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A 2D correlation Raman spectroscopy analysis of a human cataractous lens

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ABSTRACT

This work is a continuation of our study of a cataractous human eye lens removed after phacoemulsification surgery. There are clear differences in the lens colors that allowed for distinguishing two opaque phases in the obtained biological material: the white- and yellow-phase. The Raman spectroscopy and 2D correlation spectroscopy method were used to trace a pathologically altered human cataract lens at a molecular level. Although the Raman spectra of these two phases are relatively similar, taking advantage of 2D correlation, and considering time as an external perturbation, the synchronous and asynchronous spectra were obtained showing completely different patterns. Prominent synchronous auto-peaks appear at 3340, 2920, 1736, 1665 and 1083 cm^{-1} for the white-, and at 2929 and 1670 cm^{-1} for the yellow phase. The white phase is characterized by intensive asynchronous peaks at −(2936, 3360), −(1650, 1674) and +(1620,1678). The modifications in the water contained in the white phase structure are ahead of the changes in the protein (CH_3-groups), furthermore changes in β-conformation are asynchronous with respect to the α-structure.

The yellow phase demonstrates asynchronous peaks: +(2857, 2928), +(1645,1673), +(1663, 1679), and +(1672,1707). These illustrate concomitant modifications in the β- and unordered conformation. Both forms of cataractous human eye lens, white- and yellow-phases, are degenerate forms of the eye lens proteins, both are arranged in a different way. The main differences are observed for the amide I, methyl, methylene and O–H vibrational band region. The effect of Asp, Glu and Tyr amino acids in cataractous lens transformations was observed.

1. Introduction

The lens of the human eye is a soft, transparent organ of lenticular structure. It is built with lens fibers and placed in the bag of the eye. The central part is made of tightly packed, non-nucleus cells, which are filled with lens fibers – transparent proteins called crystallins [1,2]. During its lifetime a lens grows on the periphery, whereas cells of the central part are never overwritten or converted, what makes them the oldest cells of the human body [2–4]. The chemical composition and chemical properties of the eye's lens changes with age. A protein lens contains 33% of wet weight, of which 90% is crystallin, set out as α-, β- and γ-crystallins [5]. Other proteins are cytoskeletal proteins, membrane proteins, transportation proteins or enzymes [2,6]. In the lens crystallin of mammals stands out subunits: αA, αB, βB1, βB2, βB3, βA3/A1, βa2, βA4; γA, γB, γC, γD, γE, γF, and γS, which differ because of their genetic organization, the way of their expression and participation in several diseases [7,8]. The distribution of the proteins through the lens is uneven, reaching maximum values in the dense nuclear region [6]. Fifty percent of the proteins of the human eye belong to an α-crystallin [8]. The α-crystallin fulfills the function of a molecular chaperone, in addition to being a structural protein, while β- and γ-crystallins are only structural proteins [7]. The α-crystallin plays an important role, being responsible for transparency and insolubilization. Its chaperone-like function is to protect proteins

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from aggregation and environmental stress throughout life. With age the contents of each of the crystallin conformations change, reducing the amount of α-crystallin and increasing simultaneously the amount of β- and γ-crystallin. The eye lens contains more proteins and less water compared to other cells of the body, however, the mean nuclear water content in healthy lenses significantly increases with age [5,9]. The protein has no influence on the lipid hydrocarbon chain structure. The arrangement of lipids tend to increase with age and the development of cataracts [10]. Then, also light scattering increases and the lens loses transparency, flexibility, protective functions and optical properties [3,11]. A decrease of α-crystallin chaperone activity may contribute to protein aggregation in the lens. In such cases, loss of lens properties and a cataract is unavoidable [5,7,12].

A cataract is the most common pathological condition of a lens. It involves partial or complete clouding of the lens and causes loss of the primary function of the lens of the eye, which is its transparency (Fig. 1). There are several types of cataracts: congenital (capsularis front and rear, nuclear, perinuclear, total and membranous), primary (senile cataracts associated with aging) and secondary (pathological cataracts – associated with systemic disease caused by other diseases of the eye, traumatic, toxic, residual). The most common type of cataract is age-related, accounting for 80% of cases. Senile cataracts can occur after 40 years of age, although generally it is detected after 50–60 years of age. Congenital changes instead are caused by factors such as chromosomal abnormalities, fetal damage during organogenesis, metabolic disorders or intrauterine infections. Secondary cataracts also take various forms, for instance: diabetic and myotonic [2,13]. Diabetes is a common disease in the community, that may cause retina and lens diseases changing the secondary structure of human lens capsules [13]. A longer history of diabetes usually results in a more advanced cataract [13,14].

