Proteoglycans Contain a 4.6 Å Repeat in Macular Dystrophy Corneas: X-Ray Diffraction Evidence

Andrew J. Quantock,* Gordon K. Klintworth,‡ David J. Schanzlin,* Malcolm S. Capel,§ Mary Ellen Lenz,¶ and Eugene J-M.A. Thonar¶

*Anheuser-Busch Eye Institute, Department of Ophthalmology, St. Louis University School of Medicine, St. Louis, Missouri; ‡Duke University Eye Center, Durham, North Carolina; §National Synchrotron Light Source, Brookhaven National Laboratory, Upton, New York; and ¶Departments of Biochemistry and Internal Medicine, Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois, USA

ABSTRACT Synchrotron x-ray diffraction patterns from macular corneal dystrophy (MCD) corneas contain an unusual reflection that arises because of an undefined ultrastructure with a periodic repeat in the region of 4.6 Å. In this study, we compared the wide-angle x-ray diffraction patterns obtained from four normal human corneas and four MCD corneas. Moreover, portions of two of the MCD corneas were pretreated with a specific glycosidase to shed light on the origin of the 4.6 Å reflection. None of the normal corneas produced an x-ray reflection in the region of 4.6 Å, whereas all four of the MCD corneas did (MCD type I at 4.65 Å and 4.63 Å, MCD type II at 4.63 Å and 4.67 Å). This reflection was diminished after incubation of the MCD tissues with either chondroitinase ABC or *N*-glycanase. The findings indicate that glycosaminoglycans or proteoglycans contribute to the unusual MCD x-ray reflection and hence most likely contain a periodic 4.6 Å ultrastructure. Furthermore, the results imply that periodic 4.6 Å MCD ultrastructures reside in either intact, unsulfated lumican molecules and regions of the CS/DS-containing molecules or in a region of a hybrid macromolecular aggregate formed by the interaction of the two molecules.

INTRODUCTION

Glycosaminoglycans and proteoglycans play many physiologically essential roles. Those proteoglycans that reside in connective tissues are particularly important because of their influence on the ultrastructure and biomechanics of the extracellular connective tissue matrix. Seemingly simple defects of the glycosaminoglycan or proteoglycan metabolism in various connective tissues—most notably cartilage and cornea—underlie such disabling conditions as osteoarthritis, rheumatoid arthritis, disc degeneration, and the corneal mucopolysaccharidoses. One affliction whose pathogenesis has been linked to an erroneous glycosaminoglycan metabolism is the potentially blinding human condition macular corneal dystrophy (MCD) (Klintworth, 1994; Quantock, 1994). Classically, this autosomal recessive inherited trait presents within the first decade of life and becomes symptomatically advanced by the third decade. The clinical course involves the cornea becoming progressively cloudy, with initial, central, superficial deposits eventually extending posteriorly and peripherally (Klintworth, 1980). The resultant visual disturbance is most successfully treated with a corneal transplantation.

Opacification of the corneal stroma in MCD is due ultimately to a breakdown in the regular organization of the stromal collagen fibrils—an essential condition for tissue transparency (Maurice, 1957). Typical MCD ultrastructural abnormalities include an accumulation of glycosaminoglycan (Klintworth and Vogel, 1964; Morgan, 1966; Hassell et al., 1980; Meek et al., 1989) and fibrillogranular deposits (Snip et al., 1973; Edward et al., 1990; Quantock et al., 1993b) in the stromal matrix, the presence of stromal lacunae (Meek et al., 1989), occasional pockets of large diameter collagen fibrils (Quantock et al., 1993b), and a compaction of the stromal collagen fibrils (Quantock et al., 1990), resulting in a thin central MCD cornea (Ehlers and Bramsen, 1978; Donnenfeld et al., 1986).

The normal human corneal stroma contains two main types of proteoglycan, lumican and decorin. Lumican consists of a core protein to which one or two side chains of keratan sulfate (KS) are covalently attached (Gregory et al., 1982; Midura and Hascall, 1989; Blochberger et al., 1992), and decorin consists of a core protein and a glycosaminoglycan side chain that is a mixture of chondroitin sulfate (CS) and dermatan sulfate (DS) (Axelsson and Heinegard, 1975; Midura and Hascall, 1989; Li et al., 1992). Both types of proteoglycan are intimately associated with the stromal collagen fibrils (Scott and Haigh, 1985; Meek et al., 1986) and play important, although not yet totally clear, functional roles in governing stromal architecture (Borcherding et al., 1975; Scott and Haigh, 1985; Hahn and Birk, 1992; Rada et al., 1993; Cornuet et al., 1994).

Immunochemical criteria dictate that MCD patients fall into one of two main subgroups that are clinically indistinguishable (Yang et al., 1988; Edward et al., 1988). Briefly, the cornea and serum of the majority of MCD patients contain no normally sulfated KS, a glycosaminoglycan easily identified by a monoclonal anti-KS antibody in an enzyme-linked immunosorbent assay (ELISA) (Klintworth

Received for publication 5 September 1995 and in final form 3 January 1996.

Address reprint requests to Dr. Andrew J. Quantock, Anheuser-Busch Eye Institute, Department of Ophthalmology, St. Louis University School of Medicine, 1755 South Grand Blvd., St. Louis, MO 63104. Tel.: 314-577-8660; Fax: 314-771-0596; E-mail: quantoaj@sluvca.slu.edu.

