# A STUDY OF GELATIN BY IR SPECTROSCOPY

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Samples of freshly prepared soluble, naturally aged soluble, and insoluble gelatins (an accumulation of glue taken from a wooden sculpture of the XVth century) before and after heating at 210°C have been investigated by IR spectroscopy. The IR spectra of the samples before heating consisted of the characteristic spectra of collagen or gelatin although there were differences in the 1380–1460 $cm^{-1}$ region of the spectra. After the samples had been heated, the IR spectra of the soluble gelatins differed from those of the unheated samples. The changes in the IR spectra are discussed in connection with the probable structural rearrangements taking place in the natural ageing of gelatin.

According to the literature [1–3], on heating above 140°C gelatin passes into an insoluble state with the formation of a structure characterized by the presence of a large number of interchain cross-linkages. As a rule, this fact is studied in model experiments in which a sample of freshly-prepared gelatin is subjected to the action of high temperatures. At the same time, a unique possibility of investigating samples of gelatin that have undergone the process of long-time natural ageing during which the gelatin may become insoluble exists. The amino acid compositions of samples of soluble and insoluble gelatin found in a wooden sculpture of the XVth century have been given previously [4]. The aim of the present investigation was to elucidate the structural features of soluble and insoluble (natural-aged and freshly-prepared) gelatins by IR spectroscopy.

Three types of samples of dried gelatin were studied: 1) gelatin obtained by steaming fragments of calfskin at 60°C; 2) soluble gelatin extracted from an accumulation of glue taken from a wooden sculpture of the XVth century; and 3) insoluble gelatin extracted from an accumulation of the same glue. To analyze possible structural changes, the samples were subjected to the action of elevated temperatures, after which their spectral characteristics before and after heating were compared.

In the IR spectra of the samples in the 4000-400 $cm^{-1}$ region (Fig. 1 shows the 1800–1200 $cm^{-1}$ region) there are the following absorption bands characteristic for collagens or gelatin [5, 6], ($cm^{-1}$): amide A, ∿3310; amide B, ∿3070; amide I, 1655; and amide II ∿1540. When the spectra of samples 1–3 before the action of heat are compared, attention is attracted by the 1460–1380 $cm^{-1}$ region, since certain differences can be seen in it: characteristic for sample 1 is a doublet at 1457 and 1400 $cm^{-1}$ (Fig. 1, curve 1a); for sample 2, a doublet at 1457 and 1400 $cm^{-1}$ with a shoulder at 1390 $cm^{-1}$ (Fig. 1, curve 2a); the soluble gelatin extracted from the ground of a picture by Rubens has a similar IR spectrum [7]), while in the case of sample 3 there is a band at 1450 $cm^{-1}$ with a shoulder at 1400 $cm^{-1}$ (Fig. 1, curve 3a). As is well known [8], in the spectral region considered, the bands of the deformation vibrations of —C—H groups and the symmetrical stretching vibrations of —C=O groups of ionized $—COO^-$ groupings appear.

In order to understand the reason for the differences, particularly between the spectra of the samples of soluble gelatins (1, 2) and the insoluble gelatin (3) they were subjected to the action of heat. As has been mentioned, when it is heated above 140°C gelatin gradually changes into the insoluble state with the formation of a structure characterized by the presence of a large number of interchain cross-linkages [1–3], while on further heating (205–211°C) supercontraction is observed – an irreversible process due to the breakdown of the triple-stranded sections of the macromolecules into individual chains [3]. In the process of heating, water is eliminated from the gelatin, and when the amount of residual

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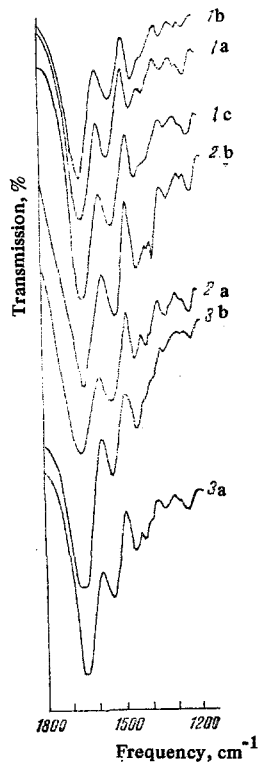


