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STRUCTURAL CHANGES IN THE PROTEIN AND CARBOHYDRATE COMPONENTS OF GLOMERULAR BASEMENT MEMBRANE IN AMINONUCLEOSIDE NEPHROSIS

NICHOLAS A. KEFALIDES AND LEILA FORSELL-KNOTT

La Rabida-University of Chicago Institute and Department of Medicine, University of Chicago, Chicago, Ill. (U.S.A.)

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SUMMARY

The glomerular basement membranes of normal and amino nucleoside nephrotic rats were analyzed chemically. Glomerular basement membrane from nephrotic rats contained significantly less hydroxylysine, hydroxyproline and glycine than the normal. The collagen extracted from glomerular basement membrane of nephrotic rats with 5% trichloroacetic acid contained 1/3 less hydroxylysine, and hydroxyproline and 2/3 more lysine and 1/3 more proline than the normal. No differences were noted in total carbohydrate. However, the glycopeptide from nephrotic rats had a ratio of glucose: galactose: hydroxylysine = 2:1:1 compared with a ratio of 1:1:1 from normal rats. These structural differences could account for the functional alterations of nephrotic glomerular basement membrane.

INTRODUCTION

The nephrotic syndrome has been induced experimentally in rats by the administration of puromycin aminonucleoside[1]. In the presence of marked proteinuria and cellular damage, the glomerular basement membrane shows no morphologic changes during the acute phase of the disease[2].

The collagen component of basement membranes contains unusually high amounts of hydroxylysine[3-5]. Initial studies on the chemical composition of glomerular basement membrane from normal and puromycin aminonucleoside-treated rats revealed a decrease in the hydroxyproline and hydroxylysine content of the latter group[9]. Since the neutral hexose unit of basement membranes from the glomerulus[3,6], from lens capsule[3,7] and choroid plexus[8] is composed of glucosylgalactose linked to hydroxylysine it was of interest to investigate the nature of this glycopeptide in glomerular basement membrane of rats rendered nephrotic with puromycin aminonucleoside.

The present study shows that in the glycopeptide unit from glomerular basement membrane of nephrotic rats the ratio of glucose:galactose:hydroxylysine = 2:1:1, compared with the ratio of 1:1:1 in the normal.

MATERIALS AND METHODS

Male albino rats (Sprague–Dawley), weighing 160–180 g, were used in this study. In three separate experiments, 100, 200 and 200 rats were used. Puromycin aminonucleoside (a gift of Lederle Laboratories Division, American Cyanamide Corporation) was injected subcutaneous(1.5 mg/100 g body weight) as a 0.5% aqueous solution daily for 11 days. Control rats for each experiment were injected with an equivalent volume of distilled pyrogen-free water. All rats were fed Purina Chow and allowed to drink tap water *ad libitum*. Rats were sacrificed 1 week following the last injection, the kidneys removed and stored at —25°.

Glomerular basement membrane was prepared according to the method of KRAKOWER AND GREENSPON[10].

Collagen from glomerular basement membrane was extracted with 5% trichloroacetic acid at 80° according to a previous method[4].

Preparation and purification of glycopeptides. The glycopeptides were prepared by alkaline hydrolysis after a modification of the method described by BUTLER AND CUNNINGHAM[11]. 100 mg glomerular basement membrane were incubated with 7 ml of 2 M NaOH at 100° for 30 h in a sealed tube with slow stirring. The mixture was centrifuged at 34800 × *g* for 30 min and the supernatant solution neutralized with 2 M HCl. The precipitate formed was removed by centrifugation and after the supernatant was concentrated to dryness *in vacuo*, 2 ml of 0.1 M HCl were added and the solution passed through a Dowex-50, 8X(H^+) column (1 cm × 35 cm). The column was washed with 10 vol. of distilled water and the glycopeptides eluted with 10 vol. 1.5 M NH_4OH. The NH_4OH eluate was concentrated *in vacuo* and the glycopeptides separated by gel filtration using two Sephadex G-10 columns (1 cm × 112 cm) linked in series. Elution was carried out with 0.1 M pyridine–acetic acid buffer (pH 5.0) and 2-ml fractions were collected. On each fraction total hexose and ninhydrin-positive material were estimated. A major hexose-containing peak emerged in both the control and nephrotic rat groups. However, the hexose peak from the nephrotic glomerular basement membrane emerged between 80 and 106 ml (Fig. 1B), while the one from the control glomerular basement membrane emerged between 96 and 120 ml (Fig. 1A). The major hexose peak was pooled and concentrated. This peak will subsequently be referred to as glycopeptide peak.

