Adsorption of Proteins From Artificial Tear Solutions to Contact Lens Materials

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A series of polymers and copolymers of 2-hydroxyethyl methacrylate (HEMA) and methyl methacrylate (MMA) were synthesized in order to find surfaces that would adsorb minimal amounts of protein. The adsorption of albumin, lysozyme and immunoglobulin G from a three-way mixture of these proteins in isotonic buffered saline to the polymers was measured using 125I-labeled proteins. Apparently high protein uptake on copolymers rich in HEMA was found to be due to sorption of unbound 125I by the polymers. 125I sorption by the polymers was minimized by dialysis of the protein solution to remove unbound 125I iodide and inclusion of 0.01 M sodium iodide to block uptake of residual 125I iodide. Using these improved protocols, minimal total protein uptake was observed on copolymers containing 50% or more HEMA. The majority of adsorbed protein on all p(MMA-HEMA) polymers was albumin. Total protein uptake was greatest on pMMA. Commercial contact lenses composed of copolymers of HEMA and N-vinyl pyrrolidone (NVP) or acrylamide (AAm) adsorbed small amounts of all proteins whereas copolymers of methacrylic acid (MAAc) and HEMA adsorbed much larger quantities of lysozyme. These results indicate that protein uptake by contact lens materials varies greatly with polymer composition. Artificially high “adsorption” can occur if precautions are not taken to prevent uptake of unbound 125I. Invest Ophthalmol Vis Sci 29:362-373, 1988

One of the problems encountered with contact lens use is the formation of proteinaceous deposits on the lens from the tear fluid.1-3 The deposits cloud the lens, cause the wearer discomfort, and may be responsible for a variety of inflammatory conditions. Giant papillary conjunctivitis,4 corneal vascularization and infections all may be induced or aggravated by lens deposits.5 The development of lens materials exhibiting minimal protein adsorption is therefore desirable.

Hydrogels have been studied extensively as contact lens materials due to wearer comfort and the relatively good oxygen permeability of hydrogels compared to hard contact lenses made of poly(methyl methacrylate). The adsorption of proteins from tear fluid to contact lenses made of hydrogels has been studied from single protein solutions,6-9 diluted human tear fluid,10 and a protein mixture simulating tear fluid.11,12 These studies, using either radiolabeled proteins10,11,13 or Fourier Transform Infrared (FTIR) spectroscopy,6-8,12 showed that protein adsorption ranged from ng/cm2 to /μg/cm2.

In an effort to decrease protein adsorption, hydrogels have been modified by copolymerization of 2-hydroxyethyl methacrylate (HEMA) with other polymers. The adsorption of albumin, lysozyme and immunoglobulin G (IgG) from a protein mixture simulating tear fluid to a series of polymers and copolymers of methyl methacrylate (MMA) and HEMA has been studied previously in this laboratory.14 The amount of lysozyme and albumin adsorbed to copolymers containing more than 50% HEMA was apparently much higher than to copolymers containing less than 50% HEMA, in contrast to observations of decreased protein adsorption to hydrogels reported by other investigators.14-18 In the present study the reasons for this apparent high protein adsorption were reexamined. Unbound 125I in the protein solutions was readily sorbed by HEMA-rich copolymers. Sorption of unbound 125I to these polymers could be prevented by removal of the unbound 125I from radiolabeled protein solutions by dialysis. Blockage of polymer binding sites by addition of nonradioactive iodide to the buffer was also effective in preventing unbound 125I sorption. Artificially high levels of protein adsorption can result on hydrogels if these steps are not used.

Using these improved protocols, minimal total
protein uptake was observed on copolymers containing 50% or more HEMA. The majority of adsorbed protein on all p(MMA-HEMA) polymers was albumin. Commercial contact lenses composed of copolymers of HEMA and N-vinyl pyrrolidone (NVP) or acrylamide (AAm) adsorbed small amounts of all proteins whereas copolymers of methacrylic acid (MAAc) and HEMA adsorbed much larger quantities of lysozyme.

Materials and Methods

General

The buffer used in the adsorption experiments was 0.01 M sodium citrate, 0.01 M sodium phosphate, 0.12 M NaCl, 0.02% sodium azide, pH 7.4 (CPBSz). When 0.05 M NaI was included in the buffer used in the experiments in Figure 4 the amount of NaCl was reduced to yield a buffer of the same ionic strength as CPBSz. For the experiments in Figure 3, NaI was added directly to CPBSz. The buffer used in the protein penetration experiments was 0.01 M sodium phosphate, 0.15 M NaCl, pH 7.4 (PBS). All salts were reagent grade. Iodine-125 NaI was purchased from Amersham (Arlington Heights, IL). Fluorescein isothiocyanate (FITC) (isomer I, code 12008) was purchased from Becton, Dickinson and Co. (Rutherford, NJ). Sephadex G-25 (medium) was purchased from Pharmacia Fine Chemicals, Inc. (Piscataway, NJ).

Egg white lysozyme (3X crystallized, code L-6876) and insulin (bovine pancreas crystalline, code 15500) were purchased from Sigma Chemical Company (St. Louis, MO). Bovine albumin (crystallized, code 81-001-2) and immunoglobulin G (electrophoretically pure, code 64-140) were purchased from Miles Laboratory, Inc. (Naperville, IL).