Restoration of normal visual acuity or improvements in cataracts can occur only through surgery. Nowadays, there are applied two operating methods: extracapsular cataract extraction by push kernel after the removal of the anterior lens capsule and the most widely used method of removing cataracts, phacoemulsification surgery. Phacoemulsification is a procedure in which a cloudy lens nucleus is broken into small pieces using ultrasounds emitted by a device. Under local anesthesia, the tip of the device is introduced by making a small incision in the sclera. The clouded lens nucleus is crushed by the action of ultrasounds, and then using this same device the tip fragments are sucked and immediately the synthetic lens is placed. The wound seals itself under the influence of intraocular pressure, it does not require sutures and takes only several minutes. In both surgical methods, after the removal of the turbid lens the synthetic lens is introduced as a replacement [2].

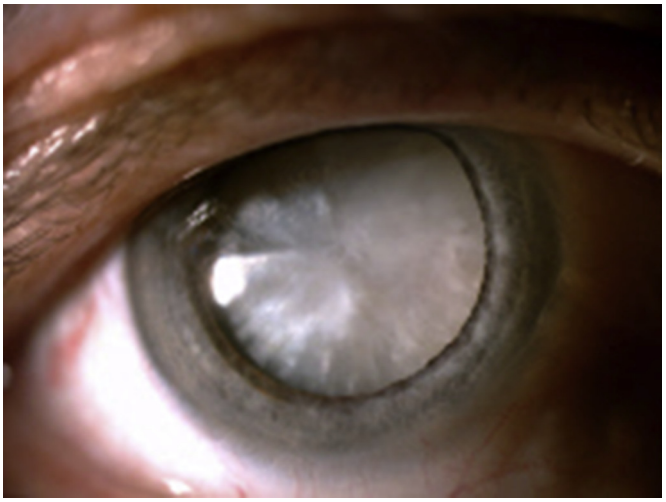

Fig. 1. Human eye cataract lens before phaco surgery – (Fot. by P. Chaniecki).

The application of Raman spectroscopy as a noninvasive structural tool has greatly enhanced our understanding of lens pathological processes at a molecular level [6,9–21].

The aim of this study is to determine what changes in the protein conformation of the cataractous human eye lens obtained by phacoemulsification are observed and also any changes to both phases as a function of time. Clear differences in the colors allowed for the recognition of the white and yellow phases. It is of interest how the participation of secondary structures in the peptide bond vibrations characterizes these eye lens proteins phases.

2. Experimental

2.1. Samples

In our study three human eye lenses were systematically tested after modern cataract surgery – phacoemulsification. The lens pieces, which were emulsified with an ultrasound and removed from the eye, were afterwards placed on the spectrophotometer stage to be measured. From each of the fragmented lenses there was chosen *ca.* 6 spots to analyze.

2.2. FT-Raman spectroscopy

The spectra were measured using the Thermo Scientific Nicolet NXR 9650 FT-Raman spectrometer module with a Micro-Stage Microscope, resolution was set to 4 cm^{-1}.

Samples were excited with a 1064 nm line of Nd:YAG laser applying power of 400 mW for the fragmented lenses, for each measurement 400 scans were added. The measurement parameters were chosen experimentally.

Factory supplied software was used to develop the spectra (Thermo Scientific OMNIC v.7.3 and Renishaw WiRE v. 2.0).

2.3. 2D FT-Raman correlation

The generalized 2D correlation analysis using the Noda method [22–24] was performed using FT-Raman spectra as input data for generating the correlation maps. The study was carried out for four years after phaco surgery, so the time was regarded as an external perturbation in 2D correlation. 2Dshige, v.1.3 software was employed [25].

3. Results and discussion

3.1. FT-Raman spectroscopy

Following the first observation, for each lens after phaco surgery differences in the colors, white- and yellow-fraction, were specified (Fig. 2). Raman band positions for both phases are relatively similar. A summary of the positions of the bands and their assignments is listed in Table 1. However, slight differences were also observed in the spectra of both phases. The dissimilarities in the spectra of the individual samples may be caused by individual features, types of cataracts, concomitant diseases and other reasons. Besides a small difference in the positions of the maxima peaks and a clearer appearance of bands in the range 1200–1100 cm^{-1} for the yellow-phase, the white -phase has a higher noise level (Figs. 3 and 4). The averaged spectra of the white- and yellow-fractions of the lenses after phaco surgery at the range 3550–400 cm^{-1} are presented in