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Features of infrared spectra of blood serum of patients with multiple myeloma

© D.A. Chernyshev[1], E.S. Mikhailets[1], L.V. Plotnikova[1], A.D. Garifullin[1,2], A.Yu. Kuvshinov[2], S.V. Voloshin[2,3], A.M. Polyanichko[1,4]¶

[1] St. Petersburg State University, St. Petersburg, Russia
[2] Russian Scientific Research Institute of Hematology and Transfusiology of FMBA of Russia, 191024 Saint-Petersburg, Russia
[3] Kirov Military Medical Academy 194044 Saint-Petersburg, Russia
[4] Institute of Cytology of RAS, 194064 Saint-Petersburg, Russia
¶ e-mail: a.polyanichko@spbu.ru

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A comparative analysis of infrared (IR) spectra of blood serum of healthy donors and patients with multiple myeloma (MM) is carried out. The MM is characterized by hyperproduction of paraprotein, which manifests in a change in the IR absorption spectrum profile in the mid-IR range. It is shown the most significant deviations in spectra are observed for the samples of secretory MM near Amide I and Amide II′ bands. As a result of the comparative analysis of IR spectra of blood serum in the region of $1700-1350\,cm^{-1}$ 9 parameters were identified, which average values in samples taken from patients with secretory MM form are significantly different from the same parameters of healthy donor serum.

Keywords: IR spectroscopy, multiple myeloma, blood serum, secondary structure of proteins.

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Introduction

Today multiple myeloma (MM) accounts for 10% of all hematological cancers and about 1% of all oncological diseases [1]. According to SEER(US Surveillance Epidemiology and End Results Programme), African Americans have approximately twice higher incidence of multiple myeloma (MM) than White patients. This disease is manifested predominantly in older people [2,3]. The number of newly diagnosed cases is increasing every year. Thus, in 2016 a bit more than 30 000 new myeloma cases were recorded in the USA, which is 10 000 greater than in 2009 [4,5]. And in 2020 more than 32 000 new MM cases were diagnosed [6]. Currently there are no reliable methods of drug treatment for MM, and the existing therapy is aimed first of all at symptom relief and slowing of disease development [7].

Multiple myeloma is a tumor disease of plasma cell arising in the bone marrow and spreading over the body via the blood stream. Multiple myeloma belongs to the group of paraproteinemic leukemia diseases, which are characterized by the ability to produce and secrete immunoglobulins or their fragments — paraproteins [8–10]. Several types of this pathology are distinguished: MM with M-protein secretion in large quantities, characterized by the type of M-protein, as well as a rarer non-secreting MM, when no paraprotein hyperproduction is observed [11]. The M-proteins can be immunoglobulins of G-, A-, D-, M- types or their fragments — κ- or λ-light chains known as Bence Jones proteins [3].

Timely MM diagnosis is one of important factors that define bot the approaches to therapy and the possible response to treatment. However, the diversity of clinical implications [3,9,12] that may accompany the MM development complicates the diagnosing at earlier stages of the disease [3,13,14]. With this context, the need arises to search for new simple and affordable methods for primary screening of a wide range of patients in order to identify signs of the MM development. One of promising tools to detect various pathologies can be the infrared spectroscopy [15–20]. This study presents a comparative analysis of infrared spectra of blood serum of patients with multiple myeloma and healthy donors.

Materials and methods

Samples of serum

In this study serum samples from patients with MM, which are under the supervision of the hematology clinic of the Russian Research Institute of Hematology and Transfusiology (St. Petersburg, Russia) were analyzed. Samples were taken by the staff of the Russian Scientific Research Institute of Hematology and Transfusiology in accordance with the standard clinical protocol described earlier in [18]. S-Monovette tubes (Sarstedt, Germany) with clotting activator were used to obtain serum samples. The

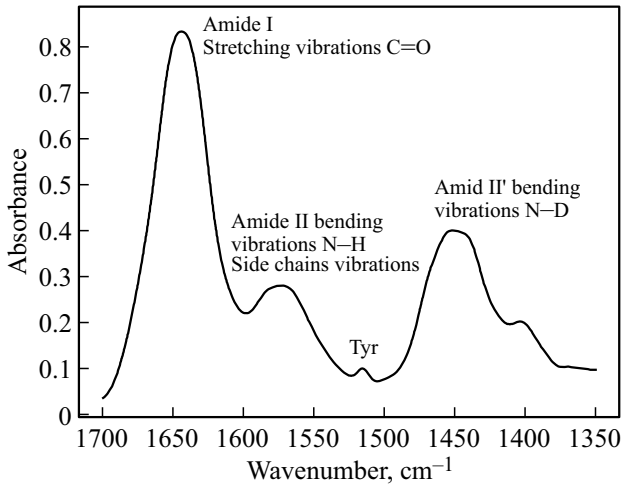

Figure 1. Absorption spectrum of blood serum in the range of 1700−1350 cm^{-1}. Band assignment is performed on the basis of studies of [24,25,27].