Polymers

Methyl methacrylate (MMA) was obtained from Polysciences, Inc. (Warrington, PA) and vacuum-distilled before use; 2-hydroxyethyl methacrylate (HEMA) in highly purified form was received from Hydromed Sciences, Inc. (New Brunswick, NJ). Ethylene glycol dimethacrylate (EGMA), azobisobutyronitrile (AIBN) and ethylene glycol (EG) were obtained from Polysciences, Inc. and used as received. Benzoyl peroxide (BOP) was purchased from J. T. Baker Co. (Phillipsburg, NJ). N,N-dimethyl formamide (DMF), spectral grade, was obtained from Aldrich (Milwaukee, WI). Silastic medical grade tubing was procured from Dow Corning (Midland, MI).

Three different lots of polymers ranging in composition from 100% pMMA through 100% pHEMA were synthesized. Lot A polymers and copolymers were polymerized in the absence of solvent ("bulk polymerized") using 5% EGMA (cross-linking agent) and AIBN as catalyst as described previously. Briefly, cross-linked polymer slabs 5 cm x 8 cm x 0.2 cm were formed by polymerization in a cell faced with Mylar film (Dupont, Wilmington, DE) and separated by a gasket fashioned from Silastic tubing. Polymers were cured in the cell in an oven at 45°C. Disks of 1 cm diameter were cut on a specially adapted milling machine at high speed (approximately 2500 rpm) with the polymer slabs affixed to a clean Plexiglass table with double-faced tape. Although the samples were not water-cooled while cutting, the cut surfaces were chemically identical to the uncut surfaces when examined by electron spectroscopy for chemical analysis (ESCA). The polymers were characterized with respect to equilibrium water content as described previously.

Lot B copolymers containing 60% or 80% HEMA and polymers containing 100% HEMA were bulk polymerized using the same methods described for lot A polymers except that 4% EGMA was used and BOP (5 mg/ml) replaced AIBN as the catalyst. In addition the thickness of the polymers was varied from 0.02 to 0.2 cm by using gaskets made from selected diameters of Silastic tubing.

Lot C polymers were of similar composition and thickness as lot B polymers except that they were polymerized in a solvent ("solution polymerized") instead of bulk polymerized and contained half the amount of crosslinker. The copolymers containing 60% or 80% HEMA were polymerized in Mylar faced molds from solutions containing 40% DMF. The cured polymers were removed from the molds and the DMF replaced with water in a succession of 2 to 3 hr methanol/water washes in which the concentration of methanol was 40, 25, 10 and 0%. Polymers composed of 100% HEMA were polymerized between glycerol coated glass plates separated with stainless steel shims from solutions containing 18% EG, 18% H2O, 12 mg/ml (NH4)S2O8, and 5 mg/ml Na2S2O5. After overnight polymerization at ambient temperature, the polymer sheets were soaked in several changes of water over several days to remove traces of unreacted monomer, catalyst, ethylene glycol and glycerol. Lot B and C polymers were cut into rectangles of approximately 1 cm x 2 cm.

The composition of all polymers is expressed as volume percent of monomer less crosslinker, catalyst and any other components present during polymerization. Lot B and C polymers were used only in the experiments measuring protein penetration into polymers with fluorescent and radiolabeled proteins. Lot A polymers were used in all other experiments.

All polymers were cleaned ultrasonically in reagent grade petroleum ether and methanol as described...
previously, blotted dry between filter paper, and placed under vacuum over dessicant for at least 1 week. Prior to each experiment, the polymers were removed from vacuum storage and equilibrated for at least 1 week in CPBSz at room temperature and then transferred to fresh buffer. This ensured complete hydration and elution of any remaining unreacted chemicals. The evening before an adsorption experiment, the polymers were placed in CPBSz or CPBSz containing NaI. The next morning the buffer was replaced with freshly degassed buffer and the samples equilibrated at 37°C prior to adsorption.

**Protein Solutions**

A protein mixture with a composition similar to human tear fluid was prepared by mixing lysozyme (1.20 mg/ml), albumin (BSA) (3.88 mg/ml) and immunoglobulin G (IgG) (1.61 mg/ml) in buffer. The individual proteins were dissolved in CPBSz to make stock solutions and clarified by centrifugation if necessary. The concentration was determined from the absorbance at 280 nm using Ecm° = 2.64, 0.60, and 1.38 for lysozyme, BSA and IgG, respectively. Equal volumes of each protein solution were mixed to form a concentrated solution and the absorbance at 280 nm determined for the mixture. The mixture was then dialyzed for at least 24 hr at 4°C with two buffer changes. The concentration of the dialyzed protein solution was determined spectrophotometrically and adjusted by the addition of buffer to a concentration of about 1,000 cpm/μg and the protein solution was then dialyzed for at least 24 hr at 4°C with two buffer changes. The concentration of the dialyzed protein solution was determined spectrophotometrically and adjusted by the addition of buffer to a concentration twice (2×) that used during adsorption. That which was removed from the protein solution by dialysis or gel filtration is referred to as free Na125I and is assumed to be noncovalently bound to the protein. Na125I added to buffer is also referred to as free Na125I.

