INFRARED SPECTRA OF SOME PROTEOGLYCANS

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Infrared (IR) spectroscopy, which reveals certain groups and bonds in various substances that are difficult or impossible to detect by chemical methods [5, 7-9, 13], has found little application in the investigation of proteoglycans. Moreover, as a rule, in IR-spectroscopic studies of these biopolymers, large fragments of their macromolecules of unknown composition have been used [6, 10, 14-16].

The object of the present investigation was to study IR absorption spectra of potassium salts of hyaluronic acid (HUA), soluble (nonaggregating) protein-chondroitin-keratin sulfate (PCKS) of cartilage, and two heparin fractions, one containing three (HP-3) and the other, four (HP-4) sulfuric acid residues per dimer of the polymer.

EXPERIMENTAL METHOD

HUA and PCKS were isolated from human umbilical cords [1] and bovine tracheas [2] respectively. HP-3 and HP-4 were isolated from a total heparin preparation [3, 4]. The methods used enabled highly purified and minimally altered preparations of the above-mentioned proteoglycans to be obtained. Potassium glucuronate and N-acetyl-D-glucosamine were commercial preparations.

IR spectra were obtained from dry preparations of proteoglycans and monomers mixed with KBr in the ratio of approximately 1:300. Tablets 13 mm in diameter were pressed in vacuo with a force of 10 t. The spectra were recorded at room temperature in a Model 577 Perkin-Elmer spectrophotometer in the 200-4000 cm^{-1} waveband. The width of the slit was chosen to allow for a signal to noise ratio of not less than 100:1.

The scanning speed was 50 $cm^{-1} \cdot min^{-1}$.

EXPERIMENTAL RESULTS

Analysis (Table 1) showed that the HUA preparations consisted of equivalent amounts of residues of N-acetyl-D-glucosamine and glucuronic acid and were not contaminated with sulfoglycosaminoglycans. PCKS contains N-acetyl-D-glucosamine (monomer of keratin sulfate) and N-acetyl-D-galactosamine (an amino-sugar of chondroitin sulfates). N-acetyl-D-galactosamine and glucuronic acid are present in this proteoglycan in equivalent proportion, but the content of sulfate groups is equivalent to the total number of amino-sugars. These proportions, and also the presence of sialic acid in the preparation, prove that during the preparation of PCKS its macromolecules did not undergo degradation. The content of hexuronic acids in HP-3 and HP-4 is the sum of the glucuronic and iduronic acids [3]. Since the problem of the nature of the amino-sugars of heparin has not yet been solved [17], their content also is represented as the total. The absence of galactosamine in these preparations is evidence that neither heparin fraction was contaminated by sulfoglycosaminoglycans.

Two absorption bands are present in the IR spectrum of potassium glucuronate (Fig. 1): 1600 and 1410 cm^{-1}, and a shoulder also is observed at 1370 cm^{-1}, all of which are due to symmetrical and asymmetrical oscillations of the $[-C\langle{}^{O}_{O}]^-$ group. In the spectrum of N-acetyl-D-glucosamine there is a band at 1645 cm^{-1}, known as "amide I" [11, 12], due to complex oscillations of the carbonyl group, in which the C–N bond and the

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TABLE 1. Results of Analysis of HUA, PCKS (in mmoles/g of the preparation), HP-3 and HP-4 (in mmoles/g anion)

Preparation	Nitrogen	Amino-sugar			Hexuronic acids (D)	Sulfate groups (E)	Sialic acids	A/D	C/D	E/C
		glucosamine (A)	galactosamine (B)	total content (C)						
HUA	2,11	2,40	0,00	—	2,21	0,00	0,00	1,09	—	—
PCKS	3,05	0,21	1,24	1,45	1,18	1,43	0,03	1,08	1,23	1,01
HP-3	1,20	—	0,00	1,41	1,45	4,19	0,00	—	0,97	2,97
HP-4	1,19	—	0,00	1,32	1,42	5,20	0,00	—	0,93	3,94

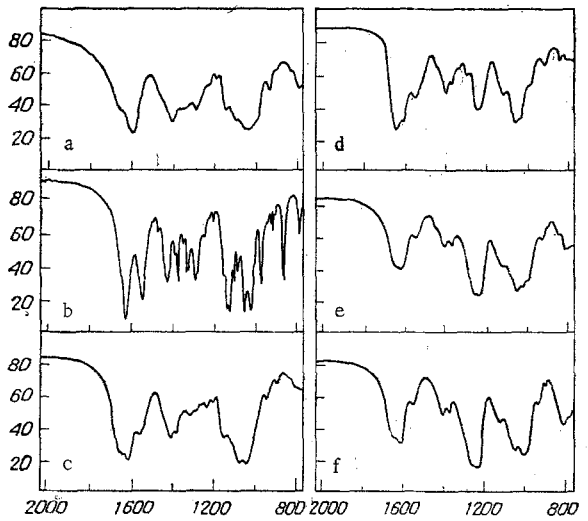

Fig. 1. IR spectra of potassium glucuronate (a), N-acetyl-D-glucosamine (b), HUA (c), PCKS (d), HP-3 (e), and HP-4 (f). Abscissa, wave number (in cm^{-1}); ordinate, extinction (in %).

