Analysis of Glycoprotein Deposits on Disposable Soft Contact Lenses

Paul C. Tripathi and Ramesh C. Tripathi

By using gel electrophoresis, as well as Western blotting with specific antibodies or with the lectin concanavalin A, we characterized the types and amounts of proteins that are deposited on 58% ionic and 38% nonionic water-content disposable soft contact lenses (DSCLs) worn for 1 to 21 days by asymptomatic subjects with mild to moderate myopic refractive errors. The total amounts of protein eluted from the lenses ranged from 0.1 to 80 μg/lens. The amount of protein deposited on 58% water-content lenses was greater than that on 38% water-content DSCLs. We did not find a strict correlation between the amount of protein deposited and the duration of wear for either type of lens. The major polypeptide fractions detected had apparent molecular weights of 14, 17, 21, 30, and 60 kD. The fractions at 14 kD-bound antibodies specific for human lysozyme, and those at 17 kD corresponded to prealbumin. The 60 kD fraction included IgG heavy chains. The identity of the fractions at 21 kD and 30 kD is unknown. Because oligosaccharide side chains on the proteins attract microbes and facilitate their adherence, knowledge about the types of carbohydrate moieties in lens deposits can provide a rational approach to inhibiting or reversing microbial infection. Invest Ophthalmol Vis Sci 33:121-125, 1992

Proteins form a major portion of the surface deposits found on hydroxyethyl methacrylate (HEMA) contact lenses. Therefore, they contribute significantly to the physicochemical properties of the lens surfaces as well as to contact lens spoilage. Ultimately, this can lead to ocular intolerance by causing clinical complications and their sequela. Recently, attention has been focused on the chemical nature of individual proteins and on their interaction with different contact lens polymers. Adsorption of protein by contact lenses appears to depend largely upon the binding characteristics of the protein molecules in vivo. Protein deposits also act as bacterial ligands and may precipitate a host of microbe-induced clinical complications. The purpose of our present investigation was to identify the types and amounts of proteins deposited on DSCLs of high (58%) and low (38%) water content that had been worn for various periods. We present evidence that suggests spoilage of disposable soft contact lenses (DSCLs) does not occur strictly as a function of period of wear. Also, we discuss the nature and likely source of DSCL protein deposits and their clinical implications.

Materials and Methods

Contact Lenses

We analyzed 43 DSCLs worn by asymptomatic patients who had mild to moderate myopic refractive errors (-1 to -4 diopters). The lenses, made of HEMA polymer of 38% nonionic (SequenCe, Bausch and Lomb, Rochester, NY) or 58% ionic (Acuvue; Vistakon, Jacksonville, FL) water content, were obtained commercially. After a designated period of wear and immediately upon removal with clean, washed fingers or with suction extractors, the lenses were macerated mechanically in approximately 1 ml of ice-cold solubilization buffer with 100 full turns of a glass microhomogenizer. The volume of the buffer was brought to approximately 300 μl by concentration on centricon 3 kD cut-off filters (Amicon, Danvers, MA). To determine if the filters had
trapped any protein, we incubated selected Amicon filters for 30 minutes in solubilization buffer, and the eluates were analyzed in the same manner.

Total Protein Determination

Samples of 100 µl aliquots were used for measuring the total amount of protein removed from the lenses. The total protein eluted was measured by the folin phenol method with bovine serum albumin as the standard. 15

SDS-PAGE Analysis

The eluted proteins were separated by SDS-PAGE and stained with silver nitrate on either full-size gels or a Phast minigel system (Pharmacia-LKB, Piscataway, NJ), as described previously. 16 The relative quantities of individual polypeptide fractions were estimated by scanning with a laser densitometer at 612 nm and 0–4.0 optical density (Ultroscan-XL; Pharmacia-LKB).

Western Blotting Experiments

For immunoblotting with antibodies, the polypeptides separated on unstained gels were transferred by diffusion onto nitrocellulose paper of 0.2 µm (Schleicher and Schuell, Keene, NH). For lectin binding, the transfer was achieved electrophoretically by application of 60 V current for 3 hr in 20 mM Tris-HCl with 20% methanol after the method of Towbin and Gordon. 17 The nitrocellulose was blocked overnight in 3% bovine serum albumin.

Individual lanes were cut from the nitrocellulose sheets and incubated in primary antibody [rabbit anti-human lysozyme (0.06 mg/ml) or rabbit anti-human prealbumin (0.06 mg/ml)] or in the lectin concanavalin A (50 to 200 µg/ml). 18 All incubations were performed in 0.15 M NaCl, 0.05 M Tris-HCl, pH 7.5 for 2 to 4 hours. After three washes in Tris-HCl buffer, the strips were incubated in peroxidase-conjugated secondary antibody (swine anti-rabbit IgG, 0.08 mg/ml) for 1 hr. For controls, we omitted the primary antibody or lectin and incubated the strips in peroxidase-conjugated secondary antibody only.

Results

The total protein eluted from individual-worn DSCLs ranged from 0.1 to 80 µg/lens (Fig. 1). Within one day of wear, five specific polypeptide fractions, with approximate molecular weights (MWs) of 14, 17, 21, 30, and 60 kD, were present in the eluates from all DSCLs. On SDS-PAGE, we detected no protein bound to the Amicon Centricon concentrators nor to unworn lenses.

More than 50% of the total silver-stained protein was detectable in two fractions, at MWs of 14 and 17 kD, under reducing conditions (Fig. 2). Analysis by laser densitometry showed that there was no strict relationship between the relative intensities of the polypeptide fractions and the period of wear (Figs. 3a, b).

