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LASER RAMAN SPECTROSCOPIC STUDY OF A DIABETIC CATARACTOUS LENS

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Raman spectrum of a diabetic cataractous lens was measured and compared with the spectrum of a normal lens. These spectra showed an appreciable difference in the relative intensity of the tyrosine doublet near 840 cm^{-1}, indicating that the strength of hydrogen bonding by some tyrosyl residues changed upon lens opacification.

Ocular lens is a transparent organ consisting of many lens fibers. Main dry components of lens fiber are three lens proteins named α-, β-, and γ-crystallins. A cataract is clinically defined as an opacity of the lens which impairs vision. The causes and processes of lens opacification have been investigated for many years mainly from biochemical aspects.[1)] However it is also very important to understand lens opacification from physico-chemical aspects such as protein conformational change. Although the derangement of lens proteins has been considered to be responsible for cataracts,[1-3)] little information is available concerning the structural change of lens proteins.

Raman spectra of intact lens have been extensively investigated since Yu and East[4)] and Schacher and Solin[5)] reported the first Raman spectra of lens in 1975. The secondary structure of main lens proteins can be monitored *in situ* by measuring Raman spectrum and, at the same time, changes in the Raman spectra of different portion of the lens reflect changes in the relative concentrations of α-, β-, and γ-crystallins.[4-9)] Raman data also include informations about protein subgroups such as tryptophan, tyrosine, and sulfhydryl.[8,10,11)] Thus, by measuring Raman spectrum of a cataractous lens it may be possible to understand lens opacification in connection with protein conformational change.

The present work has another important aspect. A comparison of the Raman spectrum of a cataractous lens with that of a normal lens is a significant step to approach the clinical application of Raman spectroscopy in the field of cataract research.[12)] In this letter, we report the Raman spectra of diabetic cataractous lenses, comparing with the spectra of normal lenses. This is the first time that the spectrum of a pathogenically induced cataractous lens is presented.

The animals used for all experiments were male SD strain rats. For diabetic cataract experiment, two rats weighing 90 grams were intravenously injected with alloxan (45 mg/kg). The rats were previously fasted for 24 hours. The animals were kept in an air-conditioned room and fed ad. lib. Body weight of the diabetic rats displayed less than twice increase after six months. Cataract was developed in both eyes apparently 4 months after alloxan injection. The nuclear opacity of the lens was clearly noticed when the rats was destroyed. The glucose level of the rats elevated up to 650 mg/100 ml. The lens was excised out then transferred into a cuvette cell. The lens was placed on the cell bottom and covered with a tris buffered balanced salt solution containing 5.5 mM glucose.[13) The osmolarity of the solution was 291 mOsm. Both lenses of each rat were employed for Raman measurement.

Raman spectra were recorded with a JEOL 400 D laser Raman spectrophotometer equipped with a HTV R585 photomultiplier tube. The 488.0 nm laser line used for Raman excitation was from a Spectra-Physics model 164 Ar laser. The sample was illuminated by the laser beam from the bottom of the cell and scattered light was collected at 90° to the incident beam. A narrow black tape slit was stuck on the front of the cell to selectively collect the Raman scattering light from the nuclear portion of lens. In the case of the cataractous lens the laser beam was focused on the opaque nucleus. Peak frequencies were calibrated using the spectrum of indene and are believed to be accurate to ±1 cm^{-1} for well resolved bands. The Raman spectra were repeated twice for the 300-1800 cm^{-1} region and at least three times for the 700-950 cm^{-1} region and showed good reproducibility.

Fig. 1 (a) compares the Raman spectrum of the nuclear portion of a normal lens (rat, 6 months of age) with that of a diabetic cataractous lens (rat, 7 months of age). Fig. 1 (b) is the enlargement of 700-950 cm^{-1} region of the spectra shown in Fig. 1 (a). The spectra in Fig. 1 (a) are very similar to each other in the region of 900-1700 cm^{-1}. The amide I and III bands did not exhibit a significant shift between the spectrum of normal lens and that of cataractous lens, suggesting that the secondary structure of main lens proteins did not change upon lens opacification. On the contrary to the amide I and III regions, an appreciable change in the relative intensity was observed in the 830-850 cm^{-1} region (Fig. 1(b)). The intensity ratio of the tyrosine doublet (I_{853} : I_{831}) changed from ca. 1 : 0.85±0.02 (normal lens) to ca. 1 : 1.00±0.03 (cataractous lens). The change is rather small, but reproducible. The intensity ratio was calculated by comparing peak heights. The baselines were estimated as shown in Fig. 1 (b). The intensity ratio was measured for normal rat lenses of various ages (6 and 10 weeks and 5 and 6 months old) and for diabetic cataractous lenses of 6 and 7 months old. For normal rat lenses the intensity ratio of tyrosine doublet did not change upon aging. The ratio was almost identical between diabetic cataractous lenses of 6 and 7 months old. Therefore the relative intensity difference observed between normal and diabetic cataractous lenses can not be explained by aging effect. The most prominent phenomena bringing about lens opacification is the aggregation of α-, β-, and γ-crystallins.[2,3) The aggregation may induce a change in the environment of

