LOW-FREQUENCY RAMAN SPECTROSCOPY OF HUMAN HAIR KERATINS

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Abstract. Low-frequency Raman lines characterizing vibrations of elements of the secondary structure of fibrillar proteins (keratins) are identified. Experiments with unpigmented human hair are performed in two configurations: with excitation radiation focused coaxially with the hair and perpendicularly to it. Based on polarization sensitivity, the bands at frequencies of 150 and 221 cm^{-1} are assigned to vibrations of the α-helical structures of keratins. Spectral interval of 270-340 cm^{-1} is assigned to vibrations of β-structures.

Keywords: *low-frequency Raman microspectroscopy, keratins, protein secondary structure, polarization sensitivity, human hair*

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INTRODUCTION

Human hair consists mainly of fibrillar proteins - keratins [1]. Three structural components can be distinguished in the hair: cuticle, cortex and medulla. The cortex is

formed from left-handed superspirals of α-keratins, which are organized into microfibrils. The microfibrils form macrofibrils that are oriented predominantly along the hair axis and are embedded in a low molecular weight matrix [2]. The stability of this structure is maintained mainly by disulfide bridges, the concentration of which is about 1.5 mmol/g [3]. The cortex is surrounded by a protective sheath, the cuticle, which is 2-3 μm thick and is formed by keratins whose secondary structure consists mainly of β-sheets, β-hairpins, and disordered elements [2]. The inner layers of the cuticle also contain superspirals of α-keratins [4]. A characteristic feature of the cuticle is a higher content of disulfide bonds (2.1 mmol/g) compared to the cortex [3]. Thus, hair is a convenient molecular system for studying and comparing the vibrational bands of the secondary structure of keratins, including in the low-frequency (LF) (50-480 cm^{-1}) spectral range.

Raman microspectroscopy (MS) has been successfully used to study the molecular structure of human hair since the beginning of the 21st century [5]. The micron spatial resolution of the method allows us to distinguish between cuticle and cortex spectra. In the fingerprint interval (OP interval), the secondary structure of proteins is mainly characterized by the amide I (1650-1680 cm^{-1}) and amide III (1230-1300 cm^{-1}) lines [5].

Of particular interest are the broad LF lines, which are often attributed to collective vibrations of the molecule skeleton or intermolecular vibrations [6-7], but there is still no unambiguous interpretation of the CR lines in this interval. At present, there are many studies devoted to determining the causes of LF oscillations of protein molecules. It has been revealed that such vibrations are sensitive to conformational

changes of the protein molecule. For example, a comparative analysis of the CR spectra of native and denatured collagen showed that the difference in the intensities of the CR lines in the 200-300 cm^{-1}band may be due to changes in the tertiary structure of the protein [8]. The effect of disulfide bond breaking on the tertiary structure of chymotrypsin and albumin is discussed by comparing the CR spectra in the OD and LF intervals [9]. There are disagreements in the interpretation of the frequency band 100-190 cm^{-1}. In some works the authors claim that the CR lines in this interval characterize the vibrations of α-helices [10-12], and in others they are attributed to the vibrations of β-structures [13-15]. The above-mentioned actualizes the problem of identification of LF bands in the CR spectra of proteins.

The aim of the work is to identify low-frequency CR lines characterizing vibrations of elements of the secondary structure of human hair keratins.

MATERIALS AND METHODS

Gray human hair was used in the experiments. The samples were washed in distilled water and dried at room temperature. The donor had not used any hair cosmetics for a week prior to sample collection. The absence of pigment in the hair ensured negligible thermal effects on the hair during measurements of the CR spectra and minimized the intensity of the broadband fluorescence background.

Measurements were performed in two experimental configurations: horizontal and vertical. The horizontal configuration involves focusing the excitation radiation on the lateral surface of the hair. In the vertical configuration, the excitation radiation is

focused normal to the end face of the hair. A free cross-section of the hair was obtained by freezing and then fracturing it in liquid nitrogen [16].