The protein solution and the polymers were thermally equilibrated for 1 hr at 37°C prior to adsorption. Adsorption was initiated by adding an equal volume of 2× protein solution to the buffer covering the polymers and mixing by gentle repipetting. After two hours at 37°C, adsorption was terminated by simultaneously dilution and removal of the protein solution with 20 volumes of buffer. In this procedure, called dilution-displacement, the sample container was tightly capped with a rubber stopper containing two glass tubes. Buffer flowed through one tube into the bottom of the sample container, mixed with the solution, and the diluted solution flowed out of the top of the container through the second tube which was flush with the bottom of the stopper. This technique avoids the exposure of polymers to an air-protein solution interface.

Following the dilution-displacement rinse, the polymers were placed in fresh CPBSz in new counting tubes, and the radioactivity measured to obtain "initial rinse" data. On the next day, the polymers were again put in fresh CPBSz in new counting tubes and recounted to obtain "overnight soak rinse" data. Overnight soak rinse data is presented unless stated otherwise. Radioactivity was measured with either a Searle 1025 or a T.M. Analytic (Elk Grove Village, IL) Model 1185R gamma counter. Corrections for background and radioactive decay were made. Surface concentration of protein adsorbed to polymers was calculated by dividing the net radioactivity retained by the polymers by the specific activity and the planar equivalent surface area of the sample. The surface area for each polymer was determined to an accuracy of ±2% by averaging the diameters and thicknesses (measured with a caliper) of three samples and then calculating the average geometric surface area.

**Protein Penetration**

Fluorescein labeled protein was prepared by overnight room temperature incubation of 10 mg of protein with 2 mg of FITC dissolved in 10 ml of 0.1 M sodium borate, pH 9. Unreacted FITC was separated from labeled protein by chromatography on Sephadex G-25. Polymers were incubated with FITC-labeled protein solution for various times and examined for fluorescence under an ultraviolet lamp.

**Sorption of Free 125I**

Sorption of 125I was measured by performing the adsorption experiments with a solution of Na125I used in place of the protein solution. The total radioactivity of the Na125I solution was about the same as that used in a typical protein adsorption experiment (ie about 20 μCi/ml), but the amount of 125I was believed to be 100 times higher than the free 125I present in experiments using undialyzed radiolabeled protein. This assumption is based on the observation that 0.8% of the radioactivity of a protein sample gel filtered on Biogel P-4 after an adsorption experiment eluted in the "salt" peak as free 125I.
Surface Area Effects

The surface area of polymers was examined by several techniques. Scanning electron micrographs of the tops and bottoms of polymer disks were taken with a JEOL 25 (Tokyo, Japan) scanning electron microscope at ×2000 magnification to determine surface heterogeneity. Since the machined edges of polymer samples appeared rough to the naked eye, the possibility that the edges could "trap" protein during rinsing and thereby increase the amount of protein measured on the polymers was examined by comparing the retained radioactivity per unit area in the edges and center of the sample. This was accomplished by punching a hole through the center of the sample with a cork borer and counting the radioactivity in the edge and center cuts. A final method for examining the surface area of the polymers was to convert the surfaces of the polymers to the same chemical form without changing their surface topography by sputter-coating with a gold and palladium alloy. Albumin adsorption to the metallized polymers was then measured.

Results

Surface Area Effects

The increased protein adsorption to HEMA rich polymers observed previously\(^{11}\) was not due to enhanced surface area of the polymers. Scanning electron microscopy of the surfaces of several hydrophilic copolymers showed that they were uniformly smooth. In addition, the retained radioactivity per unit area was the same in the rough edges and center cuts of polymers. Finally, a set of polymers made with 20 to 90% pHEMA and then metallized had relatively constant albumin adsorption (Fig. 1) indicating that the surface area of the polymers was essentially the same. The lower adsorption to the metallized 100% pHEMA surfaces was probably caused by diffusion of water through imperfections in the metal coating resulting in polymer swelling and metal delamination.

Sorption of Free \(^{125}\)I

The high levels of lysozyme and albumin adsorption to the hydrophilic polymers observed previously\(^{11}\) was probably due to the sequestration of free \(^{125}\)I by the polymers. When the adsorption experiments were performed in the presence of Na\(^{125}\)I and the absence of protein, the hydrophilic polymers sequestered iodide when measured after the initial rinse (Fig. 2A, top curve) and even after an overnight soak rinse (Fig. 2B, top curve). The iodide that remains with the polymers after the overnight soak rinse is apparently bound tightly to the polymer matrix since very little iodide was removed after soaking the polymers in buffer for 1 month.

The existence of specific binding sites for iodide on the polymer matrix was examined by repeating the

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Fig. 1. Albumin adsorption from a solution of albumin, lysozyme, and IgG to gold-palladium coated p(HEMA-MMA) copolymers. Individual measurements are plotted.

