PROTEOGLYCAN–PROTEIN INTERACTION

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One of the many unique features of proteoglycans (PG) other than hyaluronic acid is that their macromolecules contain sulfate and carboxyl groups. However, the biological necessity for the presence of two acid groups, greatly different in their degree of dissociation, in one molecule has not yet been adequately explained [1, 2, 9]. The study of acid (with an unsubstituted proton of their carboxyl groups) and normal Na^+, K^+, Ca^{++}, and Mg^{++} salts of several PG has shown that the conformational properties of the macromolecules of these biopolymers depends on the nature of the cation and also on the anionic groups of the given biopolymers with which the cations are linked [3, 8]. Hence the need to study the salt-like compounds of PG with proteins, for the role of electrovalency interactions between PG and proteins in tissues of the body is not yet known.

The aim of this investigation was to study electrovalent complexes of lysozyme, the structure of which was established in [13], with chondroitin sulfate (ChS), a natural mixture of ChS-6 and ChS-4, protein–chondroitin–keratin sulfate (PChKS), cartilage PG aggregates (CPGA), and two fractions of heparin (HP), one of which contains three (HP-3), the other four (HP-4) sulfuric acid residues per repeating disaccharide unit [12]. The chosen PG differ from each other in their glycosaminoglycan components and in the quantity of protein covalently bound with it [1, 9].

METHODS

Compounds of lysozyme (crystalline preparation from hens' egg from Reanal, Hungary) with PG [3–8] were obtained by mixing a solution of the protein with a solution of acid forms of PG (pH 4.0–5.0) in the absence of salts. Acid forms of PG were prepared by treating solutions of the potassium salts with the H^+-form of a cation-exchanged resin (Amberlite IR-124; from Serva, West Germany). The residue of the complex thus formed was centrifuged (1500 g), washed to remove unreacted components with water, dehydrated with ethanol, washed with ether, dried *in vacuo* over $CaCl_2$ and paraffin, and then dried over phosphoric anhydride. The nitrogen content in the complexes was determined by the micro-Kjeldahl method. Preparations of normal K^+ salts of the PG chosen for study [5–7] and the acid salts of the CPGA–guanidine complex [7] were used for comparison.

Infrared (IR) spectra were obtained from dry preparations of PG and their complexes with lysozymes, mixed with KBr in the ratio of 1:300. Tablets 13 mm in diameter were pressed with a force of 10 t. The spectra were recorded at 20°C on a Perkin-Elmer spectrophotometer (model 577) within the frequency range of 4000–400 cm^{-1}. The signal to noise ratio and the scanning speed were 100:1 and 50 $cm^{-1} \cdot min^{-1}$ respectively.

RESULTS

The nitrogen content in the PG–lysozyme complexes was lower than in the given protein but higher than in PG (Table 1). The ChS–lysozyme complex contained the smallest quantity of PG (calculated in %), whereas PChKS–lysozyme and CPGA–lysozyme complexes contained the most; the PG content in the two last complexes, moreover, was similar. Differences in the composition of the complexes were due to the relatively larger quantity of sulfate and carboxyl groups in the ChS than in PChKS and CPGA, which contain more of the protein components and, in addition, keratan sulfate, which has no hexuronic acid residues [9]. HP-3 contains fewer sulfate groups than HP-4; correspondingly, HP-3 binds with lysozyme in larger quantities than HP-4.

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TABLE 1. Results of Analysis of PG and Their Complexes with Lysozyme

Complex	Quantities taken, mg		Volume of mixture, ml	Yield of insoluble complex, mg	Nitrogen content, %		PG content in complex, by calculation, %
	PG (K^+ salt)	lysozyme			PG	complex	
ChS–lysozyme	25,0	50,0	30,0	52,0	2,20±0,01	10,30±0,05	4,40
PChKS–lysozyme	50,0	100,0	40,0	56,0	4,25±0,03	5,80±0,07	9,20
PGA–lysozyme	50,0	100,0	40,0	127,0	4,40±0,15	5,55±0,07	9,40
HP-3–lysozyme	25,0	50,0	20,0	38,0	1,20±0,02	6,24±0,05	8,50
HP-4–lysozyme	25,0	50,0	20,0	60,0	1,20±0,02	7,00±0,03	7,70
PGA–guanidine	—	—	—	—	4,40±0,05	7,90±0,05	95,10

Legend. Nitrogen content in lysozyme 14.60 ± 0.06%.

