INFRARED SPECTROSCOPIC STUDY OF OCULAR PROTEOGLYCANS

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Infrared (IR) spectroscopy of tissues can reveal the presence of proteoglycans in them and also the state and character of interaction between the macromolecules of these biopolymers [4, 6, 9]. Proteoglycans in tissues and the special morphological formations of the eye determine their structure and physical properties, which are essential for the physiological functions they perform.

The aim of this investigation was to study proteoglycans of the vitreous body, cornea, sclera, and lens of the rabbit eye by direct IR spectroscopy.

EXPERIMENTAL METHOD

The vitreous, cornea, sclera, and lens (together with its capsule) of the rabbit eye were freeze-dried and taken separately from each eye for IR spectroscopy. The IR absorption spectra were obtained from dry, minced tissues, mixed with KBr in the ratio of 1:300. Tablets 13 mm in diameter were pressed with a force of 10 tons. The spectra were recorded at 20°C on a "Perken-Elmer" Model 577 spectrophotometer in the 4000-400 cm^{-1} region. The signal to noise ratio was 100:1. To identify absorption bands in the IR spectra of the above-mentioned materials, besides tables we also used reference IR spectra obtained with highly purified and high polymer preparations of normal Na^+-salts of hyaluronic acid (HUA), a natural (soluble) protein-chondroitin-keratan-sulfate (PCKS) complex, and proteoglycan aggregates (PA), isolated from animal tissues. The results of the analysis are given in Table 1. The reference preparations and all the test materials were prepared and the IR spectra recorded under identical conditions [3, 4, 7].

EXPERIMENTAL RESULTS

A wide absorption band with maximum at 3450 cm^{-1}, due to symmetrical and asymmetrical valency oscillations of hydroxyl, methyl, and other groups, was present in the IR spectra of the vitreous, cornea, sclera, and lens of the rabbit eye (Fig. 1). The shoulder at 2950 cm^{-1} and the band at 2850 cm^{-1}, expressed to different degrees in these spectra, were induced by the same oscillations of methylene and valency oscillations of the imino group respectively. All spectra contained a strong band at 1650-1600 cm^{-1} of overlapping absorptions of the carboxylate ion, and valency and asymmetrical deformation oscillations of the acetamide group of N-acetyl-hexosamines (amide I). Deformation oscillations of the imino group and valency oscillations of C—N at 1550-1520 cm^{-1} (amide II) were weaker in the spectrum of the vitreous and stronger in the spectrum of the sclera than in spectra of the cornea and lens. A band at 1400-1370 cm^{-1} of overlapping deformation oscillations of the methyl group and weak valency oscillations of R—COO^--amino acids was relatively stronger in the spectrum of the lens than in the other spectra. A band at 1400 cm^{-1} of combined valency oscillations of the C—O bond and two-dimensional deformation oscillations of the OH-group was represented in all spectra and differed only in amplitude. A band of symmetrical valency oscillations of sulfate groups at 1240 cm^{-1} and a wide band, with maxima at 1450 and 1390 cm^{-1}, of asymmetrical S = O and C—O—S groups, were well marked in the spectra of the cornea, sclera, and lens. In the spectrum of the vitreous at these frequencies there was only a weak shoulder at 1350-1240 cm^{-1}. In all spectra of the ocular structures studied there were a band at 870-810 cm^{-1} of valency oscillations of sulphur-containing groups and a band

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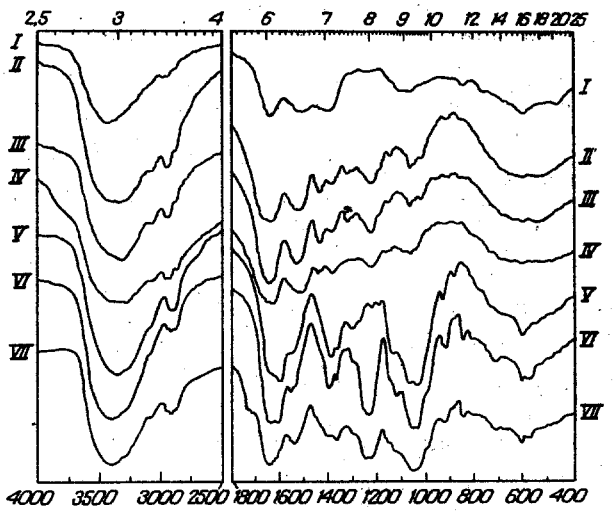

Fig. 1. IR spectra of vitreous (I), cornea (II), sclera (III), lens (IV), HUA (V), PCKS (VI), and PA (VII). Abscissa: below — wave numbers (in cm^{-1}); above — wavelength (in μ); ordinate, transmittance (in per cent).

