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PMR INVESTIGATION OF GELATIN AND ITS COMPLEX FORMATION WITH NICKEL AND COBALT

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Compounds of metals with macromolecular ligands play a large role in biological processes [1]. In the investigation of the compounds, the methods of high-resolution NMR (for solutions) and wide-line NMR (for solid compounds) are particularly promising; in a number of cases they make it possible to identify individual donor groups of the macromolecule and to determine the site of metal bonding [2]. The object of the present research—gelatin—is a comparatively simple protein of collagen origin, containing functional groups which are capable of bonding with metals. The properties and structure of gelatin have been described in detail in [3].

In [4], solutions and gels of gelatin in D_2O were investigated by the PMR method. By this method it was possible to make an identification of individual amino acid residues and to calculate the theoretical spectrum of gelatin, which practically coincided with the experimental spectrum. Information on the complex formation of gelatin is very limited. It is indicated in [5] that lanthanum bonds with gelatin at a pH below 6 through the carboxyl groups; copper forms chelates which include carboxyl and amino groups; zinc forms complexes including imidazole and other functional groups; the formation of bonds with COO^- groups has been established for cobalt [4].

The objective of the present work was to investigate aqueous gelatin solutions by the high-resolution PMR method, identify the signals from residues of individual amino acids in the gelatin, and study the changes which take place in the spectrum when the pH of the solution is increased or the temperature is increased, and when metal salts are added.

In our work we used gelatin of the "Food" grade, whose amino-acid composition was determined after splitting by the paper chromatography method of [6]: glycine, 23; proline, 16.2; hydroxyproline, 11.47; glutamic acid, 12.11; aspartic acid, 7.15; alanine, 9.34; lysine, 3.42; leucine, 2.90; valine, 2.10; serine, 3.70; threonine, 1.51; phenylalanine, 2.24; isoleucine, 1.36; hydroxylysine, 1.07; histidine, 1.02; tyrosine, 0.80; and arginine, 0.58%.

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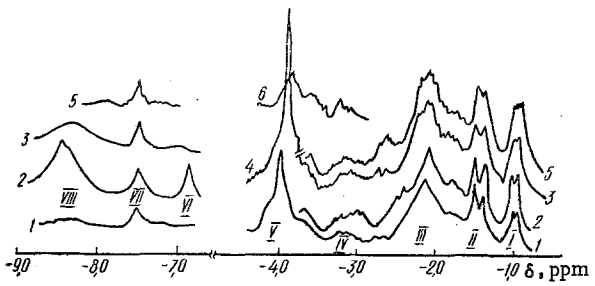


Fig. 1. PMR spectra of gelatin at various pH values (I-VIII, basic signal groups): 1) D_2O, pH = 4.3; 2) H_2O, pH = 1.5; 3) H_2O, pH = 7.1; 4) D_2O, pH = 8.0; 5) H_2O, pH = 11.4; 6) D_2O, pH = 11.0.

The gelatin solution (10% by wt.) was prepared as follows: the sample of gelatin was covered with H_2O or D_2O for 40 min to swell it, then it was heated in a water bath at 50-60°C until complete solution occurred. The cobalt nitrate and nickel nitrate which were used in our work were recrystallized by the procedure of [7] to obtain the salts $Co(NO_3)_2 \cdot 6H_2O$ and $Ni(NO_3)_2 \cdot 6H_2O$. For our work we prepared 0.1 M solutions of these nitrates in H_2O or D_2O. The PMR spectra of the gelatin were taken in the pH range from 1 to 12 and at temperatures from 20 to 90°C. The studies were carried out on an RYa-2310 spectrometer. Benzene was used as an external standard; chemical shifts were determined with respect to tetramethylsilane.

In aqueous solutions, in the acid region (see Fig. 1), gelatin gives a set of signals in high field with respect to water (−1 to −4.5 ppm) and three signals in low field, one of which (−7.5 ppm) should be assigned to the CH groups of aromatic rings (phenylalanine*) and two to protons bonded to nitrogen atoms (−6.8 and −8.5 ppm). The correctness of the assignment of the latter signals is confirmed by a study of the spectra in D_2O: in these spectra these signals have a considerably lower intensity and disappear on expiration of a certain time. These signals also disappear in aqueous solutions when the pH of the solution is raised above 7-8 or when the temperature is raised above 60°C, which may be explained by an increase in mobility of the NH_n-group protons and an averaging of their signals with the water signal.

