INFRARED SPECTROSCOPIC STUDY OF ARTICULAR CARTILAGE AND SYNOVIAL MEMBRANE

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A distinguished feature of cartilage and synovial membrane is that they contain large quantities of proteoglycans, which determine to some extent the structure and functions of these tissues [1, 6]. On the basis of the results of a study of the infrared (IR) spectra of individual proteoglycans isolated from various tissues, their salts, and their protein complexes, which can be used as reference substances [3-5], articular cartilage and synovial membrane were investigated by the method of direct IR tissue spectroscopy. This is essential for a more penetrating study of the state and interrelations of proteoglycan macromolecules of articular cartilage and synovial membrane, which share certain morphological and functional characteristics, and together they are involved in various pathological processes [7, 12].

EXPERIMENTAL METHOD

Cartilage and synovial membrane were obtained from the knee and elbow joints of persons (aged 19-60 years) dying from pneumonia and other diseases without any joint lesions, and synovial membrane was obtained from the same joints of patients with rheumatoid arthritis after synovectomy. The affected membrane was first divided into zones of active inflammation and regression of this process, which were identified on the basis of morphological criteria [6]. Cartilage and synovial membrane from the bovine knee joint also were investigated. All the tissues were dehydrated in ethanol and washed with ether, traces of which were removed in vacuo over paraffin and $CaCl_2$, and preserved in vacuo over phosphoric anhydride. IR spectra were obtained from dry minced tissue preparations mixed with KBr in the ratio of 1:300. Tablets 13 mm in diameter were pressed under a force of 10 t. The spectra were recorded at 20°C on a spectrophotometer (Perkin-Elmer, model 577) in the 4000-200 cm^{-1} region. The slit width was chosen to give a signal/noise ratio of 100:1. The scanning speed was 50 $cm^{-1} \cdot min^{-1}$. The ratio between the amplitudes of the maxima of several bands of grouped (characteristic) absorbances of the test material was calculated graphically from the recorded spectra; this is not a quantitative analytical method, but merely gives an idea of the ratio between percentages of absorbance between individual groups and bonds in the particular spectra analyzed [8].

EXPERIMENTAL RESULTS

A strong band of absorption in the 3700-2600 cm^{-1} region (maxima 3350 cm^{-1}) of overlapping symmetrical and asymmetrical valency oscillations of free hydroxyl, methyl, methylene, NH_2, and other groups and bonds, was present in the IR spectra of all tissue studied (Fig. 1). Within the limits of this region of the spectrum there was also a distinct shoulder of valency oscillations of N-H-bonds at 3120-3060 cm^{-1}, a band at 2950 cm^{-1}, and a weak shoulder at 2800 cm^{-1} of symmetrical and asymmetrical valency oscillations of the methyl group. In spectra of bovine cartilage bands were present at 2600 and 1780 cm^{-1}, possibly due to oscillations of SH= and C=O groups, respectively. In all spectra there was a strong band at 1650 cm^{-1} (Amide-I – A_1), which had two maxima in the bovine spectra (1650-1630 cm^{-1}), consisting of overlapping absorbances of asymmetrical oscillations of the carboxylate ion (1610-1550 cm^{-1}) and absorbance of C=O of primary and secondary amides. Except in the spectrum of bovine cartilage, a band at 1550 cm^{-1} (Amide-II – A_2) of combined valency deformation oscillations of N-H and C-N bonds also was present. Combined valency C=O and deformation OH oscil-

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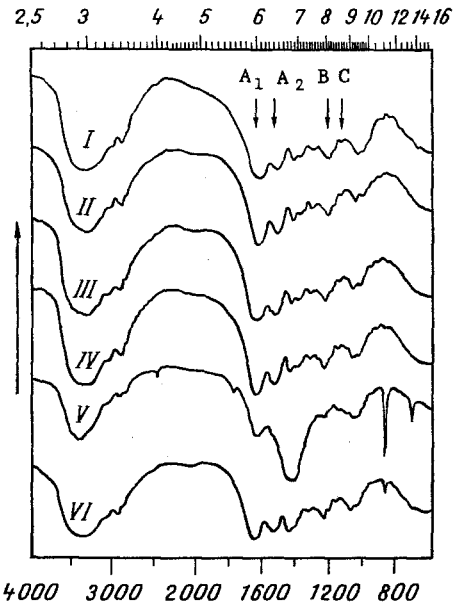

Fig. 1. IR spectra of normal cartilage (I) and synovial membrane (II) of the human knee joint, of zones of active inflammation (III) and its regression (IV) of the synovial membrane of the same joints, and of cartilage (V) and synovial membrane (VI) of the bovine knee joint. Abscissa: bottom – wave number (cm^{-1}), top – wavelength (μ); ordinate, transmittance (%).