Raman spectra were measured on a DXR Raman Microscope (Thermo Scientific) with an excitation wavelength of 780 nm and a power of 24 mW. Depolarized radiation (degree of polarization ~0.1) was focused onto the sample using an Olympus MPlan N 100X/0.90 BD objective lens (working distance 0.21 mm) into a spot with a diameter of 0.9 μm. Spectra were measured in the range 50-3500 $cm^{-1}$with a spectral resolution of 4 cm^{-1}. The lateral and axial resolutions of the microspectrometer were 1 and 2 μm, respectively. The accumulation time of one spectrum was equal to 30 min.

The correction of the background signal when comparing the CR spectra was performed using the NewCompare program, which minimizes the standard deviation between the spectra by multiplying one of the spectra by a constant and adding a polynomial of a given degree to it [17]. After correction, the spectra were smoothed over 20 points using the Savitsky-Golei algorithm [18], after which the R(ν)-transformation was applied [19].

RESULTS AND DISCUSSION

Fig. 1 shows the CR spectra of human hair cuticle measured in horizontal and vertical configurations. The spectra in Fig. *1b* are shifted vertically for ease of comparison. The differences are related to the polarization sensitivity of the CR lines of the α-helixes. In the vertical configuration, the field strength of the excitation radiation is predominantly perpendicular to the axes of the α-helixes, while in the

horizontal configuration it is directed equally likely along the axes of the α-helixes and perpendicular to them [20].

In the OD interval, significant differences are seen at frequencies 560, 935, 1316, and 1652 $cm^{(-1)}$ (Fig. 1). The line at ~560 cm^{-1}can characterize both out-of-plane bending vibrations of the C=O group (amide VI) [21] and vibrations of disulfide bridges in the trans-gosh-trans conformation [22]. At 935 cm^{-1}frequency, skeletal vibrations of the N-C_α-C polypeptide chain, valence vibrations of C-N, N-$C_\alpha$groups and pendulum vibrations of CH_3groups occur [22]. The frequency of 1316 cm^{-1}corresponds to C_α-H vibrations [5]. The band with a maximum at frequency 1652 cm^{-1}(amide I) corresponds to peptide bond vibrations [1, 22]. The indicated spectral differences are manifested at frequencies characterizing vibrations of α-helical structures.

In the LF interval, the differences in the bands at frequencies 110-165 and 200-240 $cm^{-1}$are pronounced (Fig. *1a*). In the CR spectra measured in the horizontal configuration, i.e., in the presence of a field component parallel to the spiral axes, the intensity of I_{CR}broad lines at frequencies around 150 and 221 $cm^{(-1)}$ is much larger. Since the differences in the OD and LF intervals should correlate, the lines at 150 and 221 cm^{-1}can be attributed to vibrations of the α-helix structures of keratins.

The results are in agreement with published data obtained for various α-helical proteins. Lines near the frequency of 150 $cm^{-1}$are observed in the CR spectra of bovine albumin [23], human albumin [24], and collagen [10]. In the CR spectra of α-helical poly-L-alanine, the line at 120 $cm^{-1}$is attributed to the torsional vibrations of C_α-C and N-$C_{(\alpha)}$ [12], while the line at 159 cm^{-1}characterizes the out-of-plane bending vibrations

of the N-H group and also the strain vibrations of C-N-$C_{(\alpha)}$and N-$C_{(\alpha)}$-C [11]. A comparative analysis of the CR spectra of the α-helix protein lysozyme and the β-protein chymotrypsin showed that the line intensity at 150 cm^{-1}increases with increasing concentration of α-helices in the protein [10].

The data on the frequency band 200-240 cm^{-1}also confirm its belonging to vibrations of α-structures. The CR lines near the frequency of ~200 cm^{-1}belong to vibrations of amide VII [25]. In the CR spectra of α-helical poly-L- alanine and polyglycine, lines at frequencies 209 $cm^{-1}$and 217 cm^{-1}were attributed to C-N-$C_{\alpha}$and C=O vibrations [11]. Oscillations at 240 cm^{-1}in the CR spectra of α-helical lysozyme and myoglobin [26] characterize symmetric valence or torsional vibrations of the polypeptide chain.

