ade by cimetidine potentiated stimulation of AC activity of rat gastric mucosal cells by tetragastrin. Potentiation of the stimulating action of tetragastrin by cimetidine was not an experimental error, because cimetidine did not affect basal AC activity.

Histamine and tetragastrin probably act through different receptors to stimulate AC of rat gastric mucosal cells.

LITERATURE CELLS

1. R. I. Bersimbaev, Yu. M. Konstantinov, S. V. Argutinskaya, et al., Biokhimiya, 40, 570 (1975)
2. V. L. Voeikov, V. M. Denisov, and S. P. Pozdnyakov, Bioorg. Khim., 2, 1672 (1976).
3. G. I. Dorofeev, L. A. Kozhemyakin, and V. T. Ivashkin, Cyclic Nucleotides and Adaptation of the Organism [in Russian], Leningrad (1978).
4. M. Becker and H.-J. Ruoff, Experientia, 35, 781 (1979).
5. H. T. Debas, Fed. Proc., 36, 1933 (1977).
6. T. P. Dousa and R. R. Dorois, Gastroenterology, 73, 904 (1977).
7. R. A. Levine and A. Washington, Gastroenterology, 64, 863 (1973).
8. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
9. J. Nafrady and M. Wolleman, Biochem. Pharmacol., 26, 2083 (1977).
10. B. Simon and H. Kather, Digestion, 16, 175 (1977).
11. A. Soumarmon, A. M. Cheret, and M. J. M. Lewin, Gastroenterology, 73, 900 (1977).
12. W. J. Thompson, G. C. Rosenfeld, and E. D. Jacobson, Fed. Proc., 36, 1938 (1977).
13. W. J. Thompson, L. K. Chang, and E. D. Jacobson, Gastroenterology, 72, 244 (1977).
14. Tsung-Min Lin, G. L. Southard, and G. F. Spray, Gastroenterology, 70, 733 (1976).

COMPARATIVE STUDY OF INFRARED SPECTRA OF GLYCOSAMINOGLYCANS AND THEIR MONOMERS

S. M. Bychkov, V. N. Bogatov, and S. A. Kuz'mina

UDC 547.963-1.06:543.422.4

During the identification of certain absorption bands in the infrared (IR) spectra of glycosaminoglycans difficulties often arise because the oscillation frequencies of the different groups and bonds of these components of proteoglycans may overlap to some extent [1, 5-10, 12].

In the investigation described below, in an attempt to overcome these difficulties the IR spectra of hyaluronic acid (HUA), protein-chondroitin-keratan sulfate (PCKS), proteoglycan aggregates (PA), and monomers from which glycosaminoglycans of the proteoglycans most widely distributed in animals are composed [2], were compared.

EXPERIMENTAL METHODS

Glucuronic acid and its potassium salt, glucosamine, galactosamine (hydrochlorides of both), N-acetylglucosamine, N-acetylgalactosamine, and the ammonium salt of N-acetylneuraminic acid used in the experiments were from Serva, West Germany. Mixtures of monomers for spectroscopic investigations were composed of equivalent quantities of each of them. Potassium salts of HUA (from human umbilical cords), PCKS, and PA (from the bovine trachea) were isolated by methods described previously [1, 3, 4].

IR spectra were obtained by the use of dry preparations mixed with KBr in the ratio of 1:300 and pressed into tablets 13 mm in diameter under a pressure of 10 t. Spectra were recorded on a Perkin-Elmer model 577 spectrophotometer in the 4000-200 cm^{-1} region at room tem-

KEY WORDS: infrared spectra; glycosaminoglycans; glucuronic acid; N-acetylhexosamines; N-acetylneuraminic acid.

Research Laboratory of Biological Structures, Ministry of Health of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 4, pp. 442-445, April, 1981. Original article submitted September 12, 1980.

0007-4888/81/9104-0476$07.50

© 1981 Plenum Publishing Corporation

perature. The signal to noise ratio was not less than 100:1. The scanning speed was 50 $cm^{-1} \cdot min^{-1}$.

