Optical analysis of middle-molecular mass molecules of blood of individuals suffering from myocardial ischemia

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Abstract

The normalized absorbtion and excitation luminescence spectra of middle mass molecules (MMM) obtained from the blood of individuals suffering from myocardial ischemia, show significant changes in the microenvironment of tryptophan fragments in the composition of peptide components in comparison to the spectra of MMM obtained from the blood of healthy individuals.

Key words: middle mass molecules, optical analysis, myocardial ischemia.

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Introduction

The study of the molecular processes of the pathogenesis of myocardial ischemia is the most actual problem in modern cardiology. It is known that an insufficient supply of oxygen to the myocard leads to the activation of free radical processes [1], of lipid peroxidation and consequently, to serious biochemical and morphological changes in the heart muscle, and also causes destructive processes that are accompanied by an increase in ischemia endotoxins [2]. Since ischemia toxins are among the middle mass peptides (MMM) of the blood [3], a study of their nature and physiochemical properties are of significant interest in cases of myocardial ischemia. We have shown that the amount of middle molecular mass molecules in the blood of individuals suffering from myocardial ischemia is twice as that in the norm.

Experimental methods

Optical absorption spectra were recorded using a spectrophotometer "Specord" (Germany). Emission spectra and excitation luminescence spectra were obtained using the equipment with two double graphing monochromators and detected by a photomultiplier. For exitation of luminescence a N_2-laser was used ($h\nu$=3.68eV). A halogen lamp was also used for obtaining the excitation luminescence spectra, correcting the spectral distribution in lamp emission, sensitivity of photomultiplier and transmission of monochromator.

Contents MMM (medium mass molecules) of the blood plasma of healthy and patient persons were detected according to Nicolaechicu [4]. Spectral characteristics of converting in electrical file, were obtained using a programming packet "WinDig". The luminescence and excitation luminescence spectra were measured by the luminating image found with the help of lamps overleaped with quarted balloon capability 240 Wt with spectre of measurement capturing UV part correction of spectral imaging performed on PC.

Absorption, emission and excitation luminescence spectra were examined using a Peak Fit program to determine spectral position bands maxima, their relative intensities and bandurithes.

Discussion

Absorption spectra of medium mass molecules isolated from the serum of a healthy man's blood and the one from the serum of the blood of the person who suffers from ischemic heart disease are shown in Fig.1.

Within these spectra it is possible to note an intensive band of absorption with the maximum of 290nm. In a shorter wave part of the spectrum it is possible to see some components within a pre-wave decrease of the very intensive band of absorption with a maximum value in the area, which is less than 220nm. Absorption in this area of spectrum is associated with by electronic transitions in peptides.

The absorption band at 290nm is related with hytherocyclic indolil rings of tripthophane residues in peptides with thyrosin or phenilalanine [3].

Absorption spectra shown in Fig.1 was measured at similar concentrations of medium mass molecules.

The absorption spectra of value of absorption of MMM of the person who suffers ischemic heart disease is much more intensive as compared with the value of absorption of a healthy person. This difference can be used for elaboration of a method of diagnostics of the ischemic heart disease.

In order to realise a more detailed comparison of spectrums we have researched their further processing.

Data concerning the spectrum of absorption for a person who suffers an ischemic heart disease are shown in table 1. The proper appearance of the spectra resolved into gausses can be seen in fig.2 (for a person who suffers an ischemic heart disease) and in fig.3 (for a healthy person).

From the comparison of the data, suggested in tables 1 and 2 it is possible to make the following conclusions:

1. it has been shown that all the absorption bands of MMM taken from individuals suffering from myocardial ischemia, are twice as intensive as in the case of healthy individuals that is a shift in the absorption maximum, of 1nm toward the spectra of

Table 1. Spectral parameters of absorption of a patient.

№	Intensitya. un.	maximum position, nm	mid-width, nm	energy,eV
1	136.64	247.8	14.9	5
2	6.17	252.5	1.86	4.91
3	10.27	258.65	2.51	4.79
4	9.24	264.87	2.05	4.68
5	5.7	269.13	1.22	4.61
6	56.12	291.63	13.66	4.25

Table 2. Spectral parameters of the bands of absorption of a healthy person.