0006-3495/96/04/1966/07 $2.00

et al., 1986; Thonar et al., 1986); these patients are designated as suffering from MCD type I. The primary defect in MCD type I is assumed to occur in the synthetic pathway of the KS-containing proteoglycans (Klintworth and Smith, 1977; Hassell et al., 1980; Nakazawa et al., 1984) and may involve an incomplete glycosaminoglycan sulfation process (Hassell et al., 1980; Nakazawa et al., 1984; Midura et al., 1990). Some MCD patients demonstrate detectable levels of normally sulfated KS in their serum and/or cornea and are classified as MCD type II sufferers (Yang et al., 1988; Edward et al., 1988). As most of the KS-containing molecules present in serum are products of the degradation of proteoglycans in cartilage, the absence of normally sulfated KS in the serum of patients with MCD type I also indicates that KS in cartilage is not normally sulfated, a contention that has now been supported by direct analysis of a cartilage biopsy from a patient with MCD type I (Edward et al., 1990). Some studies have documented that MCD patients synthesize abnormal CS/DS; larger than normal and oversulfated CS/DS proteoglycans have been documented in MCD type I corneas (Nakazawa et al., 1984), and a MCD type II cornea has been reported to produce smaller than normal DS proteoglycans (Midura et al., 1990). In addition, histological work has suggested that CS/DS proteoglycans accumulate and aggregate in both MCD types I and II corneas (Meek et al., 1989; Quantock et al., 1990).

Corneal stroma produces an x-ray diffraction pattern, the wide-angle component of which relates to the arrangement of atoms and groups of atoms into molecules, and of collagen molecules into fibrils (Meek et al., 1991). Previously we documented a wide-angle x-ray diffraction ring arising from a periodicity in the region of 4.6 Å that was unique to corneas with MCD (Quantock et al., 1992). Since that time, a similar x-ray reflection has been documented in scarred rabbit cornea (Rawe et al., 1994), another tissue in which abnormally sulfated preoteglycans are known to exist (Hassell et al., 1983). In our previous work, we postulated that proteoglycan or glycosaminoglycan structures could be the origin of the unusual 4.6 Å reflection in MCD corneas, but could offer only circumstantial evidence for our contention (Quantock et al., 1992). The present study attempts to identify the molecule or molecules that contain 4.6 Å periodic ultrastructures in MCD corneas.

MATERIALS AND METHODS

Normal corneas

The four normal human corneas were obtained post-mortem from a 91-year-old male with no known ocular problems and a 82-year-old female with a history of cataracts. Eyes were enucleated within 6 h of death and stored in a moist chamber. They were examined on the synchrotron within 5 days of enucleation.

MCD patients

Four patients with a clinical diagnosis of MCD were included in the study. Blood was drawn from each patient and the serum isolated. The concentration of antigenic KS in serum was measured by a previously described ELISA (Klintworth et al., 1986; Thonar et al., 1986), which uses an anti-KS monoclonal antibody (1/20/5-D-4, a gift from Dr. Bruce Caterson, University of North Carolina, Chapel Hill, NC) specific for a highly sulfated epitope on KS chains. Based on the results of the analyses, two of the patients (case 1, age 22; case 2, age unknown) were classified as having MCD type I (<10 ng KS/ml). The other two patients (case 3, age 37; case 4, age 27) had normal levels of antigenic KS in serum (284 ng KS/ml and 273 ng KS/ml, respectively) and thus were classified as having MCD type II.

Enzyme digests of MCD corneas

Small corneal portions (~2 mm^2) were dissected from the two MCD type I specimens and one of the MCD type II specimens. These frozen specimens were thawed and fixed for 25 min at 4°C in 2.5% formalin in a 25 mM sodium acetate buffer. Several histochemical investigations (Scott and Haigh, 1985; Meek et al., 1989; Quantock et al., 1990) have shown that this fixation protocol prevents stromal proteoglycans from leaking out of the cornea during immersion in an enzyme buffer. After a brief wash in the sodium acetate buffer, specimens were incubated for 5 h at 40°C in a buffer (Tris, 0.25 M) containing 0.5 mg/ml bovine serum albumin, 0.33 M sodium acetate, 0.5 M sodium chloride, 0.01% type II-S soybean trypsin inhibitor, 5 mM acid-free EDTA, and 2.5 mM benzamidine, pH 8.0 (Scott and Haigh, 1985), with or without an enzyme. A portion of one of the MCD type I specimens (case 1) was incubated in the buffer containing chondroitinase ABC (2.5 units/ml; Sigma Chemical Co., St. Louis, MO), an enzyme that degrades CS/DS chains into their constituent disaccharides. A portion of the other MCD type I cornea (case 2) was incubated in the enzyme buffer alone to check that glycosaminoglycans did not leak out of the stroma of their own accord, and a portion of one of the MCD type II corneas (case 3) was incubated in the buffer containing *N*-glycanase (5 units/ml; Genzyme Corporation, Cambridge, MA), an enzyme that cleaves asparagine-linked carbohydrate chains at β-aspartylglycosylamine bonds and thus releases otherwise intact KS chains from the core protein to which they are covalently bound. After the incubations and three 5-min washes in the Tris buffer, the specimens were stored at −80°C. After synchrotron analysis all of the incubated and non-incubated corneal specimens were digested with papain. The concentrations of antigenic KS (quantified by the previously described ELISA), total sulfated glycosaminoglycan as quantified by the dimethylmethyllene blue method (Chandrasekhar et al., 1987), and collagen (measured as hydroxyproline; Häuselmann et al., 1994) in the digest were measured to determine what effect each specific glycosidase had on CS/DS and KS in the tissue.

