Permeation of Ficoll and Ficoll Sulfate through Glomerular Basement Membrane: Effects of Molecular Size and Charge

by

Glen R. Bolton

Submitted to the Department of Chemical Engineering
in May 28, 1998 in Partial Fulfillment of
the Requirements for the Degree of
Doctor of Philosophy

Abstract

The first step in urine formation is the filtration of blood plasma across the walls of glomerular capillaries. The mechanisms by which the glomerular capillary wall selectively filters plasma solutes can be studied by comparing the sieving curves of tracer molecules of varying size and charge. The properties of ficoll, a crosslinked co-polymer of sucrose and epichlorohydrin, make it ideal for use as a tracer macromolecule. It has a rigid spherical structure, varies in radius from to 100 Å, is physiologically inert, and can be sulfated to provide an anionic analog. The goal of this thesis research was to synthesize and characterize ficoll sulfate and use it in collaborative tracer experiments investigating the structure and function of kidney capillaries.

Ficoll sulfate was synthesized by reacting ficoll with chlorosulfonic acid in the presence of pyridine. The effects of reaction time, reactant concentration and temperature on the degree of product sulfation were determined. Increasing the ficoll concentration reduced the degree of sulfation and allowed fluorescent labeling of ficoll sulfate for detection purposes. The ficoll sulfate size distributions were comparable to those of ficoll. The anionic character of ficoll sulfate was determined using ion exchange chromatography and negatively charged membranes. Ficoll sulfate exhibited little or no binding to BSA.

Preparative size exclusion chromatography was used to fractionate ficoll sulfate into four relatively monodisperse fractions to gain information about the structure of ficoll sulfate. The dispersion of ficoll sulfate during size exclusion chromatography was modeled to optimize the column dimensions and operating strategy. The weight-average molecular weight was determined by static light scattering. Stokes radii were measured by dynamic light scattering and size exclusion chromatography. The relationships between molecular weight and radius, and sulfur content and radius were determined. Sulfur content was independent of molecular size, indicating that sulfates are distributed evenly throughout the volume of the molecules.

The ability of the normal glomerular capillary wall to restrict the passage of anionic macromolecules, relative to uncharged tracers of similar size and configuration, has been demonstrated in vivo using a variety of test molecules. This suggests that the glomerular capillary wall normally possesses fixed negative charges. The extent to which the glomerular basement membrane (GMB) contributes to charge-selectivity of the gloomerular capillary wall has been controversial. To reexamine this issue, the size- and charge-selectivity of filters made from isolated rat GBM were assessed using polydisperse ficoll and ficoll sulfate

as test macromolecules. Filtration experiments using isolated GBM were performed by Dr. Barbara Daniels at the Univesity of Minnesota. Glomerular basement membrane was isolated from rat kidneys by mincing, sieving and detergent lysis. The GBM pieces were consolidated into a layer at the base of an ultrafiltration cell. The sieving coefficients of ficoll sulfate were not different from those of ficoll at physiological ionic strength, although the values for ficoll sulfate were depressed at low ionic strength. These results confirm that the GBM possesses fixed negative charges, but suggest that its charge density is insufficient to confer significant charge-selectivity under physiological conditions, where electrostatic interactions are relatively well screened. The sieving coefficients of ficoll sulfate and ficoll were elevated similarly in the presence of BSA, which could be explained as the combined effect of non-specific physical factors. The more novel of these, which seems not to have been recognized previously in physiology, is the ability of finite concentrations of one macromolecule (e.g., BSA) to augment the transmembrane passage of a second, tracer macromolecule (e.g., ficoll), via intermolecular repulsions. The view that BSA does not affect the intrinsic properties of the GBM was supported by the absence of an effect on the hydraulic permeability of isolated GBM. The sieving coefficient of BSA was roughly half that of ficoll or ficoll sulfate of similar Stokes-Einstein radius. Given the finding of negligible charge-selectivity, the differenece may be attributed to the nonspherical shape of albumin. The results suggest that, to the extent that isolated GBM is similar to GBM in vivo, the charge-selectivity of the glomerular capillary wall must be due to the endothelial and/or epithelial cell layers.

An accurate solute charge density estimate is required to model electrostatic effects on macromolecular transport. The charge density of ficoll sulfate was determined from measurements of its diffusion across track-etched polycarbonate membranes, having negatively charged straight cylindrical pores. The charge density of ficoll sulfate was determined by fitting the experimental data to a transport model that used a linearized version of the Poisson-Boltzmann equation to describe the electrical potential. The effect of using the linearized Poisson-Boltzmann equation was found to be relatively small by solving the full equation for an isolated ficoll sulfate molecule. Using this method, the number of charges per ficoll sulfate molecule was less than the number of sulfate groups.

The applicability of a number of theoretical models to glomerular basement membrane permeability data were evaluated. Water flow models that assumed the GBM was composed of a single fiber size did not accurately predict hydraulic permeabilities. It is unlikely alternative fiber arrangements would improve the predictions of the single fiber models. Three models that assumed the GBM was composed of two fiber sizes accurately predicted the hydraulic permeability for a range of small/large fiber volume ratios. It is hypothesized that these models are superior because they account for the heterogeneity of the fibers that make up the GBM. Single fiber models of partitioning and diffusion did not accurately predict ficoll and ficoll sulfate sieving coefficients in GBM. Hetero-fibrous membrane models of macromolecular transport would likely provide more accurate predictions.

Thesis Supervisor: William M. Deen
Title: Carbon P. Dubbs Professor of Chemical Engineering, MIT.