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Research Article

Molecular parameters other than size and charge are likely to influence the filtration of macromolecules across the glomerular filter. We have studies, therefore, the glomerular permeability of macromolecules with widely different molecular configuration such as horseradish peroxidase, a plant glycoprotein with an isoelectric point in the physiologic pH range, and dextran, an uncharged sugar polymer of D-glucopyranose. Simultaneous fractional clearances were determined for both test macromolecules in five Wistar-Furth rats. The results indicate that for a molecular radius of 28.45 A, as measured by gel filtration, the sugar polymer has a fractional clearance of 0.483 on the average, exceeding that of the protein tracer, with a value of 0.068, by a factor less than 7. We conclude that other molecular parameters such as shape, flexibility, and deformability play important roles in the transport of macromolecules across the extracellular matrix that constitutes the glomerular filter.

Glomerular Permeability of Macromolecules

EFFECT OF MOLECULAR CONFIGURATION ON THE FRACTIONAL CLEARANCE OF UNCHARGED DEXTRAN AND NEUTRAL HORSERADISH PEROXIDASE IN THE RAT

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ABSTRACT Molecular parameters other than size and charge are likely to influence the filtration of macromolecules across the glomerular filter. We have studied, therefore, the glomerular permeability of macromolecules with widely different molecular configuration such as horseradish peroxidase, a plant glycoprotein with an isoelectric point in the physiologic pH range, and dextran, an uncharged sugar polymer of D-glucopyranose. Simultaneous fractional clearances were determined for both test macromolecules in five Wistar-Furth rats. The results indicate that for a molecular radius of 28.45 Å, as measured by gel filtration, the sugar polymer has a fractional clearance of 0.483 on the average, exceeding that of the protein tracer, with a value of 0.068, by a factor >7. We conclude that other molecular parameters such as shape, flexibility, and deformability play important roles in the transport of macromolecules across the extracellular matrix that constitutes the glomerular filter.

INTRODUCTION

The filtration of macromolecules across the glomerular capillary wall is determined by the structural characteristics of the filter (1–12), glomerular hemodynamics (13–21), and molecular parameters of the solute. Molecular determinants include the molecular radius (22–25) and the net molecular charge (26–30). The fractional clearance of macromolecules decreases with increasing molecular radii but for the same molecular size, polyanions are selectively restricted and the filtration of polycations is enhanced. For the same molecular radius, the fractional clearances of proteins are generally much lower than those of the chemically different sugar polymers dextran and polyvinylpyrrolidone (24, 29). This has been attributed to differences between proteins and sugars with respect to molecular charge and tubular uptake (24, 31). Proteins used for clearance studies are usually anionic and are reabsorbed by tubules to a variable extent; the sugar polymers are uncharged and tubular reabsorption is insignificant. However, molecular determinants other than charge and size may be responsible for the different behavior of proteins and sugar polymers. Thus, fractional clearances of anionic, neutral and cationic horseradish peroxidase determined by us in the Wistar-Furth rat and corrected for tubular reabsorption (29) are respectively much lower than published values for anionic, uncharged and cationic dextrans obtained in the Munich-Wistar rat (25, 26, 30). Because the discrepancy in these two sets of results may be attributable to differences in species and clearance methodology, we now report fractional clearances of uncharged dextrans and neutral horseradish peroxidase that were determined simultaneously in the Wistar-Furth rat.

METHODS

Tracers. Horseradish peroxidase was purchased from Sigma Chemical Co., St. Louis, Mo. (type II) and purified as described (29).

[^{3}H]Dextran with a mean 20,000 mol wt and a 273.3-mCi/g sp act was obtained from New England Nuclear (Boston, Mass.).

Inulin was obtained from Arnar-Stone Laboratories, Inc. (Mount Prospect, Ill.) as a 10% solution.

Clearance experiments. Wistar-Furth rats, known to be resistant to mast cell degranulation induced by horseradish peroxidase or uncharged dextrans (32), were used for the present experiments. They were anesthetized by an intraperitoneal injection of Inactin (Promonta AG, West Germany) at a 100-mg/kg dose and placed on a heated operating table. One jugular vein was exposed and cannulated with two polyethylene tubings, PE 10 (Clay-Adams, Parsippany, N. J.) for

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the infusion of solutions. The left femoral artery was cannulated with a polyethylene tubing, PE 50, connected to a pressure transducer (Statham model P-23-Dc, Statham Medical Instruments, Hato Rey, Puerto Rico) for recording of arterial pressures by means of a polygraph (model 79D, Grass Instruments Co., Quincy, Mass.). The left ureter was exposed via a small abdominal incision and cannulated with a short polyethylene tubing, PE 10. Tracheostomy was routinely performed at the beginning of procedure.