Synchrotron x-ray diffraction

Synchrotron x-ray diffraction patterns were obtained at the National Synchrotron Light Source (Brookhaven National Laboratory, New York). The specimen-detector distance was 22 cm, the wavelength of radiation was 1.0936 Å, and the energy of the storage ring was 2584 MeV. A 10 × 10 cm two-dimensional, multi-wire, position-sensitive detector, interfaced with a programmable histogramming frame store, was used to record diffraction images from corneal specimens (Capel, 1993). Images were acquired over 60-s intervals and corrected for detector response inhomogeneities. The patterns revealed no angular dependence and, thus, were circularly averaged about the beam center to yield radial diffraction profiles. Background radial profiles from empty sample holders were similarly obtained and subtracted from corneal profiles after being scaled for differences in incident flux, sample transmission, and instrumental dead time. The 51.5 Å lamellar spacing of cholesterol myristate was used to calibrate the system. Radial profiles were obtained from the four untreated MCD specimens, the two companion enzyme-digested portions of MCD specimens 1 and 3, the corneal portion from specimen 2 that had been incubated in the enzyme buffer alone, and the four normal human corneas. To ensure against storage-induced artifacts, one of the freshly excised normal human

corneas was examined in a slightly dehydrated state and another after freezing and thawing.

RESULTS

The radial intensity profiles of the x-ray diffraction patterns from all four normal human corneas (Fig. 1) demonstrate a main peak at approximately $S = 0.055$ Å^{-1}. In each case this peak arises because of the regular spacing of collagen molecules in the corneal stroma; converted to real space, this S value corresponds to an intermolecular Bragg spacing of 18.2 ± 0.9 Å (the confidence limits are quoted as the accuracy with which the position of a peak could be measured, converted to real space). To be consistent with previous work (Meek et al., 1991; Meek and Leonard, 1993) herein we quote values for the intermolecular Bragg spacing. This does not take into account the mode of packing of the collagen molecules. If one assumes pseudohexagonal packing, the most common arrangement for a noncrystalline assembly of rodlike molecules (Maroudas et al., 1991), the intermolecular Bragg spacing should be multiplied by a factor of 1.11 to obtain a value for the actual spacing of the collagen molecules. This factor becomes 1.15 if hexagonal packing is assumed, and 1.12 if liquid-like packing is assumed (Meek et al., 1991; Worthington and Inouye, 1985). As the S value increases past the intermolecular peak the radial intensity of the x-ray diffraction patterns from the normal human corneas increases smoothly. The lowest radial intensity profile in Fig. 1 is from a cornea that had been air-dried for approximately 30 min; the profile demonstrates a lower overall radial intensity as S increases, presumably because of less water scatter. The broad shoulder evident on this lowest radial intensity profile in the region of $S = 0.22$ Å^{-1} was not due to a sharp "extra" reflection. The second highest radial intensity profile in Fig. 1 is from a normal cornea that had been frozen then thawed; profiles on either side of it derive from corneas that had not been frozen. The fact that the frozen and nonfrozen radial intensity profiles are similar strongly suggests that no freezing artifacts were induced in the MCD corneas that would give rise to spurious x-ray reflections. This is in line with previous x-ray work on bovine cornea carried out at the synchrotron in Daresbury, England (Meek et al., 1991; Fullwood and Meek, 1994).

The radial intensity profiles from the four MCD specimens (Fig. 2) have profiles similar to those from normal cornea (Fig. 1), except that the position of the intermolec-

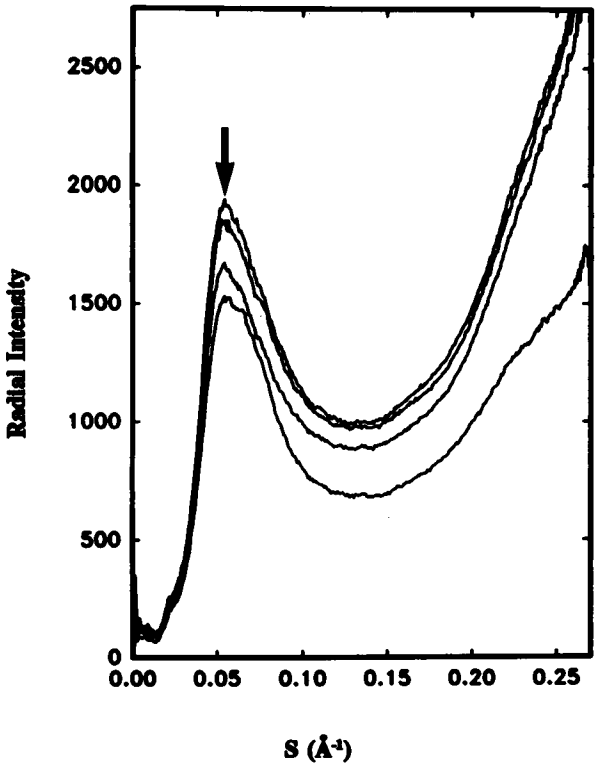

FIGURE 1 The radial intensity profiles of the four x-ray diffraction patterns from four normal human corneas. The main peak at approximately 0.055 Å^{-1} (*arrow*) arises from the regular spacing of the collagen molecules in the corneal stroma; converted to real space, this S value corresponds to an intermolecular Bragg spacing of 18.2 Å ± 0.9 Å. The lowest profile—from a cornea that had been air-dried for approximately 30 min—presumably has a lower overall radial intensity because of less water scatter.