EXPERIMENTAL RESULTS

A wide band of valence (symmetrical and asymmetrical) oscillations of free hydroxyl (3670-3500 cm^{-1}), methine (2900-2880 cm^{-1}), and certain other groups, is present in the spectrum of glucuronic acid in the 3670-3500 cm^{-1} region. The well-defined band with a maximum at 1705 cm^{-1} corresponds to valence oscillations of the carbonyl group of the carboxyl radical. The band at 1440-1395 cm^{-1} was due to combined valence oscillations of the carbonyl and in-plane deformational oscillations of the hydroxyl group of the same carboxyl radical [6, 7]. Bands at 1150 and 1125 cm^{-1}, difficult to identify, also could be distinguished in the spectrum (Fig. 1a). Replacement of the proton of the carboxyl group of glucuronic acid by potassium, increasing the reduced mass of the molecule, considerably reduced the number of absorption bands in the spectrum. In the high-frequency region of the spectra of this salt bands at 3400 and 2900 cm^{-1} of valence oscillations of hydroxyl and methine groups also were present. A band at 1600 cm^{-1} of asymmetrical valence oscillations of the carboxylate ion appeared [8]. The band at 1420 cm^{-1} belonged to valence oscillations of the carbonyl of this group. Instead of bands at 1150 and 1125 cm^{-1}, only two inflections remained (Fig. 1b).

In the spectra of glucosamine and galactosamine (hydrochlorides) bands at 3700 and 2500 cm^{-1} correspond to oscillations of methylene and other groups specified in the description of spectra of glucuronic acid and its potassium salt. Within the same frequency range there are stepwise bands of valence oscillations of the NH_3-group. Bands at 1610, 1680, and 1540 cm^{-1} belong to deformational valence oscillations of that group in the glucosamine spectrum. In the galactosamine spectrum, however, the first of the above-mentioned bands lies in the same frequency region, instead of the second band there are only inflections at 1595-1590 cm^{-1}, and the third is shifted to 1505 cm^{-1}. The spectra of these amino sugars also differ from one another in other frequency regions. The intensity of absorption in the 3670-3100 cm^{-1} interval of glucosamine was less than for galactosamine. These bands in each of the above spectra differ in structure. Instead of peaks at 1150 and 1125 cm^{-1}, present in the spectrum of the former, a band of average intensity at 1150 cm^{-1} and a doublet at 1140-1130 cm^{-1} are observed in the spectrum of the latter (Fig. 1c, d).

Acetylation of hexosamines at the amino group caused the appearance of a doublet at 2920-2905 cm^{-1} and an inflection at 2900 cm^{-1} in the spectra, due to a methyl group, for this and the methylene group differ somewhat in the frequencies of their valence oscillations [6]. In other respects the wide band in the high-frequency interval of the spectra of the acetylated hexosamines was due to oscillations of the same groups as are present in hexosamines with an unacetylated amino group (Fig. 1e, f). The band at 1625 cm^{-1} (amide I) in the spectra of acetylated hexosamines was due to combined oscillations of the carbonyl group of the acetyl residue and C—N, C—C—O, and C—N—R bonds [6, 7, 11]. The bands at 1550 and 1575 cm^{-1} (both amide II) respectively in the spectra of N-acetylglucosamine and N-acetylgalactosamine were caused by deformational valence oscillations of the imino group. The acetyl group in these spectra was characterized by absorption bands at 1460, 1380, and 1360 cm^{-1}. The first band was caused by asymmetrical, the second by symmetrical deformational oscillations of the methyl group, the third by deformational valence oscillations of the acetyl group as a whole [6]. Besides that mentioned above, the other difference between the spectra of N-acetylglucosamine and N-acetylgalactosamine is that in the first case one wide band appeared with maxima at 1130 and 1120 cm^{-1} (i.e., characteristic bands for unacetylated glucosamine at 1150-1125 cm^{-1} overlap), whereas in the second case a band at 1150 cm^{-1} and doublet at 1120-1100 cm^{-1} are observed. The differences between the structures of the bands at 3670-3100 cm^{-1} in the spectra of glucosamine and galactosamine still remained in the spectra of the acetyl derivatives of these amino sugars, probably on account of their isometric character.