taken blood samples were kept in tubes for 20 − 30 min at room temperature (18−24°C), then centrifuged for 15 min at a speed of 3000 rot/min in a Heraeus Labofuge 200 centrifuge (Thermo Scientific, USA). Before spectroscopic studies, the main portions of samples were frozen and stored at a temperature of −30°C.

In this study samples of 25 healthy donors, 36 patients with MM were analyzed. non-secretory MM form was diagnosed in 8 patients. There were 17 male and 19 female patients. Patients of this group were in the age from 44 to 82 years, average age was 61 years.

IR spectroscopy

Absorption spectra in the mid-IR range (4000−800 cm^{-1}) were recorded using a Tensor 27 (Bruker) IR Fourier-spectrometer equipped with a low-noise MCT-detector (detector of HgCdTe) cooled with liquid nitrogen. Optical paths were purged with dry nitrogen at a flowrate of at least 10 l/min. All spectra were recorded with a resolution of 2 cm^{-1} and averaged over 128 accumulations. Prior to the measurement, blood serum samples were lyophilized with intermediate isotopic substitution of the solvent by D_2O described elsewhere [18,21]. Samples were studied in D_2O solutions using demountable BaF_2 liquid cells with an optical path length of 50 μm. The spectra were primary processed and analyzed using the software supplied with the instrument, as described earlier [22,23].

Results and discussion

The absorption the mid-IR range is due to vibrations of atoms in molecule, which are accompanied by a change in the dipole moment of the bond or chemical group. The absorption of protein molecules in this spectral range is due, first of all, to vibrations in the peptide bond. These vibrations are manifested in the spectra as a number of wide bands, known as „Amide“ bands. Vibrations of C=O and N−H bonds are very sensitive to the change in polypeptide conformation, which allows the absorption spectra of proteins to be used for the analysis of their secondary structure. The most informative from this point of view is the analysis of Amide I band, which is due predominantly to vibrations of the carbonyl group and observed in spectra in the region of 1650 cm^{-1}.

In practice, the recording of IR absorption spectra of aqueous solutions of proteins is complicated by the strong absorption of water (H_2O) in this spectral range. Therefore, the measurements are traditionally carried out after the isotopic substitution of solvent by the heavy water (D_2O) that has a transparency window in the range of Amide I vibrations.

To conduct the comparative analysis, spectra of blood serum samples of healthy donors and patients with MM were recorded. Typical absorption spectrum of blood serum is shown in Fig. 1. In this spectrum a number of absorption bands are clearly distinguished, which are referred to both the polypeptide backbone vibrations and the vibrations of groups in side chains of amino-acid residues [24–27]. The dominating Amide I band of the spectrum is located in the 1700−1600 cm^{-1} range of wavenumbers; this band is due to vibrations of carbonyl groups within the polypeptide backbone. The region of 1600−1500 cm^{-1} contains a superposition of vibrations of side chains and the residue contribution of Amide II band that corresponds to deformation vibrations of groups of the N−H peptide bond located in internal regions of protein molecules where exchange processes of H↔D with the solvent are constrained. A band with its maximum at 1517 cm^{-1} is separately distinguished in this range; this band can be assigned to vibrations of the side chain of tyrosine [24,26]. With the isotopic substitution of solvent, due to the exchange processes in the solution, hydrogen atoms in groups of the N−H peptide bond are mainly substituted by deuterium as well and form the N−D group. As a result, the corresponding Amide II band shifts approximately by 100 cm^{-1} toward lower wave numbers, to 1500−1400 cm^{-1}, and is named as Amide II′ [25].

