Effects of Plasma Proteins on the Sieving of Macromolecular Tracers in the Kidney

by

Matthew Jordan Lazzara

Submitted to the Department of Chemical Engineering
on January 7, 2003, in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

ABSTRACT

The ultrafiltration of plasma in the mammalian glomerulus is the first step in the processing of blood by the kidney. Proper functioning of this process is critical to the kidney's ability to effectively eliminate waste and retain desirable substances. The glomerular barrier has long been regarded as both a size and charge selective screen for plasma solutes. The origin of this selectivity is found in the unique three-layered structure of the glomerular capillary wall (GCW), consisting of a fenestrated endothelium, the interdigitating foot processes of the glomerular epithelium, and the shared glomerular basement membrane (GBM). The selectivity properties of the GCW have commonly been probed by measuring the sieving coefficients of a variety of tracers, both proteins and exogenous polymers, across the intact glomerular barrier and across isolated components of the GCW. It was found previously that the sieving coefficients of the tracers Ficoll and Ficoll sulfate across isolated GBM were greatly elevated when BSA was present at physiological levels (Bolton et al. 1998). It was suggested that most of this increase was the result of steric interactions between BSA and the tracers which increased tracer partitioning from the bulk into the GBM. Such an effect, if present, would have important implications for the interpretation of macromolecular sieving studies, both *in vivo* and *in vitro*. The goals of this thesis research were to model the effect of an abundant protein on the partitioning of a dissimilar tracer molecule, to incorporate that effect into models for glomerular sieving, and to test the partitioning model by measuring the effect of protein concentration on the partitioning of protein and Ficoll in agarose gels.

The theoretical effects of solute size on partition coefficients in straight pores or randomly oriented fiber matrices have been investigated previously for very dilute solutions, where solute-solute interactions are negligible, and also for more concentrated solutions consisting of spherical solutes of uniform size. For concentrated solutions it has been found that steric and other repulsive interactions among solutes increase the partition coefficient above the dilute limit. To extend the results for porous or fibrous media to include concentrated mixtures of solutes with different sizes or shapes, we used an excluded volume approach. In this formulation, which describes steric interactions only, partition coefficients were computed by summing all volumes excluded to a solute molecule by virtue of its finite size, the finite size of other solutes, and the presence of fixed obstacles (pore walls or fibers). For a mixture of two spherical solutes, the addition of any second solute at finite concentration increased the partition coefficient of the first solute. That increase was sensitive to the size of the second solute; for a given volume fraction of the second solute, the smaller its radius, the larger the effect. When the total volume fraction of solutes was fixed, an increase in the amount of a second, *smaller* solute increased the partition coefficient of the first

solute, whereas an increase in the amount of a second, *larger* solute had the opposite effect. Results were obtained also for oblate or prolate spheroidal solutes and for fibrous media with multiple fiber radii. For constant total fiber volume fraction, an increase in the amount of a second, *smaller* fiber decreased the partition coefficient of a spherical solute, whereas an increase in the amount of a second, *larger* fiber had the opposite effect. Overall, the theory suggests that the introduction of heterogeneity, whether as mixtures of solute sizes or mixtures of fiber sizes, may cause partition coefficients to differ markedly from those of uniform systems.

Using the excluded volume partitioning model, the theory for the sieving of macromolecular tracers was extended to account for the presence of a second, abundant solute. Using that theory, we returned to the experimental data of Bolton et al. (1998) and attempted to model the effect of protein concentration on Ficoll sieving. The osmotic reduction in filtrate velocity caused by an abundant, mostly retained solute will also tend to elevate the tracer sieving coefficient. The osmotic effect alone explained only about one third of the observed increase in the sieving coefficients of Ficoll and Ficoll sulfate, whereas the effect of BSA on tracer partitioning was sufficient to account for the remainder. At physiological concentrations, predictions for tracer sieving in the presence of BSA were found to be insensitive to the assumed shape of the protein (sphere or prolate spheroid). The effect of plasma proteins on tracer partitioning is expected to influence sieving not only in isolated GBM, but also in intact glomerular capillaries *in vivo*.

To test the predicted effects of solute concentration on the equilibrium partitioning of single macromolecules and macromolecule mixtures, measurements of the equilibrium partition coefficients of BSA and four narrow fractions of Ficoll were made in agarose. Solutions of each test macromolecule were equilibrated with a known volume of gel, final liquid concentrations measured, and partition coefficients calculated by applying a material balance. The partition coefficient of each molecule was measured under dilute conditions and under conditions where BSA was present at concentrated levels. All measurements were made for two different gel solid volume fractions (4 and 6%). As expected, the partition coefficients decreased with increasing gel solid volume fraction and with increasing molecular size. Increasing BSA concentration caused an increase in the partitioning of BSA itself and that of all four sizes of Ficoll. This effect was most significant for the largest molecules. A subset of the measurements repeated at a higher ionic strength demonstrated that electrostatic interactions were unimportant. The experimental results were compared with predictions generated from the excluded volume partitioning theory. Agarose was represented as a randomly oriented array of cylindrical fibers, BSA was modeled as a prolate spheroid, and Ficoll was treated as a sphere. Comparisons of the theoretical predictions with the experimental data produced generally good agreement, indicating that steric interactions among solute molecules and between solute molecules and gel fibers could explain the partitioning behavior.

Thesis Supervisor: William M. Deen
Title: Carbon P. Dubbs Professor of Chemical Engineering and Bioengineering