Fig. 2 shows the CR spectra of cuticle and cortex measured in the vertical configuration of the experiment, i.e., when the excitation radiation is focused on the hair end. The spectra in Fig. *2b* are shifted vertically for ease of comparison. The change in the intensities of I_{CR}in the OD interval (Fig. 2) during the transition from cuticle to cortex is due to a decrease in the concentration of S-S (510 $cm^{-1)}$, C-S (665 $cm^{(-1)}$), and S=O (1040 $cm(^{-1})$) bonds [5]. It is important to note the spectral differences associated with different concentrations of keratin secondary structure elements in cuticle and cortex. The increase in the intensity of I_{CR}lines in the CR spectrum of cortex is observed at frequencies 560, 935, 1316, 1652 cm^{-1}, which is due to the higher content of α-helices. At the same time, in the CR spectrum of cuticle the lines at 1247 and 1671 $cm^{(-)\ (1)}$are more intense due to the increased concentration of β-structures and disordered elements [22]. The increase in the intensities of I_{CR}lines at 750, 1340, and

1615 cm^{-1}during the transition from cuticle to cortex corresponds to an increase in the concentration of tryptophan amino acid residues [5,21].

In the LF interval, we also observe a redistribution of the intensities of I_{CR}lines in the transition from cuticle to cortex: an increase in the intensity of $I_{(CR)}$lines at frequencies 150 and 221 $cm^{-1}$is accompanied by a decrease in the intensity of$I_{(CR)}$in the interval 270-340 $cm^{(-1)}$(Fig. *2a*). The changes near the frequencies 150 and 221 $cm^{-1}$are consistent with those shown in Fig. *1a* and therefore characterize an increase in the α-helix content. The CR line in the interval 270-340 cm^{-1}can be attributed to the oscillations of β-structures. Indeed, characteristic lines at 300 and 330 cm^{-1}have been observed, for example, in the CR spectra of poly-L-alanine, poly-L-alanylglycine and polyglycine in the β-conformation, which have been attributed to C-N-$C_{(\alpha)}$, N-$C_{(\alpha)}$-C strain vibrations, C=O planar vibrations [27] or N-C valence vibrations [28].

Differences between the CR spectra of cuticle and cortex in the LF interval are also observed at 465 $cm^{(-1)}$(Fig. *2a*), which may be related to the higher concentration of lysine in the cortex [3, 29]. Vibrations of C-N groups can also be observed near the frequency of 465 cm^{-1}, for example, in the case of lysozyme [30].

CONCLUSION

Unpigmented human hair is a convenient system for studying the CR lines characterizing the vibrations of the secondary structure of keratins in the LF interval (50-480 $cm^{-1)}$.

The identification of LF CR lines characterizing vibrations of keratins secondary structure elements based on their polarization sensitivity was carried out. Spectral

differences appear when focusing depolarized radiation on the lateral surface of the hair and on its end. The broad lines at frequencies 150 and 221 $cm^{-1}$are attributed to vibrations of α-helixes of keratins. As a result of comparison of CR spectra of cuticle and cortex (regions of hair with different content of secondary structure elements), it was obtained that in the interval 270-340 cm^{-1}vibrations of β-structures are manifested.

The results of this work can be used as a basis for studies of conformational transitions occurring in keratins, for example, under the influence of various external factors such as heating, UV irradiation, or chemical actions.

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FIGURE CAPTIONS

Figure 1. CR spectra of human hair cuticle measured when focusing the excitation radiation on the hair end (solid curve) and when the radiation is directed perpendicular to the hair axis (curve with circles).

Figure 2. CR spectra of the cuticle (solid curve) and cortex (curve with circles) of human hair measured in the vertical experimental configuration.