The strong band in the high-frequency region of the spectrum of the ammonium salt of N-acetylneuraminic acid was due to overlapping oscillations of the same groups that caused it in the spectra of the other monomers mentioned above (Fig. 2a). Bands with maxima at 2920, 1460, and 1380 cm^{-1} and inflections at 2930-2900 cm^{-1} correspond to oscillations of the methyl group. The band at 1360 cm^{-1} was due to oscillations of the acetyl group. Bands of the carboxylate ion and amide I and amide II bands were clearly defined in the spectrum of this salt. A distinguishing feature of the spectrum of this substance was a band of valence oscillations of the free carbonyl group at 1725 cm^{-1} (Fig. 2a).

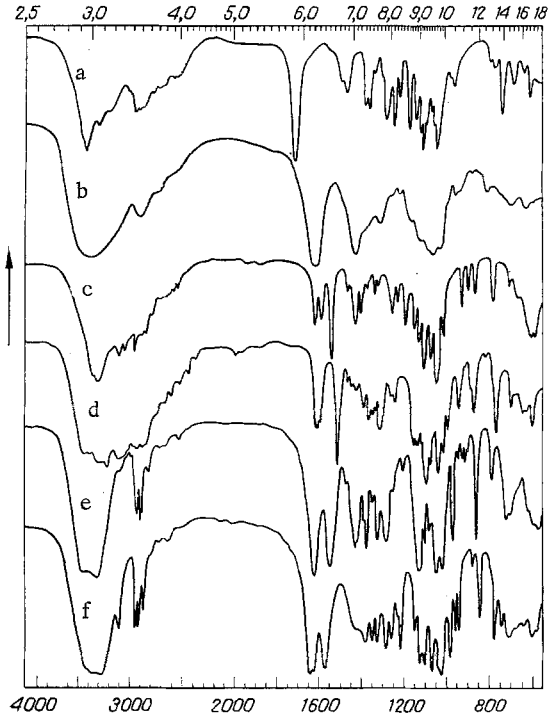

Fig. 1

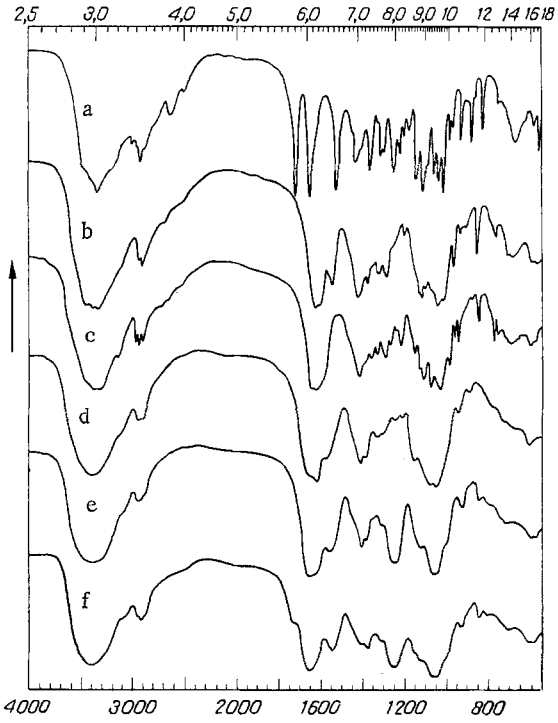

Fig. 2

Fig. 1. IR spectra of glucuronic acid (a), potassium glucuronate (b), glucosamine hydrochloride (c), galactosamine hydrochloride (d), N-acetylglucosamine (e), and N-acetylgalactosamine (f). Abscissa: below — wave numbers (in cm^{-1}), above — wavelength (in μ); ordinate, transmission (in %).