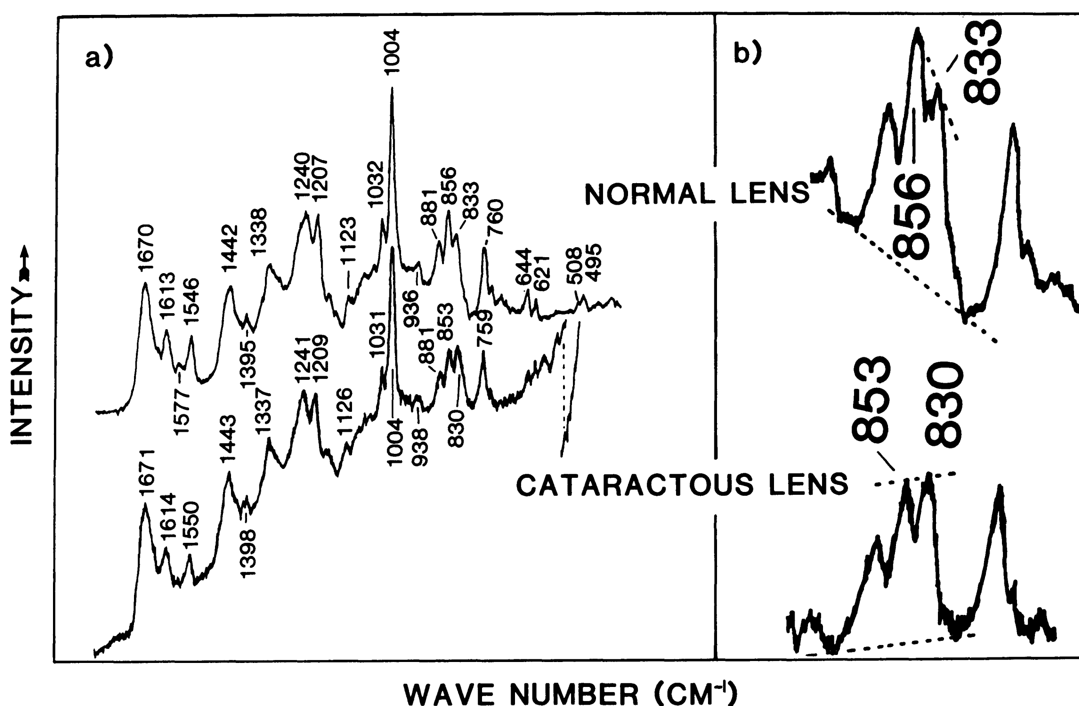

Fig. 1(a) Raman spectra of the nuclear portion of normal rat lens and diabetic cataractous rat lens. Instrumental conditions: excitation wavelength; 488.0 nm, laser power; 180 mW for normal lens, 300 mW for diabetic cataractous lens, spectral slit width; 8 cm^{-1} for normal lens, 9.5 cm^{-1} for diabetic cataractous lens. (b) Enlargement of 700-950 cm^{-1} region

tyrosine residues. The relative concentrations of α-, β-, and γ-crystallins may also vary upon lens opacification. Thus the observed change in the intensity ratio of tyrosine doublet can be interpreted by two possibilities. One possibility is the variation of relative concentrations of α-, β-, and γ-crystallins. Another possibility is the micro-environmental change of tyrosine residues.

Kuck _et al_.[11)] measured the Raman spectra of α-, β-, and γ-crystallins derived from rat lenses by chromatography. The Raman spectra of α-, β-, and γ-crystallins were different from each other in the relative intensities of several Raman bands.[11)] On the other hand, the Raman spectra in Fig. 1 are very similar to each other in the relative intensities except for the tyrosine doublet. Therefore it is rather unlikely that the change in the intensity ratio of the doublet reflects the variation of relative concentrations of α-, β-, and γ-crystallins.

It is well established that the intensity ratio of the tyrosine doublet is sensitive to the nature of hydrogen bonding or the state of ionization of the phenolic hydroxyl group.[14)] The doublet intensity ratio is ca. 0.5 : 1 for the tyrosyl residue where the OH group is strongly bound to a negative acceptor, whereas the corresponding ratio is ca. 1 : 0.8 for the tyrosyl residue forming a

moderately strong hydrogen bond to H_2O. If the OH group functions as a strong hydrogen bond acceptor, the ratio is ca. 1 : 0.4. Thus the result that the intensity ratio changed from 1 : 0.85 to 1 : 1 suggests that the nature of hydrogen bonding of some tyrosyl residues in lens proteins changes upon lens opacification. Probably some tyrosyl residues having formed a hydrogen bond to H_2O was captured by a stronger hydrogen bond acceptor upon aggregation of crystallins. Since the intensity ratio of tyrosine doublet in Fig. 1 contains contribution from tyrosines of α-, β-, and γ-crystallins, it is impossible to identify which tyrosine residues undergo the environmental change. Changes in the strength of hydrogen bonding are not correlated with the conformational change of peptide backbone as observed above. However, the changes might be correlated with the formation of disulfide bond. Unfortunately it was impossible to test this idea since the spectral quality was very poor in the S-S stretching region due to the rising background.

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