Fig. 2. Sorption of \(^{125}\)I iodide to p(HEMA-MMA) copolymers from CPBS\(_z\) (triangles) and CPBS\(_z\) + 0.01 M NaI (squares). No protein was present in these solutions. (A) Initial rinse, (B) overnight soak rinse. Individual measurements are plotted.
above experiment with 0.01 M NaI added to the buffer. The concentration of nonradioactive iodide was about 10²⁷ times higher than that of ¹²⁵I in this solution, so that the iodide binding sites should be saturated and any detectable uptake of ¹²⁵I should be in the internal fluid phase of the hydrogels. The lower curves of Figure 2 show that the amount of ¹²⁵I associated with the HEMA-rich polymers is greatly reduced but not completely eliminated in the presence of excess nonradioactive iodide. Thus, a portion of the ¹²⁵I associated with the HEMA-rich polymers appears to be bound to sites which cannot be blocked by nonradioactive iodide while the majority appears to wash out, presumably from the buffer filled inter-

stices of the gel. ¹²⁵I binding sites that cannot be blocked by nonradioactive iodide are also present on the MMA-rich polymers.

Effect of Dialysis and Iodide Concentration

Since free ¹²⁵I uptake could not be completely eliminated in the presence of excess unlabeled iodide, the amount of free ¹²⁵I in solution was reduced by dialyzing the protein mixture immediately before an adsorption experiment. In an experiment with ¹²⁵I-albumin, dialysis greatly reduced the amount of ¹²⁵I retained on polymers containing 100% or 45% HEMA (Fig. 3A, B), whereas it had no effect on a
Table 1. Lysozyme adsorption to p(MMA-HEMA) copolymers of varying thickness*

<table>
<thead>
<tr>
<th>Percent pHEMA</th>
<th>Thickness (cm)</th>
<th>Adsorption (μg/cm²)†</th>
<th>Thickness (cm)</th>
<th>Adsorption (μg/cm²)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>0.033</td>
<td>0.0298 ± 0.0033</td>
<td>0.028</td>
<td>0.0277 ± 0.0016</td>
</tr>
<tr>
<td></td>
<td>0.066</td>
<td>0.0242 ± 0.0002</td>
<td>0.058</td>
<td>0.0199 ± 0.0014</td>
</tr>
<tr>
<td></td>
<td>0.137</td>
<td>0.0300 ± 0.0028</td>
<td>0.119</td>
<td>0.0265 ± 0.0028</td>
</tr>
<tr>
<td></td>
<td>0.191</td>
<td>0.0296 ± 0.0004</td>
<td>0.201</td>
<td>0.0404 ± 0.0110</td>
</tr>
<tr>
<td>80</td>
<td>0.028</td>
<td>0.0434 ± 0.0008</td>
<td>0.036</td>
<td>0.0283 ± 0.0032</td>
</tr>
<tr>
<td></td>
<td>0.071</td>
<td>0.0440 ± 0.0036</td>
<td>0.058</td>
<td>0.0357 ± 0.0049</td>
</tr>
<tr>
<td></td>
<td>0.135</td>
<td>0.0557 ± 0.0033</td>
<td>0.124</td>
<td>0.0327 ± 0.0066</td>
</tr>
<tr>
<td></td>
<td>0.218</td>
<td>0.0721 ± 0.0056</td>
<td>0.185</td>
<td>0.0453 ± 0.0069</td>
</tr>
<tr>
<td>100</td>
<td>0.023</td>
<td>0.0689 ± 0.0066</td>
<td>0.020</td>
<td>0.0248 ± 0.0036</td>
</tr>
<tr>
<td></td>
<td>0.033</td>
<td>0.0775 ± 0.0119</td>
<td>0.036</td>
<td>0.0312 ± 0.0061</td>
</tr>
<tr>
<td></td>
<td>0.135</td>
<td>0.0584 ± 0.0001</td>
<td>0.074</td>
<td>0.0263 ± 0.0008</td>
</tr>
<tr>
<td></td>
<td>0.206</td>
<td>0.0492 ± 0.0054</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Protein adsorption was for 2 hr at 37°C. Protein adsorption was measured after the overnight soak rinse.
† Data are the average of duplicate samples ± standard deviation.

lower water content polymer (Fig. 3C). The effect of the concentration of unlabeled iodide on albumin adsorption was measured in the same experiment since preliminary experiments had shown that albumin adsorption increased on the hydrophobic copolymers in the presence of excess unlabeled iodide. Figure 3A-C shows that unlabeled iodide has no effect on albumin adsorption to either the hydrophobic or hydrophilic polymers.

The lack of an iodide effect on ¹²⁵I uptake by the polymers in this albumin adsorption experiment differs from the results presented in Figure 2 where excess iodide reduced the uptake of ¹²⁵I iodide. However, the amount of free ¹²⁵I present in the undialyzed protein solution in the albumin experiment was approximately 100 times lower than that used in the iodide experiment of Figure 2. In addition, the concentration of free ¹²⁵I was even lower in the dialyzed samples, so that very little free ¹²⁵I was present to be blocked by excess iodide. Finally, the protein present in the albumin study could have blocked many of the free ¹²⁵I binding sites, whereas no protein was present in the ¹²⁵I iodide studies.

