Origin of non-dialysable urinary glucoconjugates

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Abstract

A Gel exclusion chromatographic method was used to determine the molecular weight distribution, and therefore, the origin, of non-dialysable urinary glucoconjugates in normal men. The results showed a mixture of glucoconjugates with molecular weight ranges of 1605 to 141 000.

It is suggested that the high molecular weight forms originally constitute the glucoconjugates, and that they are probably post-glomerular in origin. These may be degraded in vivo by glucosylhydrolases to produce the low molecular weight forms. The activities of the urinary enzymes may be reduced in male type 1 diabetic patients, and this may be responsible for the reported increase in their excretion of non-dialysable urinary glucoconjugates.

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The presence of non-dialysable urinary glucoconjugates has been reported in human urine.¹⁻³ It was suggested that the urinary glucoconjugates may be used as markers for the male type 1 diabetes mellitus because of the increased excretion in this condition.^{2,3} However, the origin of the urinary glucoconjugates remains unknown. Therefore, the analysis of their molecular weight distribution was carried out to obtain information about their origin.

The correlation between the molecular weight and gel exclusion chromatography of dextrans shows the usefulness of this method for the determination of the molecular weight of glucose polymers. A direct correlation was shown between the gel chromatography findings and the molecular weight of dextrans within the range of 5400 to 147 000.⁴ This correlation may also apply to other closely related glucans of equivalent molecular weight. Therefore, a gel exclusion chromatographic method was used in this study for the determination of the molecular weight distribution of non-dialysable urinary glucoconjugates.

Methods

The subjects comprised 10 healthy male laboratory workers aged 24 to 60 years. Twenty four hour urine samples were collected in a plastic container containing merthiolate (>100 mg/dl) as preservative to prevent the growth of micro-organisms, and kept at 4°C immediately on completion.

The urine was dialysed in Visking seamless tubing, as described previously.³ The void and inclusion volumes of the chromatographic column (Pharmacia K-50 column, 99.5 × 1.6 cm containing Sephadex G100, fine grade, and with bed volume of 160 cm³) were determined using dextran blue 2000 (2 mg/ml) and 100 mM sodium chloride. The column was calibrated with dextrans (1 mg/ml in 6% aqueous glycerol (w/v)), average molecular weights 70 000 and 40 000, as markers. Ideally, three molecular weight markers should be used. These solutions were eluted with distilled water at a flow rate of 0.5-1.0 ml/minute. Fractions of 2 ml were collected; the glucose content of the dextran was released by acid hydrolysis.⁵

The calibration curve of log molecular weight against kilodalton and distribution coefficient was plotted ($K_d = V_e - V_o$, where V_e is the elution volume and V_o is the void volume).

Dialysed urine powder solution (4 ml) in distilled water (50 mg/ml), clarified by centrifugation, was applied to the column. It was eluted with distilled water at a flow rate of 0.5-1.0 ml/minute.

The fractions were collected and pooled for the analysis of the total glucoconjugates by acid hydrolysis⁵ and α -glucan by amyloglucosidase hydrolysis.⁶ All chromatographic procedures were carried out at 4°C.

Glucose was determined spectrophotometrically with hexokinase and glucose-6-phosphate dehydrogenase.⁶

Results

Satisfactory recoveries of the urinary glucoconjugates were achieved by the gel exclusion chromatography method. Thus the recovery after centrifugation to remove precipitate was 94% (determined by acid hydrolysis) or 93% (determined by amyloglucosidase hydrolysis); and after gel filtration the recovery was 92% (determined by acid hydrolysis) or 86% (determined by amyloglucosidase hydrolysis) (table 1).

The range of the molecular weight of the urinary glucoconjugates computed from the

Table 1 Recoveries of non-dialysable urinary glucoconjugates after centrifugation and gel filtration

Methods	Centrifugation	Gel filtration
Acid hydrolysis	94%	92%
Amyloglucosidase hydrolysis	93%	86%

The recoveries were based on the amount of glucose released after hydrolysis pre- and post-centrifugation and filtration.

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Table 2 Approximate molecular weight distribution of the glucoconjugates eluted from Sephadex G100 column

Fraction	Elution volume	Kilodalton range	Molecular weight range
1	48–64	0.2–0.325	141 000–70 000
2	64–80	0.325–0.525	73 000–23 900
3	80–96	0.525–0.725	23 900–7500
4	96–114	0.725–1.00	7500–1605

The range of molecular weight of the urinary glucoconjugates were computed from calibration curve of the standard dextran solutions.

Table 3 Gel filtration of non-dialysable urinary glucoconjugates on Sephadex G100 column

Elution volume (ml)	Acid hydrolysis		Amyloglucosidase	
	$\mu\text{mol glucose}$	% Total	$\mu\text{mol glucose}$	% Total
0–48	0	0	0	0
48–64	0.544	4.89	0.064	1.68
64–80	1.568	14.09	0.368	9.68
80–96	2.544	22.86	1.088	28.62
96–114	4.752	42.69	1.539	40.49
114–128	1.722	15.47	0.742	19.52
Total	11.130	100.00	3.801	100.00

The urinary glucoconjugates were distributed around a mean peak volume of 96–114 ml.

calibration graph was 1605 to 141 000 (table 2).

The elution profile of the urinary glucoconjugates showed a distribution around a mean peak elution volume from 96 ml to 114 ml, representing 42.69% of the glucoconjugates (determined by acid hydrolysis) or 40.49% of the glucoconjugates (determined by amyloglucosidase hydrolysis) (table 3). Therefore, the main components were the glucoconjugates of low molecular weight 1605 to 7500, constituting 42.69% of the total. High molecular weight glucoconjugates of 70 000 to 141 000 constitute 4.89% of the total.

Discussion

The results suggest the presence of a mixture of non-dialysable glucoconjugates (glucans) in urine. The non-dialysable urinary glucoconjugates are probably derived from the high molecular weight forms. They are probably of post-glomerular origin; and they may be degraded in vivo to produce the low molecular

weight glucoconjugates. The observation of the activities of glucosylhydrolases in dialysed urine⁷ provides the evidence in support of this theory. These enzymes were shown to affect the structure and size of the non-dialysable urinary glucoconjugates.

As a result of the activities of the glucosylhydrolases in normal urine, low molecular weight products might be formed from the urinary glucoconjugates which would be removed by dialysis. High molecular weight glucoconjugates were shown to predominate in the urine of male type 1 diabetic patients.³ These might be largely responsible for the observed increased excretion of non-dialysable urinary glucoconjugates in the diabetic patients.

In conclusion, the findings of this study imply that urinary glucosylhydrolase activities may be reduced or absent in male type 1 diabetic patients. Therefore, high molecular weight glucoconjugates may be less affected or completely unaffected by enzymatic activities, and little or no low molecular weight glucoconjugates, which can be removed by dialysis, will be formed. This may be considered a plausible explanation for the phenomenal increase in the excretion of non-dialysable urinary glucoconjugates in diabetic patients.

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