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Albumin adsorption onto surfaces of urine collection and analysis containers☆

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ABSTRACT

Background: Adsorption of albumin onto urine collection and analysis containers may cause falsely low concentrations.
Methods: We added ^{125}I-labeled human serum albumin to urine and to phosphate buffered solutions, incubated them with 22 plastic container materials and measured adsorption by liquid scintillation counting.
Results: Adsorption of urine albumin (UA) at 5–6 mg/l was <0.9%; and at 90 mg/l was <0.4%. Adsorption was generally less at pH 8 than pH 5 but only 3 cases had $p < 0.05$. Adsorption from 11 unaltered urine samples with albumin 5–333 mg/l was <0.8%. Albumin adsorption for the material with greatest binding was extrapolated to the surface areas of 100 ml and 2 l collection containers, and to instrument sample cups and showed <1% change in concentration at 5 mg/l and <0.5% change at 20 mg/l or higher concentrations. Adsorption of albumin from phosphate buffered solutions (2–28%) was larger than that from urine.
Conclusions: Albumin adsorption differed among urine samples and plastic materials, but the total influence of adsorption was <1% for all materials and urine samples tested. Adsorption of albumin from phosphate buffered solutions was larger than that from urine and could be a limitation for preparations used as calibrators.

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1. Introduction

Chronic kidney disease is a significant public health problem worldwide, and diagnosis is hampered by the lack of standardized early detection methods. Urinary albumin (UA) measurement is widely used for detection of chronic kidney disease, but albumin adsorption to collection and analysis containers may cause falsely low measurements. A joint working group of the National Kidney Disease Education Program and the International Federation of Clinical Chemistry and Laboratory Medicine identified surface adsorption onto containers as one of the pre-analytical variables that could influence interpretation of urine albumin results especially at lower concentrations important for early detection [1].

Investigators have used both chemical and physical methods including radioisotopes [2], atomic force microscopy [3], fluorescence and infrared spectroscopy [4], and ellipsometry [5] to study the interactions of proteins with surfaces. Many have studied albumin adsorption in search of promising materials for use in implantable medical devices, but the albumin concentrations used in these studies approached the concentrations in human serum or plasma, which are considerably greater than those in urine. Only a few studies have examined albumin adsorption to containers at the concentrations found in human urine. Holmberg and Hou [6] reported on the competitive adsorption of proteins in buffered mixtures onto polymer surfaces and the complex nature of this process. A few investigators have reported adsorption onto particular containers [7] and explored the use of surfactants such as Triton X-100 and Tween-20 to reduce albumin adsorption [8]. Bakker

Abbreviations: UA, urinary albumin; HSA, human serum albumin; IA, immunoturbidimetric assay; LSC, liquid scintillation counting; Sp.Ac, specific activity; HPET, hydrophilic-coated polyethylene terephthalate; PE, polyethylene, PP, polypropylene; PS, polystyrene; PET, polyethylene terephthalate.

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reported up to 90% reduction in albumin adsorption using surfactants [9]. Because the pH of urine varies widely, the influence of pH and surfactants are important considerations for measurement of albumin adsorption onto container surfaces.

2. Materials and methods

Fig. S1 (supplemental data) provides a flow chart showing the series of experimental components that are described in detail in the following sections.

2.1. Containers

We purchased urine collection containers, centrifuge tubes, storage vials, and sample analysis cups from companies indicated in Table S1 (supplemental data). The polyethylene terephthalate materials with a hydrophilic coating claiming to reduce bovine serum albumin adsorption to 1/10,000 of that for polystyrene were donated by Sumitomo-Bakelite, Japan through their U.S. subsidiary Wako.

2.2. Labeled and unlabeled human serum albumin

We used custom-labeled ^{125}I-HSA from Perkin Elmer (88–93 mCi/mg, in 0.01 mol/l sodium phosphate buffer, pH 7.4, containing 0.0027 mol/l KCl and 0.137 mol/l NaCl) to prepare mixtures of labeled and unlabeled HSA. HSA (crystallized cat # A8763-1G) was from Sigma-Aldrich Chemical Co. We assumed that ^{125}I-HSA and unlabeled HSA had the same adsorption properties.

2.3. Urine and human serum albumin solutions

For adsorption studies using urine, we collected unidentified, fresh human urine samples or used leftover, unidentified urine samples from Grady Health System or Virginia Commonwealth University Hospital using a protocol approved by the CDC Human Subjects Review Committee. Urine samples were transported and stored at 4–8 °C and used within 30 days of collection. We measured both albumin concentration and pH and used some of the samples without adjustment. We used some of the samples with very low urine albumin concentrations as baseline urine to dilute samples with higher concentrations (pooled urine). We prepared stock unlabeled HSA solutions (1 g/l) in both baseline urine and 10 mmol/l phosphate-buffered saline (PBS). From these stock solutions, we prepared dilutions to contain 5 and 100 mg/l. We adjusted these solutions to pH 4.0, 5.0, or 8.0 using 2 mol/l HCl or 2 mol/l NaOH just prior to adjusting the final volume. To examine the effects of surfactants, we prepared buffered HSA solutions to contain 0.1 v/v% Triton X-100 and 0.05 v/v% Tween-20. We stored all solutions refrigerated in small aliquots in 20 ml glass liquid scintillation counting (LSC) vials and spiked with the ^{125}I-HSA as needed to obtain specific activities of 10^7–10^{10} counts per minute/mg (cpm/mg). All chemicals were analytical grade.

