Assessment of tryptophan content in the blood oropharyngeal cancer patients after covid-19. A simple method analysis

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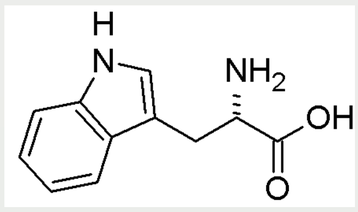
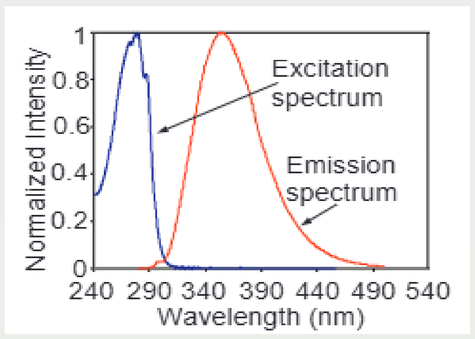
INTRODUCTION

It is known that the clinical manifestations of various diseases correspond to a number of metabolic disorders. Therefore, the determination of the content of certain metabolites in the blood is one of the traditional methods for assessing the patient's condition, the effectiveness of treatment and the prognosis of possible complications. A comprehensive assessment of the content of free amino acids in blood plasma is informative for the diagnosis of oncological diseases. However, the implementation of this method in wide practice is limited due to the need to use complex laboratory equipment and expensive reagents. Tryptophan has unique luminescent properties, which allows its quantitative determination by the fluorometric method.



Tryptophan is an α-amino acid that is used in the biosynthesis of proteins. Tryptophan contains an α-amino group, an α-carboxylic acid group, and a side chain indole, making it a polar molecule with a non-polar aromatic beta carbon substituent. Tryptophan is also a precursor to the neurotransmitter serotonin, the hormone melatonin, and *vitamin B3*



Typical excitation and emission spectra of tryptophan show that the UV source must operate over 260-290 nm wavelengths, the fluorescence peaks over 340-360 nm
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The emission spectra of tryptophan has a strong dependence on pH

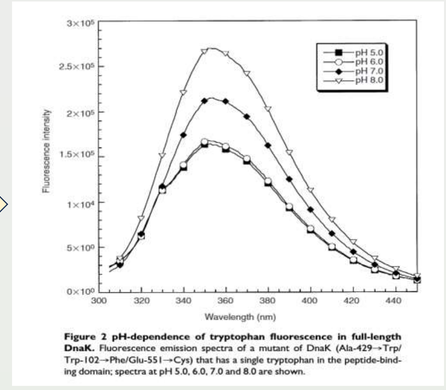

Figure 2 pH-dependence of tryptophan fluorescence in full-length DnaK. Fluorescence emission spectra of a mutant of DnaK (Ala-429→Trp/Trp-102→Phe/Glu-551→Cys) that has a single tryptophan in the peptide-binding domain; spectra at pH 5.0, 6.0, 7.0 and 8.0 are shown.

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EXPERIMENTAL METHODS

The work examined the blood plasma of 17 patients with laryngeal cancer after suffering from COVID-19 and 3 blood plasma for reference samples. Plasma samples were obtained by centrifugation at 4000 rpm for 20 minutes. A mixture of plasma from three healthy people was used as a control. Precipitation of high-molecular proteins and peptides was carried out by adding an equal volume of 10% perchloric acid and centrifugation. After that, the blood plasma samples were additionally dissolved in phosphate buffer (Na_2HPO_4), with the final pH value being 7.5.

Fluorescence spectra were measured on an Edinburgh Instruments FS 5 spectrophotometer in the wavelength range from 290 to 550 nm under excitation with a halogen lamp at a wavelength of 280 nm. To quantitatively assess the tryptophan content in blood plasma, a calibration curve was first constructed based on the fluorescence of a tryptophan solution at known concentrations in a system that was similar in composition to that used in the preparation of the samples under study.

RESULTS AND DISCUSSION

To establish optimal experimental conditions, calibration curves were constructed in buffers with different pH to determine the concentration of tryptophan.

Samples	Intensity		C Trypt, mkMol/l
e1	29165	--	1.6
e2	41566	--	2.4
e3	35366	--	2.0
c35	25712	--	1.3
c45	43976	--	2.6
c49	52906	--	3.2
c57	53530	--	3.2
d1	37995	--	2.2
d2	34844	--	1.9
d3	43353	--	2.5
d4	35787	--	2.0
d5	31474	--	1.7
d6	38197	--	2.2
d7	32957	--	1.8
d8	36411	--	2.0
d9	39983	--	2.3
d10	59208	--	3.6
d11	34322	--	1.9
d12	54591	--	3.3
d13	46503	--	2.7

Concentration dependences of tryptophan emission spectra in different buffers

pH=5,5 **pH=6,0** **pH=7,5**

BUF #1 (pH6) = 2ml Na_2HPO_4 (phosphate buffer) + 0.5ml $HClO_4$ (aqueous solution 5 %)
BUF #2 (pH7) = 2ml Na_2HPO_4 (phosphate buffer) + 0,05ml $HClO_4$ (aqueous solution 10 %)

Calibration curve for determination of the concentration of tryptophan in experimental samples, obtained from buffers with different pH

calibration curve

The experimental data show that the content of free tryptophan in the blood plasma of cancer patients indicate a different direction of changes compared to the control. It could be a synergy of different effects of two pathological processes.

- On the one hand, the intensification of tissue breakdown characteristic of cancer processes leads to changes in the composition of free amino acids in the bloodstream.
- On the other hand, it has been shown that in patients with COVID-19 and patients with oncopathology, a decrease in the level of tryptophan in the bloodstream is observed.
- The detected differences between the indicators of individual patients indicate a different ratio of the manifestation of at least these two processes.

CONCLUSIONS

The data obtained in this work indicate the feasibility of prolonged determination of the content of free tryptophan in the blood plasma of cancer patients who have suffered COVID-19 or have been exposed to other viral diseases.

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