The relative contribution of HP-3 and HP-4 to complexes with lysozyme was greater than that of ChS in its complex with this protein, due to the larger number of sulfate groups per disaccharide repeating structural unit and with the correspondingly greater molecular weight of the residues of these units in macromolecules of the HP fractions compared with ChS. The CPGA–guanidine complex contained 95% of PG, due to the low molecular weight of the base.

The IR spectra (Fig. 1) of normal K^+ salts of ChS, PChKS, CPGA, HP-3, HP-4, and CPGA–guanidine contained a wide absorption band at 3700-2800 cm^{-1} with a maximum of 3450 cm^{-1}, with inflections at 3100-2560 cm^{-1} caused by overlapping symmetrical and asymmetrical valency oscillations of methylene and free hydroxyl groups, the N-H bond, and certain other groups and bonds. Spectra of ChS, PChKs, CPGA, HP-3, and HP-4 contained bands at 1670 and 1650 cm^{-1} of overlapping oscillations of the carboxylate ion, Amide-I bands (1680 cm^{-1}), and the neighboring Amide-II (1545 cm^{-1}) valency oscillations C=O, N–H, and C–N, and also bands at 1420 and 1370 cm^{-1} of combined valency and deformation oscillations of hydroxyl groups. In the spectra of all the above-mentioned PG there were clearly defined bands at 1245-1220 and 850 cm^{-1} of valency oscillations of $R-SO_3$, S=O, and C–O–S respectively, and bands at 1070-1025 cm^{-1}, which can be classed as planar deformation oscillations of hydroxyl groups. Spectra of ChS, PChKS, CPGA, and CPGA–guanidine contained a band at 730 cm^{-1}, and spectra of HP-3 and HP-4 contained a band at 710 cm^{-1}, classed as deformation oscillations of secondary amide groups with hydrogen bonds, i.e., Amide-V [14-16].

There were no significant differences between the IR spectra of ChS, PChKS, and CPGA. The presence of a large quantity of covalently bound protein components in PChKS and CPGA compared with ChS was not reflected essentially in their spectra. This was probably due to the presence of a certain quantity of peptides (covalently bound through oligosaccharides) in the ChS macromolecules, remaining after removal of the main mass of protein from PChKS by treatment with papain, with the production of ChS from it [11]. The certain differences found between the spectra of these three biopolymers amounted essentially to the fact that the small but clear band at 1125 cm^{-1} present in the spectra of PChKS and CPGA was represented in the spectrum of ChS only by an inflection. The difference between the spectra of ChS, PChKS, and CPGA and the spectra of CPGA–guanidine was that the spectrum of the last-mentioned salt contained inflections at 3400-3000 cm^{-1}, 3200, 3100, 2960, 2940, and 2860 cm^{-1} of valency oscillations of NH-groups and a well defined inflection on oscillations of hydrogen bonds at 2800-2300 cm^{-1} [14-16]. A distinguishing feature of the spectrum of CPGA–guanidine was that it had a clear band at 1745 cm^{-1} of valency oscillations of the carbonyl group of an unionized carboxyl, from which it follows that guanidine in this salt was bound only with sulfate groups. The band at 1550 cm^{-1} (Amide-II) in the spectrum of CPGA–guanidine was stronger than in spectra of all the other PG, due to the presence of guanidine. The IR spectra of normal K^+ salts of HP-3 and HP-4 differed from those of the biopolymers described above in the exceptionally strong band at 1245 cm^{-1}, due to the presence of far more sulfuric acid residues in HP-3 and HP-4 than in ChS, PChKS, and CPGA, and also with the absence of the band at 1150 cm^{-1} present in the spectra of the last three biopolymers. Bands at 1125 and 800 cm^{-1} and an inflection at 725 cm^{-1} also were present in the spectra of these HP fractions.

The IR spectra of lysozyme were characterized by inflections at 3200, 3100, and 2780 cm^{-1} of valency oscillations of NM-groups, by distinct bands at 1450 and 1335 cm^{-1} (Amide-I and Amide-II respectively), by a relatively weak band at 1230 cm^{-1} of valency oscillations of the C=O bond of dicarboxylic amino acids, and Amide-V, i.e., by everything typical of spectra of proteins [14-16].