Acid hydrolysis. Mild acid hydrolysis of sugars was performed using 0.2 M HCl at 100°. For graded hydrolysis samples were taken for analysis at 30 min, 1, 2, 3, and 4 h. Complete acid hydrolysis was carried out with 2 M HCl at 100° for 2 h.

Chemical determinations. Quantitative determinations of amino acids, protein-bound hexose, glucose, galactose, hexosamine, sialic acid and fucose were made using the methods described elsewhere[5]. For the determination of hexose in the isolated glycopeptides the phenol method of DUBOIS *et al.*[12] was used. Reducing sugar was measured according to PARK AND JOHNSON[13].

RESULTS

The amino acid composition of the intact glomerular basement membranes appears in Table I. It will be noted that there are marked differences in their composition. The hydroxyproline, hydroxylysine and glycine content is lower in the glomerular

basement membrane from nephrotic rats. The lysine content however, is higher while the amount of proline is the same in both groups. The amino acid composition of the collagen extracted from glomerular basement membrane with 5% trichloroacetic acid appears in Table I. We note again the lower hydroxyproline, hydroxylysine and

TABLE I

AMINO ACID COMPOSITION OF RAT GLOMERULAR BASEMENT MEMBRANE

Values expressed as residues/1000 residues.

Amino acid	*Intact*		*5% trichloroacetic acid extract*	
	Control	*Nephrotic*	*Control*	*Nephrotic*
Hydroxylysine	21.8	15.5	43.0	30.0
Lysine	40.1	61.0	7.7	23.8
Histidine	20.9	18.6	2.7	2.0
Arginine	52.6	56.0	29.6	30.7
Hydroxyproline	52.0	38.0	157.0	108.4
Aspartic acid	74.2	76.0	57.4	57.5
Threonine	46.3	52.6	25.0	24.6
Serine	62.8	64.2	38.0	46.0
Glutamic acid	100.0	102.0	76.0	85.0
Proline	62.0	62.3	62.0	92.0
Glycine	200.0	154.2	322.0	307.0
Alanine	67.0	83.2	38.0	76.6
Half-cystine	20.4	17.0	11.0	10.0
Valine	43.0	46.0	27.0	25.0
Methionine	11.0	11.3	8.0	6.1
Isoleucine	32.0	33.7	18.0	17.5
Leucine	66.0	72.7	54.3	38.6
Tyrosine	9.0	10.3	2.0	2.6
Phenylalanine	19.0	25.2	21.6	17.1

TABLE II

CARBOHYDRATE COMPOSITION OF RAT GLOMERULAR BASEMENT MEMBRANE

Values expressed as g/100 g.

Carbohydrate	*Intact*		*5% trichloroacetic acid extract*	
	Control	*Nephrotic*	*Control*	*Nephrotic*
Hexose	5.0	5.2	8.5	8.7
Glucose	2.3	2.5	4.0	5.8
Galactose	1.9	1.0	4.1	3.0
Mannose	0.8	1.7	0.4	0.1
Hexosamine	0.65	0.63	N.D.*	N.D.
Fucose	0.30	0.40	N.D.	N.D.
Sialic acid	2.3	2.2	N.D.	N.D.
Ratio glucose: galactose	1.2	2.5	0.98	1.94
Glycopeptide		*μmoles/μmole hydroxylysine*		
Glucose	1	2	–	–
Galactose	1	1	–	–

*Not determined.

glycine content in the material extracted from glomerular basement membrane of nephrotic rats; however, the amount of lysine and proline is higher.