The signal of the NH_n groups in low field has approximately 4.5 times greater intensity than the signal in high field. Since the peptide protons should be deshielded to a larger extent than the protons of the NH_3^+ groups which are not involved in the peptide chain, the signal at −8.5 ppm may be assigned to NH groups of side chains, and the signal at −6.8 ppm to the NH_3^+ groups of side chains. Calculation of the number of NH groups of peptide chains (on the assumption of bonding of only α-amino groups) and of NH_3^+ groups gives the ratio 81.3:17.22, which corresponds to the ratio of the signal areas at −8.5 and −6.8 ppm.

The spectra of the nonlabile protones of gelatin solutions in H_2O and D_2O practically coincide (Fig. 1). The signal of the protons in the −4.0 ppm region which is observed in D_2O solution cannot be observed in water solution due to overlap with the water signal.

To assign the signals, we used the spectrum of a water solution of gelatin, including in it the group of signals in the −4 ppm region. In the gelatin spectrum one can discern eight groups of signals (see Fig. 1): five groups to the right of the water signal, and three groups to the left. All the signals (except VI and VIII) pertain to nonlabile protons of the amino-acid residues which are part of the composition of gelatin. To assign these signals we used our data from a study of amino acids by the PMR method [8, 9], and we also carried out additional studies of certain amino acids.

In identifying the gelatin signals we took into account the position of bands in the spectra of amino acids in the zwitterion form (Fig. 2). The upper figure in Fig. 2 gives the assignment of the band in the amino acid; the lower one, the number of protons of the given type with consideration of the amino-acid composition of the gelatin. The most intense bands in the gelatin spectrum are indicated by arrows. A comparison of the spectra of the amino acids and of gelatin offers the possibility of making an assignment of the signals from the nonlabile protons. A calculation of the number of protons corresponding to each signal and a comparison of these numbers with the relative intensities (areas) of the signals serves as a criterion of the correctness of signal assignment.

The assignment of signals, number of protons of each type, and the ratio of signal intensity which corresponds approximately to the assignment made are given in Table 1. Thus, the position of the signals of the nonlabile protons in gelatin is practically the same as in the amino acids; the formation of peptide chains with a

*Histidine should give two signals, which are not observed; probably this is due to its low concentration.

TABLE 1. Assignment of ^1H NMR Signals in Spectrum of Gelatin

Group of signals	Amino acid group	Number of protons	S*
I	δ-Leucine; γ-valine; γ and δ-isoleucine	41	1
II	β-Alanine; β- and γ-leucine; γ-threonine; δ-lysine	49	1.2
III	γ- and β-proline; glutamic acid; lysine, β-hydroxyproline; valine, isoleucine	150	4
IV	δ-Proline; β-aspartic acid; phenylalanine; histine; ε-lysine	59	1.2
V	β-Serine; α-protons of C_6H_5 in phenylalanine	126	2.0
VI		11	0.3

*The intensity of signal I is taken as unity.

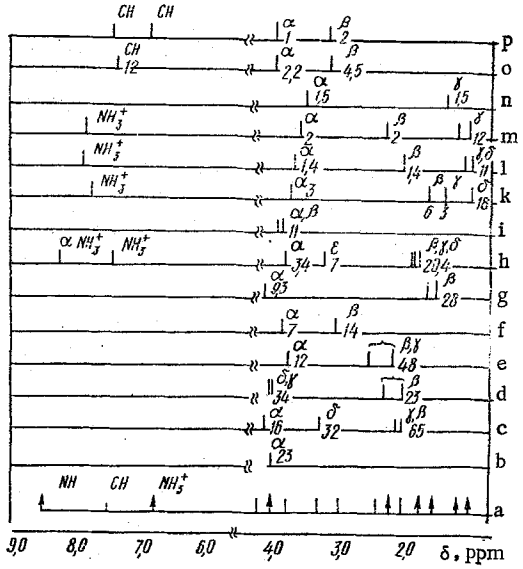


Fig. 2

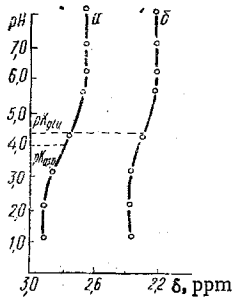
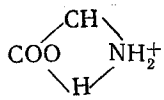


Fig. 3

Position of signals in PMR spectra of gelatin and amino acids: a) gelatin; b) glycine; c) proline; d) hydroxyproline; e) glutamic acid; f) aspartic acid; g) alanine; h) lysine; i) serine; k) leucine; l) isoleucine; m) valine; n) threonine; o) phenylalanine; p) histidine.