Protein Penetration Studies

The possibility that the relatively large amounts of albumin and lysozyme adsorption measured previously to the HEMA-rich polymers compared to the MMA-rich polymers was due to penetration of the protein into the polymer matrix was examined. Protein penetration was studied using both bulk and solution polymerized p(HEMA-MMA) copolymers (lot B and C). These polymers were slightly less crosslinked (4% vs. 5%) than the polymers used in the adsorption studies (lot A) in order to maximize the possibility of protein penetration. When pHEMA samples were incubated with fluorescein-labeled insulin or fluorescein-labeled albumin, a moderate amount of fluorescence was observed even after 3 weeks of incubation. The moderate amount of fluorescence was small compared to the intense fluorescence of polymers incubated with FITC not bound to protein, suggesting that little if any protein penetration occurred. In addition, no difference in the fluorescent uptake of the two proteins was observed at short incubation times, despite the much smaller size of insulin compared to albumin.

Although lysozyme is a larger molecule than insulin and would not be expected to penetrate the polymer matrix on the basis of the fluorescence experiments, lysozyme might undergo a shape change that allows it to fit through the polymer pores. Therefore, the effect of sample thickness on lysozyme uptake was examined. ¹²⁵I-lysozyme adsorption was measured from the protein mixture to bulk polymerized and solution polymerized polymers (60%, 80%, and 100% pHEMA) made in four thicknesses varying from approximately 0.2 to 2 mm. If lysozyme completely penetrated the polymer matrix during a typical 2 hr adsorption experiment, then larger quantities of lysozyme would be measured on the thicker polymers. No dependence of lysozyme uptake on thickness was observed for polymers containing 60% or 100% HEMA, although an increase in lysozyme adsorption was noted on the thickest polymers containing 80% HEMA (Table 1). Depletion of lysozyme from the protein solution was only 0.0355% based on a maximum adsorption of 0.08 µg/cm² (Table 1) and a rectangular sample of 1 x 2 cm. Thus, both the fluorescence and thickness study indicate that lysozyme penetration into the hydrophilic polymer matrix did not occur during routine 2 hr adsorption experiments.
Fig. 4. Adsorption of (A) albumin (triangles) and IgG (diamonds) and (B) lysozyme (squares) and total protein (circles) to p(HEMA-MMA) copolymers from a dialyzed solution of albumin, lysozyme, and IgG. Adsorption was measured in two separate experiments from protein solutions prepared in CPBSz containing 0.01 M NaI (open symbols) or 0.05 M NaI (closed symbols). The amount of lysozyme remaining bound after an additional overnight soak rinse (reverse triangles) was used to calculate the total amount of protein adsorbed. The average adsorption of duplicate (closed symbols) or triplicate (open symbols) samples is plotted. Error bars are two standard deviations in length.

Protein Adsorption Without Free $^{125}$I Sorption

The adsorption of albumin, lysozyme, and IgG to the p(HEMA-MMA) copolymers was performed using dialyzed protein solutions prepared in CPBSz containing 0.01 M NaI (open symbols) or 0.05 M NaI (closed symbols). The amount of lysozyme remaining bound after an additional overnight soak rinse (reverse triangles) was used to calculate the total amount of protein adsorbed. The average adsorption of duplicate (closed symbols) or triplicate (open symbols) samples is plotted. Error bars are two standard deviations in length.

Desorption Studies

The percent of each protein desorbing during the overnight soak rinse of the polymers used in one experiment shown in Figure 4 (solid symbols) is shown in Figure 5. While the amount of adsorption varied among the three proteins (Fig. 4), approximately the same fraction of each protein desorbed. In addition, the relative desorption increased with HEMA content in the polymer. Similar desorption results were obtained from the polymers used in the other experiment that is shown in Figure 4. The increase in desorption with HEMA content was not due to free $^{125}$I.
desorption since free $^{125}$I should have been removed from the protein solution during dialysis. The absence of free $^{125}$I was confirmed qualitatively by the initial rinse data of the HEMA-rich polymers which was much lower (5–10-fold) than the values obtained in the presence of free $^{125}$I.

**Adsorption to Contact Lenses and Other Polymers**

The adsorption of albumin, lysozyme, and IgG to several other polymers as well as commercial contact lenses was measured using dialyzed protein solutions and buffer containing excess unlabeled iodide. Table 2 shows that pHEMA prepared in this laboratory or as a commercial contact lens adsorbed very little protein, in agreement with the data already presented. Copolymers of HEMA and MMA, N-vinyl pyrrolidone (NVP), or acrylamide (AAm) also adsorbed small amounts of protein with adsorption decreasing as the percentage of HEMA increased. Copolymers containing methacrylic acid (MAAc), however, adsorbed large quantities of lysozyme, probably due to electrostatic interaction of the positively charged lysozyme molecule with the negatively charged acid groups.

