Vol. 216, No. 1, 1995 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 2, 1995 Pages 183-189

EFFECT OF DIABETIC DURATION ON THE SECONDARY STRUCTURES OF THE HUMAN LENS CAPSULES IN DIABETIC CATARACTS

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Received September 14, 1995

The decrease in triple helix content from 16% to 9% and in ß-turn content from 45% to 33%, combined with markedly increased random coil structure (16%) can be found in the secondary structure contents of lens capsule of the premature cataractous patients with longer diabetic history. However, almost the same peak position and secondary structure content are observed in the curve-fitted IR amide I band of lens capsules of both non-diabetic controls and premature cataractous patients with shorter diabetic history, even if the cataracts are senile. The present study emphasizes the duration of diabetes as a major cause to alter the secondary conformation of the cataractous human lens capsules in diabetic patients.

Aging and diabetes are the major powerful risk factors of cataract formation, in which diabetes mellitus could rapidly accelerate the opacification of lens (1-2). Either polyol accumulation or non-enzymatic glycosylation of lens proteins has been proposed as the possible cataractogenic mechanism for diabetic cataracts (3-4). Several changes of collagen in connective tissues have been observed, such as the decrease in physico-chemical properties (solubility, elasticity, mechanical strength and permeability) and the increase in chemical properties (thermal stability and enzymatic resistance) (5-6), and can be related to the significant increase in the cross-linked glycosylated collagen content. Hyperglycemia is also found to accelerate this non-enzymatic glycosylation of lens proteins in diabetic patients (7-9). Glycosylation-induced structural changes in bovine lens crystalline has been examined to cause a change in tertiary but not secondary structure of the molecule (10).

Glycosylation of lens capsule has been studied both *in vitro and in vivo*, (11-12). It has been found to be significantly higher in diabetic lens cap-

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0006-291X/95 $12.00

sules than in the non-diabetic ones (13). The lens capsule was mainly constructed by collagen IV, which can increase with non-enzymatic glycosylation to a greater extent in diabetes to a progressively thicken lens capsule (12, 14-15). This glycosylated collagen IV can reduce to interact with other basement membrane proteins in diabetes mellitus due to weakened binding affinity caused by structural alteration (16). It also appears to have direct effects on the chemical alterations of collagen structure since the conformation of collagen IV can be directly affected by early glycation products (17-18), but little attention has been paid to this topic.

The purpose of this study was to investigate the possible conformational changes in human lens capsules of diabetic patients with different diabetic histories. The effect of diabetic duration on the secondary structure of the lens capsules in diabetic cataracts was found.

MATERIALS AND METHODS

Anterior lens capsules were isolated from surgically removed cataractous lens obtained at the time of intracapsular cataract extraction in ten premature patients (male, age range: 44-75 years) with different diabetic histories. Normal lens capsule as non-diabetic control was obtained from two normal male persons with traumatic lens subluxation after accident (age: 38 and 52 years). The dried lens capsules were measured by FT-IR microscopic spectrometer (Micro FTIR-200, Jasco, Japan) equipped with an MCT detector using the ordinary transmission method, as described in our previous studies (19-20). Amide I band in IR spectra was used to predict the conformation of peptide bond structure (21). Second-derivative spectral analysis and Fourier self-deconvolution were done to verify the peak position and assignment of the IR spectra (22-25). The secondary structure and composition of these lens capsules were estimated quantitatively by curve fitting program. The proportion of a particular structure was computed to be the fractional area (%) of the corresponding peak, divided by the sum of the areas of all the peaks having their maximum between 1700-1600 cm^{-1}. Results are given as a mean, and the number of patients is given in parenthesis (Table I).

RESULTS

According to the peak position of FT-IR spectra of the above human lens capsules (Fig. 1 and Table I), conformation of these lens capsules had two presentations: the first one was seen in two non-diabetic controls (age: 38 and 52 years) and eight premature patients (age range: 44-72 years) with 1-10 years of diabetic history; the other group included two premature cataractous patients (age: 68 and 75 years) with 30 and 32 years of diabetic history, respectively.

