COMPARATIVE STUDY OF INFRARED SPECTRA OF PROTEOGLYCANS

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In previous investigations the infrared (IR) spectra of various salts of many individual proteoglycans were studied [1, 2, 5, 6] and it was shown that these biopolymers can be revealed in the tissues by IR-spectroscopy [4, 9, 10]. Meanwhile, some absorption bands of several proteoglycans remained unidentified in the spectra thus obtained. The aim of the present investigation was to identify these bands in the IR spectra of different individual proteoglycans by comparative analysis of their IR spectra.

EXPERIMENTAL METHOD

Individual preparations of hyaluronic acid (HUA), of the natural hyaline cartilage complex of protein – chondroitin–keratan sulfate (PCKS), and proteoglycan aggregates (PA) of the same tissue and of two individual fractions of heparin, one of which contains three (HP-3) whereas the other contains four (HP-4) sulfuric acid residues, calculated per aminosugar contained in the fraction [7]. Commercial preparations ("Sigma") of chondroitin-4-sulfate (CS-4), chondroitin-6-sulfate (CS-6), dermatan sulfate (DS), and glucuronic acid also were used. All proteoglycan preparations for spectroscopy were used in the form of normal Na^+-salts, and glucuronic acid was given in the form of the K^+ salt.

IR absorption spectra were obtained from dry preparations of the test substances, mixed with KBR in the ratio of 1:300. Tablets 13 mm in diameter were pressed under a force of 10 tons in vacuo. The IR spectra were recorded at 20°C on a Perkin-Elmer model 577 spectrophotometer in the region 4000-400 cm^{-1}. The signal to noise ratio was 100:1. The scanning speed was 50 $cm^{-1} \cdot min^{-1}$ [8].

EXPERIMENTAL RESULTS

Detailed structures of IR spectra of glucuronate, HUA, CS-4, CS-6, DS, PCKS, PA, HP-3, and HP-4 in the 4000-1500 cm^{-1} range were analyzed in detail previously [1, 2, 6]. In the present investigation, the range between 1500 and 600 cm^{-1} of the IR spectra of these proteoglycans was studied more thoroughly. Within this range are concentrated a number of characteristic grouped absorptions, typical of individual proteoglycans (Fig. 1).

Bands at 1420-1400 cm^{-1} and a shoulder at 1370 cm^{-1} of combined valency oscillations of the carboxylate group (-COOH) were found in the IR spectra of glucuronate in all the above-mentioned proteoglycans. The structure R—C—O—CR was clearly revealed as absorption bands at 1150 and 1000 cm^{-1} in the spectra of glucuronate and HUA, whereas in the spectra of the remaining proteoglycans these oscillations were represented by a more or less clearly defined weak shoulder at 1150 cm^{-1}. The same also applies to the mixture of CS-4, CS-6, and DS. Absorption at 1125 cm^{-1} was absent in the spectra of glucuronate and HUA but was present in the spectra of sulfate-containing proteoglycans. In the spectra of these proteoglycans absorption at this particular frequency was present in the form of a well defined shoulder (CS-4, DS, mixture of CS-4, CS-6, DS, and PCKS) or a clearly defined band (CS-6, PA, HP-3, and HP-4). Since absorption at 1125 cm^{-1} was absent in the spectra of glucuronate and HUA, which do not contain sulfate, but in spectra of all the sulfate-containing proteoglycans this absorption, to some degree or other, was invariably present, there is every reason to ascribe this absorption to the presence of sulfate groups in the proteoglycans. Absorption of two-dimensional deformation waves of primary

