Protein secondary structure analysis of serum from patients with oncohematological diseases

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Abstract. One of the promising approaches for diagnosing oncohematological diseases is infrared spectroscopy of blood serum. In this work secondary structure of blood serum proteins of patients with multiple myeloma, chronic lymphocytic leukemia and healthy donors was studied using IR spectroscopy. As a result of the study, it was found that the secondary structure of blood serum proteins in patients with chronic lymphocytic leukemia does not change in comparison with healthy donors. In contrast, patients with multiple myeloma have significant differences in the secondary structure composition of serum proteins compared to healthy donors. We conclude, that IR spectroscopy makes it possible to distinguish serum of healthy donors and patients with multiple myeloma, leading to the potential applicability of this approach to the diagnosis of multiple myeloma.

1. Introduction

The development of effective approaches for the diagnostics and treatment of oncological diseases today is one of the most important tasks of medicine. Multiple myeloma is a blood cancer, which is characterized by the accumulation of large amount of malignant B-lymphocytes in the bone marrow and blood the bone marrow producing a monoclonal immunoglobulin (M-protein) [1, 2]. Overproduction of the M-protein leads to a change in the average parameters of protein secondary structure in blood serum. Therefore, the analysis of the secondary structure of serum proteins gives us the opportunity to reveal the disproportion in the protein composition in the serum [3]. We used infrared spectroscopy, as one of the most sensitive techniques for secondary structure analysis [4, 5]. In this study, the applicability of this approach for the diagnosis of multiple myeloma (MM) and chronic lymphocytic leukemia (CLL) was tested.
2. Materials and methods
In this study we analyzed FTIR spectra of serum samples from patients with multiple myeloma (MM) and chronic lymphocytic leukemia (CLL) provided by the hematology clinic of the Russian Research Institute of Hematology and Transfusiology (St. Petersburg, Russia), where blood serum samples from MM, CLL patients and healthy donors were collected and characterized. In total, 25 samples of blood serum from healthy donors, 20 from patients with MM and 10 from patients with CLL were studied. The samples were studied in D$_2$O solutions using Tenor27 FTIR spectrometer (Bruker) in demountable CaF$_2$ cells with an optical path length of 50 μm and a resolution of 2 cm$^{-1}$ according to the method described earlier [3, 6]. The primary processing and analysis of the spectra was carried out using the software supplied with the instrument.

3. Results and discussion
Peptides and proteins contain numerous amide groups in their structure, the vibrations of which are highlighted in the vibrational spectra of proteins. The characteristic frequencies of these vibrations are located near 1650, 1540 and 1240 cm$^{-1}$ and usually are called the vibrations of Amide I, Amide II and Amide III, respectively. The form of these vibrations depends on the change in the length of the C = O peptide bond, the angle CNH and the length of the CN bond [7].

In the obtained absorption spectra, we analyzed Amide I (1700–1600 cm$^{-1}$) band, which is a superposition of vibrations corresponding to different conformations of the polypeptide chains. These vibrations provide information about α-helices, β-structures of various types, and disordered regions in the protein [3]. The relationship between the position of the bands and the secondary structure was established using Table 1.

| Secondary structure | Range (cm$^{-1}$) |
|---------------------|------------------|
| α-helix             | 1648 – 1657      |
| β-sheet              | 1618 – 1641      |
| Turn                | 1670 – 1695      |
| Disordered structure| 1642 – 1657      |

Table 1. Correspondence of the position of the main bands in the degradation of "Amide I" to different types of the secondary structure of the polypeptide chain [8-12].

To decompose the Amide I band into components, the spectrum of the second derivative was analyzed as described earlier [8, 13]. The contribution of each type of the secondary structure was estimated as the area under the corresponding contour in ratioed to the total area of the Amide I band. This approach has been widely used earlier to determine the secondary structure of various proteins [3, 6, 13, 14]. We applied similar approach to analyze secondary structure composition of total serum protein. The results of Amide I band decomposition for samples from healthy donors and patients with MM and CLL are shown in Figures 1-3 and the corresponding secondary structure assignment is summarized in Table 2.
Figure 1. Decomposition of the Amide I band of a MM serum spectrum: bands 1, 4, 5 - β-sheets; band 2 - α-helix; band 3 – turns.

Figure 2. Decomposition of the Amide I band of a healthy donor serum spectrum: band 1 - α-helix; bands 2, 4 - β-sheets; band 3 – turns.
Figure 3. Decomposition of the Amide I band of a CLL serum spectrum: band 1 - α-helix; bands 2, 4 - β-sheets; band 3 – turns.

Table 2. The percentage of certain types of secondary structures in serum samples from healthy donors, patients with MM and CLL.

| Secondary structure | Donors | MM     | CLL     |
|---------------------|--------|--------|---------|
| α-helix             | 48 ± 1 | 36 ± 6 | 48 ± 1  |
| β-sheet             | 44 ± 1 | 52 ± 4 | 44 ± 1  |
| Turns               | 8,1 ± 0,2 | 12 ± 3 | 8,2 ± 0,2 |

Based on the obtained results differences in the secondary structure composition of total serum protein in samples from healthy donors and from the patients with MM were revealed. The most characteristic difference is a decrease in the proportion of α-helical regions by 10-12% and an increase in the content of β-structures by 5-8% in patients with MM compared to healthy donors. At the same time, comparison of the secondary structures of blood serum proteins from healthy donors and patients with CLL did not reveal any differences, which suggests that this disease does not affect the secondary structure composition of serum proteins.

4. Conclusion
Thus, it can be concluded that the suggested approach is not suitable for the diagnosis of CLL. Further research to reveal additional spectroscopic criteria are required. As for the diagnosis of MM, the described approach demonstrated rather good results. However, in its current state, the method also has some limitations. In particular, the absence of the pathological M-protein in serum, which is
typical for the non-secreting form of multiple myeloma (less than 5% of the total number of MM cases), does not allow one to detect such a disease using secondary structure analysis. Therefore, for the application of this approach for the diagnosis of MM, certain improvements are also required.

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