As already mentioned above, the shape of Amide I band is very sensitive to the secondary structure of proteins [20]. Due to the fact that the MM development is accompanied by a significant change in the protein composition of blood serum [8–10], the analysis of Amide I band allows detecting statistically significant differences in average values of main parameters of the secondary protein structure in the blood serum of patients with diagnosed MM and healthy donors [18,28]. However, this approach is not fully convenient for wide screening because it requires quite labor-intensive processing of each spectrum separately, and it is suitable for automatic analysis to a limited extent. At the same time, to classify samples of patients with MM and healthy donors, no numerical values of parameters of the

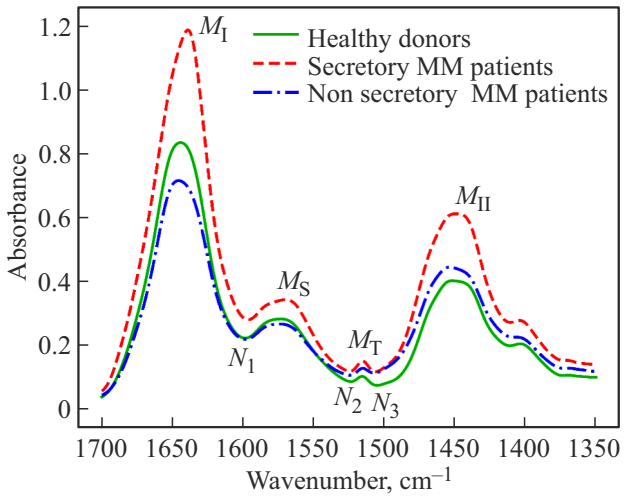

Figure 2. Example of IR absorption spectra of blood serum of a healthy donor (solid line), a patient with secretory myeloma (dotted line), and a patient with non-secretory myeloma (dash-dotted line). The figure shows points of the spectra chosen for the comparison.

secondary protein structure are required. Instead, using the high informativeness of IR spectra, one can try to identify in them various features, which are typical for each group of samples. These features can be the presence or absence of certain bands, positions of their maxima/minima, relative intensities of bands, etc.

Fig. 2 shows typical spectra of blood serum of healthy donor, as well as spectra of patients with secretory and non-secretory MM forms. In this study, we have limited ourselves to the analysis of spectra in the wavenumber range of $1700-1350\,\mathrm{cm}^{-1}$ because earlier it was this range that was identified as the most informative when analyzing differences in the protein composition of blood and serum [18,28,29]. It is worth noting that although the differences in absolute absorption values observed in the spectra reflect objective changes in the protein composition of the blood serum during the development of MM in an individual patient, however, they cannot be used as an independent reliable criterion for classifying samples obtained from different people. In this study the recorded IR spectra were analyzed first of all for the presence of simple characteristic features, such as positions of the most explicit maxima/minima and their corresponding absorption values (Fig. 2), which would allow classifying samples of patients with MM and those of healthy donors.

For clarity, all the ratios between the chosen spectral parameters obtained in this study are normalized to the same ratios for healthy donors. The parameter ratios obtained for each spectrum were averaged with similar ratios for all samples of each group (healthy donors, secretory and non-secretory MM samples). Average values for all parameters demonstrating significant differences between samples of healthy donors and patients with MM are summarized in the table. Root-mean-square deviation for the corresponding group of samples is given as the error.

The samples of secretory MM demonstrate the most significant differences from healthy donors for the parameter ratios located near Amide I and Amide II′ bands. At the same time, ratios of different parameters demonstrate a variety of changes. In particular, ratios of Amide I and Amide II′ absorption bands to the absorption of MS side chains increased in MM samples and were $M_I/M_S = 1.16 \pm 0.02$ and $M_S/M_{II} = 1.15 \pm 0.03$, respectively. On the contrary, the ratio of the M_S side chain absorption band to the M_T tyrosine absorption band for secretory MM samples was lower than that for donors, $M_S/M_T = 0.87 \pm 0.03$. Generally, the obtained results (see the table) make it possible to describe the differences in the spectra of blood serum samples from patients with MM and healthy donors by nine main parameters. The obtained results can be illustrated using a radial diagram (Fig. 3, *a*), which gives a visual representation of the magnitude and direction of changes in the main parameters, which exhibit differences in the spectra of secretory MM and healthy donors.

We have failed to find statistically significant differences in the selected parameters for samples of non-secretory MM (Fig. 3, *b*). This result is caused by two circumstances. First, the similarity of spectra of non-secretory MM and healthy donor samples in the region of vibrations of Amide I–Amide II reflects the similarity of protein composition of these samples. Second, due to the less prevalence of non-secretory MM form, this group contains a considerably lower number of samples, which resulted in a noticeably larger error in determining the spectral parameters to be analyzed. Nonetheless, it must not be ruled out that with sufficient accumulated statistics reliable differences can be identified for this group of samples as well.