During the surgical procedure, which lasted 25–30 min, the animals received a constant infusion of 0.034 ml/min of mannitol solution, 5 g/100 ml. After the placement of catheters, a 1-ml bolus of a solution containing 10 g/100 ml of inulin and 50 μCi/ml of [^{3}H]dextran in isotonic saline was given via one of the indwelling intravenous catheters followed by a constant infusion of 0.05 ml/min. Mannitol was discontinued and replaced by 0.15 M NaCl solution at a rate of infusion of 0.034 ml/min via the second intravenous catheter. After an equilibration period of 40 min, the transit time between the jugular vein and the tip of the ureteral catheter was measured by lissamine green infusion. (0.15 ml of 0.15 M NaCl solution containing 1 g/100 ml of lissamine green).

At the beginning of the clearance period, saline infusion (0.032 ml/min) was discontinued and horseradish peroxidase solution (1 mg/ml) was administered as a 0.7-ml bolus (over 30 s) followed by continuous infusion at 0.034 ml/min of the same solution. Simultaneously a continuous collection of blood from the femoral artery was begun at a constant rate of 0.05 ml/min for exactly 30 min using a constant withdrawal pump (model 940, Harvard Apparatus Co., Inc., Millis, Mass.). Urine collection was initiated transit time minutes after administration of horseradish peroxidase and was continued for exactly 30 min. At the end of the urine collection, the abdominal aorta was clamped off above the origin of the renal arteries and the kidneys were perfused with 0.15 M NaCl for 3 to 4 min. The left kidney was then excised and homogenized in a tissue grinder for the determination of the reabsorbed fraction of the protein tracer.

Chemical analysis. Inulin concentration in the plasma and urine samples was determined by the anthrone reaction (33), horseradish peroxidase was measured in plasma, urine, and kidney homogenate by its enzymatic activity with 3,3'-diaminobenzidine as hydrogen donor (29, 34).

Chromatographic procedures. Gel filtration on urine and plasma samples and the proteins that served as standards was performed on a 2.5 × 60-cm column of Ultrogel AcA44 (LKB Produkter, Bromma, Sweden) with a 288-ml total bed volume and a 99.2-ml void volume, as measured by the elution of blue dextran. The fractionation range of Ultrogel AcA44 given by the manufacturer is 10,000–130,000 daltons for globular proteins. All chromatographic procedures were performed on the same column, which was standardized with proteins of known molecular radii (29). A 0.7-ml total sample volume was applied to the column; it contained 2 mg/ml blue dextran, 5 mg/ml of dextrose, and either 0.1 ml of plasma or 0.025–0.1 ml of urine. Fractions with an average volume of 3 ml (90 drops) were collected by means of a fraction collector (Gilson Medical Electronics, Inc., Middleton, Wis.). Specific activities for [^{3}H]dextran in different fractions was measured by standard scintillation procedure.

Fractional clearances for differently sized dextrans were then calculated by standard procedures. For horseradish peroxidase, the clearance was determined on the basis of the amount actually filtered by the glomerulus; this value was determined by adding the excreted amount in the urine collected during the clearance period and the amount of peroxidase reabsorbed during the same time interval (30 min), as measured in the tissue homogenate after saline perfusion, corrected for the time of exposure of the kidney to the tracer (34 min).

Rate of degradation of horseradish peroxidase in proximal tubules. 11 Wistar-Furth rats were used for these experiments. They all received 1 ml of horseradish peroxidase solution containing 10 mg/ml by intravenous injection. After 12–14 h peroxidase was no longer detectable in plasma by the sensitive diaminobenzidine assay. At this time, both kidneys were perfused simultaneously with saline for 4 min via the abdominal aorta in five animals, and the peroxidase content established in the homogenates of right and left kidneys. In six additional animals, 12–14 h after HRP administration, the left kidney was isolated and perfused via the left renal artery for 4 min and homogenized for peroxidase estimation. 3 h later the right kidney was perfused via abdominal aorta, homogenized, and analyzed for its peroxidase content. The amount of peroxidase per gram of wet tissue was then calculated. The concentration of the right kidney was then expressed as a fraction of the concentration in the left kidney in every animal.

RESULTS

The relationship between mean fractional clearance of dextran and effective molecular radius (sieving curve) is given in Fig. 1. Whole kidney glomerular filtration rate as calculated from the clearance of inulin, fractional clearance of a dextran similar in size to horseradish peroxidase, fractional clearance of horseradish peroxidase and the ratio of dextran to horseradish peroxidase clearance for five experimental animals are given in Table I. The mean value of the fractional clearance for dextran compares favorably with values reported by others in Munich-Wistar rat (25, 30); the fractional clearance of peroxidase is almost identical to the value obtained by us previously in this species (29). The peroxidase content present in the kidney homogenate after the 30-min clearance period corresponds on the average to 3.5% of the total amount present in the urine and tissue (filtered horseradish peroxidase) with a 2.0–6.4% range. As can be seen from the last

