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Vibrational microspectroscopy analysis of human lenses

C. Paluszkiewicz [a], N. Piergies [a,*], A. Sozańska [b], P. Chaniecki [c], M. Rękas [d], J. Miszczyk [c], M. Gajda [e], W.M. Kwiatek [a]

[a] Institute of Nuclear Physics Polish Academy of Sciences, PL-31342 Krakow, Poland
[b] Spectroscopy Product Division, Renishaw Sp. z o o, 02-823 Warszawa, Poland
[c] Department of Ophthalmology, 5th Military Hospital with Polyclinic in Krakow, Wroclawska 1-3, 30-091 Kraków, Poland
[d] Department of Ophthalmology, Military Medical Institute, Szaserów 128, 04-141 Warszawa, Poland
[e] Department of Histology, Jagiellonian University Medical College, Kopernika 7, 31-034 Krakow, Poland

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ABSTRACT

In this study we present vibrational analysis of healthy (non-affected by cataract) and cataractous human lenses by means of Raman and FTIR spectroscopy methods. The performed analysis provides complex information about the secondary structure of the proteins and conformational changes of the amino acid residues due to the formation of opacification of human lens. Briefly, the changes in the conformation of the Tyr and Trp residues and the protein secondary structure between the healthy and cataractous samples, were recognized. Moreover, the observed spectral pattern suggests that the process of cataract development does not occur uniformly over the entire volume of the lens.

1. Introduction

Infrared (IR) and Raman spectroscopies (RS) are commonly used for qualitative and quantitative analysis and for studying different types of samples over a wide range of temperatures and physical states [1]. The main advantage of RS is associated with rather weak scattering of water. Thus, it is successfully used for investigations of biological samples, what can be difficult in the case of IR method due to the strong water absorption [2,3]. On the other hand, IR spectroscopy offers no fluorescence interference often occurs in RS method [1]. The application of these two spectroscopic methods ensures complete information about vibrational structure of the investigated biomolecules and biomaterials [4–6]. This information is especially connected with the conformational amino acid changes and protein secondary structure variations. Moreover, the use of the microscope provides nondestructive analysis without the need to homogenize investigated samples together with more precise information about environment surrounding them [7].

The possibilities offered by IR and RS methods make them attractive tools to perform characterization of biological components of human tissues [5]. Without a doubt one of the most important human organ is eye. It is affected by diseases- and age-related changes due to the backbone and protein conformation variations [8]. Cataract is a very popular disease, which leads to cloudy or misty vision related to the decrease of eye lens transparency [9,10]. The mechanism of cataract formation is very complex and is connected with the disorders in membrane lipid peroxidation [11], oxidant-antioxidant status [12], lenticular cell death [13], and ion balance [14]. For example, products of lipids oxidation promote defragmentation of the lens proteins and damage of the membrane structure [15]. On the other hand, enzymatic antioxidants are crucial for the protection of lens against toxic radicals and oxidative stress [16]. Thus, decrease in this enzyme activity is recognized in the cataract development [17]. Opacification is also noticed as a result of defected cellular communication [18] and aggregation of proteins [19,20]. Moreover, process of lens degradation after an inflammation and injury is observed [21]. The most cases of cataract are associated with age although children may be born with this illness too. However, this congenital cataract is less common. In contrast, the age-related and secondary cataracts (associated with such diseases as glaucoma and diabetes) are the most frequently diagnosed [22].

Previously, we have presented Raman data for cataractous human lenses obtained from phacoemulsification surgery [23,24]. Our investigations revealed the usefulness of Raman spectroscopy for studying cataract disease development. Here, we present the systematic FTIR and RS studies for healthy (non-affected by cataract) and degraded (cataractous) human lenses. In this work, our interest is focused on a comparison of protein secondary structure and conformation of the amino acid residues between two aforementioned samples of lenses. It is commonly known that the formation of a human lens opacification is associated with the aggregation process of proteins [25]. Thus, the differentiation in the spectral ranges of specific amino acid residues and amide bands, is expected. Siamwiza and co-workers indicated that

* Corresponding author.
E-mail address: natalia.piergies@ifj.edu.pl (N. Piergies).

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tyrosine (Tyr) residues are sensitive for changes in the secondary structure of proteins. This is related to the formation of strong hydrogen bond with acceptor by tyrosines rather than with H_2O under the influence of appearing aggregation [26,27]. Furthermore, Kitagawa and co-workers showed that in the opaque lenses tryptophan (Trp) residues accept "exposed" conformation. Whereas, in the healthy lens the "buried" conformation is observed [28,29]. On the other hand, Yu and co-workers show in *in vivo* model that the cataract induced by UV irradiation is associated with synthesis of proteins with low —SH content or with the conversion of —SH group to moieties other than disulfide bonds [30].

2. Material and Methods

2.1. Biological Material

The both experimental materials were obtained as a result of a surgical intervention and were delivered from the Department of Ophthalmology, Military Medical Institute in Warsaw. The healthy (non-affected by cataract) human lens was received from a 42 year old female patient after accident (see Fig. 1A) and the cataractous sample was removed from 77 years old man (see Fig. 1B). These samples were cryo sectioned and placed on CaF_2 windows. Such prepared materials with a thickness of 10 μm were used for further investigations.

2.2. Raman Spectroscopy Measurements

The Raman spectra were obtained using the inVia Renishaw spectrometer equipped with a EMCCD detector (Back Illuminated, Deep Depletion CCD) and a Leica confocal microscope in combination with the water immersion objective (×60, NA = 0,75). The 785 nm excitation laser source was used for Raman imaging in the reflection mode for fingerprint region from 700 cm^{-1} to 1750 cm^{-1} with resolution of 1 cm^{-1}. The output laser power was maintained at 100 mW and the 15 second exposure time was set.