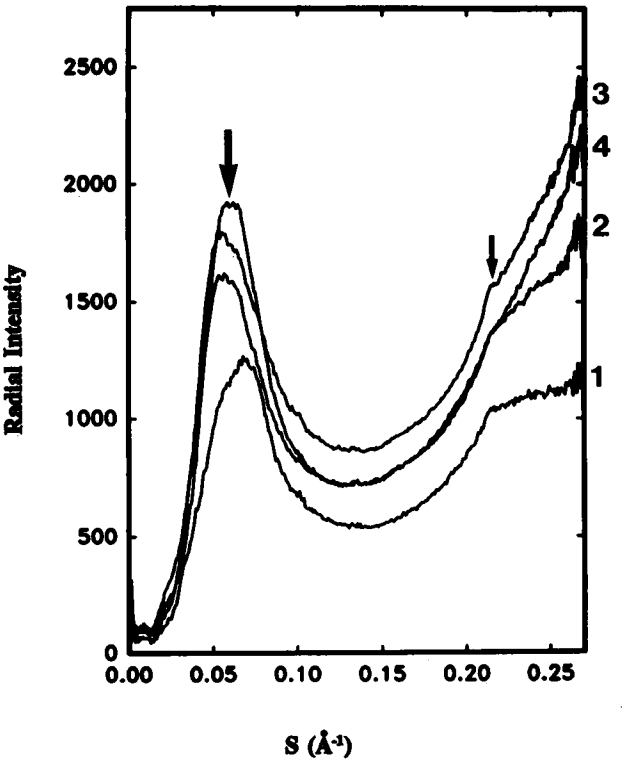

FIGURE 2 The radial intensity profiles of the four x-ray diffraction patterns from MCD specimens 1–4 have profiles similar to normal ones, except that, because of hydration differences, the position of the intermolecular peak (*large arrow*) varies. A sharp "extra" reflection (*small arrow*) is evident as a small peak/discontinuity in the region of $S = 0.21$ Å^{-1}. Converted to real space, the "extra" reflection arises from periodic repeats of 4.65 Å (case 1), 4.63 Å (case 2), 4.63 Å (case 3), and 4.67 Å (case 4).

ular peak varies within the MCD group. This is reflective of the various states of hydration, and hence the spacing of the collagen molecules, of the specimens. The Bragg intermolecular spacings for the MCD specimens (all with confidence limits of ±1.0 Å) are 14.9 Å (case 1), 16.1 Å (case 2), 18.1 Å (case 3), and 17.6 Å (case 4). As with the normal corneas, the MCD specimen that was allowed to become somewhat dehydrated (case 1) demonstrates a relatively low radial intensity as S increases.

Unlike the x-ray diffraction patterns from the normal corneas, the patterns from all four MCD specimens contain a sharp "extra" reflection. This reflection varied in intensity but is evident as a small peak/discontinuity in the region of $S = 0.21$ Å^{-1} on the radial intensity profiles from all four MCD specimens (Fig. 2). Converted to real space, the "extra" reflection arises from periodic repeats of 4.65 Å and 4.63 Å (MCD type I) and 4.63 Å and 4.67 Å (MCD type II). Previous corneal x-ray work carried out at the synchrotron in Daresbury (Quantock et al., 1992) occasionally documented a faint MCD reflection arising from a periodicity of approximately 9.6 Å; this was marginally more prominent at lower tissue hydrations. Two of the present MCD x-ray patterns, recorded on a different experimental set-up in Brookhaven, NY (MCD corneas 1 and 3) suggest a faint reflection at around $S = 0.102$ to 0.105 Å^{-1} (real space periodicity of approximately 9.5 to 9.8 Å), but because of their very low intensity it was difficult to analyze them further.

Figs. 3 through 5 display the individual radial intensity profiles from three of the MCD corneas along with the profiles from their companion MCD corneal portion, which had been incubated in the enzyme buffer with or without a particular glycosidase (the height discrepancy between the traces is probably indicative of variable specimen hydration, as is the difference in the position of the intermolecular peaks). The 4.6 Å MCD reflection is still evident on the x-ray pattern (Fig. 3) derived from the portion of specimen 2 (MCD type I) that had been incubated in the enzyme buffer with no enzyme present. Subsequent biochemical analysis (Table 1) of this tissue indicated that, as predicted, fixation in 2.5% formalin before incubation in the enzyme buffer was sufficient to prevent glycosaminoglycans leaking out of the cornea. Unlike the x-ray pattern from the buffer-treated portion of specimen 2 (MCD type I) (Fig. 3), we found that the 4.6 Å reflection was diminished (Fig. 4) after pretreatment of a portion of specimen 1 (MCD type I) with chondroitinase ABC, indicating that CS/DS glycosaminoglycans are involved in the expression of the reflection. The effectiveness of the enzyme in degrading the CS/DS chain was confirmed by biochemical analysis of the chondroitinase ABC-treated specimen, which showed that more than 90% of the sulfated CS/DS glycosaminoglycans were lost from the tissue (Table 1). The result of the analyses of specimen 3 (MCD type II) treated with *N*-glycanase is more difficult to interpret. It is worth noting that although the MCD type II cornea contained some normally sulfated KS chains, these were present at only 10–20% the concentration found in normal age-matched corneas, a finding consistent with previous observations (Quantock et al., 1992). Treatment of a portion of specimen 3 (MCD type II) with *N*-glycanase, which separates the KS chains from the core protein of lumican but leaves the chains otherwise intact, did not cause a significant decrease in the tissue content of antigenic KS (Table 1). However, we did notice a significant diminution in the 4.6 Å reflection (Fig. 5), even though the released KS chains were retained in the tissue. In this

