Effect of Surface Chemistry on Protein Interaction with Hydrogel Contact Lenses

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ABSTRACT

Protein is one of major classes of contaminations for hydrophilic contact lenses. The absorption of individual proteins from single protein solutions in vitro on different lens materials has been measured. The effect of water content and surface chemistry of the lens in terms of ionic characteristics on the interaction of different proteins have also been studied. A series of contact lenses with different surface and structural properties have been used to study the effect of hydrogel material on the quantity of charged or uncharged protein absorbed. The early stages of interaction and the leaching of protein from the contact lens into storage solution have been studied for proteins deposited in vitro and in vivo.

Key Words: hydrophilic contact lenses, adsorption, spoliation, biological activity, hydrogels

INTRODUCTION

The natural habitat of most proteins is an aqueous environment. When a protein solution contacts another phase (either a solid, liquid, or a gas) with which it is immiscible, protein molecules tend to accumulate at the interface between the two phases. This tendency has a great effect on various natural and technological processes. Adsorption of proteins takes place almost instantaneously when a solid surface comes into contact with most biological fluids [1]. The protein film being formed may then act as a substratum for subsequent adhesion of other components such as eukaryotic cells or microorganisms.

Protein adsorption is the overall result of various types of interactions between the different components present in the system, that is, the sorbent surface, the protein molecules, the solvent (water) and any other solutes such as low-molecular mass ions.

The surface of a protein is often complex in nature, with different characteristics such as hydrophilicity and ionic charges [2]. The fact that many real surfaces are heterogeneous together with the complex nature of the protein surface complicates the prediction of how a protein interacts with a surface.

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One major factor influencing protein adsorption is the surface energy, and it has been reported that hydrophobic surfaces adsorb more protein than hydrophilic ones [3]. Prediction of the effect of surface charge on protein adsorption is not straightforward. Proteins with net charge similar to the surface might bind through local patches of the opposite charges. Norde [3] suggests that the driving force in this case is an increase in entropy, due to conformational changes of the protein resulting in the loss of secondary structure. He points out that the groups at the surface of a protein are the ones most likely to interact with a solid surface, although interior groups might be exposed through conformational changes. In the case of proteins with strong internal coherence, "hard" proteins, structural rearrangements do not significantly contribute to the adsorption process. The "soft" proteins which have lower structural stability will adsorb even under unfavourable conditions due to the structural rearrangements.

Certain ions can bind to specific sites in proteins and thereby change the molecules' adsorption behaviour. For example, the binding of insulin to zinc ions increases the amount adsorbed onto chromium surfaces [4]. Binding of small molecules such as fatty acids to proteins may also influence the amount of adsorbed protein [5]. The effect of temperature on adsorption is not always predictable. The increases [6] and decreases [7] in the adsorption with increasing temperature have been reported.

Protein adsorption has an impact on the performance of many processes; its effect may be beneficial or detrimental. In fouling of heat exchangers, ultrafiltration membranes and other process equipment, for example, protein adsorption needs to be minimized [8]. However, chromatography [9] and immunoassays [10] require the binding of the protein.

One major area where protein interaction with solid surfaces is of interest is in the field of biocompatible materials. Soft and hard tissue implants [11] and blood compatible materials [12] have been investigated. Adsorption and adhesion from tear fluids [13] and saliva [14] have also been studied.

Analysis of protein adsorption onto polymer surface is important in biotechnological and biomedical fields. The eye is a unique body site for the study of protein interactions with biomaterials because of its ease of access and deceptive complexity of the tears. It is, for example, easy to introduce a contact lens into the tear and take it out in few minutes without surgery and causing any trauma to the patient.

The use of contact lenses for either vision correction and cosmetic reasons or as a route for the controlled drug delivery, has significantly increased recently. Currently, 60 million people requiring vision correction wear some type of hydrophilic soft contact lenses.

The widespread use of hydrophilic contact lenses has demonstrated the problem of lens spoilage. Polymer deterioration and protein deposition will change the optical quality and permeability of the lenses and may reduce wear tolerance [15]. The protein adsorbed is also a primary layer for the subsequent adsorption of other proteins and tear components. Most of the previous studies have concentrated on the proteinaceous deposits on the surface of the lens. The presence of proteins inside the hydrogel matrix, however, has received little attention.

This work describes the in vitro surface deposition of individual proteins onto different lens materials. The materials have been used to study the effect of hydrogel structure on the nature and quantity of protein adsorbed into lens matrix and the biological activity of the protein which, subsequently leaches out into, for example, storage solution from in vitro and in vivo spoiled lenses.

**EXPERIMENTAL**

**Materials and Methods**

The major tear proteins, albumin, lactoferrin and lysozyme used together with a group of other proteins (ribonuclease, myoglobin, insulin and ferredoxin which enable the effect of size and charge to be studied), as presented in Table 1. A
Table 1. Characteristics of some proteins used for in vitro deposition.