Fig. 3. Displacement of PMR signals in spectra of aspartic (a) and glutamic (b) acids as a function of pH of solution.

definite disposition of the amino acid residues is reflected only in a broadening of the signals. Marked changes are observed for the NH_n groups: The mobility of the protons in these groups is considerably less in gelatin than in the amino acids. Signals of the NH and NH_3^+ groups are observed at room temperature and are not averaged with the signals of the water protons up to pH $\simeq$ 7, while in the amino acids the signals of the NH_3^+ groups are detected only at a pH less than 4 and at temperatures of 0-10°C. Differences are also observed in the position of these signals: the signals of the NH groups are shifted toward lower field by 0.5 ppm, and signals of the NH_3^+ groups are shifted to higher field by 0.5-1.0 ppm with respect to the signals of these groups in amino acids. The lack of coincidence in the positions of the NH_3^+ group signals may be explained by the fact that in the amino acids these groups are part of  cyclic structures [8]; in gelatin the formation of such structures does not take place; the formation of chain structures is possible.

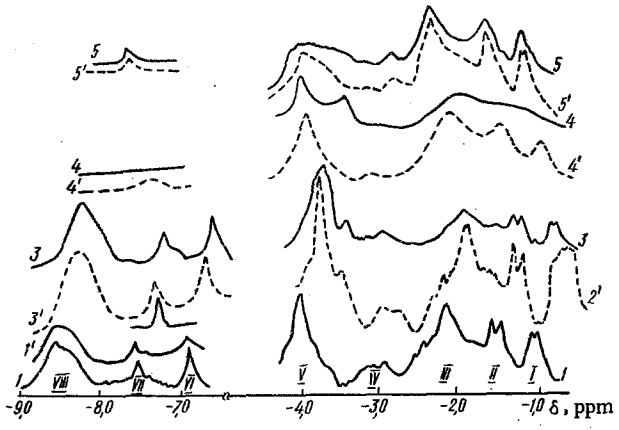


Fig. 4. PMR spectra of 10% gelatin solution on addition of $Co(NO_3)_2$: 1) H_2O, pH = 2.0; 1') pH = 6; 2-5) PMR spectra of gelatin with addition of 0.0125 M Co^{2+}; 2) D_2O, pH = 2.9; 3) H_2O, pH = 5.0; 4) H_2O, pH = 8.0; 5) H_2O, pH = 14.0; 2'-5') same, with addition of 0.0075 M Co^{2+}; 2') pH = 2.9; 3') pH = 5.0; 4') pH = 8.0; 5') pH = 14.0.

When the pH of the solution is raised (≥7) or the temperature is raised (≥60°C), the signals of the NH_n groups disappear, which is associated with an increase in the lability of the protons. Changes are also observed in the −3 to −2 ppm region. At a pH of 3-5, the signals of the CH_2COOH groups in aspartic and glutamic acids are shifted (Fig. 3), which is caused by dissociation of the carboxyl groups; at higher pH values (>7), the signals of ε-lysine and δ-proline are shifted, as a result of the dissociation of $\overset{+}{N}H_n$ groups bonded to these fragments.

The results obtained on the displacement of signals made it possible to calculate the dissociation constants of the COOH groups of aspartic and glutamic acids in gelatin for the formulas for the averaged signal

$$\delta_{av} = \delta_A N_A + \delta_{HA} N_{HA}$$

(δ_A and δ_{HA} are the chemical shifts, and N_A and N_{HA} are the mole fractions of the dissociated and undissociated forms): $pH_{asp} = 4$, $pH_{glu} = 4.4$. The values given are somewhat higher than for the free acids: $pK_{asp} = 3.7$; $pK_{glu} = 4.28$ [11].