Table II shows the FT-IR amide I component bands of two representative examples for each human lens capsule of non-diabetic controls and premature patients with different diabetic histories. The results suggest that the peak position and fractional band areas of FT-IR amide I component bands of human lens capsules are identical between two-diabetic controls, between eight premature patients with shorter diabetic history, between these two, and between two premature patients with longer diabetic history. These evidences

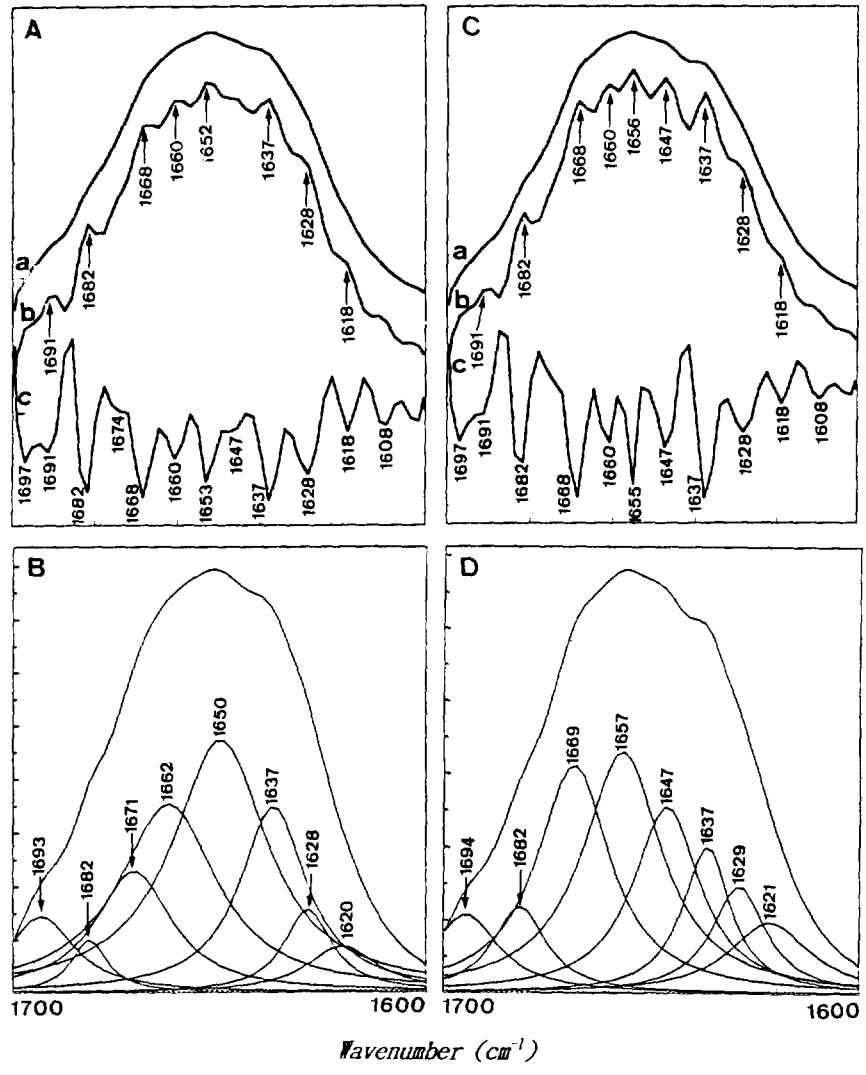

Fig. 1. The original (a), deconvoluted (b), secondary-derivative (c) and curve-fitted (B, D) IR spectra of amide I band of human lens capsules for nondiabetic control (A, B) and premature patient with longer diabetic history (C, D).

indicate that there was no significant difference between data of the different place scanned in each sample and within each group, implying the better reproducibility and precision of this quantitative FT-IR analysis.

