Analysis of the secondary structure of blood serum proteins from patients with multiple myeloma

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Abstract. Infrared spectroscopy of biomolecules is one of the few methods, which combine a relatively simple measurements and a possibility of highly informative structural analysis. The most high-demand field of such investigations is the identification of various pathologies accompanied by changes in the biomolecular structure. In this study the comparative analysis of the secondary structure of blood serum proteins from patients with multiple myeloma and healthy donors was performed using infrared spectroscopy. There is a tendency for reduce the proportion of α-helices in the secondary structure of blood serum proteins in patients with multiple myeloma compared to healthy donors. Thus, infrared spectroscopy reveals difference in the secondary structure of blood serum proteins in patients with multiple myeloma and healthy donors.

1. Introduction
Multiple myeloma (MM) is a blood disease, which is accompanied by the production of monoclonal immunoglobulin by plasma cells. MM accounts for about 1% of all types of cancers and about 10% of hematologic cancers. MM is characterized by the proliferation of a single clone of plasma cells, which may produce and secrete a homogeneous monoclonal immunoglobulin [1]. The monoclonal immunoglobulin is commonly referred to as an M protein. The M protein acts as a “tumor” marker and that is useful for diagnosis and disease monitoring. In addition to the increased amount of M-protein in blood serum an increase in β2-microglobulin levels is also observed. However, the level of serum albumin in patients with MM is usually reduced. Ninety percent of myeloma patients have a reduction of one of the uninvolved immunoglobulins [1, 2]. Such dramatic changes in the protein profile of the serum noticeably affect its secondary structure composition, which can be monitored, using infrared (FTIR) spectroscopy.

In this study we compared MM samples with those from healthy donors, and the patients with chronic lymphocytic leukemia (CLL). CLL is classified as a lymphoproliferative disorder characterized by the relentless accumulation of mature B-lymphocytes showing a peculiar immunophenotype in the peripheral blood, bone marrow, lymph nodes and spleen. An increase in the
amount of tyrosine kinase, CD23 and β2-microglobulin usually observed in the blood serum from patients with CLL [4].

The aim of this study is to analyze the secondary structure composition of blood serum proteins in patients with multiple myeloma in comparison with patients with chronic lymphocytic leukemia and healthy donors using the FTIR spectroscopy.

2. Materials and methods

2.1. Serum samples
Blood samples were collected in Russian Scientific Research Institute of Hematology and Transfusiology from the patients, prepared according to the standard procedure. To obtain the samples of the serum S-Monovette tubes (Sarstedt, Germany) with clotting activator were used. The collected blood samples rested in the tubes for 30 minutes at the room temperature to allow complete interaction with the clotting activator, followed by centrifugation for 15 min at 3000 rpm (Thermo Scientific Heraeus Labofuge 200, USA). The collected serum was used for the EF analysis within 2 hours. The rest of the samples were frozen (–30 °C) and kept for the spectroscopic analysis. In this study we analyzed 45 samples from patients with multiple myeloma (40 liquid and 5 freeze dried), 7 samples from patients with chronic lymphocytic leukemia and 65 liquid samples from healthy donors (55 liquid and 10 freeze dried).

2.2. IR spectroscopy
The ATR absorption spectra in the mid-infrared region (4000 — 400 cm⁻¹) were obtained using Nicolet 8700 FTIR spectrometers (Thermo Scientific, United States) equipped with an artificial diamond ATR cell as described elsewhere [5, 6]. The ATR correction was performed using the software supplied by the manufacturer. The spectra of each sample were recorded with a resolution of 2 cm⁻¹ and averaged by 256 accumulations. For liquid serum samples the spectrum of saline was subtracted [6] followed by 15-point Savitsky-Golay smoothing.

3. Results and discussion
Serum samples obtained from patients with MM and from healthy donors have been analysed using attenuated total reflectance (ATR) Fourier transformed infrared spectroscopy (FTIR) in the mid-IR region. The vibrations of C=O and N–H bonds (peptide bonds) of the peptide bonds manifest themselves in the spectra as several strong bands known as the “amide” bands. These vibrations are sensitive to changes of the polypeptide backbone conformation, which allows using the absorption spectra of proteins for the analysis of their secondary structure [6-8].

The most reliable results in determination of the protein secondary structure by IR spectra are provided by the analysis of the superposition of the vibrations forming the amide I band in the region of 1700-1600 cm⁻¹. The number of vibrations and the position of the corresponding bands in the absorption spectra were determined from the analysis of their second derivatives [9]. The contribution of each type of the secondary structure was estimated from the contribution of the corresponding contour to the decomposition of the particular spectrum (Table 1).