lations were represented by bands at 1450 and 1370 cm^{-1}, overlapping with deformation oscillations of methylene (1480 cm^{-1}) and methyl (1370 cm^{-1}) groups. In spectra of bovine cartilage, however, the A_2 band merged with bands at 1450 and 1370 cm^{-1}, and a strong region of overlapping absorptions appeared in the 1600-1300 cm^{-1} region with a maximum at 1425 cm^{-1}, possibly due to the presence of large quantities of a protein component in this cartilage. Deformation oscillations of NH and valency oscillations of C–N were represented in the spectra, except that of bovine cartilage, by weak shoulders at 1305 and 1290 cm^{-1}. A band at 1240 cm^{-1} of valency oscillations of sulfate groups and of the carbonyl group of carboxyls of dicarboxylic amino acids (1230-1215 cm^{-1}) was present in the spectra of all tissues studied. The same remark also applies to unidentified bands at 1150 and 1125 cm^{-1} and to a band with maxima at 1070 and 1025 cm^{-1}, and also to a shoulder at 950-900 cm^{-1} of primary and secondary alcohol groups. Bands at 870-850 cm^{-1}, which could be ascribed to valency oscillations, depending on the nature of the cation, and to S=O and C–O–S groups [3-5, 13], were represented in all spectra, but were strongest in the spectrum of bovine cartilage, which also had a band of deformation oscillations of secondary amide groups at the hydrogen bond (Amide-V). This last band in the remaining spectra overlapped with a band in the 840-400 cm^{-1} region.

All groups (characteristic) absorbances present in the articular cartilage spectrum were present in the IR spectra of human synovial membrane, from normal individuals and from patients with rheumatoid arthritis, the only difference being that in the former, instead of a band at 1125 cm^{-1}, there was only a corresponding shoulder, or this band overlapped with a band of complex structure with maxima at 1075 and 1025 cm^{-1} and a shoulder at 1050 cm^{-1}. Spectra of zones of active inflammation and regression of the synovial membrane were identical with each other and also with the spectrum of normal synovial membrane. Differences were found between the IR spectrum of bovine synovial membrane and of bovine articular cartilage. Bands at 2600, 1780, and 705 cm^{-1}, which were present in the spectrum of bovine cartilage, were absent from the spectrum of this synovial membrane, but absorptions of A_2 were found at 1450 and 1370 cm^{-1}, and the band at 870 cm^{-1} was much smaller that in the spectrum of bovine cartilage. Hence it follows that the structures of the IR spectra of human and bovine synovial membranes are more similar to one another than the structures of the IR spectra of the articular cartilage of these two species.

The IR spectra of cartilage and synovial membrane of the human elbow were completely identical with those of the knee joint.

Comparison of the values of the maxima of the absorption bands of A_1 and A_2, of A_1 and the 1240 cm^{-1} band (B) and A_1 and the 1150 cm^{-1} (C) band shows more clearly the similarities and differences between the IR spectra of the various tissues studied (Fig. 1; Table 1). In spectra of bovine cartilage the ratio of A_1 and A_2 was not calculated, for in this case the A_2 band lies within the interval of several overlapping absorptions (see above). Values of the ratio A_1/A_2 in the spectra of all the tissues studied did not differ significantly from one

TABLE 1. Correlation between Maxima of Group (characteristic) Absorption Bands of IR Spectra of Articular Cartilage and Synovial Membrane (M ± m)

Species	Tissue	A_1/A_2	A_1/B	A_1/C
Human	Cartilage (n = 5)	1,10±0,05	1,30±0,05	1,53±0,07
	Synovial membrane (n = 5)	1,12±0,02	1,43±0,06	2,30±0,09
	zone of active inflammation (n = 9)	1,18±0,07	1,43±0,05	2,14±0,08
	zone of regression (n = 9)	1,13±0,02	1,55±0,10	2,17±0,38
Bovine	Cartilage (n = 4)	—	1,42±0,04	1,73±0,10
	Synovial membrane (n = 4)	1,11±0,09	1,42±0,02	2,04±0,07

another. This was also true of the A_1/B ratio, but in absolute terms it was a little higher than the first ratio. In the spectra of cartilage of both species the A_1/C ratio was virtually identical, and it was less than this ratio in spectra of the synovial membrane, in which there were likewise no differences in the values of this ratio between different samples of the same membrane. The lower value of the A_1/C ratio in spectra of cartilage than in those of synovial membrane of the two species is perhaps due to the comparatively smaller content of protein components in the cartilage, not forming an integral part of the proteoglycan macromolecule, and a correspondingly larger fraction of proteoglycans than in the synovial membrane [6].

