B and O. Fig 3 shows an example of the variety of patterns that may be observed with these lectins.

GLYCOPROTEINS IN FIBROBLAST MEMBRANES

Membrane-rich extracts of fibroblasts, both treated and untreated with sialidase, from six cystic fibrosis patients were examined using up to nine different lectins after SDS-PAGE (table 2). None of the lectins used revealed any cystic fibrosis specific patterns, though the complexity varied considerably with the different lectins.

Discussion

SDS gel electrophoresis of the membrane associated proteins of the fibroblast and red cell failed to show any difference in protein and glycoprotein profile between cystic fibrosis patients and controls. The red cell membrane changes previously reported⁷ were detected using a very different electrophoretic system followed by the protein stain aniline blue

black, but were in any case not present in all cystic fibrosis patients and were not specific for cystic fibrosis. Our failure to show any abnormality of fibroblast glycoproteins is at variance with the reports of abnormalities in the molar ratios of monosaccharides in the cell surface glycoproteins of cystic fibrosis fibroblasts,^{8,9} but consistent with the findings of Harris and Bramwell¹⁴ who used three lectins (CONA, WGA, and *Lotus tetragonolobus*) to detect glycoproteins after SDS gel electrophoresis.

Both SDS gel electrophoresis and isoelectric focusing were used to examine the 'soluble' glycoproteins of plasma and urine. No reproducible disease specific differences were detected. The RCA-I binding components detected in one experiment in plasma of three cystic fibrosis patients may have been an artefact or could conceivably correspond to one of the previously described cystic fibrosis factors present in plasma²⁰ which are known to be somewhat labile.

Our inability to demonstrate any generalised or specific glycan changes in the cystic fibrosis material suggests that if the primary genetic defect is due to a mutation of a gene involved in processing glycoproteins (such as a glycosyltransferase or a glycosidase), the gene is not expressed in the tissues we have examined. It would be interesting to extend this type of study to examine less accessible material, such as pulmonary secretion, duodenal fluid, and sweat, which are more clearly associated with the pathology of this disease.

Although we have not demonstrated any disease specific changes in the course of this study, we have identified considerable person-to-person and sample-to-sample variation, some of which is attributable to known genetic polymorphisms, namely PUM and ABO. This variability illustrates some of the difficulties encountered in making a sensible interpretation of data derived from a screening approach to the analysis of a genetic disorder of unknown aetiology, and the value of conducting such a study against a background knowledge of the variation present in healthy persons.

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