№	Intensity,a. un.	maximum position, nm	mid-width, nm	energy, eV
1	77.92	247.72	26.66	5.01
2	3.29	248.3	1.86	4.99
3	5.63	253.28	1.95	4.9
4	10.02	259.7	2.87	4.77
5	7.22	266	1.86	4.66
6	4.76	269.7	1.12	4.6
7	25.603	292.89	12.27	4.23

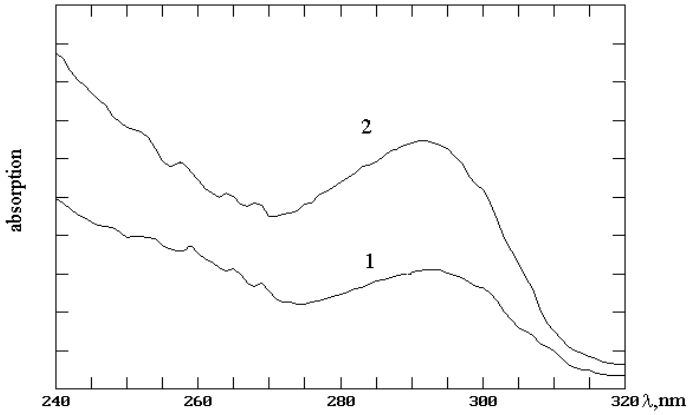

Fig.1. Absorption spectrums of the solution of medium mass molecules of blood of a healthy person (1) and patient suffering ischemia (2).

Table 3. Spectral parameters of the absorption bands of the molecules of tryptophane, phenilalanine and tyrosine.

	tryptophane	phenilalanine	tyrosine
Long wave, nm	271	253.9	274.4
	278.4	257.3	-
	287.5	263.3	-

MMM taken from individuals suffering from myocardial ischemia;

2. it was determined that all the bands of absorption, of a person, are approximately twice as intensive as those of a healthy person;
3. half-width of absorption in the area of 290nm is less than a half-width of the band of absorption in the area less than 290nm;
4. in a pre-wave recession of the short-band intensive band of absorption maximum of λ=220nm, of a healthy person, a supplementary component at λ=245nm is observed, which is not detected in the case of a patient.

Fig.4 shows normalized spectra with the maximum of absorption of 290nm. In fig.5 the difference between them is shown.

It is seen that a different spectrum of absorption is differently signed. It certifies that in the areas of approximately 270-290nm and 240-270nm and 290-320nm the concentration of

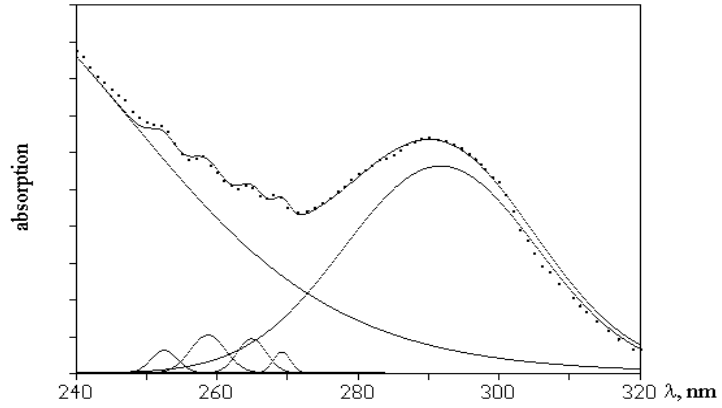

Fig.2. Resolution in gausses of absorption spectra of a patient.

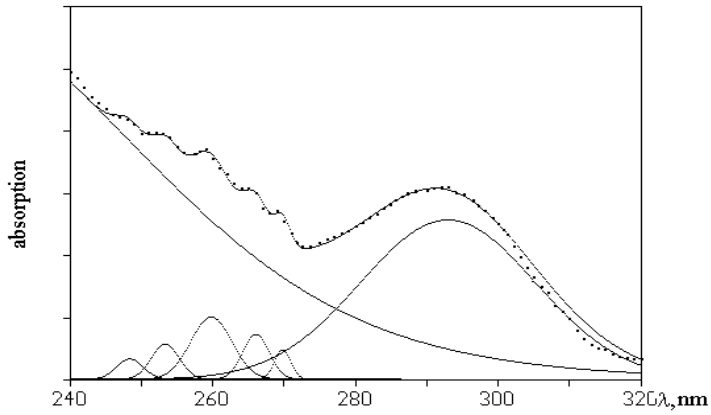

Fig.3. Decomposition in gausses of absorption spectra of a healthy person.

medium mass molecules of a patient and healthy persons relevantly prevails. Especially interesting is the area of absorption at of λ>300nm, in which different spectra indicate the existence of an absorption band at λ=315nm. For identification of the absorption spectrums MMM of blood spectrums of absorption of tryptophane, tyrosine and the phenilalanine were measured additionally.