**Discussion**

The apparent high levels of protein adsorption on copolymers rich in HEMA reported previously were found to be due to sorption of free $^{125}$I by the polymers. Experiments with protein free buffers containing $^{125}$I iodide suggested that the iodide diffused into the polymer matrix, since large amounts of radioactivity were taken up by the high water content HEMA rich copolymers and the majority of this radioactivity could be removed by an overnight soak rinse. The $^{125}$I remaining, however, was adsorbed to the polymers since no more radioactivity was removed by further rinsing. The inclusion of nonradioactive iodide in experiments with $^{125}$I iodide greatly reduced the amount of $^{125}$I bound to the polymers indicating that there were specific binding sites for iodide on the polymers. However, binding of free $^{125}$I was not completely abolished, suggesting that there were additional $^{125}$I binding sites that were not specific for iodide.

The amount of free $^{125}$I that can diffuse into the HEMA-rich polymers during a protein adsorption experiment is significant. Assuming 0.8% free $^{125}$I (measured in one sample of undialyzed protein solution), a protein concentration of 3.88 mg/ml, a specific activity of 1.000 cpm/µg, a polymer disc of 1.1 cm diameter and 0.16 cm thickness, and a water content of 30% for 100% pHEMA, and assuming the free $^{125}$I diffused into the water phase of the polymer to the same concentration present in the bulk phase, then $1.45 \times 10^{-3} \mu$Ci of free $^{125}$I would be present in
Table 2. Protein adsorption to contact lens materials†

<table>
<thead>
<tr>
<th>Material‡</th>
<th>Albumin (µg/cm²)</th>
<th>Immunoglobulin G (µg/cm²)</th>
<th>Lysozyme (µg/cm²)</th>
<th>Total (µg/cm²)</th>
<th>Water content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pMMA</td>
<td>0.278 ± 0.025</td>
<td>0.101 ± 0.009</td>
<td>0.144 ± 0.018</td>
<td>0.523 ± 0.032</td>
<td>2.3</td>
</tr>
<tr>
<td>p(60HEMA/40MMA)</td>
<td>0.133 ± 0.006</td>
<td>0.038 ± 0.009</td>
<td>0.024 ± 0.003</td>
<td>0.215 ± 0.011</td>
<td>13.8</td>
</tr>
<tr>
<td>pHEMA</td>
<td>0.032 ± 0.006</td>
<td>0.026 ± 0.008</td>
<td>0.010 ± 0.001</td>
<td>0.068 ± 0.014</td>
<td>39</td>
</tr>
<tr>
<td>p(80HEMA/20NVP)</td>
<td>0.027 ± 0.002</td>
<td>0.016 ± 0.003</td>
<td>0.023 ± 0.002</td>
<td>0.066 ± 0.004</td>
<td>47</td>
</tr>
<tr>
<td>p(60HEMA/40NVP)</td>
<td>0.028 ± 0.001</td>
<td>0.021 ± 0.002*</td>
<td>0.100 ± 0.071*</td>
<td>0.149 ± 0.071*</td>
<td>49</td>
</tr>
<tr>
<td>p(40HEMA/60NVP)</td>
<td>0.029 ± 0.005</td>
<td>0.021 ± 0.000</td>
<td>0.227 ± 0.036*</td>
<td>0.277 ± 0.036*</td>
<td>49</td>
</tr>
<tr>
<td>p(90HEMA/10MAAc)</td>
<td>0.036 ± 0.009</td>
<td>0.040 ± 0.017</td>
<td>390 ± 5</td>
<td>390 ± 5</td>
<td>35</td>
</tr>
<tr>
<td>p(70HEMA/30MAAc)</td>
<td>0.038 ± 0.007</td>
<td>0.106 ± 0.059</td>
<td>374 ± 34</td>
<td>374 ± 34</td>
<td>42</td>
</tr>
<tr>
<td>p(80HEMA/20AAM)</td>
<td>0.016 ± 0.002</td>
<td>0.010 ± 0.001</td>
<td>0.153 ± 0.009</td>
<td>0.179 ± 0.009</td>
<td>58</td>
</tr>
<tr>
<td>1. Wesley-Jessen Lens</td>
<td>0.122 ± 0.024</td>
<td>0.0043 ± 0.0009</td>
<td>0.0065 ± 0.0064</td>
<td>0.133 ± 0.025</td>
<td>30</td>
</tr>
<tr>
<td>2. B &amp; L Soflens</td>
<td>0.108 ± 0.013</td>
<td>0.018 ± 0.003</td>
<td>0.013 ± 0.006</td>
<td>0.139 ± 0.015</td>
<td>38</td>
</tr>
<tr>
<td>3. Hydrocurve II Lens</td>
<td>0.069 ± 0.012</td>
<td>0.0082 ± 0.0008</td>
<td>8.20 ± 0.98</td>
<td>8.28 ± 0.98</td>
<td>55</td>
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<td>4. Cooper PermaLens</td>
<td>0.110 ± 0.012</td>
<td>0.018 ± 0.003</td>
<td>15.5 ± 2.2</td>
<td>15.5 ± 2.2</td>
<td>71</td>
</tr>
<tr>
<td>5. Cooper Duragel Lens</td>
<td>0.458 ± 0.077</td>
<td>0.032 ± 0.007</td>
<td>0.106 ± 0.023</td>
<td>0.569 ± 0.081</td>
<td>75</td>
</tr>
<tr>
<td>6. Vistamar</td>
<td>0.0208 ± 0.0070</td>
<td>0.0064 ± 0.0016</td>
<td>19.6 ± 3.3</td>
<td>19.6 ± 3.3</td>
<td>58</td>
</tr>
<tr>
<td>7. Lunelle</td>
<td>0.224 ± 0.009</td>
<td>0.0218 ± 0.0016</td>
<td>0.0213 ± 0.0019*</td>
<td>0.267 ± 0.009</td>
<td>70</td>
</tr>
<tr>
<td>8. Hydrocurve Elite</td>
<td>0.084 ± 0.055</td>
<td>0.084 ± 0.055</td>
<td>0.084 ± 0.055</td>
<td>0.084 ± 0.055</td>
<td>55</td>
</tr>
</tbody>
</table>