carbon atoms of the C–C–O and C–N–R bonds also participate. Near to it is another band ("amide II") at 1550 cm^{-1}, due to deformation oscillations of the N–H group [11, 12].

The HUA spectrum is characterized by the presence of a band with complex structure with maxima at 1645 and 1615 cm^{-1}; it is the result of the absorption of a carbonyl group, acetymide residue ("amide I"), and groups of COOK which corresponds to the structure of a repeating dimer of a given biopolymer. This also agrees with the presence in the HUA spectrum of 1550 cm^{-1} bands ("amide II").

In the spectrum of PCKS, just as in that of HUA, there are clearly defined bands at 1645, 1615, and 1550 cm^{-1}, due to the same groups as in HUA. A distinguishing feature of the PCKS spectrum is the strong band at 1240 cm^{-1}, due to asymmetrical valency oscillations of the sulfate group residue [10, 14-16].

Bands at 1410, 1370, and 1150 cm^{-1}, belonging to glucoronate, are well defined in the spectra of HUA and PCKS, in which the last of them is represented only by a shoulder. In the spectra of PCKS, a band difficult to identify is observed at 1125 cm^{-1}.

In the spectra of HP-3 and HP-4 bands at 1615, 1550, and 1240 cm^{-1} are stronger in HP-4 than in HP-3. The structure of the 1240 cm^{-1} band in these cases is rather more complex than in PCKS, because of the weakness of its maxima at 1230 and 1250 cm^{-1} (which are clearer in HP-3) and of the shoulder at 1300 cm^{-1}, which can be ascribed to symmetrical valency oscillations of sulfamido groups contained in heparin, but absent in PCKS. Bands at 1410, 1370, and 1125 cm^{-1} are observed in the spectra of HP-3 and HP-4, but the 1150 cm^{-1} band is not found in them. Since this last band is present in the spectra of HUA and PCKF, its discovery by some workers in the spectra of certain heparin preparations is evidence that those preparations contain the above-mentioned proteoglycans and others [5, 6]. The absence of the 1150 cm^{-1} band in the spectra of HP-3 and HP-4, however, shows that the method of obtaining these fractions [4] entirely frees them from ballast proteoglycans.

Differences between the spectra of HP-3 and HP-4 reflect not only differences in the quantitative proportions of the structural components of the macromolecules of these fractions, but also qualitative differences

between them, in agreement with the very considerable difference in the anticoagulation activity of HP-3 and HP-4 [3, 4]. The relative content of organic components is greater in the HP-3 anion than in the HP-4 anion, and the bands at 1615 and 1550 cm^{-1} are weaker in the spectra of HP-3. The maximum at 1250 cm^{-1} is clearer in the spectrum of HP-3 than in that of HP-4. The presence of the "amide I" and "amide II" bands in the spectra of the HP-3 and HP-4 preparations not contaminated by other proteoglycans is probably due to the presence of a certain number of N-acetyl groups in heparin, as is confirmed by other physical methods [10]. However, this is a problem for further investigation, which is particularly necessary in view of evidence that heparin contains mannose derivatives as structural components [17].

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FUNCTIONAL CHARACTERISTICS OF NERVE ENDINGS ISOLATED FROM THE BRAIN BY HAJOS' METHOD

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Isolated nerve endings (synaptosomes) provide a unique opportunity for the direct study of mechanisms of the fundamental processes lying at the basis of synaptic function: biosynthesis and secretion of mediators, accumulation of mediators in vesicles, uptake of the extracellular mediator by the terminal.

The solution of these problems requires the possession of synaptosome preparations free from impurities and preserving the initial level of functional activity as far as possible. The most widely used method of isolation of synaptosomes [6] satisfies these demands partially, but is time-consuming. In many cases, the long duration of fractionation required is unacceptable, and for that reason investigators are content with the initial stages of fractionation and work with the P_2 fraction of unpurified synaptosomes, in which synaptosomes proper account for up to 55% of the total (as protein). To allow for interference caused by the impurities, an additional series of experiments is set up.

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