We consistently observed a greater accumulation of protein on high-water-content lenses (58%; n = 8; range of total protein, 0.8 to 80 µg/lens) than on low-water-content DSCLs (38%; n = 8; range of total protein, 0.1 to 60 µg/lens) worn for comparable lengths of time by the same subject (Fig. 1). However, no qualitative differences in the polypeptides were found (Fig. 4).

In all samples analyzed, the two major polypeptide fractions, at 14 and 17 kD, had affinity for Con A (Fig. 5). Based upon the size and apparent N-glycosylation, we analyzed these two fractions further by using spe-
specific antibodies. The fraction at 14 kD comigrated with pure lysozyme and reacted positively with the antibody against human lysozyme. This latter reaction was specific and was comparable to results obtained with pure lysozyme. However, there was also a detectable reaction with a minor fraction at approximately 60 kD. The 17 kD polypeptide fraction reacted weakly with antibody specific for human prealbumin. We also observed a reaction product at approximately 60 kD. In separate experiments, the minor 60 kD fraction was found to react with the secondary antibodies (swine anti-rabbit IgG).

**Discussion**

The results of the lectin binding studies suggest that the two major polypeptide fractions eluted from the DSCLs contain N-linked carbohydrate chains, probably of a complex or hybrid type. In an earlier histochemical study, Klotz et al. reported that three different brands of extended-wear soft contact lenses bound FITC-conjugated Con A. However, the histochemical method used did not clarify the nature of the molecules that bound to the lectin. We now have evidence that at least some of the Con A binding observed was a result of glycoproteins that have subunit MWs of 14 and 17 kD. These characteristics are consistent with the known sizes and carbohydrate contents of two major tear proteins: lysozyme and tear specific prealbumin.
The presence of lysozyme at 14 kD was confirmed by Western blotting, which showed it to be the major silver-stained protein deposited on DSCLs. Some glycoproteins react only weakly with silver nitrate and, therefore, may not be detected under the conditions used in our study. However, most soluble glycoproteins in tears have some affinity for Con A and should be detectable by the silver-staining approach or by use of the lectin.

That the 17 kD fraction adsorbed onto DSCLs may contain tear-specific prealbumin is supported by the following observations: (1) this fraction had migration patterns characteristic for tear specific prealbumin\textsuperscript{20,21}; (2) it reacted, albeit weakly, with antibodies against prealbumin; and (3) tear specific prealbumin with a MW of 17 kD is known to be the major protein in tears. Our findings, however, do not rule out that additional, minor polypeptides were deposited on the DSCLs at the MWs of 14 or 17 kD.

Earlier, we reported the presence of a fraction at 30 kD in proteins eluted from both 38% and 58% DSCLs even after one day of wear by asymptomatic patients.\textsuperscript{12} This finding is now confirmed by investigators in Europe who examined Vistakon (42% Etafilcon and 58% hydration) lenses after two days of wear.\textsuperscript{22} Because the 30 kD fraction is an undefined component of normal tears, its deposition on ionic DSCLs is conceivable. Whether it represents a soluble mucous glycoprotein, a reduced fraction of a higher molecular weight protein, or an unknown specific protein remains to be investigated.

We consistently observed that, on immunoblotting, the 60 kD fraction bound anti-lysozyme and anti-prealbumin antibodies. Our result indicated that this reaction is probably mediated by the secondary antibody. By omitting the primary antibody (anti-lysozyme or anti-tear-specific prealbumin), we were able to corroborate this hypothesis. Therefore, we conclude that the 60 kD fraction includes IgG heavy chains, which have an approximate MW of 57 kD under reducing conditions and which are known to be components of tears.\textsuperscript{20,21}

Emphasis has been placed on the roles of total water content and the ionic composition of the lens matrix in determining the characteristics and time course of spoilage of HEMA lenses.\textsuperscript{11} Because of the importance of charge interactions for the adherence of protein to contact lenses,\textsuperscript{4,14} we believe that information about the types of oligosaccharide side chains on deposited proteins is very important. Oligosaccharide moieties can contribute to the formation of mixed protein-lipid deposits as well as act as receptors for bacteria in the early stages of their adherence to the lens surface.\textsuperscript{14,23,24}

During the course of colonization of the HEMA lens matrix by microbes, the exposed sugar moieties of proteins deposited on the lens surface may provide sites for initial attachment. Sugars on the surface of the contact lens also would ensure a convenient source of carbon for bacteria or fungi. Consequently, greater knowledge about the types of carbohydrate chains on the deposited proteins could result in a rational approach to inhibiting or reversing microbial infection. This might include inhibition with analog saccharides or enzymatic treatment with specific glycohdirolases.\textsuperscript{14}

Our finding that there is no direct correlation of the time of wear with the amount of protein deposited onto DSCLs highlights the importance of specific patient-related factors in lens spoilage. Reports also have indicated that the accumulation of a polypeptide presumed to be lysozyme on DSCLs of high water content increases with wearing times of up to one week.\textsuperscript{25} However, in this study no objective quantitative analysis was performed. Some factors that may account for variability in the amount of deposited protein are the structure and composition of the tear film, the cleaning regimen used, compliance with manufacturers' instructions, environmental exposure to pollution or other irritants, and, perhaps, genetic microheterogeneity in the structure of tear proteins among individuals.

Key words: antibodies, gel electrophoresis, immunoglobulin G, lectins, lysozyme, prealbumin, polypeptide fractions, western blotting

Acknowledgment

The authors appreciate the technical help and critique of Drs. Brenda J. Tripathi and Charles B. Millard.

References