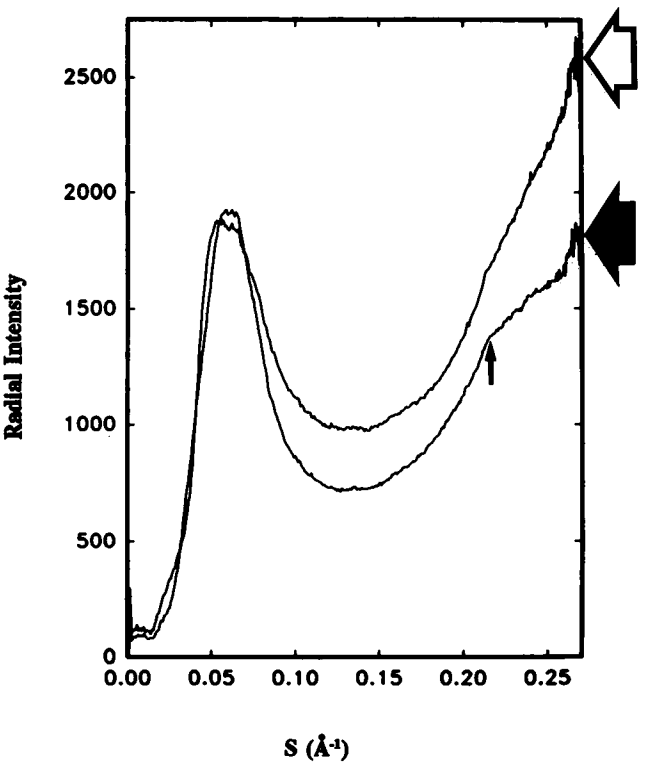

FIGURE 3 The radial intensity profiles of the x-ray diffraction patterns from an untreated MCD type I specimen (case 2; *large solid arrow*) and its companion portion, which had been incubated in the enzyme buffer alone (*large open arrow*). The 4.6-Å MCD reflection (*small solid arrow*) is still evident after the incubation.

TABLE 1 The relative proportions of collagen, antigenic keratan sulfate, and total glycosaminoglycan in MCD corneas before and after treatment with specific glycosidases

Specimen	GAG (ηg) / Collagen (μg)	AgKS (ηg) / Collagen (μg)	AgKS (% GAG)	MCD subtype
1	25.0	<0.10	<0.3	I
1*	<2.1	<0.10	<0.9	
2	25.6	<0.10	<0.3	I
2‡	27.0	<0.10	<0.2	
3	28.7	0.96	3.3	II
3§	26.4	1.04	3.9	
4	43.9	3.85	8.8	II

GAG, total glycosaminoglycan; AgKS, antigenic keratan sulfate.
*Specimen incubated in chondroitinase ABC.
‡ Specimen incubated in enzyme buffer alone.
§ Specimen incubated in *N*-glycanase.

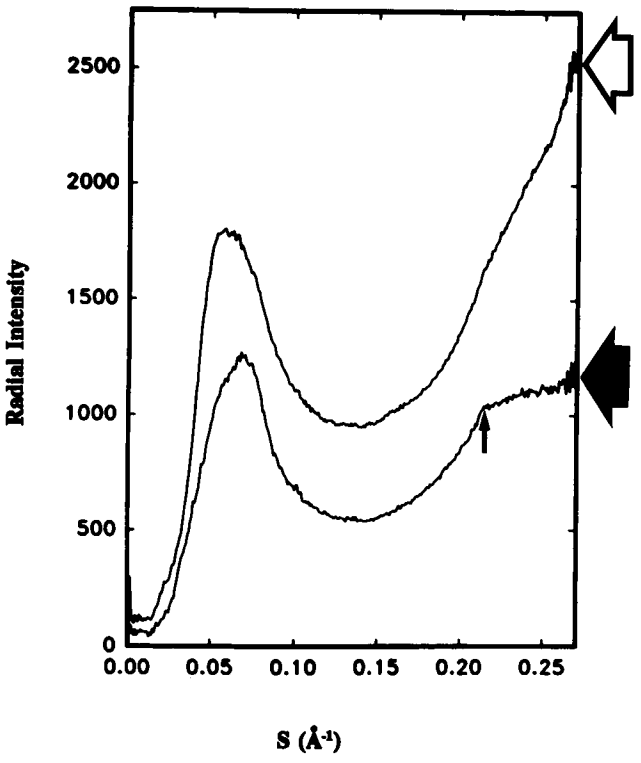

FIGURE 4 The radial intensity profiles of the x-ray diffraction patterns from an untreated MCD type I specimen (case 1; *large solid arrow*) and its companion portion, which had been incubated with chondroitinase ABC (*large open arrow*). The 4.6-Å MCD reflection (*small solid arrow*) is diminished after the incubation.

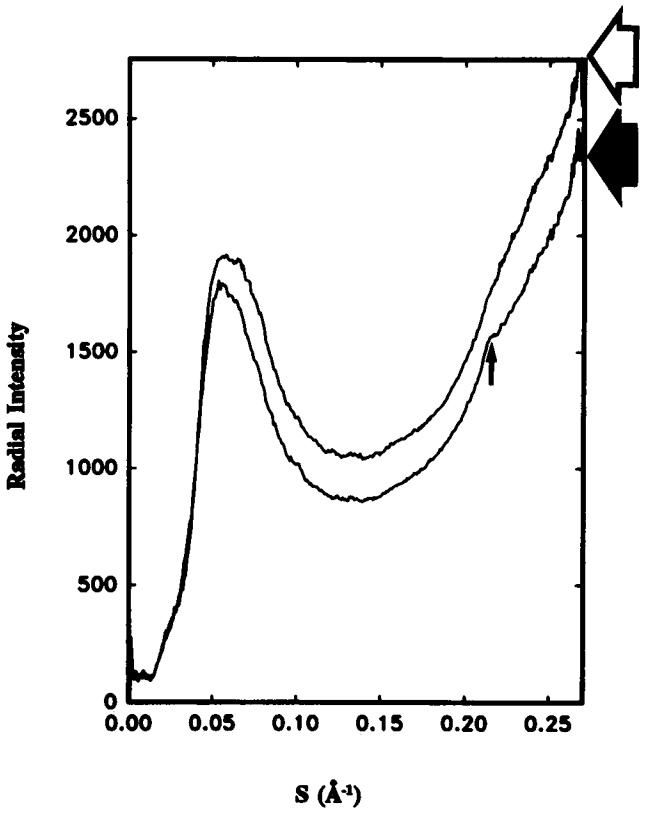

FIGURE 5 The radial intensity profiles of the x-ray diffraction patterns from an untreated MCD type II specimen (case 3; *large solid arrow*) and its companion portion, which had been incubated with *N*-glycanase (*large open arrow*). The 4.6-Å MCD reflection (*small solid arrow*) is diminished after the incubation.

case it is important to note that the incubation of this portion of specimen 3 (MCD type II) with *N*-glycanase was not associated with a marked decrease in the CS/DS content (Table 1).