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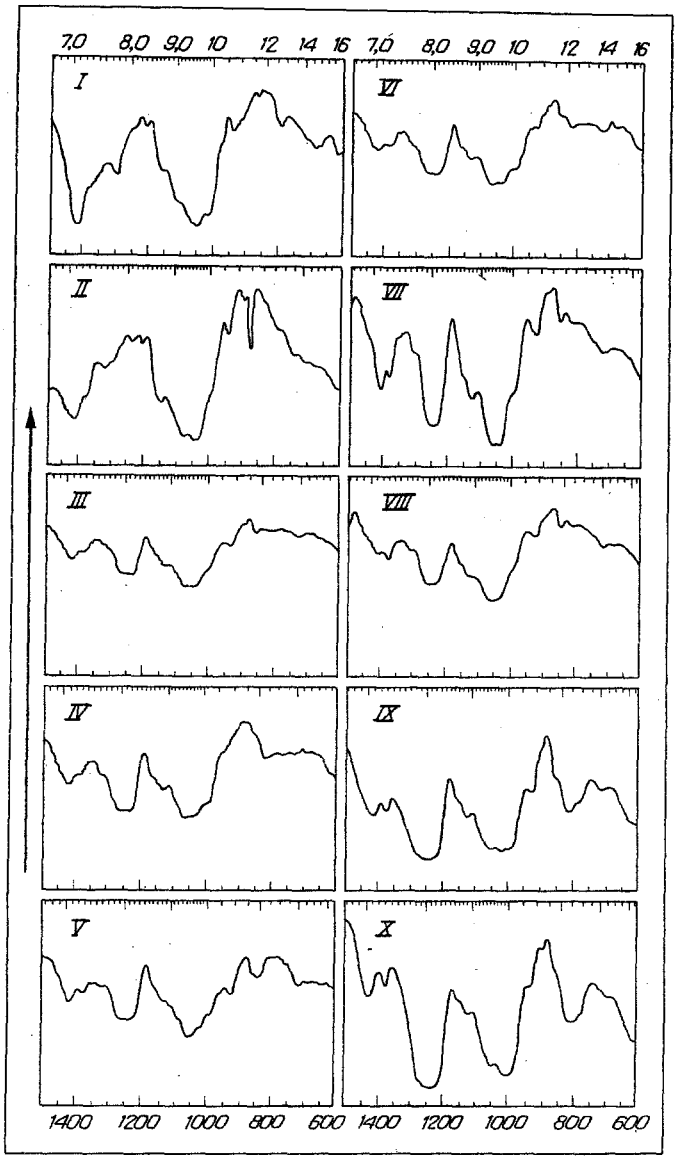

Fig. 1. IR absorption spectra of proteoglycans. Bottom horizontal axis — wave numbers (in cm^{-1}); top horizontal axis – wavelengths (in μ); vertical axes – absorption (in %). I) Glucuronate; II) HUA; III) CS-4; IV) CS-6; V) DS; VI) mixture: CS-4, CS-6, and DS; VII) PCKS; VIII) PA; IX) HP-3; X) HP-4.

and secondary alcoholic hydroxyl groups in the glucuronate spectrum was represented by band at 1050 cm^{-1}, but in the HUA spectrum these waves appeared in the form of a band with maxima at 1070 and 1040 cm^{-1}. In the CS-4, CS-6, DS, PCKS, and PA spectra, absorption in this region was even more intensive and was equal to the sum of the absorptions of the hydroxyl groups indicated above and absorptions of valency oscillations of the S—O group. The intensity of these absorptions was particularly high in spectra of HP-3 and HP-4; in the HP-4 spectrum, moreover, this band had two maxima – at 1040 and 1000 cm^{-1}. In the spectra of glucuronate and of all the proteoglycans studied there was a band at 950-920 cm^{-1}, caused by nonplanar deformation oscillations of any hydroxyl group of the carboxylic acids. The hydrogen bond formed between the carboxyl and acetamide groups, and also the sulfonamide (in HP-3 and HP-4) groups, whose absorption was concentrated in the 750-700 cm^{-1} region, was found in the spectra of CS-4, PCKS, PA, a mixture of CS-4, CS-6, and DS, and HP-3 in the form of a small band, whereas in HUA, CS-6, DS, and HP-4 spectra this bond was present in the form of a distinct shoulder at the above-mentioned frequencies. The presence of the hydrogen bond mentioned above in proteoglycan macromolecules also has been confirmed by other methods [5, 11].

Individual proteoglycans, together with other distinguishing features, differ also in the structure of the hexuronic acids contained in their macromolecules [3, 5]. The IR spectra of HUA, CS-4, CS-6, PCKS, and PA, however, which contain a glucuronic acid residue in the composition of their macromolecule, and of the spectra of DS, HP-3, and HP-4, which contain glucuronic and iduronic acid residues, are indistinguishable in the grouped absorptions of these hexuronic acids. It follows from the results of comparative analysis of the IR spectra of these proteoglycans that the previously unidentified absorptions at 1150 and 1125 cm^{-1}, with respect to which the spectra of the nonsulfated and sulfated proteoglycans differ, can be ascribed to the grouped absorptions mentioned above. This may be of great importance when the method of indirect IR spectroscopy is used to study proteoglycans contained in the tissues without isolation of these biopolymers from them [4].

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