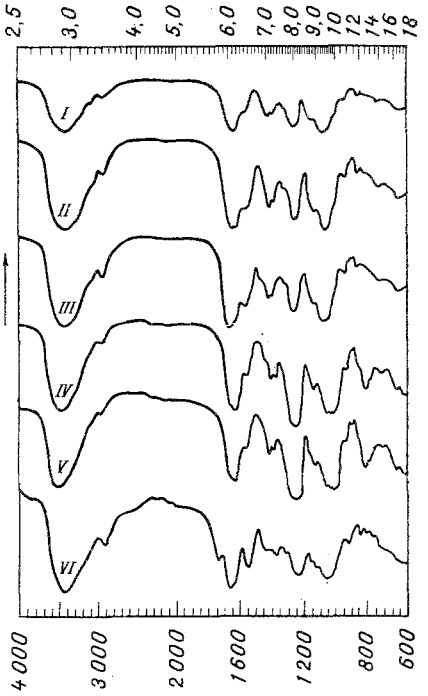

Fig. 1. IR spectra of normal K^+ salts of natural mixture of isomers 4 and 6 of ChS (I), PChKS (II), CPGA (III), HP-3 (IV), HP-4 (V), and CPGA–guanidine (VI). Here and in Fig. 2, abscissa: on right, wavelength (in μ), on left, wave numbers (in cm^{-1}); ordinate, transmittance (in %).

The IR spectra of complexes of ChS, PChKS, CPGA, HP-3, and HP-4 with lysozyme (Fig. 2) were characterized by a decrease in the number and weakening of the intensity of the inflections in the region of high frequencies (3700-2600 cm^{-1}) relative to the spectrum of lysozyme, due to a decrease in their contribution to the complexes. The spectra of the complexes had strong absorption bands at 1650 and 1545 cm^{-1} (Amide-II); the latter, moreover, was much stronger than in the spectra of all the PG investigated, due to a relative increase in the number of N–H and C–N bonds in the complexes on account of addition of lysozyme. The reduction in the intensity of the band at 1225 cm^{-1} in the spectra of the complexes compared with those of PG can be explained by a decrease in the content of sulfate groups in the complexes, which was more considerable than is evident from the spectroscopic data, for absorption in this region is partly due also to dicarboxylic amino acids present in lysozyme and the protein components of PG. Within the range from 1500 to 1250 cm^{-1} of the spectra of the complexes very weak absorption remained. The band at 1725 cm^{-1} of valency oscillations of the carboxyl carbonyl group was not seem in the spectra of aky of the PG-lysozyme complexes, but a strong band was present at 1650 cm^{-1}, resulting from overlapping of the absorption bands of the carboxylate ion and Amide-I. The Amide-V band (which varied in strength), shifted toward 750-745 cm^{-1} in the spectra of the complexes, can be regarded as evidence of the existence of hydrogen bonds or secondary amide groups in PG-lysozyme complexes. Instead of the bands at 1150-1125 cm^{-1} of the PG spectra of PG, inflections remained only in the ChS-lysozyme complex. A band at 1070 cm^{-1} was present in the spectra of all PG-lysozyme complexes except HP-3-lysozymes and HP-4-lysozyme complexes. Bands within the interval 1180-800 cm^{-1} were absent from the spectra of the last two complexes, which distinguish them from spectra of the remaining complexes. Hence it follows that the greatest changes in the PG spectra, arising after their binding with lysozymes, took place in the spectra of HP-3 and HP-4. Absorption in the region of "fingerprints" (1400-900 cm^{-1}) was absent in the spectra of all the complexes, evidence of a reduction in the degree of individuality of each PG in a complex with lysozyme. The basic features distinguishing the IR spectrum of free lysozymes still remained after binding of this protein with PG.