Thus, as a result of the comparative analysis of IR spectra of blood serum in the region of $1700-1350\,\mathrm{cm}^{-1}$ 9 parameters were identified, which average values in samples taken from patients with secretory MM form are significantly different from the same parameters of healthy donor serum. We believe that the obtained results are indicative of promising potential of the IR spectroscopy method for the development of a methodically simple, quick and low-cost technique of screening to detect signs of MM development.

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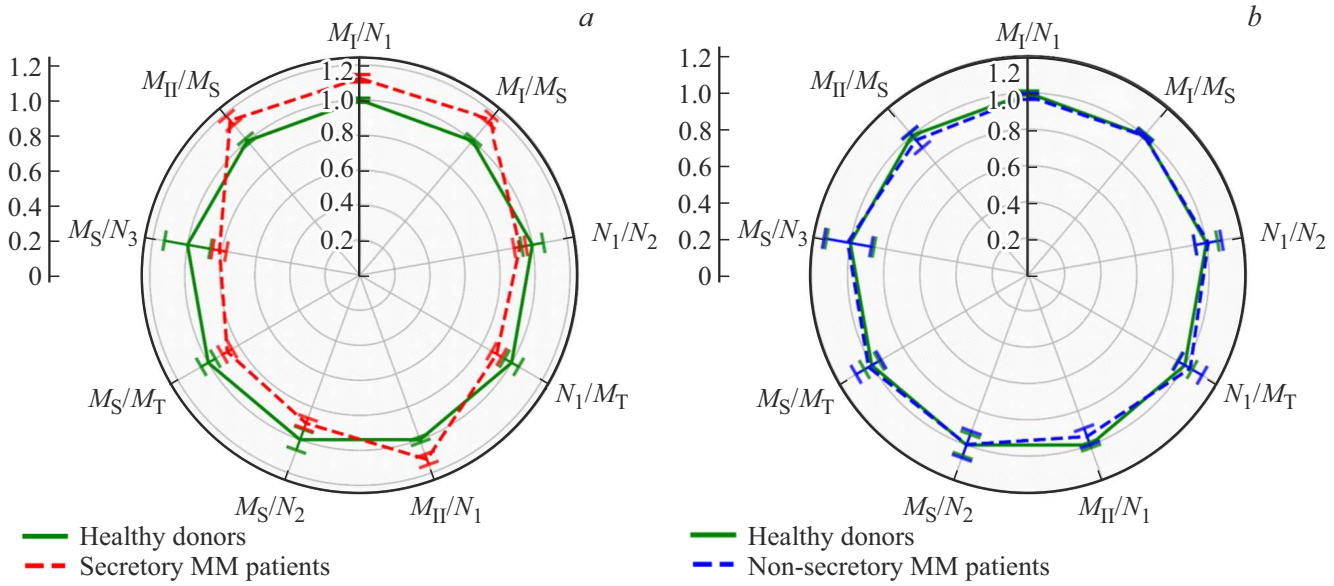

Figure 3. Radial diagram showing ratios between the chosen spectral parameters (table) for the blood serum of healthy donors (solid line) and patients (dashed line) with diagnosed: (*a*) secretory MM form, (*b*) non-secretory MM form.

Normalized* values of absorption ratios in spectral maxima and minima for samples of healthy donors and patients with MM

Ratio	Healthy donors	Secretory MM	non-secretory MM
M_I/N_1	1.00 ± 0.02	1.12 ± 0.02	0.98 ± 0.02
M_I/M_S	1.00 ± 0.01	1.16 ± 0.02	1.00 ± 0.01
N_1/N_2	1.00 ± 0.06	0.93 ± 0.03	1.01 ± 0.08
N_1/M_T	1.00 ± 0.05	0.9 ± 0.03	1.03 ± 0.09
N_1/M_{II}	1.00 ± 0.02	1.12 ± 0.03	0.95 ± 0.05
M_S/N_2	1.00 ± 0.06	0.90 ± 0.03	1.00 ± 0.08
M_S/M_T	1.00 ± 0.05	0.87 ± 0.03	1.02 ± 0.08
M_S/N_3	1.0 ± 0.1	0.81 ± 0.04	1.0 ± 0.1
M_S/M_{II}	1.00 ± 0.02	1.15 ± 0.03	0.97 ± 0.06

* The presented values are normalized to the values of corresponding ratios for healthy donors.

Conflict of interest

The authors declare that they have no conflict of interest. This work does not contain any studies with involvement of human beings as a subject of investigations.

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