DISCUSSION

The main reflection in the wide-angle x-ray diffraction pattern from corneal stroma arises because of the regular spacing of the type I and type V collagen molecules that constitute the stromal collagen fibrils (Meek et al., 1991). In this study the intermolecular Bragg spacings for all four normal human corneas (Fig. 1) are consistent at 18.2 Å (±0.9 Å). A recent synchrotron x-ray diffraction study (Meek and Leonard, 1993) quoted a value of 16.3 ± 1.0 Å for the intermolecular Bragg spacing of normal human cornea at around physiological hydration. The higher values in the present study are probably reflective of our use of corneas freshly excised from cadaver eyes; our clinical experience shows that, typically, corneas in this condition are highly edematous (50% to 100% thicker than normal). Meek and colleagues (Meek et al., 1991) recently showed that when a cornea dehydrates below physiological hydration, the initial water comes from between the collagen fibrils, not within them. Consequently, in the present study, the fact that the cornea which was somewhat dehydrated (Fig. 1) has an intermolecular Bragg spacing similar to that of the hydrated corneas is not surprising.

It is remarkable, especially considering the variation in the intermolecular Bragg spacings between the MCD corneal specimens (Fig. 2), how constant the position (4.63 to 4.67 Å) of the unusual MCD x-ray reflection remains. Its presence in x-ray patterns from all four MCD specimens confirms our previous observations (Quantock et al., 1992, 1993b) that the existence of the 4.6 Å x-ray reflection is independent of both corneal hydration and MCD subtype. The absence of the 4.6 Å reflection in x-ray patterns obtained from normal corneas cannot be attributed to the advanced age of the donors compared to the MCD patients. Extensive synchrotron x-ray diffraction studies of normal and dystrophic (Fullwood et al., 1992; Quantock et al., 1992, 1993a) human corneas have shown that the 4.6 Å MCD x-ray reflection is not expressed by any non-MCD cornea, regardless of age.

Previously we reasoned (Quantock et al., 1992) that the undersulfation of KS in MCD is likely the major cause of the 4.6 Å reflection; however, the present analysis (Table 1, Fig. 4) clearly indicates that the contribution to this reflection by CS/DS glycosaminoglycans is at least as great. This is especially true in MCD type I, where the unique, periodic

4.6 Å MCD ultrastructures seem predominantly to reside in chondroitinase ABC-susceptible CS/DS glycosaminoglycans. From the results obtained here, it is not possible to state unequivocally whether the unsulfated form of lumican contributes to the 4.6 Å reflection in these MCD type I corneas.

The effect of *N*-glycanase incubation on the disruption of the periodic 4.6 Å ultrastructures in a portion of specimen 3 (MCD type II) provides some interesting insights regarding the possible architecture of stromal proteoglycans in this condition. Pretreatment with *N*-glycanase caused a significant attenuation of the 4.6 Å x-ray reflection; this attenuation, however, was not accompanied by a significant decrease in the content of antigenic KS chains in the tissue. We can safely assume that, because (as expected) CS/DS-bearing proteoglycans were not significantly depleted by *N*-glycanase incubation (Table 1), these molecules could not, by themselves, contribute greatly to the 4.6 Å reflection produced by this particular MCD type II specimen. However, *N*-glycanase is known to cleave the β-aspartylglycosylamine bond between KS and the core protein of lumican but leaves the KS chains otherwise intact. Therefore, our findings are consistent with the interpretation that neither individual KS chains nor individual core proteins of lumican are as effective as intact lumican molecules in expressing the 4.6 Å reflection.

Our present data indicate that glycosaminoglycans or proteoglycans (or aggregates thereof) possess a 4.6 Å periodicity in MCD corneas that is not seen in normal corneas. We cannot, however, offer any information as to the orientation of the periodic 4.6 Å ultrastructures; they could exist along glycosaminoglycan chains, between glycosaminoglycan chains, or in any manner of other orientations. Furthermore, it remains to be seen whether or not the 4.6 Å x-ray reflection in scarred rabbit corneas (Rawe et al., 1994) arises because of abnormally sulfated proteoglycans.

Previous workers (Nakazawa et al., 1984) have suggested that, in MCD, the CS/DS proteoglycan is probably incorporated into the extracellular deposits of the unsulfated KS proteoglycan, although, as yet, no conclusive evidence exists to prove this supposition or to specify what form the incorporation might take. Nevertheless, this contention finds some support in histochemical studies that have provided clear evidence that the stromal matrix of MCD type I and type II corneas contains numerous lacunae in which large proteoglycan aggregates abound (Meek et al., 1989; Quantock et al., 1990, 1993b). Although the exact nature of these proteoglycan aggregates remains to be clarified, they are known to contain a CS/DS component (Meek et al., 1989). The data presented here—that, independently, both chondroitinase ABC and *N*-glycanase cause an attenuation of the 4.6 Å reflection in MCD type I and type II corneas—raises the intriguing possibility that some form of interaction between the CS/DS-bearing proteoglycans and the intact, undersulfated KS-bearing lumican may underlie the expression of the 4.6 Å reflection in MCD.

The authors thank Dr. Keith M. Meek for helpful comments.