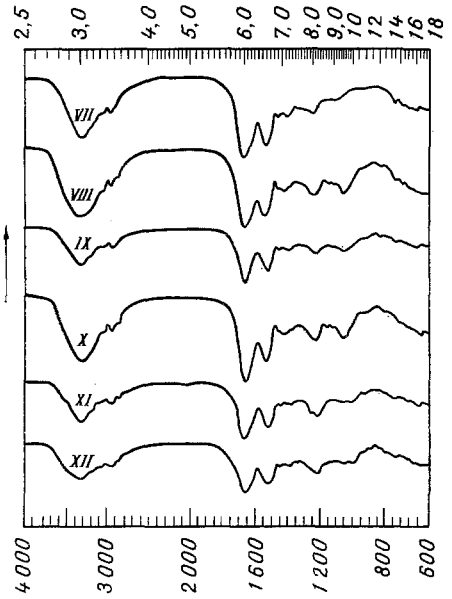

Fig. 2. IR spectra of lysozyme (VII) of complexes of lysozyme with ChS (VIII), PChKS (IX), CPGA (X), HP-3 (XI), and HP-4 (XIII)

PG-lysozyme complexes, insoluble under the conditions of their formation, are thus macrocomplexes of normal salt type. The protein or polypeptide component of PG, covalently bound with glycosaminoglycans, evidently does not play an essential role in the formation of the electrovalent PG-lysozyme complex. IR spectra of complexes of lysozyme with ChS, PChKS, CPGA, HP-3, and HP-4, containing different amounts of the protein component, differ mainly in certain aspects of the structures of the glycosaminoglycans present in the composition of the given PG. This was confirmed also by the general character of dependence of complex formation of ChS, PChKS, and unfractionated heparin with lysozyme on the reaction and ionic strength of the medium [10, 11]. The possibility cannot be ruled out that electrovalent compounds, formed very rapidly, precede and facilitate the course of subsequent nonelectrovalent interactions between PG and other proteins [2]. PG is probably contained in the tissue in the form of ionic compounds, both with inorganic cations and also with proteins and low-molecular-weight organic bases (histamine etc.). Under these circumstances the sulfate and carboxyl groups of the same PG macromolecule may be bound with cations of different nature. The CPGA–guanidine complex extracted from hyaline cartilage with a 4M solution of guanidine hydrochloride is an acid salt in which the carboxyl groups remain free. It may be that carboxyl and sulfate groups of CPGA in the tissues are connected with different cations. Because of the presence of anionic groups of varied nature in PG, complexes of the same PG may differ in their composition, solubility, degree of dissociation, and physiological properties [10, 17].

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SPIN PROBE STUDY OF CHANGES IN SURFACE POTENTIAL OF PLASMA LIPOPROTEINS FROM PATIENTS WITH ISCHEMIC HEART DISEASE

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The plasma lipoproteins (LP) are the principal transport system of exogenous and endogenous lipids in the blood. Much clinical evidence has now been obtained to show that an increase in the plasma concentration of low-density (LDL) and very-low-density (VLDL) lipoproteins and a fall in the concentration of high-density lipoproteins (HDL) promote deposition of lipids in the arterial wall and the development of atherosclerotic changes in the vessels. Experimental results suggest that this is linked with the ability of LDL to transport lipids, including cholesterol (ChS) into cells of the vessel wall and the ability of HDL to accept ChS from cell membranes [2, 3]. Disturbance of the operation of this lipid transport mechanism leads to anomalies in lipid metabolism and ultimately to the development of atherosclerosis. It is not yet clear what is the mechanism triggering disturbances of interaction between LP and the cell membrane. One possible cause of this phenomenon is modification of the surface of LP, leading to a change in the surface charge density and the surface potential of LP. It has been shown, for instance [7], that feeding rabbits with ChS for 4 months leads to lowering of the negative surface charge of VLDL and LDL, without any change in the surface charge of HDL. Changes also have been found in the surface charge of LP in hyper-α-lipoproteinemia [6]. However, the problem of whether the surface potential of LP is changed in patients with ischemic heart disease (IHD) remains unsolved.

In the present investigation by the spin probe method the surface potential of the main classes of human plasma LP and its changed in IHD, a clinical manifestation of atherosclerosis, were studied.

METHODS

LDL and HDL (subfractions HDL_2 and HDL_3) were isolated from the blood plasma of patients with IHD and of healthy blood donors on the Zh-62 ultracentrifuge (USSR) in the RU-50 rotor as described previously [12]. The diagnosis of IHD was based on clinical manifestations of angina, ECG changes, and the results of graded physical exertion tests. The isolated LP were dialyzed for 24 h at 4°C against a 5 mM solution of Tris-HCl, pH 7.3. The LP concentration was determined on the basis of their phospholipid content [15]. EPR spectra were recorded on an E-4 radiospectrometer (Varian, USA) at 37°C [5]. The following imonoxyl radicals:

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