In fig.6 a luminescent spectrum of a patient who suffers an ischemic heart disease is shown. It has been obtained during stimulation of the solution of the molecules of medium mass of blood from the area of absorption blends (λ>300nm).

Using the Peak Fit program the main spectral parameters were found (see table 5).

Luminescence spectral parameters of a patient person were as follows: intensity 0.0379 a. un; maximum position 363.06nm; mid-width 21.78 nm; energy 3.41 eV.

The analysis of the data indicates that

Table 4. Luminescence spectral parameters of bands of a patient.

№	Intensity, a. un	maximum position, nm	mid-width, nm	energy, eV
1	0.665	217.6	4.15	5.7
2	0.25	224.4	2.04	5.53
3	0.287	242.29	4.47	5.12
4	0.15	250.7	3.34	4.95
5	0.45	261.7	9.67	4.74
6	0.71	292.9	20.07	4.23
7	0.7	321.3	8.7	3.86

Table 5. Spectral parameters of bands of excitation of a healthy person.

№	Intensity, a. un	maximum position, nm	mid-width, nm	energy, eV
1	0.77	211.97	4.64	5.85
2	0.43	221.6	3.01	5.6
3	0.31	240.8	4.96	5.15
4	0.36	255.4	2.85	4.86
5	0.36	260.9	2.36	4.75
6	0.39	267.8	2.85	4.63
7	0.68	286.9	10	4.32
8	0.98	318.9	11.79	3.89

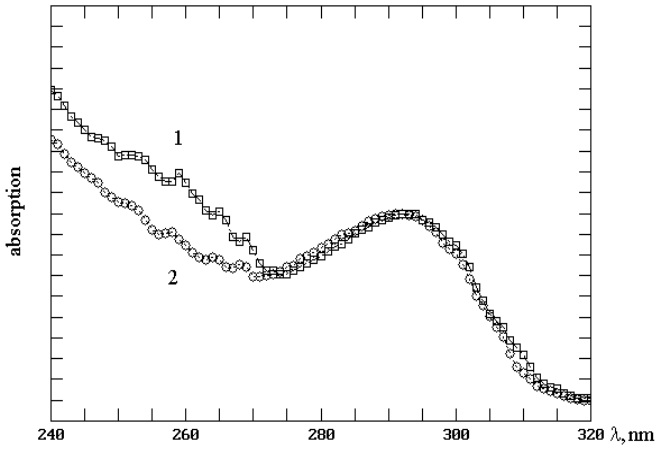

Fig.4. Difference of the normalized I_{290} absorption spectrums of a healthy person (1) and a patient who suffers ischemia (2).

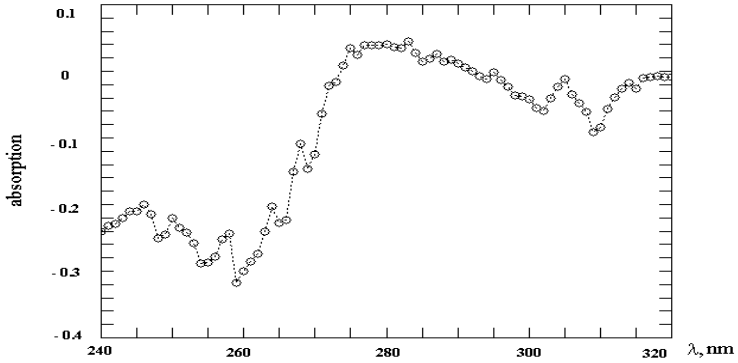

Fig.5. Difference of the normalized spectra of absorption (a patient -a healthy person).