2.4. Adsorption and retention measurements

We conducted adsorption studies by incubating container materials with urine samples or buffered HSA solutions at room temperature (23.0 ± 2.0 °C), for specific time periods (usually 300 min). Disks (1.032 cm diameter, 0.836 cm^2) were punched from plastic materials when possible and 100 μl of solutions was placed on the surface. Instrument sample cups were filled with 200 μl of solutions which covered an area of approximately 1.43 cm^2. After incubation we transferred the solution, five water rinses, and the pipet tip to a glass LSC vial and the material (disk, cone, sample cup, or sample vial) to a separate counting vial. To calculate "total counts" we added the counts for both vials and used that total to calculate specific activity. This approach allowed us to measure both the albumin retained in solution and the albumin adsorbed onto the material. For some experiments, we only counted the material (disk, cup or vial) and prepared two separate vials using an equal volume of the solution incubated on the material (either 100 or 200 μl) to determine specific activity. For containers that could not be cut or transferred to LSC vials, we used the latter approach. In all cases, we added 10 ml of liquid scintillation cocktail (Ultima Gold, Perkin Elmer) to each vial and counted for 1 min with a Perkin Elmer Tri-carb 3100TR Liquid Scintillation Counter using a protocol for ^{125}I. The counter was calibrated weekly with ^{3}H and ^{14}C reference solutions.

We calculated specific activity (Sp.Ac.) according to the following equation:

$$\text{Sp.Ac.}(\text{cpm/mg}) = \text{counts}(\text{cpm})/(\text{Vol}\ [\text{ml}] \times C_b[\text{mg/ml}]).$$

Vol is the volume counted (100 μl for disks and 200 μl for cones, sample analysis cups or vials) and C_b is the total concentration of albumin (labeled + unlabeled). We calculated the albumin concentration in the labeled solution from data provided on the certificate of analysis. We measured the albumin concentration in both urine and buffered HSA solutions by immunoassay (IA) prior to spiking with ^{125}I-labeled HSA, then calculated the total albumin concentration for use in the equation above. We measured albumin concentrations using IA with a Roche Hitachi 912 Clinical Analyzer using Roche Tina-Quant reagents, calibrators, and controls according to the manufacturer's instructions. We also measured buffered HSA solutions with and without the surfactants to determine whether surfactants interfered with the albumin IA. We calculated the surface adsorption for each disk, cup, or other container according to the following equation:

$$\text{Surface adsorption}(\text{mg}) = \text{counts}(\text{cpm})/(\text{Sp.Ac.}[\text{cpm/mg}]).$$

To further support our ^{125}I estimates of albumin retention in urine samples, we measured albumin concentrations in urine samples using IA before and after 10 serial transfers from one container to the next over 300 min (30 min intervals per container) in both polystyrene and hydrophilic-coated sample vials.

To measure the effects of pH on albumin concentration, we mixed 2 urine samples to obtain an albumin concentration of approximately 10 mg/l. We adjusted the pH of duplicate 3 ml aliquots to values from 4.0 to 9.5 in increments of 0.5 pH units with microliter quantities of 2 mol/l NaOH or 2 mol/l HCl. After overnight refrigeration, we measured the albumin concentrations by IA; then re-adjusted each aliquot to pH 5.7 (the pH of the original pooled urine sample) and re-measured the albumin concentration.

Experiments to examine rinsing efficiency, effects of specific activity on adsorption measurements, and kinetics of adsorption are described in the Supplemental Data File. Because we were storing our samples in glass counting vials, we compared adsorption in glass and polystyrene counting vials. We also compared adsorption onto materials from the sides, bottoms, and tops of a single polypropylene container and compared adsorption onto surfaces of two types of 15 ml centrifuge tubes (hydrophilic-coated PET and polystyrene). We measured adsorption onto glass and polystyrene counting vials and conical bottoms of hydrophilic-coated PET and polystyrene centrifuge tubes. We compared surface adsorption onto disks cut from the bottom, sides, and top of one polypropylene urine collection cup. These data are also shown in the Supplemental Data File.

2.5. Experimental design

We used an approach outlined in Introduction to Design of Experiments with JMP, Third Edition published by the SAS Institute [10], to develop the experimental design. We selected albumin concentrations (5 and 100 mg/l), pHs (4.0, 5.0 and 8.0), and times (0.5 and 5.0 h), and used published surfactant concentrations for Triton X-100 (0.1%) and for Tween-20 (0.05%).

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