Fig. 2. IR spectra of ammonium salt of N-acetylneuraminic acid (a), and of mixture of equivalent quantities of potassium glucuronate with N-acetylglucosamine (b) and with N-acetylgalactosamine (c), HUA (d), PCKS (e), and PA (f). Legend as to Fig. 1.

A band at 955 cm^{-1} due to out-of-plane deformational oscillations of the hydroxyl group [6] was present in the spectra of all monomers of glycosaminoglycans studied.

Within the range 1350-1000 cm^{-1} of the spectra of acetylated amino sugars and neuraminic acid there were several narrow bands which could be ascribed to two-dimensional deformational oscillations of primary and secondary alcohol hydroxyl groups that have so far been little studied [6]. The number of these bands in the spectrum of potassium glucuronate was less than in the spectra of glucuronic acid and of aceylated amino sugars, because of the greater value of the reduced mass of the salt (see Figs. 1a-f and 2a).

The number of separate absorption bands in spectra of mixtures of potassium glucuronate with N-acetylglucosamine or N-acetylgalactosamine was considerably reduced. One strong band with several maxima, the number and wave numbers of which depended on the composition of the mixture, appeared in the 1350-1000 cm^{-1} region. This was explained by summation of the individual oscillations that coincide to some degree in different monomers (Fig. 2b, c). A doublet at 2920-2905 cm^{-1}, an inflection at 2900 cm^{-1}, and a band with two maxima, the resultant of superposition of valence oscillations of the carboxylate ion and amide I, could be distinguished in the spectra of the mixtures. The band at 1425 cm^{-1} in these spectra was the sum of the valence oscillations of carbonyl and asymmetrical deformational oscillations of the methyl group of the acetyl residue. This was due to the much greater width of this band than in the spectra of aceylated hexosamines, and disappearance of the band at 1380 cm^{-1}. Identification of bands at 1360 and 955 cm^{-1} in these cases was not difficult. The greatest dependence of spectra of the mixtures on their composition was observed in the 1150-1000 cm^{-1} region. In the spectrum of a mixture of potassium glucuronate with N-acetylglucosamine there was a band with maxima at 1110, 1100, and 1005 cm^{-1}, whereas in spectra of a mixture of this same salt with N-acetylgalactosamine a wider band was present, with inflections at 1150, 1100, and 980 cm^{-1} and maxima at 1070-1020 cm^{-1} (Fig. 2b, c).

Particular features could thus be found in the spectra of mixtures of monomers, in the absence of chemical interaction between them, which were not found in spectra of the individual

monomers but were due to summation of overlapping waves. Differences in the band structure of amino sugars associated with their isomerism also were preserved in the structures of the mixtures.

The spectra of HUA, PCKS, and PA differed from spectra of mixtures of monomers by the great reduction in the number of bands, due partly to an increase in the reduced mass of the macromolecules of these biopolymers. The band at 3400 cm^{-1} in the spectra of HUA, PCKS, and PA is the sum of overlapping oscillations identified in the spectra of the monomers. The doublet at 2920-2880 cm^{-1} merged into one band with the maxima at the same frequencies. Bands amide I, amide II, 1400 cm^{-1} (combined, as in the spectra of the mixtures), 1380, and 940-925 cm^{-1} were clearly defined in all three spectra. In this spectrum in the 1300-950 cm^{-1} region, unlike that of the mixture of potassium glucuronate with N-acetylglucosamine, only weak bands appeared at 1305, 1225, and 1200 cm^{-1}, but in the 1180-950 cm^{-1} region there was a strong band with an inflection at 1150 cm^{-1} and maxima at 1080-1030 cm^{-1} (Fig. 2d). In the spectrum of PCKS, however, there was an ill-defined inflection at 1150 cm^{-1}, and a distinct inflection at 1125 cm^{-1}, probably due to N-acetylneuraminic acid contained in this biopolymer, the spectrum of which has a pointed band in this region (Fig. 2a) and a wide band at 1050 cm^{-1}. Since absorption in the 1350-1000 cm^{-1} region is due to two-dimensional deformational oscillations of primary and secondary alcohol hydroxyl groups, and absorption of valence oscillations of the =C–O–C– bond are concentrated in the 1150-1160 cm^{-1} interval, it can be tentatively suggested that the above distinguishing features of the spectra of HUA and PCKS are due to the presence of glucoside bonds in these biopolymers. It does not seem likely that all the particular features of the spectra of HUA and PCKS within this range can be explained purely by summation of overlapping frequencies. Further investigations into this problem are needed.