Comparison of the IR spectra of articular cartilage and synovial membrane thus obtained with spectra of individual proteoglycan preparations [3-5] shows that the principal group (characteristic) absorption band in the 4000-300 cm^{-1} region, belonging to these biopolymers, were clearly represented in the spectra of the two tissues. The presence of a large quantity of protein components, and of mineral and other substances, of course, introduces a number of changes into the structure of the tissue spectra which we obtained. However, these changes are not particularly significant, for isolated individual proteoglycans themselves contain covalently or otherwise bound protein components, and also mineral cations [1, 2, 6]. Unidentified absorption bands at 1150 and 1125 cm^{-1}, but typical for proteoglycans, are interesting in principle. In the IR spectra of normal Na^+, K^+, and Ca^{++} salts of hyaluronic acid (HUA) there is a weak but distinct band at 1150 cm^{-1}, whereas the band at 1125 cm^{-1} is absent. In the spectra of the same salts of protein-chondroitin-keratan-sulfate (PCKS) and of aggregates of proteoglycans consisting of HUA, PCKS (insoluble fraction), and binding protein, only a shoulder is present at 1150 cm^{-1}, but a band at 1125 cm^{-1} is present. In the spectra of the other individual heparin fractions studied no band is present at 1150 cm^{-1} but there is a shoulder at 1125 cm^{-1}, the prominance of which depends on the nature of a cation bound with the sulfate and carboxyl groups of heparin [3-5]. The band at 1150 cm^{-1} and shoulder at 1125 cm^{-1} are present in spectra of articular cartilage of the two species, from which (and also from all other spectral parameters) it follows that IR spectroscopy reveals the presence of hyaluronate and PCKS in this tissue, in which they exist mainly in aggregate forms [1, 6]. In the IR spectra of normal human synovial membrane, in zones both of active inflammation and of its regression in rheumatoid arthritis, and also in the spectrum of bovine synovial membrane a distinct band at 1150 cm^{-1} and a weak shoulder at 1125 cm^{-1} are present. This fact, and also the presence of a fairly strong band in these spectra at 1240 cm^{-1} suggest that the predominant proteoglycans in the synovial membrane, both under normal conditions and in rheumatoid arthritis, are hyaluronate, heparin, and, probably, heparin sulfate. The presence of mast cells, synthesizing heparin, in synovial membrane is in full agreement with this conclusion [2, 12]. In relation to bovine synovial membrane, as additional confirmation of the above conclusion, the intensity of the 870 cm^{-1} band in its spectrum was much weaker than that in the spectrum of cartilage, evidence of a smaller quantity of proteoglycans of the PCKS type, containing only ester-bound sulfate, but also of a high content of sulfate bound with the amino group of the glucosamine of heparin [13], which is found in this tissue.

Qualitative identity of the structure of the IR spectra of zones of active inflammation and regression in the synovial membrane in rheumatoid arthritis and of normal synovial membrane indicates that biosynthesis of primary repeating structures of glycosaminoglycan components of proteoglycan macromolecules in this tissue and in this pathology is not disturbed, in agreement with the results of studies of synovial membrane undertaken from different aspects and by other methods [9-11]. One result of these pathological processes may be a change in the degree of polymerization of the proteoglycans, and in the rate of their synthesis and breakdown, etc., etc.

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STRUCTURAL HETEROGENEITY OF CHROMATIN PREPARATIONS AT THE LEVEL OF DNA TOPOLOGY

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The view that genomes of eukaryotic cells contain circular supercoiled DNA domains has been confirmed in the last decade [7, 9]. Chromatin preparations, which are model systems of the eukaryotic genetic apparatus, are convenient objects with which to study the topology of the genome under normal and pathological conditions, although they have not yet been used for this purpose. The view that domains with circular DNA ought to be preserved, at least partially, in chromatin preparations [2] was confirmed by the present writers by rheologic analysis of the effect of the dye ethidium bromide (EB), intercalating into DNA, on chromatin in a deproteinizing solution [3].

The aim of this investigation was to study the structural heterogeneity of chromatin preparations at the level of DNA topology, using the method of chromatin fractionation [1], by means of which chromatin fibrils containing linear and circular DNA could be separated.

EXPERIMENTAL METHOD

Chromatin was obtained from calf thymus by washing the tissue homogenate 4 times in 0.025M Na_2—EDTA + 0.075 M NaCl (pH 8.0) to remove ribonucleoproteins. The chromatin residue was washed once in a mixture of 0.15M NaCl + 0.7 mM Na-phosphate buffer (pH 7.0) and was dispersed in the same medium and homogenized for 50 and 100 sec, equal samples of the chromatin suspension being withdrawn at intervals for analysis. The chromatin preparations (C_{DNA} = 200 μg/ml) were transferred into 0.7 M NaCl in Na-phosphate buffer, with the addition of an equal volume of 1.25 M NaCl into Na-phosphate buffer. The solution was poured into a test tube (50 cm^3, internal diameter 28 mm) and a glass rod (diameter 5 mm), revolved by an electric motor at a speed of 60 rpm for 90 min, was lowered into it coaxially. Under these circumstances some of the chromatin was adsorbed on the rod and removed together with it from the solution. Solutions of unadsorbed chromatin and also the initial chromatin sus-

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