Figures 1-A and 1-C present the original (a), deconvoluted (b) and second-derivative (c) IR spectra of human lens capsules of the premature patients with longer diabetic history (>30 year) and non-diabetic control or diabetic patients with shorter history (≤10 year). Figures 1-B and 1-D demonstrate the final fitting results of these samples. Obviously, the predominant bands at 1637, 1650 and 1662 cm^{-1} in the spectra of non-diabetic controls or diabetic patients with shorter history were observed and assigned to triple helix, a

Table I Frequency Assignments and Fractional Band Area for Amide I Components of Human Lens Capsules of Non-diabetic Controls and Premature Patients with Different Diabetic History

Non-diabetic control (Age : 38 and 52 years) (n=2)			Diabetic lens capsule (Age range: 44-72 years) (History: 1-10 years) (n=8)			Diabetic lens capsule (Age: 68 and 75 years) (History: 30 and 32 years) (n=2)		
A	B	C	A	B	C	A	B	C
1620	5.65	ß-sheet	1620	6.44	ß-sheet	1620	7.66	ß-sheet
1628	8.07	ß-sheet	1628	7.19	ß-sheet	1628	7.75	ß-sheet
1637	16.92	Triple helix	1637	15.57	Triple helix	1637	9.32	Triple helix
1651	23.85	α-helix + random	1651	24.69	α-helix + random	1647	16.26	random
						1658	24.76	α-helix
1662	21.15	ß-turn	1662	21.83	ß-turn			
1670	12.95	ß-turn	1670	12.39	ß-turn	1670	21.61	ß-turn
1681	4.74	ß-turn	1681	5.33	ß-turn	1681	5.93	ß-turn
1693	6.83	ß-turn	1693	6.56	ß-turn	1693	6.70	ß-turn

A; Wavenumber (cm^{-1}), B; Area (%) which was mean value obtained by curve-fitting, estimated error ±10%, C; Assignment.

combination of α-helix and random coil, and ß-turn structures, respectively. Further components and its assignments present within the amide I band are listed in Table I. However, the principal bands at 1637, 1647, 1657 and 1669 cm^{-1} in the IR spectra of the premature patients with longer diabetic history were found to be related to the triple helix, random coil, α-helix and ß-turn structures, respectively, in which the appearance of random coil structure at 1647 cm^{-1} and the disappearance of one ß-turn structure at 1662 cm^{-1} were markedly pronounced. Table I also indicates a significant decrease in triple helix content (from 16% to 9%) and ß-turn content (from 45% to 34%), and a remarkable increase in random coil structure (16%) in the IR spectra of lens capsule of the premature patients with longer diabetic history, as compared with the non-diabetic controls or diabetic patients with shorter history.

Table II Reproducibility and Precision of FT-IR Amide I Component Bands of Two Examples for Each Human Lens Capsule of Non-diabetic Controls and Premature Patients with Different Diabetic Histories

Non-diabetic control		Premature patient with short diabetic history		Premature patient with longer diabetic history	
Example 1	Example 2	Example 1	Example 2	Example 1	Example 2
1620 (5.52)	1619 (5.67)	1620 (6.18)	1621 (6.58)	1620 (7.56)	1621 (7.76)
1628 (8.14)	1628 (8.10)	1628 (7.55)	1629 (6.93)	1628 (7.65)	1629 (7.85)
1637 (16.79)	1637 (17.04)	1637 (15.54)	1636 (15.54)	1637 (9.42)	1637 (9.22)
1651 (23.81)	1651 (23.56)	1650 (24.85)	1651 (24.73)	1647 (16.46)	1646 (16.06)
1662 (21.16)	1661 (21.14)	1661 (21.79)	1662 (21.86)	1657 (24.96)	1658 (24.56)
1670 (12.86)	1670 (13.05)	1670 (12.41)	1669 (12.29)	1669 (21.58)	1670 (21.64)
1681 (4.86)	1681 (4.63)	1681 (5.40)	1681 (5.30)	1681 (5.76)	1681 (6.11)
1693 (6.86)	1693 (6.81)	1693 (6.28)	1694 (6.77)	1693 (6.61)	1693 (6.80)

* Wavenumber (area), estimated quantitatively by curve fitting program.