Additions of cobalt(II) and nickel(II) salts were made to a 10% gelatin solution at gelatin:metal ion ratios from 25:1 to 25:5 by volume (the concentrations of the metal salt solutions were 0.1 M). In the investigated solutions, the concentrations of the metal ion were $2.5 \cdot 10^{-3}$, $5.0 \cdot 10^{-3}$, $7.5 \cdot 10^{-3}$, $1.0 \cdot 10^{-2}$, and $1.25 \cdot 10^{-2}$ mole/liter. The molecular weight of the gelatin was about $6 \cdot 10^4$; consequently, the approximate molar concentration of the gelatin was $1.7 \cdot 10^{-3}$ M, and the concentration calculated for the functional groups (COO^-, NH_2, or NH) was approximately 40 times as large. Thus, in our experiments there was always an excess of the functional groups, which made available to the metal the opportunity to "select" for bonding those groups for which it had the larger affinity.

Investigation of the spectra of gelatin with additions of Co(II) and Ni(II) was carried out in the pH region from 3 to 14. On addition of a cobalt salt, changes are observed in the spectrum of gelatin which depend on the concentration of the cobalt and the pH of the solution (Fig. 4).

At pH 2.9, a shift of all the gelatin signals and of the water signal in the direction of higher field takes place, plus a broadening of the group I signals. Changes in the signals of the CH_2COOH groups of aspartic and glutamic acids are not observed. On the basis of these data it may be suggested that in the acid region cobalt forms compounds of the solvate type with water and the nitrogen of the amino groups; the CH_2COOH groups of aspartic and glutamic acids do not take part in complex formation.

At pH 5, the signals of the main groups of nonlabile protons of the aliphatic amino acids are broadened, but are not displaced as compared with the signals at pH ≃ 3. The signal of the α-CH protons is broadened, and the signals of the CH_2COOH group are displaced, their position being different than in the spectrum of gelatin at pH ≃ 5; consequently, these groups are bonded with cobalt. The signals of the NH and NH_3^+ groups and of the C_6H_5 groups of phenylalanine are shifted; i.e., the cobalt(II) also forms bonds with the nitrogen atoms.

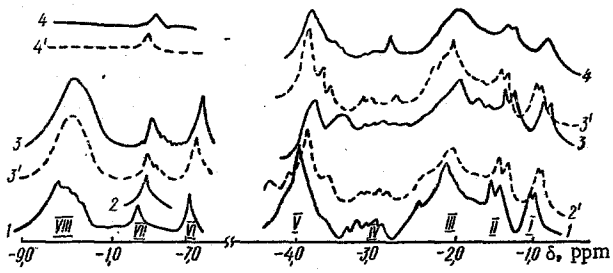


Fig. 5. PMR spectra of 10% gelatin solution on addition of $Ni(NO_3)_2$: 1) spectrum of gelatin at pH = 2.0; 2-4) PMR spectra of gelatin containing added 0.0125 M Ni^{2+}; 2) pH 3.0; 3) pH 5.0; 4) pH 8.0; 2'-4') spectra of gelatin containing 0.0075 M Ni^{2+}; 2') pH 3.0; 3') pH 5.0; 4') pH 8.0.

At pH 8 and a cobalt concentration of $0.75 \cdot 10^{-3}$, the signal of the α-CH protons is narrowed, the signals of the groups which are remote from the peptide bond are broadened, and so are those of the C_6H_5 group. At a cobalt concentration of $1.25 \cdot 10^{-2}$ M the C_6H_5 signal disappears; the signal of the α-CH protons is split into two, and the signals of groups III, II, and I are so broadened that the sharp separation into groups disappears. Consequently, at pH 8 a selectivity of Co^{2+} ions with respect to various amino acids is manifested. The absence of broadening of the α-CH signal at $C_{Co} = 0.75 \cdot 10^{-2}$ M indicates that complex formation takes place initially basically with respect to side chains (COO^-, NH_2 groups) with splitting out of protons. The disappearance of the phenylalanine signal on increase in Co^{2+} concentration indicates that under these conditions phenylalanine is completely bonded. The appearance of a second broadened signal of the α-CH groups in higher field at a C_{Co} of $1.25 \cdot 10^{-2}$ M is connected with the formation of cobalt bonds with the NH an C =O groups; the intensity ratio of the two α-CH signals indicates that a third of the NH groups forms these bonds.

At pH 14, the spectra differ from those observed at pH $\simeq$ 8 in that again the signals of groups I, II, III, and VI are displayed, plus the signals of aspartic acid. The signals of the α-CH groups are sharply broadened. These changes may be explained by complex formation at high pH values, mainly through the NH and C =O groups of gelatin.