Fig. 1. IR spectra of gelatin (1800–1200 $cm^{-1}$ region); modern soluble before (1a) and after (1b) heating at 210°C; modern soluble after UV irradiation (1c); aged soluble before (3a) and after (3b) heating.

moisture is less than one percent by weight the gelatin becomes practically insoluble [9]. It may be assumed that heating models to some degree the process of natural ageing and that in samples of natural gelatin greater structural changes take place than in samples of insoluble gelatin. The insolubility of sample 3 is in fact probably due to a large number of intra- and interchain covalent bonds in the gelatin that was present on the surface of the accumulation of glue and was subjected to the more intense action of external forces than the interior part of the gelatin, which remained soluble (sample 2).

In actual fact, after the samples had been heated to 210°C (i.e., to a temperature at which an irreversible change takes place in the structure of soluble gelatin [3]), the IR spectrum of the samples changed considerably (Fig. 1, curves 1, 1b, 2b, and 3b). In particular, in the spectra of the heated samples of soluble gelatin (1b) and (2b), the amide II band had shifted into the 1530 $cm^{-1}$ region and the contour of the amide I band had changed somewhat. The shift of the amide II band and the change in the contour of the amide I band are usually explained by the elimination of water molecules from the samples [5, 9]. These are most probably water molecules bound to the main polypeptide chains of the gelatin, for example, with the oxygen of –C=O groups "directed sideways" [11].

More considerable changes were observed in the 1560–1380 $cm^{-1}$ region where, in the spectrum of gelatin of sample 1, the band at 1400 $cm^{-1}$ disappeared and in the spectrum of sample 2 the intensity of the band at 1400 $cm^{-1}$ likewise decreased considerably and a separate narrow band appeared at 1390 $cm^{-1}$. The IR spectrum of sample 3 was practically unchanged in this region (Fig. 1, curve 3b). Such differences between the IR spectra of the samples must apparently be considered from the aspect of possible small structural modifications connected with changes in the side chains of the molecules under the action of high temperatures on the gelatin.

The acidic and basic functional groups determine many of the physical properties of a collagen fiber [10, 11]. It must be mentioned that glutamic and aspartic acids together make up a considerable proportion of the amino acids of collagen and gelatin – averaging 15% of the amino acid composition [4, 10-12]. Since some of the water molecules interact

with the side chains of the polypeptide chains of the gelatin, including the carboxy groups [11], the action of a high temperature on the samples must lead to the elimination of these water molecules, which may be accompanied by, for example, the conversion of —COOH groupings into the —$COO^-$ form, or conversely. This, in its turn explains the disappearance of the band at ∿1400 $cm^{-1}$ in the IR spectra of samples 1 and 2. It may be assumed that on the elimination of water from samples 1 and 2 the number of —$COO^-$ groupings decreases, since the water content of sample 3 was so small that the action of the temperature did not lead to any appreciable elimination of it, and the number of —$COO^-$ groupings in this sample was inconsiderable. The absence of changes in the quantitative water content of sample 3 is also shown by the above-mentioned invariability of the positions of the amide I and amide II bonds in the spectra of heated insoluble gelatin. It does not appear possible to assign the band at 1390 $cm^{-1}$ in the IR spectrum of heated aged soluble gelatin (sample 2).

A decrease in the intensity of the band at ∿1400 $cm^{-1}$ was also observed in the IR spectrum of gelatin (sample 2) that had been subjected to ultraviolet irradiation (source of light with a wavelength of 254 nm; Fig. 1, curve 1c). It is known that the irradiation of gelatin by light with a wavelength greater than 240 nm leads to the photopolymerization of the molecules, and in this process, apparently, it is the amino acids of the nonhelical sections of the molecules that are primarily involved [13]. Judging from the decrease in the intensity of the band at ∿1400 $cm^{-1}$, the process of photopolymerization is accompanied by structural rearrangements with the probable elimination of water molecules.