† After exposure to simulated tear fluid for 2 hr at 37°C followed by an initial rinse and a further overnight soak rinse. Amounts adsorbed are the average ± standard deviation of triplicate samples for pMMA, p(60HEMA/40MMA), and materials 1-8, and of quadruplicate samples for all other polymers. In several cases denoted by an * the number of samples is one less.

‡ Materials 1–8 are commercial contact lenses. The exact formulations are proprietary but the major chemical components were as follows: 1 and 2 polyHEMA; 3, HEMA-MAAc-dimethyl-3-oxobutyl acrylamide copolymers; 4, HEMA-MAAc-NVP terpolymer; 5, unknown; 6, HEMA-MAAc copolymers; 7, MMA-NVP copolymers; 8, unknown.

The fluid phase of the polymer. This amount of radioactivity corresponds to 0.580 µg/cm² of protein. Although these calculations are approximate and the majority of free ¹²⁵I may diffuse out of the hydrogel during the overnight soak rinse, they suggest that even trace amounts of ¹²⁵I in the protein solution could contribute significantly to the radioactivity measured on the polymers and lead to incorrect interpretation of protein adsorption behavior.

When protein solutions were dialyzed to remove free ¹²⁵I, lower protein adsorption values were measured on the HEMA-rich polymers. In a few experiments dialysis did not affect adsorption measured after the overnight soak rinse (compare albumin adsorption in Fig. 3; IgG adsorption in Fig. 4 and Fig. 2 from reference 11) but this may have been because of extremely small amounts of free ¹²⁵I initially present in the protein solution.

The inclusion of nonradioactive iodide in concentrations as high as 0.05 M in dialyzed protein solutions did not affect the adsorption of protein to the p(MMA-HEMA) polymers. Excess iodide did, however, reduce the binding of free ¹²⁵I to the polymers in the absence of protein. Since free ¹²⁵I has been observed to bind to gold and platinum (Pat Lew, personal communication) as well as the p(MMA-HEMA) polymers, it may bind to many other surfaces studied for protein adsorption. Therefore, the inclusion of excess iodide in the buffer during dialysis and adsorption of ¹²⁵I-labeled proteins is recommended. In addition, nonradioactive iodide may disrupt noncovalent interactions between free ¹²⁵I and proteins allowing more effective removal of the radionuclide during dialysis.

When the adsorption of albumin, lysozyme, and IgG to p(MMA-HEMA) copolymers was measured using precautions to prevent free ¹²⁵I sorption, the amount of lysozyme and albumin bound to the HEMA-rich polymers was much lower than originally reported,¹¹ whereas the amount of albumin bound to the MMA-rich polymers increased. The total amount of protein bound to polymers was minimal at pHEMA concentrations greater than 50%. These results agree with decreased protein adsorption to hydrogels observed by other investigators.¹⁰-¹⁸,²² Desorption of all three proteins increased as the water content of the polymers rose, in agreement with increased desorption of proteins from hydrogels noted by others.¹⁷,²³,²⁴ The preferential adsorption of albumin to the hydrophobic polymers, however, is in contrast to observations in other laboratories that albumin, unlike most proteins studied, adsorbs preferentially to hydrogels. This preferential adsorption has been observed from plasma¹⁵,²⁵,²⁶ as well as with single protein solutions.²⁷,²⁸ Horbett et al.¹⁵ however, reported a "U"-shaped albumin adsorption curve from plasma with minimum adsorption at intermediate copolymer concentrations and maximum adsorption at both 100% polyethylmethacrylate (pEMA) and 100% pHEMA.

The total amount of protein bound to the p(MMA-HEMA) copolymers after the initial rinse
was similar to the amount calculated for a monolayer. This is in contrast to adsorption from single protein solutions to pHEMA measured by ATR-FTIR spectroscopy, where adsorption amounted to several monolayers. The amount of protein reported to be bound in these other studies is approximately 15, 20 and 75 times higher for lysozyme, albumin and IgG, respectively, than that reported in this study. The larger amounts adsorbed undoubtedly reflect lack of competition with other proteins as well as less protein removal during a short static rinse (usually 1 min or 1 hr) compared to our dynamic rinse and overnight soak rinses.