2.3. FTIR Spectroscopy Measurements

FTIR experiments were done using the Bruker Spectrometer Vertex70v in a transmission mode. The spectrometer was equipped with microscope in combination with 15xIR objective and FPA detector. The spectra were taken in the range from 3800 cm^{-1} to 900 cm^{-1} with the resolution of 4 cm^{-1}. 2D FTIR maps were collected from the selected 180 μm × 180 μm areas with a 2 μm spatial resolution.

3. Results and Discussion

Fig. 1C and D show stained sections of healthy and cataractous human lenses, respectively. The first human sample indicates compact structure while the second one shows its fragmentation. The Raman spectra of healthy (A) and cataractous human lenses (B) are presented in Fig. 2. In these spectra occur bands due to the Amide *I* (~1670 cm^{-1}) [31,32], tryptophan residue [Trp] (~1614 cm^{-1}, ~1584 cm^{-1}, ~1338 cm^{-1}, ~876 cm^{-1}, and ~757 cm^{-1}) [26,33–35], phenylalanine residue [Phe] (~1604 cm^{-1}, ~1584 cm^{-1}, ~1208 cm^{-1}, ~1174 cm^{-1}, ~1031 cm^{-1}, and ~1003 cm^{-1}) [33,35] vibrations. Also Amide II (~1549 cm^{-1}) [5,23], CH_2 deformation (1447 cm^{-1}) [24], Amide III (~1239 cm^{-1}) [21], C—N stretching (1157 cm^{-1} and 1126 cm^{-1}) [16], C—C stretching (935 cm^{-1}) [24] and tyrosine residue [Tyr] (~1208 cm^{-1}, 854 cm^{-1}, and 830 cm^{-1}) [26] bands can be observed. Both spectra show changes in the relative intensity of bands especially associated with the Tyr and Trp residue modes. As it was discussed in the introduction part, upon the protein aggregation related to the opacification formation of human lens, the some

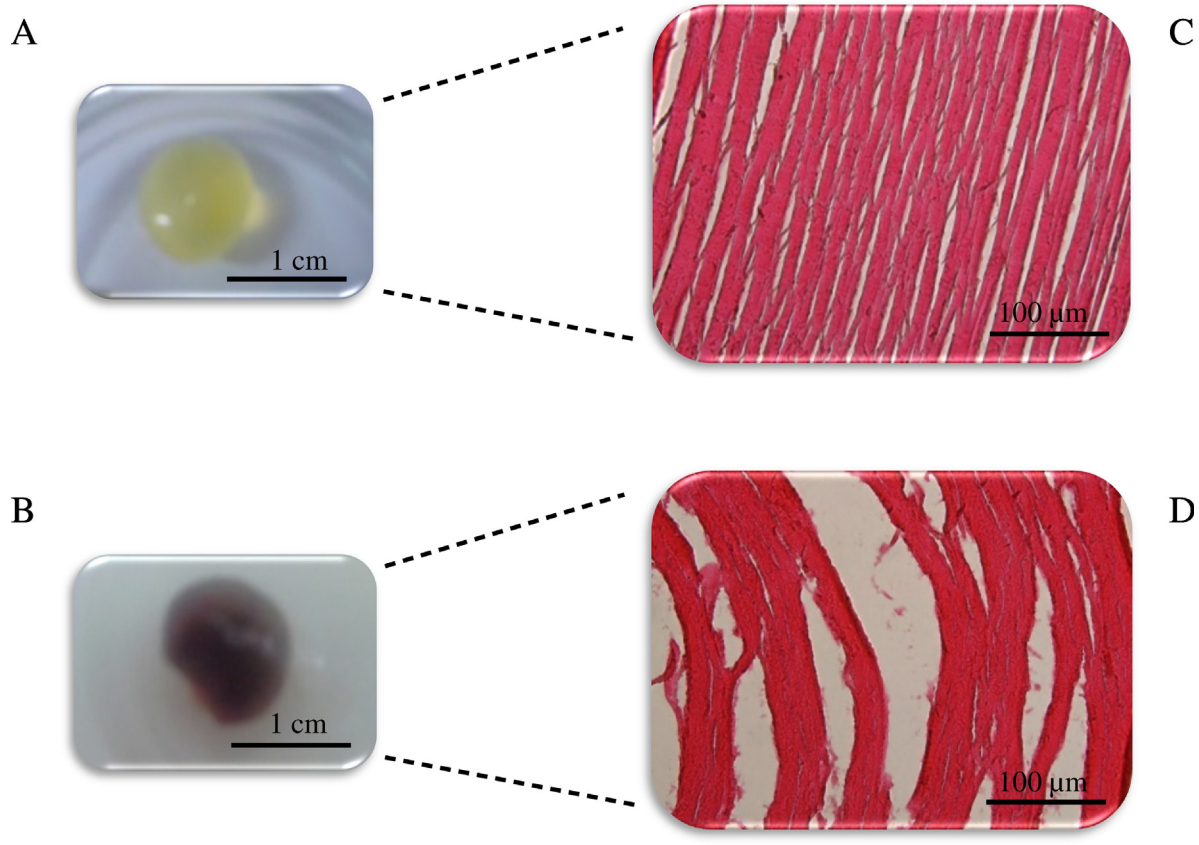

Fig. 1. Images of (A) healthy and (B) cataractous lenses together with the microscopic images of the stained tissue sections (C and D, respectively) with fibers visible as elongated cytoplasm of epithelial cells. Coloration comes from protein reach content (crystallin protein).