<table>
<thead>
<tr>
<th>Name</th>
<th>Source</th>
<th>Molecular weight (Daltons)</th>
<th>Relative charge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysozyme</td>
<td>Chicken Egg</td>
<td>12,600</td>
<td>(+++)</td>
</tr>
<tr>
<td>Albumin</td>
<td>Human serum</td>
<td>66,000</td>
<td>(-----)</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>Bovine colostrum</td>
<td>74,000</td>
<td>(+)</td>
</tr>
<tr>
<td>Ribonuclease</td>
<td>Bovine pancreas</td>
<td>13,000</td>
<td>(+++)</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>Horse heart</td>
<td>17,000</td>
<td>(+)</td>
</tr>
<tr>
<td>Insulin</td>
<td>Bovine pancreas</td>
<td>6,000</td>
<td>(0)</td>
</tr>
<tr>
<td>Ferredoxin</td>
<td>Spinach</td>
<td>12,000</td>
<td>(-----)</td>
</tr>
</tbody>
</table>

group of representative contact lenses from FDA (Food and Drug Association) groups I-IV were also used to compare different lens materials (Table 2). The proteins were all purchased from Sigma Chemical Company and the lenses were supplied by their companies (Table 2) as free gifts. An U-2000 spectrophotometer (Hitachi) was used for all the UV measurements.

The UV absorbances at 280 nm were measured by UV spectroscopy. The protein deposited lenses were rinsed once with distilled water and placed at the bottom of a UV cell filled with distilled water (Figure 1). The quantity of the protein deposited into them was calculated using an unworn lens of the same type and power as the background. A standard curve was obtained using individual protein solutions with known concentrations and was used to measure the quantity of each protein deposited. In the case of in vivo spoiled lenses the standard curve was obtained using an artificial tear solution made of proteins and electrolytes with concentrations similar to that of natural tear. The spectrophotometer was calibrated to give the quantity of deposited protein in milligrams per lens (lens surface area = 1.4 cm²).

The proteins were then extracted from contact lenses by cutting the lenses into quarters and shake them vigorously in 1.0 mL of ReNu™ solution for at least 3h. This was found to be the most effective way of extracting the proteins from spoiled lenses.

The activity of lysozyme in the extraction solutions was measured using a method based on the decrease in the turbidity of a Micrococcus lysodeikticus at 450 nm [16]. The activity of ribonuclease was measured by a modification of Kuntiz spectrophotometric assay [17]. The method is based on the reactivity of the enzyme on yeast ribonucleic acid in a buffer environment.

The effects of the equilibrium water content and the surface charge of the contact lens material were studied using some poly(2-hydroxyethylmethacrylate) lenses with varying cross-link density, that is, water content and methacrylic acid (MAA) content. Increase in the proportion of methacrylic acid (1–5%) caused an increase in the concentration of negative charges in the polymer.
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Table 2. The characteristics of some commercial contact lenses (USAN: United States Accepted Name).

<table>
<thead>
<tr>
<th>Company</th>
<th>USAN Name</th>
<th>Lens group</th>
<th>% Water content</th>
<th>Chemical composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydron</td>
<td>Polymacon</td>
<td>I</td>
<td>38</td>
<td>HEMA</td>
</tr>
<tr>
<td>Vistakon</td>
<td>Surfilcon A</td>
<td>II</td>
<td>74</td>
<td>MMA-VP</td>
</tr>
<tr>
<td>CIBA Vision</td>
<td>Buflcon A</td>
<td>III</td>
<td>45</td>
<td>HEMA-DA-MA</td>
</tr>
<tr>
<td>Lunelle</td>
<td>Etatlicon A</td>
<td>IV</td>
<td>58</td>
<td>HEMA-MA</td>
</tr>
<tr>
<td>Vista Optics</td>
<td>Vifilcon</td>
<td>IV</td>
<td>55</td>
<td>HEMA-PVP</td>
</tr>
</tbody>
</table>

Abbreviations: HEMA[2-hydroxyethylmethacrylate], VP[vinylpyrrolidone], MA[methacrylic acid], DA[diacetone acrylamide] and PVP[polyvinylpyrrolidone].

RESULTS AND DISCUSSION

Group IV lenses, such as Etafilcon and Vifilcon, which are high water content ionic lenses that contain a significant negative charge are well known to absorb significant quantities of protein. In order to establish the relative uptake of the three main tear proteins, the uptake profile for lysozyme, lactoferrin and albumin was developed as a function of time. The results are shown in Figure 2.

From a comparison of size and charge of the protein (Table 1), it is apparent that both size and charge may be influential. Since lactoferrin is both larger and less charged than lysozyme, its lower level of uptake may be affected by either or both. In order to investigate these factors separately, the uptake of a series of proteins having similar size to lysozyme but with different charges were studied. Figure 3 shows the relative deposition rates of lysozyme, ribonuclease, myoglobin, ferredoxin together with insulin which has no charge but is significantly smaller than lysozyme. This figure clearly demonstrates that it is the charge rather than the size of lysozyme that produces its high level of uptake.