The amount of protein associated with polymers while still in the presence of protein solution ("in situ adsorption") is probably higher than that measured after the rinsing step since some loosely adsorbed protein can be removed by the rinse. Presumably, the in situ adsorption is more relevant to the in vivo use of contact lenses since the lenses are bathed in tear solution during wear. On the other hand, the amount of protein bound after rinsing is important both in the practical problem of removing protein deposits from lenses and in understanding the relationship between strength of protein binding and chemical composition of biomaterials. The initial protein molecules that adsorb and are presumably in closest contact with the polymeric surface may experience conformational changes that result in denaturation of the protein and its consequent irreversible adsorption, a possibility that is suggested by ATR-FTIR analysis of tear protein adsorption to pHEMA. Subsequent protein molecules may adsorb on top of this protein monolayer and may not undergo drastic structural changes so that they become reversibly adsorbed. Our data support this idea since the amount of protein adsorbed decreased after rinsing to levels consistent with a close packed monolayer. The protein in this monolayer, however, was bound with different affinities as evidenced by the removal during additional overnight soak rinses of 10 to 20% more of the original amount of protein adsorbed. These different binding strengths could be due to differences in protein-polymer interactions or interactions between the proteins themselves. For example, some proteins may be bound to the polymer indirectly through interactions with proteins bound directly to the polymer.

Analysis of protein deposits on soft contact lenses has shown that lysozyme is the predominant protein deposited on pHEMA and other lenses in vivo. These results seem surprising in light of our studies showing approximately three times more albumin adsorbed to 100% pHEMA than lysozyme. However, the in vivo lenses have been treated quite differently from our samples by undergoing repeated exposure to tear fluid and a protein-air interface as well as a cleaning and sterilization regime involving solvents, enzymes and heat. Furthermore, the methacrylic acid (MAAc) content of the lenses in other studies may have been significant since MAAc is a common contaminant of HEMA. MAAc causes high levels of lysozyme adsorption.

The generally higher levels of lysozyme adsorption to the commercial lenses probably results from electrostatic interaction with methacrylic acid present in many contact lenses. Also, it is possible that lysozyme penetrates into the polymer matrix of less highly cross-linked polymers or polymers with extremely high water contents. Manufacturers of commercial lenses may be able to decrease lysozyme deposits by decreasing polymer pore size or cross-linking the surface of the lens and using polymers free of methacrylic acid.

Penetration of lysozyme, albumin, and insulin into the hydrophilic p(MMA-HEMA) copolymers used in this laboratory did not occur when penetration was measured on solution and bulk polymerized polymers using fluorescent and radiolabeled proteins. The average pore size of solution polymerized pHEMA has been estimated at 5 Å so no protein penetration would be expected. However, bulk polymerized pHEMA may have average pore sizes of about 30 Å so it is possible that lysozyme (30 Å diameter) or insulin (28 Å diameter) could have penetrated the polymer. Lysozyme penetration into HEMA was observed by Refojo and Leong, but their polymers may have contained larger pores than ours since no cross-linking agent other than the amount normally found in redistilled HEMA was present during polymerization of their polymers. In addition Refojo and Leong dissolved lysozyme in water rather than buffer and the protein may have denatured with time, undergoing shape changes that allowed it to penetrate the polymer matrix. Penetration of 125I-BSA into pHEMA has been observed by Holly and Owen, but their results may have been due to penetration of free 125I into the polymer or build up of layers of denatured protein on the surface of the hydrogel resulting from repeated passage of the polymer through an air-water interface.

The significance of the adsorption measurements made here for clinically related contact lens protein deposition problems has not been directly addressed by this work. However, a model of the in vivo adsorption process might be constructed that considers the common use of relatively viscous contact lens wetting solutions. A lens with such a solution cover-
ing its surface would, upon insertion into the eye, adsorb its initial layer of protein without the lens passing through an air-water interface. Thus, the initial layer of protein formed in these experiments may have a relationship to the initial layers formed in vivo; ie in our studies the polymer was not passed through the air-water interface and the adsorption experiments were performed in competition with other proteins. Subsequent protein adsorption may be affected by the drying of the tear film that has been frequently observed on the outer surface of contact lenses. Other factors that must be considered in comparing the experiments presented here to actual clinical contact lens deposition include the side of the lens studied (inside versus outside), shear induced by the eyelid, and sterilization methods used (peroxide and heat).

In summary, we have shown that free 125I is present in preparations of 125I-labeled proteins and that the free 125I absorbs to polymers and copolymers of MMA and HEMA. Most of the free 125I can be removed from the proteins by dialysis and the inclusion of nonradioactive iodide in the buffer further reduces problems due to specific binding of free 125I. Using these precautions tear proteins were found to adsorb minimally to p(MMA-HEMA) copolymers made with 50% or more HEMA. Penetration of protein into the polymer matrix did not appear to occur. Because of low protein adsorption these hydrophilic copolymers may be useful materials for contact lenses if an appropriate copolymer that increases oxygen permeability is included. The copolymers of (MMA-HEMA) alone may not be suitable for contact lenses but they provide a good material to work with and improve. Since the lower water content copolymers are stronger than those composed of 100% HEMA, they can be made thinner to increase oxygen permeability. Furthermore, chemical additives may be able to increase the oxygen permeability.

**Key words:** protein adsorption, tear proteins, contact lenses, polymers, hydrogels

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