Figures 1 and 2 shows typical amid I band and its decomposition to the hidden peaks.
Table 1. Band assignments of the subcomponent bands of the amide I absorption [8, 10, 11]

| Structure          | Wavenumber Range  |
|--------------------|-------------------|
| β-sheet            | 1620-1640 cm⁻¹    |
|                    | 1680-1690 cm⁻¹    |
| Associated β-layer | 1615-1628 cm⁻¹    |
| Random coil        | 1640-1650 cm⁻¹    |
| α-helix            | 1650-1658 cm⁻¹    |
| Loop               | 1669-1680 cm⁻¹    |

Figure 1. Amid I bands and hidden peaks of IR spectra

A – liquid sample of blood serum in patients with multiple myeloma; B – liquid sample of blood serum in healthy donor (green line – α-helix, blue line – β-sheet)
The analysis of liquid samples revealed decreasing amount of $\alpha$-helices in the MM samples (60 % for the healthy donors, 55 % for patients with MM and 65 % for patients with CLL). The increase in the number of $\beta$-sheets in serum proteins in patients with multiple myeloma in comparison with healthy donors and patients with CLL was also observed (42 % vs. 37 % and 34 %, respectively). In addition, the increased number of associated $\beta$-layers in serum proteins in patients with multiple myeloma was noted (Table 2). The latter most likely indicates increasing intermolecular interactions between immunoglobulin molecules in serum from MM patients.

![Figure 2](image_url)

**Figure 2.** Amid I bands and hidden peaks of IR spectra

A– freeze dried sample of blood serum in patients with multiple myeloma; B – freeze dried sample of blood serum in healthy donor (green line – $\alpha$-helix, blue line – $\beta$-sheet, orange line – random coil)

Thus, the secondary structure composition of the proteins in the serum of patients with MM differs from healthy donors and patients with CLL. At the same time, samples from the patients with CLL,
having only minor alterations from healthy donors in serum composition, demonstrate protein structure similar to one of healthy donors, which confirms the reliability of our analysis.

The results of the analysis of the freeze dried samples (from 5 patients with MM and 10 healthy donors) demonstrate significantly differ from the corresponding liquid MM samples accompanied by increasing amount of the random coils. However, the secondary structure of serum proteins in healthy donors remained almost unchanged.

Table 2. Secondary structure type content in liquid samples of blood serum

| Type            | Wave number | Donors | Multiple myeloma | Chronic lymphocytic leukemia |
|-----------------|-------------|--------|------------------|-----------------------------|
|                 |             | Mean, % | Mean, % | Mean, % | Mean, % | Mean, % | Mean, % | Mean, % | Mean, % |
| α-helix         | 1650-1658 cm⁻¹ | 60,69 | 0,07 | 55,46 | 0,11 | 64,98 | 0,08 |
| β-sheet         | 1620-1640 cm⁻¹ | 23,35 | 0,03 | 29,00 | 0,08 | 24,00 | 0,07 |
|                 | 1680-1690 cm⁻¹ | 1,99 | 0,01 | 1,77 | 0,01 | 1,00 | 0,01 |
| Associated β-layer | 1615-1628 cm⁻¹ | 5,71 | 0,03 | 6,29 | 0,03 | 4,16 | 0,01 |
| Loop            | 1669-1680 cm⁻¹ | 5,92 | 0,02 | 5,25 | 0,02 | 4,83 | 0,01 |
| Random coil     | 1640-1650 cm⁻¹ | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |

SD – standard deviation

The number of α-helices which remain the most common type of secondary structure was 57 % vs. 36 % in healthy donors and patients with multiple myeloma, respectively. The number of random coils in samples of serum in patients with MM was approximately 18 %, whereas there were no random coils in samples of serum in healthy donors (Table 3). Such a behavior might indicate that the changes in M-protein structure, observed above for the liquid samples, resulted in considerable changes in their condensation and/or aggregation ability, clearly manifested in the structure of the dried samples.

Table 3. Secondary structure type content in freeze dried samples of blood serum

| Type            | Wave number | Donors | Multiple myeloma |
|-----------------|-------------|--------|------------------|
|                 |             | Mean, % | Mean, % | Mean, % | Mean, % | Mean, % | Mean, % | Mean, % |
| α-helix         | 1650-1658 cm⁻¹ | 57,39 | 0,04 | 35,52 | 0,15 |
| β-sheet         | 1620-1640 cm⁻¹ | 25,08 | 0,02 | 21,00 | 0,16 |
|                 | 1680-1690 cm⁻¹ | 2,30 | 0,01 | 3,43 | 0,01 |
| Associated β-layer | 1615-1628 cm⁻¹ | 5,14 | 0,02 | 10,09 | 0,05 |
| Loop            | 1669-1680 cm⁻¹ | 8,14 | 0,01 | 7,96 | 0,04 |
| Random coil     | 1640-1650 cm⁻¹ | 0,00 | 0,00 | 18,20 | 0,21 |

SD – standard deviation
4. Conclusion
Thus, application of the FTIR spectroscopy allows one to monitor structural changes in the structure of serum proteins and spectroscopically discriminate serum of healthy donors and patients with multiple myeloma. We suggest that, the hyperproduction of M-protein might increase intermolecular interactions between immunoglobulin molecules in serum of MM patients.

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