This research was carried out, in part, at the National Synchrotron Light Source, Brookhaven National Laboratory, which is supported by the U.S. Department of Energy, Division of Materials Sciences and Division of Chemical Sciences (user grant 93-X-729 to AJQ; DOE contract no. DE-AC02-76CH00016). Financial support from National Institutes of Health grants AG04736 (EJMAT), AR39239 (EJMAT), EY00146 (GKK), EY08249 (GKK), and EY05722 (GKK), and an unrestricted grant to the Anheuser-Busch Eye Institute (DJS) by Research to Prevent Blindness, Inc., New York, are gratefully acknowledged.

REFERENCES

Axelsson, I., and D. Heinegard. 1975. Fractionation of proteoglycans from bovine corneal stroma. *Biochem. J.* 145:491–500.

Blochberger, T. C., J-P. Vergnes, J. Hempel, and J. R. Hassell. 1992. cDNA to chick lumican (corneal keratan sulfate proteoglycan) reveals homology to the small interstitial proteoglycan gene family and expression in muscle and intestine. *J. Biol. Chem.* 267:347–352.

Borcherding, M. S., L. J. Blacik, R. A. Sittig, J. U. Bizzell, M. Breen, and H. G. Weinstein. 1975. Proteoglycans and collagen fibre organization in human corneoscleral tissue. *Exp. Eye Res.* 21:59–70.

Capel, M. S. 1993. X12B—a facility for time-resolved x-ray diffraction for biology and macromolecular systems at the NSLS. *Sync. Rad. News.* 6:22–27.

Chandrasekhar, S., M. A. Esterman, and H. A. Hoffman. 1987. Microdetermination of proteoglycans and glycosaminoglycans in the presence of guanidine hydrochloride. *Anal. Biochem.* 161:103–108.

Cornuet, P. K., T. C. Blochberger, and J. R. Hassell. 1994. Molecular polymorphism of lumican during corneal development. *Invest. Ophthalmol. Vis. Sci.* 35:870–877.

Donnenfeld, E. D., E. J. Cohen, H. J. Ingraham, S. A. Poleski, E. Goldsmith, and P. R. Laibson. 1986. Corneal thinning in macular corneal dystrophy. *Am. J. Ophthalmol.* 101:112–113.

Edward, D. P., E. J-M. A. Thonar, M. Srinivasan, B. Y. T. J. Yue, and M. O. M. Tso. 1990. Macular dystrophy of the cornea. A systemic disorder of keratan sulfate metabolism. *Ophthalmology.* 97:1194–1200.

Edward, D. P., B. Y. T. J. Yue, J. Sugar, E. J-M. A. Thonar, N. SunderRaj, E. L. Stock, and M. O. M. Tso. 1988. Heterogeneity in macular corneal dystrophy. *Arch. Ophthalmol.* 106:1579–1583.

Ehlers, N., and T. Bramsen. 1978. Central thickness in corneal disorders. *Acta Ophthalmol.* 56:412–416.

Fullwood, N. J., and K. M. Meek. 1994. An ultrastructural, time-resolved study of freezing in the corneal stroma. *J. Mol. Biol.* 236:749–784.

Fullwood, N. J., S. J. Tuft, N. S. Malik, K. M. Meek, A. E. A. Ridgway, and R. J. Harrison. 1992. Synchrotron x-ray diffraction studies of keratoconus corneal stroma. *Invest. Ophthalmol. Vis. Sci.* 33:1734–1741.

Gregory, J. D., L. Coster, and S. P. Damle. 1982. Proteoglycans of rabbit corneal stroma. Isolation and partial characterization. *J. Biol. Chem.* 257:6965–6970.

Hahn, R. A., and D. E. Birk. 1992. β-D-Xyloside alters dermatan sulfate proteoglycan synthesis and the organization of the developing avian corneal stroma. *Dev. Biol.* 115:383–393.

Hassell, J. R., C. Cintron, C. L. Kublin, and D. A. Newsome. 1983. Proteoglycan changes during restoration of transparency in corneal scars. *Arch. Biochem. Biophys.* 222:362–369.

Hassell, J. R., D. A. Newsome, J. H. Krachmer, and M. Rodrigues. 1980. Macular corneal dystrophy: failure to synthesize a mature keratan sulfate proteoglycan. *Proc. Natl. Acad. Sci. USA.* 77:3705–3709.

Häuselmann, H. J., R. J. Fernandes, S. S. Mok, T. M. Schmid, J. A. Block, M. B. Aydelotte, K. E. Kuettner, and E. J-M. A. Thonar. 1994. Phenotypic stability of bovine articular chondrocytes in long-term cultures of alginate. *J. Cell Sci.* 107:17–27.

Klintworth, G. K. 1980. Research into the pathogenesis of macular corneal dystrophy. *Trans. Ophthalmol. Soc. UK.* 100:186–194.

Klintworth, G. K. 1994. Disorders of glycosaminoglycans (mucopolysaccharides) and proteoglycans. *In* The Pathobiology of Ocular Disease: A Dynamic Approach. G. K. Klintworth and A. Garner, editors. Marcel Dekker, New York. 855–892.

Klintworth, G. K., R. Meyer, R. Dennis, A. T. Hewitt, E. L. Stock, M. E. Lenz, J. R. Hassell, W. J. Stark, K. E. Kuettner, and E. J-M. A. Thonar. 1986. Macular corneal dystrophy: lack of keratan sulfate in the serum and cornea. *Ophthalmic Paediatr. Genet.* 7:139–143.

Klintworth, G. K., and C. F. Smith. 1977. Macular corneal dystrophy: studies of sulfated glycosaminoglycans in corneal explant and confluent stromal cell cultures. *Am. J. Pathol.* 89:167–182.