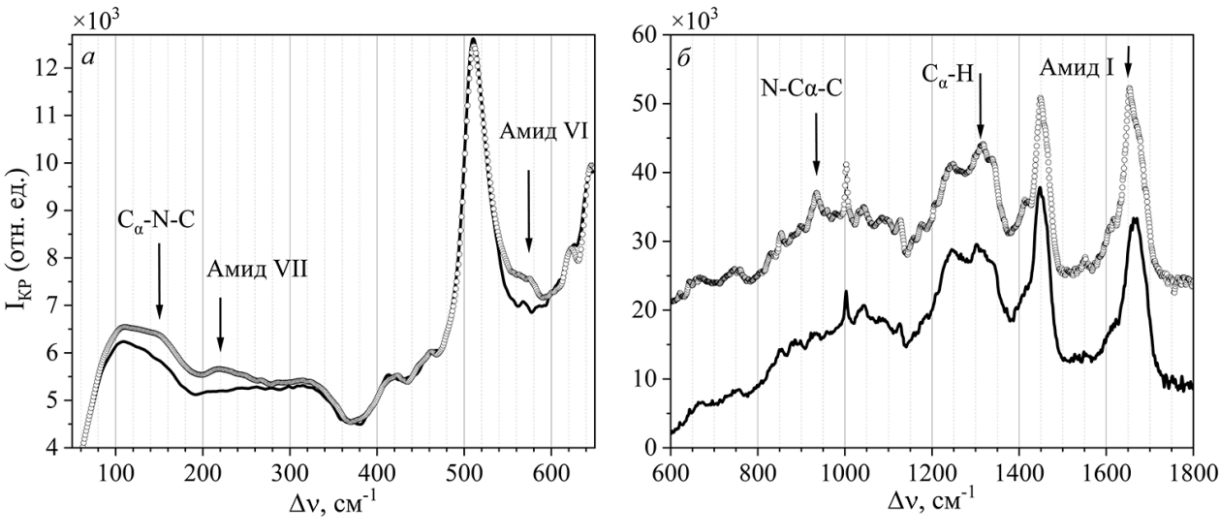

Fig. 1.

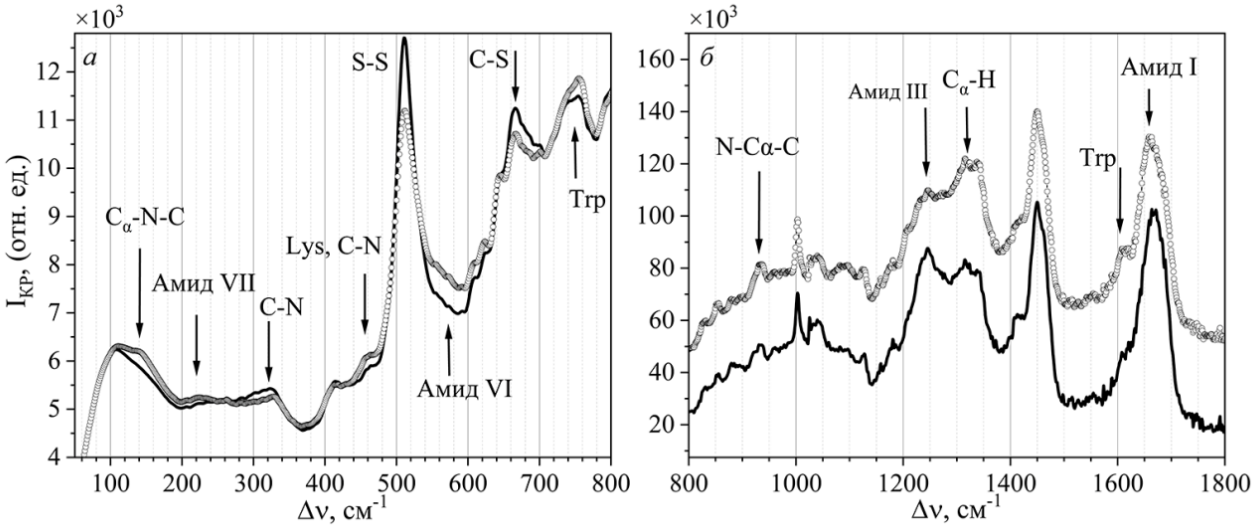
×10^3
а
S-S
C-S
Trp
Cα-N-C
Амид VII
C-N
Lys, C-N
Амид VI
I_КР, (отн. ед.)
Δν, см^-1
×10^3
б
Амид III
Cα-H
Амид I
N-Cα-C
Trp
Δν, см^-1

Fig. 2.