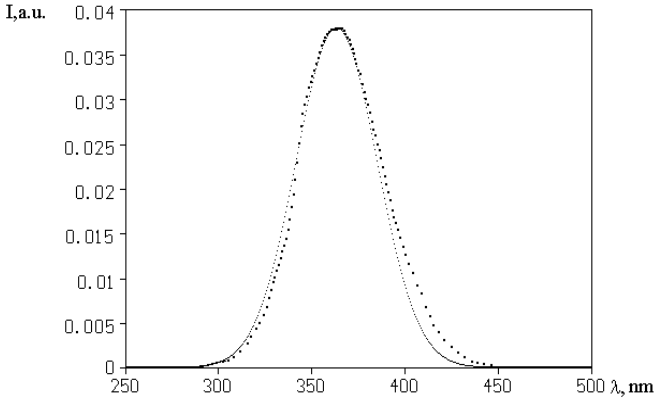

Fig.6. Luminescence spectral parameters of a patient who suffers an ischemic heart disease.

luminescence takes place in the area with the maximum of λ=363.06nm. Its intensity is small. Luminescence is insignificant. Therefore why it was impossible to find the difference between luminescent spectra of a patient and healthy persons.

Excitation luminescence spectra of MMM blood, separated from the serum of blood from a healthy person and a person who suffers an ischemic heart disease are shown in fig.7. Several intensive bands are observed in the spectrum. In fig.7 we can see that a patient spec-

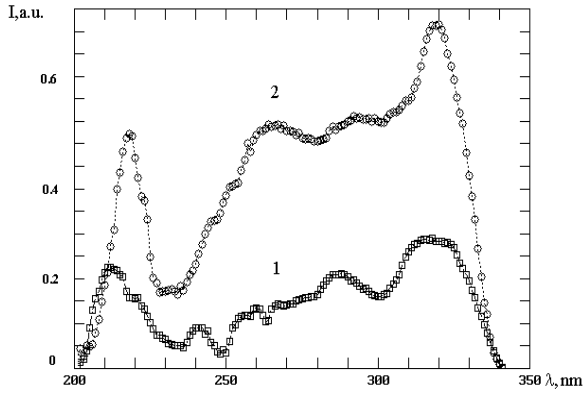

Fig.7. Spectra of excitation of luminescence for a patient (2) and healthy persons (1).

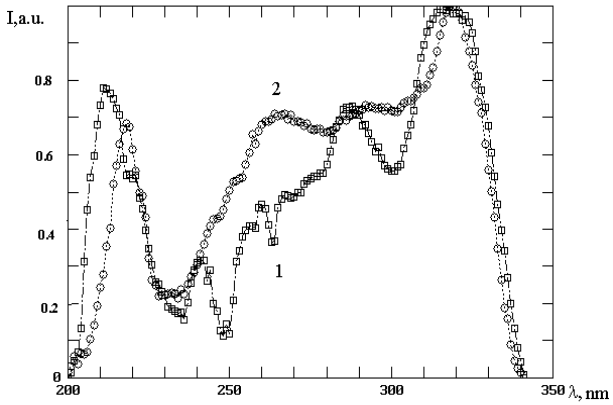

Fig.8. Resulted spectra of excitation of luminescence of a patient (2) and healthy persons (1)

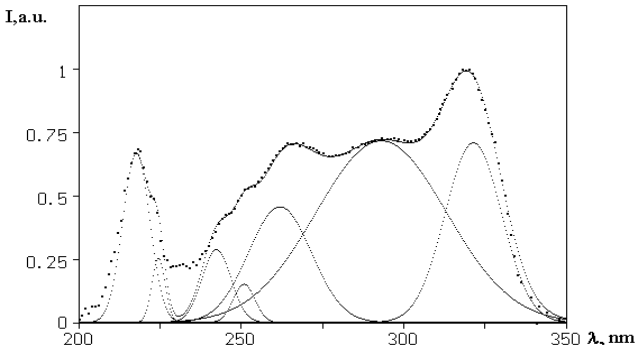

Fig.9. Decomposition in gausses of spectrums of excitation of luminescence of a patient.

trum is more intensive. Comparing excitation spectrums of luminescence and absorption a strong correlation between them is observed.

For more detailed comparison of the spectrums we examined their further processing. First both spectra were normalized with respect to I_{max}. The plots of normalized spectra are shown in fig.8.

The results of processing luminescence excitation spectra for a person who suffers an ischemic heart disease and a healthy one are shown in tables 4 and accordingly.

In order to carry out further processing a program Peak Fit was used, spectral characteristics thus being obtained.