Klintworth, G. K., and F. S. Vogel. 1964. Macular corneal dystrophy: an inherited acid mucopolysaccharide storage disease of the corneal fibroblast. *Am. J. Pathol.* 45:565–586.

Li, W., J-P. Vergnes, P. K. Cornuet, and J. R. Hassell. 1992. cDNA clone to chick corneal chondroitin/dermatan sulfate proteoglycan reveals identity to decorin. *Arch. Biochem. Biophys.* 296:190–197.

Maroudas, A., E. Wachtel, G. Grushko, E. P. Katz, and P. Weinberg. 1991. The effect of osmotic and mechanical pressures of water partitioning in articular cartilage. *Biochim. Biophys. Acta.* 1073:285–292.

Maurice, D. M. 1957. The structure and transparency of the cornea. *J. Physiol. (Lond.).* 136:263–286.

Meek, K. M., G. F. Elliott, and C. Nave. 1986. A synchrotron x-ray diffraction study of bovine cornea stained with cupromeronic blue. *Coll. Relat. Res.* 6:203–218.

Meek, K. M., N. J. Fullwood, P. H. Cooke, G. F. Elliott, D. M. Maurice, A. J. Quantock, R. S. Wall, and C. R. Worthington. 1991. Synchrotron x-ray diffraction studies of the cornea with implications for stromal hydration. *Biophys. J.* 60:467–474.

Meek, K. M., and D. W. Leonard. 1993. Ultrastructure of the corneal stroma: a comparative study. *Biophys. J.* 64:273–280.

Meek, K. M., A. J. Quantock, G. F. Elliott, A. E. A. Ridgway, A. B. Tullo, A. J. Bron, and E. J-M. A. Thonar. 1989. Macular corneal dystrophy: the macromolecular structure of the stroma observed using electron microscopy and synchrotron x-ray diffraction. *Exp Eye Res.* 49:941–958.

Midura, R. J., and V. C. Hascall. 1989. Analysis of the proteoglycans synthesized by corneal explants from embryonic chicken. II. Structural characterization of the keratan sulfate and dermatan sulfate proteoglycans from corneal stroma. *J. Biol. Chem.* 264:1423–1430.

Midura, R. J., V. C. Hascall, D. K. MacCallum, R. F. Meyer, E. J-M. A. Thonar, J. R. Hassell, C. F. Smith, and G. K. Klintworth. 1990. Proteoglycan biosynthesis by human corneas from patients with types 1 and 2 macular corneal dystrophy. *J. Biol. Chem.* 265:15947–15955.

Morgan, G. 1966. Macular dystrophy of the cornea. *Br. J. Ophthaloml.* 50:57–67.

Nakazawa, K., J. R. Hassell, V. C. Hascall, S. Lohmander, D. A. Newsome, and J. Krachmer. 1984. Defective processing of keratan sulfate in macular corneal dystrophy. *J. Biol. Chem.* 259:13751–13757.

Quantock, A. J. 1994. An erroneous glycosaminoglycan metabolism leads to corneal opacification in macular corneal dystrophy. *Glycosylation Dis.* 1:143–151.

Quantock, A. J., K. M. Meek, A. E. A. Ridgway, A. J. Bron, and E. J-M. A. Thonar. 1990. Macular corneal dystrophy: reduction in both corneal thickness and collagen interfibrillar spacing. *Curr. Eye Res.* 9:393–398.

Quantock, A. J., K. M. Meek, and E. J-M. A. Thonar. 1992. Analysis of the high-angle synchrotron x-ray diffraction patterns obtained from macular dystrophy corneas. *Cornea.* 11:185–190.

Quantock, A. J., K. M. Meek, N. J. Fullwood, and R. W. Zabel. 1993a. Scheie's syndrome; the architecture of corneal collagen and distribution of corneal proteoglycans. *Can. J. Ophthalmol.* 28:266–272.

Quantock, A. J., K. M. Meek, E. J-M. A. Thonar, and K. K. Assil. 1993b. Synchrotron X-ray diffraction studies of atypical macular corneal dystrophy. *Eye.* 7:779–784.

Rada, J. A., P. K. Cornuet, and J. R. Hassell. 1993. Regulation of corneal collagen fibrillogenesis in vitro by corneal proteoglycan (lumican and decorin) core proteins. *Exp. Eye Res.* 56:635–648.

Rawe, I. M., K. M. Meek, D. W. Leonard, T. Takahashi, and C. Cintron. 1994. Structure of corneal scar tissue: an x-ray diffraction study. *Biophys. J.* 67:1743–1748.

Scott, J. E., and M. Haigh. 1985. Small proteoglycan collagen interactions. Keratan sulphate proteoglycan associates with rabbit corneal collagen fibrils at the "a" and "c" bands. *Biosci. Rep.* 5:765–774.

Snip, R. C., K. R. Kenyon, and W. R. Green. 1973. Macular corneal dystrophy: ultrastructural pathology of corneal endothelium and Descemet's membrane. *Invest. Ophthalmol.* 12:88–97.

Thonar, E. J-M. A., R. F. Meyer, R. F. Dennis, M. E. Lenz, B. Maldonado, J. R. Hassell, A. T. Hewitt, A. T., W. J. Stark, E. L. Stock, K. E. Kuettner, and G. K. Klintworth. 1986. Absence of normal keratan sulfate in the blood of patients with macular corneal dystrophy. *Am. J. Ophthalmol.* 102:561–569.

Worthington, C. R., and H. Inouye. 1985. X-ray diffraction study of the cornea. *Int. J. Biol. Macromol.* 7:2–8.

Yang, C. J., N. SundarRaj, E. J-M. A. Thonar, and G. K. Klintworth. 1988. Immunohistochemical evidence of heterogeneity in macular corneal dystrophy. *Am. J. Ophthalmol.* 106:65–71.