DISCUSSION

Changes in characteristics of human lens capsule have been examined to evaluate their association with age, cataractogenesis and diabetes. The main alternations are morphologic, functional and biophysical, for example, increased thickness; loss of elasticity, permeability and lamination; and higher fragility (26-29). Histochemically, the lens capsule itself consists of abundant collagen IV which seems to reduce in the senile groups (14-15). The age-related changes in collagen IV, such as decreased amino acid content and alternation in the reducible cross-links, have also been observed (30-31). A progressive age-related loss of capsule elasticity has been found to be correlated with cross-linkage within the capsule's beehive-like collagenous network (32). Thus, age seems to play an important role in the chemical alteration of lens capsules. The present study, however, found almost the same peak position and secondary structure content in IR spectral amide I band of lens capsules for both non-diabetic controls and premature patients with shorter diabetic history, even if the cataracts were senile. The influence of prematuration and shorter history of diabetics on the conformational change in lens capsules seemed to be less active at the early stage of cataract formation, because the cataractogensis started from lens rather than from lens capsule.

The lens capsule has been reported to have the highest degree of nonenzymatic glycosylation in the cataractous lens, followed by the cortex and finally the nucleus (12). In addition, the anterior lens capsule of cataractous human lens is almost completely glycosylated to increase stiffening and browning (30, 33), especially in the diabetic cataractous patients when compared with the non-diabetic subjects (13). Non-enzymatic glycosylation of lens proteins can accelerate cataract formation in diabetic patients, even though they are clearly younger than the senile subjects (34). The extent of glycosylation in the diabetic lens was also found to be almost double that found in normal and senile cataractous lens (35). These strongly indicate that diabetes play a more pronounced role in non-enzymatic glycosylation than age, so that the conformational structure of collagen is modified to accelerate the cataract formation.

Several studies have found that some external factors such as drug, pressure, pH and temperature can induce the conformational changes of proteins (36-38). The major mechanism might be attributed to the changes in the intermolecular hydrogen bond interactions by these external factors, leading to the rearrangement of certain protein components and then to the modification of the secondary structure of protein. In the study of human lens capsules of premature patients with longer diabetic history, the triple helix content was estimated to decrease from 16 to 9%, because only one band at 1637 cm^{-1} was assigned to triple helix structure (19-20, 39). The peak at 1651 cm^{-1} due to α-helix with a small contribution of random coil was splitted into the two bands at 1647 cm^{-1} (random coil) and 1658 cm^{-1} (α-helix), resulting in the appearance and increase of random coil content to 16%. The alteration in

molecular structure of human lens capsule caused by the longer diabetic history might be responsible for these conformational changes (16-18), which also caused the alteration and decrease in ß-turn structure content. The original content of the peak at 1662 cm^{-1} was shared by the peaks at 1658 cm^{-1} (little contribution) and 1670 cm^{-1} (predominate) and disappeared, leading to the increase in the band area of a band at 1670 cm^{-1} in human lens capsules of premature patients with longer diabetic history.

The present study found significant differences in peak position (1647, 1658 and 1662 cm^{-1}) and secondary structure content (triple helix, random coil and ß-turn structures) in IR spectral amide I band of lens capsules between the premature patients with longer diabetic history (>30 year) and the non-diabetic controls or premature patients with shorter diabetic history (<10 year), although details as to at what advanced stage or onset of diabetic history can start to alter the conformation of lens capsule remain unclear. This study suggests that the longer history of diabetes and/or diabetic complication thus caused can alter the secondary structure of human lens capsules in diabetics, resulting in more advanced cataract.