The main difference between the spectra of HUA and PCKS lies in the presence of bands at 1245 and 850-820 cm^{-1} of valence oscillations of S=O and C–O–S groups in the spectrum of HUA (Fig. 2d-f). The pesence of O-sulfate groups in PCKS also explains certain differences in the spectra of PCKS and HUA in the 1300-950 cm^{-1} region. In addition, it must be remenbered that both amino sugars are present in PCKS [2].

A distinguishing feature of the spectrum of PA was the inflection at 1725 cm^{-1} due to oscillations of the free carbonyl group of N-acetylneuraminic acid contained in PA. Residues of this acid present in PCKS could not be detected spectroscopically in the form of this inflection. This feature evidently reflects one of the structural differences between the soluble and aggregated fractions of PCKS [2]. In all other respects the spectrum of PA was the sum of the spectra of HUA and PCKS, both components of PA. The wide band in the 1180-950 cm^{-1} region had inflections at 1150-1125 cm^{-1} (Fig. 2f).

Comparison of the IR-spectra of glycosaminoglycans with those of monomers and their mixtures thus enables identification of a larger number of bands than investigation of the spectra of these biopolymers alone. This method can be used to identify glycosaminoglycans in the presence of other biopolymers and in more complex biological structures.

LITERATURE CITED

1. S. M. Bychkov, V. N. Bogatov, and V. N. Kharlamova, Byull. Éksp. Biol. Med., No. 6, 680 (1980).
2. S. M. Bychkov and M. M. Zakharova, Vop. Med. Khim., No. 3, 227 (1979).
3. S. M. Bychkov and M. F. Kolesnikova, Biokhimiya, 34, 204 (1969).
4. S. M. Bychkov and V. N. Kharlamova, Biokhimiya, 83, 840 (1968).
5. R. G. Zhbankov, Infrared Spectra of Cellulose and Its Derivatives [in Russian], Minsk (1964).
6. A. Cross, Introduction to Practical Infrared Spectroscopy [Russian translation], Moscow (1961).
7. L. A. Kazitsina and N. B. Kupletskaya, The Use of UV-, IR-, NMR-, and Mass-Spectroscopy in Organic Chemistry [in Russian], Moscow (1979).
8. A. M. Ovsepyan, V. V, Kobyakov, and V. P. Panov, Zh. Prikl. Spectrosk., 24, 523 (1977).
9. D. Kendall (editor), Applied Infrared Spectroscopy, Van Nostrand Reinhold (1966).
10. G. Zundel, Hydration and Intermolecular Interaction, Academic Press (1969).
11. Yu. N. Chirgadze, Biofizika, 7, 523 (1962).
12. F. Högle, A. H. Olavesen, and N. C. Wickramassinghe, Nature, 272, 229 (1978).
13. T. G. Kantor and M. Schubert, J. Am. Chem. Soc., 79, 152 (1956).
14. A. C. Lloyd, K. S. Dodgson, R. G. Price, et al., Biochim. Biophys. Acta, 46, 108 (1961).
15. A. C. Lloyd and K. S. Dodgson, Biochim. Biophys. Acta, 46, 116 (1961).