Some further deductions may be made. First of all, tear specific pre-albumin, which is a significant tear component of similar size to lysozyme but without the positive charge, is unlikely to be significantly absorbed. This important tear component is not available as an isolated protein. It is only by studies shown in Figure 3 that information relating to its deposition behaviour may be deduced. Secondly, both the shape and the

Figure 2. The effect of size and charge of the protein on its uptake by a group IV lens material; △: lysozyme, ○: lactoferrin, ◆: albumin.

Figure 3. The effect of the charge of proteins on their in vitro deposition onto a group IV lens; △: lysozyme (+ + +), ◆: ribonuclease (+ + +), ▲: myoglobin (+ +), ○: insulin (0), ■: ferredoxin (———).
size of the protein appear to be influential in governing uptake. As a result, the more compact lysozyme structure, illustrated in Figure 4, permits a more efficient interaction with the material than is the case with ribonuclease which has similar size and charge.

Because of its charge, size and compact structure, lysozyme is a useful member of the family of proteins developed in studying the interaction of tear proteins with different contact lens materials. Group I-IV lens materials are defined as low water content non-ionic (I), high water content non-ionic (II), low water content ionic (III) and high water content ionic lenses (IV), respectively. A typical member of each group (Polymacon, Perfilcon, Bufilcon and Etasilcon) was used as a substrate to study the effect of lens material on lysozyme uptake. The results are shown in Figure 5. Here again the message is clear. It is the charge and not the EWC percentage that dominates the interaction.

In order to distinguish between surface adsorption and absorption into the lens material of ionic lenses, two families of materials were used. One consisted of hydroxyethylmethacrylate-methacrylic acid copolymers in which the normal (~1%) level of cross-link was used, producing a family of materials where equilibrium water content (EWC) rose from 42% at 1% MAA to 67% at 5% MAA. The second family was prepared with the same percentages of MAA but with a very high (10%) cross-link density.

This was designed to prevent the network...
from expanding and thus to inhibit lysozyme uptake into the matrix. The equilibrium water content of this family varied only between 42-48%. The results of lysozyme and lactoferrin uptake over a 14 days period are shown in Figure 6.

Two clear points emerge from Figure 6. In the case of high cross-link density materials (EWC<50%), the lysozyme and lactoferrin penetration into the lens matrix will be negligible enabling the surface concentration of these proteins on negatively charged ionic polymers to be assessed.

In the case of group IV lens materials (EWC>50%), such as Etafilcon and Vifilcon, the figure will be in the region of 150 micrograms per lens. The second point that emerges is that lactoferrin (as well as lysozyme) is both adsorbed onto the surface of group IV lens materials and absorbed into the bulk of the materials although much more slowly. This explains the slow, but progressive rise in lactoferrin uptake into Etafilcon shown in Figure 2.

The question that must now be addressed is the mobility and activity of protein deposited onto the surface and into the matrix of ionic materials. By exposing the lenses to lysozyme (or using ex in vivo lenses), measuring surface protein, extracting with conventional care systems (such as ReNu™) and measuring the protein concentration and destruction of activity (if any) can be monitored.

Figure 7 compares the extraction of total protein from the surface and bulk of in vivo and in vitro lenses (each lens was extracted into 1 mL of solution). These lenses contained very similar gross level of protein initially and, as can be seen, the protein leaches steadily from both in vivo and in vitro spoiled lenses. This suggests that there is no apparent difference in extraction of protein from worn lenses and in vitro extractions. Similarly it is quite clear that the mobility of protein diffusing from the lenses is of the same order as that diffusing into the lens (Figure 2 cf Figure 7).

By measuring the biological activity of total protein desorbed from the surface of a lens the extent to which its uptake and removal have produced denaturation may be established.

Figure 8 shows the power and sensitivity of this technique. This figure relates to lysozyme adsorbed onto a poly(2-hydroxymethacrylate) lens produced from a commercial lens blank material (EWC=37% which does not allow the penetration of protein into the matrix). The material contained appreciable residual methacrylic acid than high quality poly (2-hydroxyethylmethacrylate) lenses and accumulated over 100 mg onto the lens surface. The protein was progressively extracted into ReNu™ multi-purpose solution and both concentration and biological activity were determined.

It is clear that, within experimental errors,
whereas albumin and tear specific pre-albumin are less adsorbed.

Lactoferrin, as well as lysozyme, penetrates into the matrix of group IV lenses despite its greater size.

Lysozyme is mobile and can be leached out of the matrices of group IV lenses stored at pH>7 in, for example, ReNuTM.

The biological activity of lysozyme is not detectably changed by the absorption and desorption processes.

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REFERENCES


CONCLUSION

Both size and charge of proteins are influential in their deposition onto contact lenses.

The uptake of different proteins into the matrices of group IV lenses is more influenced by the charge of proteins than their size.

Lysozyme and lactoferrin adsorb onto the surface of group IV lenses to a high degree,

![Figure 9. The activities of lysozyme and ribonuclease leached into ReNuTM from an Etafllcon lens.](image-url)
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