REFERENCES

1. Harding, J.J. (Ed.), Cataract: Biochemistry, epidemiology and pharmacology, Chapman & Hall, London, 1991, pp. 83-124
2. Reiser, K.M. (1991) Proc. Soc. Exp. Biol. Med., 196, 17-29
3. Kinoshita, J. H. (1974) Invest. Ophthalmol., 12, 713-724
4. Stevens, V.J., Rouzer, C.A., Monnier, V.M. and Cerami, A. (1978) Proc. Natl. Acad. Sci., 75, 2918-2922
5. Robins, S.P. and Bailey, A.J. (1972) Biochem. Biophys. Res. Commun., 48, 76-84
6. Malik, N.S., Moss, S.J., Ahmed, N., Furth, A.J., Wall, R.S. and Meek, K.M. (1992) Biochim. Biophys. Acta, 1138, 222-228
7. Kohn, R.R. and Schnider, S.L. (1982) Diabetes, 31 (suppl.), 47-51
8. Kennedy, L. and Baynes, J.W. (1984) Diabetologia, 26, 93-98
9. Schleicher, E.D. and Olgemoller, B. (1992) Eur. J. Clin. Chem. Clin. Biochem., 30, 635-640
10. Liang, J.N. and Chakrabarti, B. (1981) Invest. Ophthalmol., 20 (suppl.), 129
11. Cohen M.P., Urdanivia, E., Surma, M. and Ciborowski, C.J. (1981) Diabetes, 30, 367-371
12. Oimomi, M., Maeda, Y., Hata, F., Kitamura, Y., Matsumoto, M., Baba, S., Iga, T. and Yamamoto, M. (1988) Exp. Eye Res., 46, 415-420
13. Mandel S.S., Shin, D.H., Newman, B.L., Lee, J.H., Lupovitch, A. and Drakes, G.N.N. (1983) Biochem. Biophy. Res. Commu., 30, 51-56
14. Dehm, P. and Kefalides, N.A. (1978) J. Biol. Chem., 253, 6680-6695
15. Bailey, A.J. (1987) Eye, 1, 175-183
16. Tarsio, J.F., Reger, L.A. and Furcht, L.T. (1987) Biochemistry, 26, 1014-1020
17. Means, G.E. and Chang, M.K. (1982) Diabetes, 31 (Suppl. 3) 1-4
18. Monnier, V.M. and Cerami, A. (1982) Diabetes, 31 (Suppl. 3) 57-63
19. Lee, S.M., Lin, S.Y. and Liang, R.C. (1995) Art. Cells Blood Subs. Immobil. Biotechnol., 23, 193-205
20. Lin, S.Y., Liang, R.C. and Lee, S.M. (1994) Ophthalmologica, 208, 17-22
21. Susi, H. (1972) Methods Enzymol., 26, 455-472
22. Susi, H. and Byler, D.M. (1983) Biochem. Biophys. Res. Commun., 115, 391-397

23. Lin, S.Y., Liang, R.C., Yang, C.H., Hsu, H.S. and Lin, A.T.L. (1994) Urol. Res., 22, 205-208
24. Lin, S.Y., Liang, R.C., Yang, C.H., Hsu, H.S. and Lin, A.T.L. (1994) Anal. Methods Instru., 1, 191-195
25. Lin, S.Y., Liang, R.C., Hsu, H.S. and Lin, A.T.L. (1994) Cancer Lett., 79, 221-226
26. Paterson, R.A. and Heath, H. (1967) Exp. Eye Res., 6, 233-238
27. Seland, J.H. (1974) Acta Ophthal. 52, 688-706
28. Popdimitrova, N. and Bettelheim, F.A. (1989) Ophthalmic Res., 21, 352-359
29. Fisher, R.F. (1987) Eye, 1, 184-189
30. Peczon, B.D., Peczon, J.D., Cintron, C. and Hudson, B.G. (1980) Exp. Eye Res., 30, 155-165
31. Tsilibary, E.C., Charonis, A.S., Reger, L.A., Wohlhueter, R.M. and Furcht, L.T. (1988) J. Biol. Chem., 263, 4302-4308
32. Bailey, A.J., Sims, T.J. and Light, N.D. (1984) Biochem. J., 218, 713-723
33. Andreassen, T.T. (1985) Diabetologia, 28, 687-691
34. Kasai, K., Nakamura, T., Kase, N., Hiraoka, T., Suzuki, R., Kogure, F. and Shimoda, S.I. (1983) Diabetologia, 25, 36-38
35. Ansari, N.H., Awatshi, Y.C. and Srivastava, S.K. (1980) Exp. Eye Res., 31, 9-19
36. Cadrin, M., French, S.W. and Wong, P.T.T. (1991) Exp. Mol. Path., 55, 170-179
37. Wong, P.T.T., Girons, I.S., Guillou, Y., Cohen, G.N., Barzu, O. and Mantsch, H.H. (1989) Biochim. Biophys. Acta., 996, 260-262
38. Casal, H.L., Kohler, U. and Mantsch, H.H. (1988) Biochim. Biophys. Acta., 957, 11-20
39. Ozaki, Y., Kaneuchi, F., Iwamoto, Y., Yoshiura, M. and Iriyama, K. (1989) Appl. Spectrosc., 43, 138-141