Spectrum appearance decomposed in gausses is shown in fig.9 (for a patient who suffers an ischemic heart disease) and in fig.10 (for a healthy person).

The analysis of the data shown in table 5

indicates a good correlation between excitation spectra of luminescence and absorption.

In order to conduct further analysis the difference between excitation spectra of luminescence of a patient and healthy person was examined. It testifies that in the areas of about 200-210nm and 310-350nm concentration of MMM of a patient person prevails comparing to the areas of about 240-290nm of a healthy person.

It can bee seen that a different spectrum (Fig.11) has this feature. In this spectrum the area band of in 315-320nm is clearly defined.

The spectrophotometric analysis of solutions of MMM molecules extracted from the blood of a healthy person indicated that the latter intensively absorb light in the ultraviolet range of spectrum with a maximum absorption of 290.9nm. At the same time, electron spectra of solutions of middle-mass molecules from the blood of individuals suffering from ischemia were characterised by a maximum of 291.6nm intensively twice above the norm. In the comparative investigation of fluorescence spectra, it turned out that for middle mass molecules of individuals suffering from myocardial ischemia there is a hypochromic effect of 316-317nm in relation to the solutions of a MMM of a healthy person. The presence of such an extreme short-wave structured component of the spectrum allowed to place this peptide fraction of MMM in the S class [5]. The hyperchromic effect of the area of the spectrum under discussion shows that MMM peptides in the blood of individuals

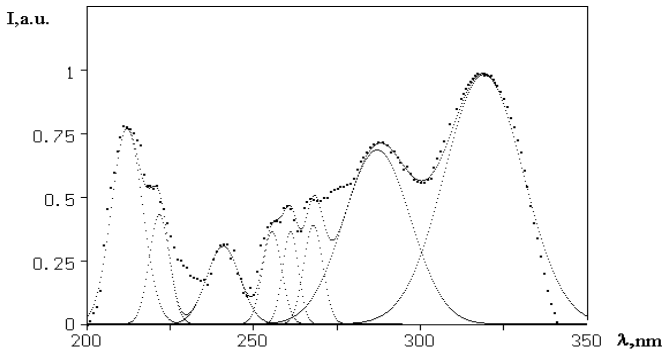

Fig.10. Decomposition in gausses of spectrums of excitation of luminescence of a healthy person.

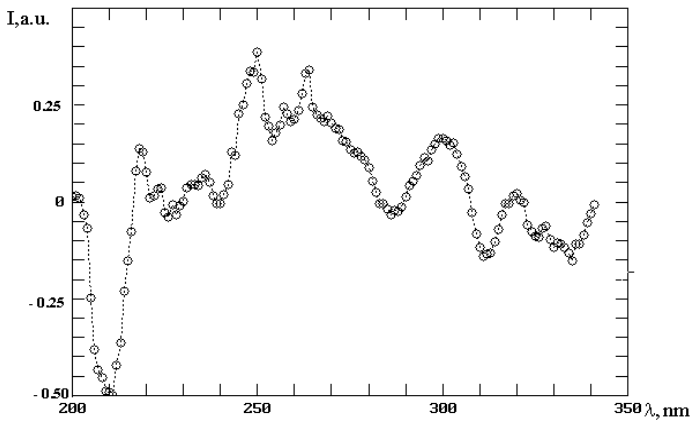

Fig.11. Difference of the normalized spectra of excitation of luminescence (patient-healthy).

suffering from myocardial ischemia differ from similar molecules within the norm by the character of the local steric microenvironment of tryptophan fragments in the peptide component.

References:

1. Diaz M., Frei B., Vita J.et. al. New England J. Med. (1997) p.337-404.
2. Baraboy V.A., Sutkovoy D.A. Oxydative-antioxidant homeostasis in norm and in pathology. Kiev: Cernobylinterinform. part1. (1997). 203p. (in Russian)
3. Oksman T.M., Dalin M.V. Pathogenesis of early postischemisia disorders. Tissue hypoxy and it's correction. Moscow: Nauka, (1981). P (in Russian)
4. Nikolaichik V.V., Moin V.M., Kirovsky V.V. et al. A metod for the detection of "medium-sized molecules", Lab. Delo. №10. (1993) p.11-18. (in Russian).
5. Burstein E.A. Protein fluorescence: nature and applications. Moscow. (1977).