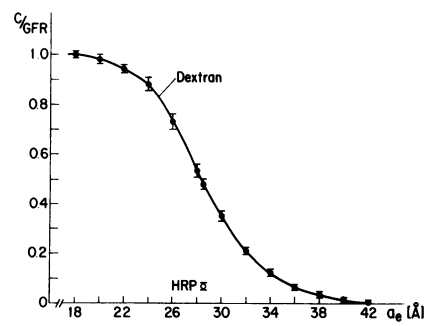

FIGURE 1 Comparison of fractional clearance (ratio of the clearance of the test substance and glomerular filtration rate, C/GFR) of uncharged dextrans (●) and native horseradish peroxidase (○) plotted as a function of the molecular radius as determined by gel chromatography. Values are given as means±1 SE (n = 5).

TABLE I

Summary of Measurements in Normal Wistar-Furth Rats during Simultaneous Infusion of Uncharged Dextrans and Native Horseradish Peroxidase

Animal	Body wt	AP	GFR	^{c}D/GFR	^{c}HRP/GFR	^{c}D/^{c}HRP
	g	*mm Hg*	*ml/min*			
1	196	100	0.521	0.468	0.091	5.128
2	196	100	0.702	0.441	0.059	7.505
3	195	100	0.576	0.438	0.067	6.536
4	205	115	0.778	0.506	0.057	8.927
5	208	115	0.686	0.562	0.066	8.483
Mean±SEM	200±2.7	106±3.7	0.653±0.046	0.483±0.023	0.068±0.006	7.316±0.685

Abbreviations used in this table: GFR, glomerular filtration rate; ^{c}D/GFR, fractional clearance of a dextran similar in size to horseradish peroxidase; ^{c}HRP/GFR, fractional clearance of horseradish peroxidase; ^{c}D/^{c}HRP, the ratio of dextran to HRP clearance.

column in Table I and from Fig. 1, the fractional clearance of dextran exceeds, on the average, that of the protein tracer by a factor of 7.3, with a range of 5.1–8.9 in five animals studied.

Degradation of enzyme activity by proximal tubule cells. The concentration of peroxidase per gram wet tissue in the right kidney relative to that in the left kidney for five animals in which right and left kidneys were perfused simultaneously had a mean value of 1.05 ±0.03 (SEM). When both kidneys were perfused sequentially, separated by an interval of 3 h, this ratio dropped to 0.76±0.03 indicating an approximate degradation rate of 9.2%/h. From this figure, it can be calculated that underestimation of horseradish peroxidase clearance because of degradation of reabsorbed tracer cannot be >0.3%.

DISCUSSION

Our results show that horseradish peroxidase, a globular protein with a net charge close to zero at physiologic pH as indicated by its isoelectric point, undergoes more restriction across the glomerular capillary wall than dextran, an uncharged polymer. Because both tracers were injected simultaneously and possess identical hydrodynamic radii, their different behavior can only be explained by: (*a*) a gross underestimation of the reabsorbed fraction of peroxidase or (*b*) differences in parameters other than size and charge. It has been shown that transtubular transport of intact proteins does not occur (35–37). Our results as well as previous studies on lysosomal degradation of horseradish peroxidase (38, 39) indicate that underestimation of reabsorbed protein was insignificant in our clearance experiments.

Horseradish peroxidase is similar in molecular configuration to rat albumin, as judged by their frictional coefficients (40, 41). The internal disulfide bridges, known to be present in horseradish peroxidase (42) as well as hydrophobic and electrostatic interactions within the molecule indicate a fairly rigid tertiary structure for horseradish peroxidase. Dextrans form loose and randomly coiled hydrated spheres in free solution (43). However, experimental evidence provided by Laurent et al. (44–46) indicates that the transport of linear polymers through gels is facilitated compared to that of globular particles with the same hydrodynamic parameters. This phenomenon is attributed to the fact that polymers preferentially move end-on through the network by a process of "reptation" (47, 48) which results in a small "effective molecular radius." The change in the rate of transport of a macromolecule through a network is a function of the probability that it will encounter an obstacle; a flexible macromolecule is less likely to meet an obstacle when compared to a rigid, globular protein (47).

The present results indicate that a dextran molecule with a hydrodynamic radius of ≅36 Å has a fractional clearance of 0.064, similar to that of horseradish peroxidase (0.068). In analogy with Laurent's (44–46) determinations, this particular 36 Å dextran has an "effective radius," equivalent to that of horseradish peroxidase, that is 28.45 Å. The calculated ratio "effective radius: hydrodynamic radius in free solution" of ≅0.79 for this dextran in our clearance experiments would indicate that the phenomenon described by Laurent et al. (44–46) in artificial gel systems has important biological implications in glomerular physiology.

We conclude, therefore, that in addition to size and net molecular charge, other macromolecular determinants such as shape and deformability play an important role in glomerular permselectivity.

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