The carbohydrate composition of the intact glomerular basement membranes and of the 5% trichloroacetic acid extracts appears in Table II. We note that the ratio of glucose: galactose in the glomerular basement membrane from nephrotic rats is twice that of the controls. No significant differences are noted in the hexosamine and sialic acid content between the control and nephrotic glomerular basement membrane, although, the fucose content is higher in the latter. Amino acid analysis of the glycopeptide peak from Sephadex G-10 (Fig. 1) revealed only hydroxylysine from both the control and nephrotic glomerular basement membrane. The ratio of glucose:galactose:hydroylysine in the glycopeptide peak was 1:1:1 for the control glomerular basement membrane, while the ratio from glomerular basement membrane of nephrotic rats was 2:1:1. Mild acid hydrolysis of the glycopeptide peak for 4 h split all the available glucose from glomerular basement membrane of both the control and nephrotic rats. Complete acid hydrolysis of the residue released galactose and hydroxylysine. The glycopeptide peak from control and nephrotic glomerular basement membrane was subjected to graded acid hydrolysis and aliquots were analyzed for reducing sugar. The results appear in Fig. 2. It can be seen that starting with the same amount of total hexose 50% more reducing sugar was released from the glycopeptide obtained from glomerular basement membrane of nephrotic rats.

The data suggest that the chemical structure of basement membrane in aminonucleoside nephrosis is altered. The decrease in hydroxylysine and hydroxyproline content is reflected in the relative increase of the lysine and proline content in the nephrotic glomerular basement membrane. Recent studies by YOSHIDA *et al.*[14] and

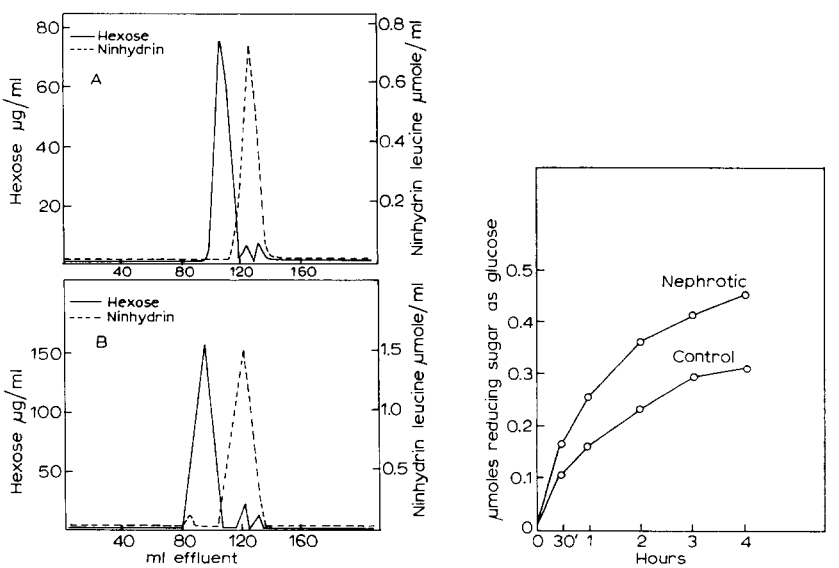

Fig. 1. Gel filtration, on Sephadex G-10, of glomerular basement membrane glycopeptides following alkaline hydrolysis and Dowex 50-X8 chromatography. A, control; B, nephrotic.

Fig. 2. Graded hydrolysis of glomerular basement membrane glycopeptides from control and nephrotic rats. Amounts equivalent to 0.62 µmole hexose were hydrolyzed with 0.2 M HCl, at 100°. Aliquots were analyzed for reducing sugar at 0 and 30 min and 1, 2, 3 and 4 h.

YOSHIDA AND METCOFF[15] show that aminonucleoside inhibits oxidative phosphorylation at the substrate level, starting from α-ketoglutarate, in kidneys of nephrotic rats. It was further shown by KIVIRIKKO AND PROCKOP[16] that for the hydroxylation of prolyl and lysyl residues on the protocollagen molecule α-ketoglutarate, atmospheric O_2, ascorbate and Fe^{2+} are required as cofactors. Aminonucleoside of puromycin could interfere with the hydroxylation of proline and lysine by inhibiting the proline and lysine hydroxylase enzyme in the kidney. The presence of a glycopeptide containing 2 moles of glucose to 1 mole galactose in the glomerular basement membrane of nephrotic rats could be the result of increased glycosyl transferase activity or of the appearance of a new enzyme. Although MOSCARELLO *et al.*[17] demonstrated that aminonucleoside stimulates glucosamine incorporation into plasma glycoproteins, our studies show no differences in the hexosamine content of normal and nephrotic glomerular basement membrane.

It is conceivable, therefore, that in aminonucleoside nephrosis peptides which differ structurally from the normal might be synthesized resulting in functional alterations of the glomerular basement membrane.

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