TABLE 1. Results of Analysis of Reference Proteoglycans (in per cent, M ± m, n = 8)

Preparation	Nitrogen	Hexosamine	Glucuronic acid	SO_4^{2-}	Protein, calculated
HUA (from human umbilical cord)	3,00±0,01	34,20±0,95	32,20±0,15	0,00	5,00
PCKS (from bovine tracheal cartilage)	4,14±0,07	28,40±0,54	28,00±0,31	11,63±0,50	14,62
PA (from bovine tracheal cartilage)	3,80±0,10	29,40±0,70	26,20±0,15	11,22±0,50	12,00

of deformation oscillations of the OH-group. Extraplanar deformation oscillations of a secondary amide group attached by hydrogen bonds (amide V) were discovered to some degree or other in the spectra of the vitreous, sclera, and lens within the interval 750-700 cm^{-1}. This particular band in the spectrum of the cornea overlapped with other, stronger absorption bands.

It must be emphasized that within the range 1400-900 cm^{-1} of IR spectra, besides the bands identified above, absorption of valency oscillations of C—C, C—O, C—N, and other bonds and various deformation oscillations were concentrated, causing overlapping of the bands and making some of them difficult to identify. The structure of this range of the IR spectra ("finger prints") reflect individual differences for each substance, including proteoglycans [3-5].

Comparison of the IR spectra of the vitreous, cornea, sclera, and lens with IR spectra of reference preparations of HUA, PCKS, and PA shows that the maximum of the overlapping absorption of the carboxylate group and amide I in the spectra of the above-mentioned ocular systems coincides with that of the spectrum of PA (1650 cm^{-1}). In the spectra of HUA and PCKS this maximum was shifted to 1600 and 1625 cm^{-1} respectively, probably due to different relative numbers of C=O groups of the carboxylate ion and the same groups of amides in macromolecules of individual proteoglycans (Table 2). The amide II band was present in all spectra. Absence of absorption bands of sulfates was clearly evident only in the spectrum of HUA. Localization of the hydrogen bond in spectra of all the above ocular structures was the same as in spectra of the reference proteoglycan preparations. IR spectra of proteoglycans contained unidentified absorption bands at 1150 and 1125 cm^{-1}, typical for these biopolymers. The HUA spectrum had a weak but distinct band at 1150 cm^{-1}, but the band at 1125 cm^{-1} was absent. In spectra of PCKS and PA, consisting of HUA and PCKS (insoluble fraction), and binding protein, a shoulder at 1150 cm^{-1} was present, but there was a band at 1125 cm^{-1}. Since weak absorption at 1150 cm^{-1} took place in the spectrum of PCKS, which does not contain HUA, but does contain keratan sulfate, it is probably typical of keratan sulfate also. In the IR spectrum of the vitreous, bands at 1150 cm^{-1} and 1125 cm^{-1} were within a wide band with

TABLE 2. Frequency of Absorptions (abs.) of Groups of Atoms in IR Spectra of Morphological Structures of the Eye and of Reference Proteoglycan Preparations

Preparation	Frequency, cm^{-1}						
	1725—1650	1550	1240	1150	1125	870—810	750—700
Vitreous	1650	Shoulder	Weak shoulder	Combined	band at 1100	+	++
Cornea	1650	++	++	++	Overlaps with abs. at 1070		Overlaps with abs. at 600
Sclera	1650	+++	++	Shoulder	Does not overlap	Shoulder	+++
Lens	1650	+++	++	—	Does not overlap	»	Shoulder
HUA	1600	Shoulder	—	+	Does not overlap	—	»
PCKS	1625	+	+++	+	Weak Abs.	+	+++
PA	1650	++	+++	Shoulder	Weak Abs.	+	Shoulder

Legend: +++) Strong absorption, ++) average, +) weak, —) no absorption.

a maximum at 1100 cm^{-1}, having a shoulder at 1150 cm^{-1}. Hence it follows that direct spectroscopy reveals the presence of HUA and of sulfated proteoglycans in this structure, and according to the chemical data, they account for two fractions of keratan sulfate and two fractions of chondroitin-4-sulfate [8, 10-12]. In the spectrum of the cornea there was a band at 1150 cm^{-1}, but absorption at 1125 cm^{-1} overlapped with a band at 1080 cm^{-1}. Considering the presence of all absorption bands of the SO_4^{2-}-group in this spectrum, it can be concluded that IR spectroscopy reveals the presence of sulfated proteoglycans in the cornea, of which the most important are proteokeratan-sulfate and proteochondroitin sulfate, present in it in the form of a single complex. The presence of a band at 1150 cm^{-1}, a shoulder at 1125 cm^{-1}, and of well-marked absorption bands of the SO_4^{2-}-group in the spectrum of the sclera indicates that this structure contains proteoglycans of the PA type. In the lens, heparan sulfate and chondroitin-4- and 6-sulfates were identified by a band in it spectrum at 1150 cm^{-1}, a shoulder at 1125 cm^{-1}, and well-marked absorption of the SO_4^{2-}-group [15].

Comparative analysis of the IR-spectra of the vitreous, cornea, sclera, and lens of the eye and IR-spectra of standard proteoglycan preparations thus revealed the various proteoglycans contained in these structures and the localization of hydrogen bonds in the macromolecules of the above-mentioned biopolymers, without the need to isolate them from the test material.

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