The results obtained permit the assumption that in the course of the natural ageing of gelatin present in prolonged contact with the air, an irreversible elimination of a large part of the water molecules takes place which is accompanied by the formation of structures with numerous intra- and interchain bonds. It is possible that glutamic and aspartic acid residues take part in the formation of the covalent bonds since γ-glutamyl bonds between the molecules are known in collagens [10—12], and the shoulder at 1400 $cm^{-1}$ in the spectrum of insoluble gelatin probable shows a small content of free —$COO^-$ groupings in the sample. Although the properties of gelatin present within the accumulation of glue have changed to a smaller degree than the surface layer, and it has remained soluble for the course of 500 years, some changes have nevertheless taken place, since, as has been shown, the IR spectra of heated modern and aged gelatins differ somewhat.

## EXPERIMENTAL

IR spectra were recorded on a Specord 75 IR instrument, using 1 mg of sample molded into a tablet with KBr (150 mg). The instrument was calibrated with the spectrum of polystyrene. The accuracy of measurement was ±5 $cm^{-1}$. After the tablets had been heated in a thermostat at 210°C for 3 h, the spectra were recorded again. The IR spectrum of sample 2 was also recorded after irradiation with ultraviolet light (SVD-120A irradiator) for 3 h; the irradiated gelatin was molded into tablets with KBr.

## SUMMARY

1. The IR spectra of samples of naturally aged soluble and insoluble gelatins, the age of which was 500 years, consist of the characteristic spectra of collagen or gelatin.

2. Appreciable differences are observed in the 1380—1460 $cm^{-1}$ region between the IR spectra of soluble (freshly prepared and aged) and insoluble gelatins: in the former two cases there are bands at 1400 and 1457 $cm^{-1}$, and in the last case a band at 1450 $cm^{-1}$ with a shoulder at 1400 $cm^{-1}$.

3. The differences in the IR spectra of samples of soluble and insoluble gelatins before and after heating indicate structural rearrangements taking place both under the action of the temperature and during the process of the natural ageing of gelatin, and also the probable formation of intra- and interchain bonds.

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# A STUDY OF THE BEHAVIOR OF HISTONE H1 AND ITS COMPLEX WITH DNA BY THE SPIN-LABEL METHOD

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The behavior of the tyrosine-72 residue of histone H1 has been studied by the spin-label method as a function of the ionic strength of the solution and of the temperature and on its interaction with DNA. It has been shown that in the formation of complexes of histone H1 with DNA the globular part of the protein not directly interacting with the nucleic acid retains a definite conformation enabling it to participate in various processes taking place in the chromatin.

In order to study the microstructure and the conformational properties of the globular part of the histone H1, we have developed a method for the selective attachment of a spin label to the single tyrosine-72 residue of the protein molecule [1].

In the present paper we give the results of an investigation of the behavior of the spin-labeled histone H1 as a function of the ionic strength of the solution and of the temperature and on its interaction with DNA. The interest in questions of the structural behavior of the globular section of histone H1 is due to the fact that, according to modern ideas, this region of the protein molecule is considered to be involved in the process of the specific recognition of twisted DNA [2]. The ESR spectrum of histone H1 of calf thymus labeled at the tyrosine-72 residue (Fig. 1a) indicates a weak immobilization of the spin label in the protein and shows that the tyrosine residue is most probably located on the surface of the globular segment of the histone.

To investigate the microenvironment and to determine the "accessibility" of the radical attached to the tyrosine-72 residue, the spin-labeled histone H1 was titrated with a 0.1 M solution of potassium ferricyanide. The change in the ESR spectra after the addition of each aliquot of the 0.1 M solution of potassium ferricyanide with a volume of 0.05–0.1 ml was followed from the broadening of the lines. The addition of potassium ferricyanide up to a concentration of $10^{-2}$ M did not lead to their broadening. According to the literature [3], such a phenomenon can be explained by the presence in the immediate microenvironment of the nitroxyl radical of a considerable accumulation of positively charged amino acid residues.

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