Additions of nickel(II) nitrate to gelatin in the same concentrations as of the corresponding cobalt salt lead to approximately the same changes in the PMR spectra, which, however, are significantly less sharply manifested (Fig. 5). In the pH 3-5 region a shift of the signals of the NH, NH_3^+, and C_6H_5 groups of phenylalanine takes place to higher field, and the C_6H_5 signal is broadened. However, at pH $\simeq$ 5, the C_6H_5 signal does not disappear, but continues to broaden up to pH 11. The signal of the α-CH groups is split already at pH $\simeq$ 5 (in the case of Co^{2+}, at 8) and at a lower Ni^{2+} concentration ($0.75 \cdot 10^{-2}$ M) than for cobalt. Thereupon the number of bonded NH groups rises with increase in nickel concentration; i.e., Ni^{2+} displays a larger tendency to form bonds with NH groups at low pH values. The signals of the β, γ, δ, and other nonlabile protons are broadened to a considerably smaller extent, which may be explained by the fact that the broadening of the signals in the case of Ni^{2+} is caused only by contact interaction and only the signals of the groups directly bonded with the donor atom are broadened.

The studies performed show that the PMR method makes it possible to detect complex formation of the paramagnetic nickel(II) and cobalt(II) ions with gelatin, and establish a difference in the complex-formation processes as a function of the nature of the metal, its concentration, and the pH of the solution, and makes it possible in some cases to pinpoint the presence of bonds with definite amino-acid residues.

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STATE OF THE WATER IN THE $SiO_2 - X_2$ AND $SiO_2 - Y$ SILICAS

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There are practically no data on the state of the water in silica crystals of the SiO_2-X_2 and SiO_2-Y type. We obtained the SiO_2-X_2 and SiO_2-Y silicas as intermediate metastable phases during the hydrothermal crystallization of amorphous silica (silica gel) into quartz in weakly basic media [1]. The SiO_2-X_2 silica was synthesized both in pure KOH or NaOH solutions and in alkaline solutions of various potassium or sodium salts; the SiO_2-Y silica, which is an analog of the natural mineral magadiite [2], was obtained only in alkaline solutions containing sodium cations. As a result of the increased ion-exchange capability of both silicas a variable amount of sodium or potassium cations, which is determined by the pH of the solution, can be contained in its composition. The cations are completely removed by washing the samples with acidic solutions. The limiting capacity of the exchange, which is observed at pH 8-9, is 1.0-1.3 meq/g independent of the exchanged cation (Na^+ or K^+). The investigation of the state of the water in the cationized and decationized forms of the SiO_2-X_2 and SiO_2-Y silicas was carried out in this work using DTA, PMR, and the thermogravimetric and IR spectroscopic methods.

The thermograms and curves of the loss in weight with heating (rate of heating 10 deg/min) were plotted on a drift indicator of a Paulik–Paulik–Erdey derivatograph; the PMR spectra were run on a low-resolution RYa-2301 radiospectrometer at an operating frequency of 16 MHz at 293 and 77°K. The IR spectra were run on a UR-20 spectrometer. The samples were prepared by pressing 4 mg of the substance to be investigated in an evacuated die with 200 mg of KBr preliminarily heated at 200°C, which enabled us to completely remove the adsorbed water from the KBr and to avoid its repeated adsorption from the air. The samples heated at the various temperatures were cooled in sealed weighing bottles over conc. H_2SO_4. The determination of the water content and structural OH groups by the PMR spectra was carried out by comparing them with the spectrum of a standard containing a known amount of $Ca(OH)_2$.

The differential curves of the loss in weight (DTG) and the differential heating curves (DTA) for samples of the SiO_2-X_2 and SiO_2-Y silicas, which differ from each other by the nature of the exchange cation, are given in Fig. 1. In the sample of the NaH form obtained by treating the original Na form of SiO_2-Y with a 0.01 N HCl solution, the sodium content comes to 75% of the limiting value. The K and Na forms were obtained in pure KOH and NaOH solutions respectively under the conditions for the hydrothermal synthesis of the corresponding silica. It is evident from Fig. 1 that the character of the dehydration and also of the weight loss at each given temperature (see Fig. 2) of the silica forms studied is governed primarily by the nature of the exchanged cation.

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