ABSORPTION AND FLUORESCENCE MODIFICATIONS OF TUMORAL TISSUE PROTEINS

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Abstract. The possible 3D alterations of proteins extracted from tumoral tissues are, ultimately, the consequence of the primary structure modifications, due to the alterations of some genes involved in the synthesis of tumor tissue proteins. The possible modifications of tumoral tissue proteins can be evidenced by absorption and fluorescence spectroscopy methods, these modifications influencing the absorption and fluorescence behavior of the protein intrinsic fluorophores (i.e., aromatic amino acids). Absorption and fluorescence measurements were performed on biochemical samples obtained from mature Wistar rats bearing Walker tumours. Both tumour tissues as well as normal tissues from the hosts were harvested in order to extract the proteins. The absorption spectra were recorded in the ultraviolet and visible ranges, between 200 nm and 700 nm, while fluorescence excitation spectra were recorded in the spectral range 200 – 320 nm, at the Tryptophan (Trp) emission wavelength: λ_{em} = 348 nm. The fluorescence emission maxima, for all samples, are situated very close to that of the free molecules of Trp in water, this meaning that, the side chains of Trp are not shielded by the matrix of the proteins extracted from tumour tissues, this suggesting a slight denaturation of tumour proteins. The experimental data, judged by the absorption and fluorescence intensities, are confirming the findings that the tumor tissues have an increased metabolic activity as compared to the normal tissues. Therefore, the protein synthesis occurring in tumoral tissues is more intense than in normal tissues. At the same time, the quality of tumoral tissue proteins seems to be different both in amino acid compositions and 3D protein folding as compared to proteins extracted from normal tissues.

Key words: absorption spectra, aromatic amino acids, fluorescence spectra, excitation spectra, tumor tissue proteins.

INTRODUCTION

The starting hypothesis/assumption of the present study was that proteins extracted from the tumoral tissues could present subtle folding modifications as compared with those extracted from different normal tissues of the tumor host. These 3D alterations, if they exist, are the result of the primary structure modifications, due to the modifications of some genes involved in the synthesis of proteins.

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These possible modifications can be revealed by absorption spectroscopy and, especially, by fluorescence methods, because these modifications could impair both the absorption and fluorescence behavior of the intrinsic fluorophores encountered into proteins (i.e., aromatic amino acids) [1].

The spectral absorption and fluorescence parameters of the aromatic fluorescent amino acids (in aqueous solutions, at pH = 7.0) are presented in Table 1.

Table 1

Absorption and fluorescence characteristic parameters of three natural fluorophores (i.e. tryptophan, tyrosine and phenylalanine) occurring in proteins (aqueous solutions at pH = 7.0). λ: wavelength; ε_{max}: molar coefficient of absorption; τ_F: fluorescence lifetime [2]

Fluorophore	Absorption λ_{max} (nm)	$\varepsilon_{max}\times10^{-3}$ ($M^{-1}cm^{-1}$)	Fluorescence λ_{max} (nm)	τ_F (ns)
Tryptophan (Trp)	280	5.6	348	2.6
Tyrosine (Tyr)	274	1.4	303	3.6
Phenylalanine (Phe)	257	0.2	282	6.4

THE INFLUENCE OF THE MICROENVIRONMENT ON THE FLUORESCENCE PARAMETERS

The molecule absorption and fluorescence are very sensitive to the molecular microenvironment and, consequently, the characteristic parameters of the fluorescence spectra (i.e., fluorescence intensity, quantum yield, λ_{max},, τ_F) are affected in a variety of ways by different parameters (e.g., pH, ionic strength, temperature of the aqueous solution) [11, 12].

As a general rule, a molecule in the first excited electronic state will present an electrical charge distribution different from that in its fundamental state (i.e., non excited state) [8].

Therefore, in this excited state the intrinsic fluorophores of proteins, that is, one or more of the three aromatic amino acids (i.e., Trp, Tyr and Phe) could interact with neighboring solvent molecules, prior to their deactivation through fluorescence pathway [7, 13].

The microenvironment fluorescence sensitivity [14] of the fluorophores can be exploited in many ways, the most common one being that of the estimation of the solvent molecule polarity.

Generally speaking, the excited state of a molecule is more polarized than that of the fundamental one. Consequently, the excited molecules manifest a propensity of interaction with the polar solvent molecules inducing an ordering of its polar molecules. The ordering of the solvent molecules is decreasing the energy of the excited states this engendering a red shift of the fluorescence spectra and

implicitly of their maxima. However, the absorption λ_{max} position, as a consequence of the solvent polarity, must be interpreted with the highest caution. For instance, if the excited molecule has no sufficiently long lifetime for a rearrangement of the neighboring solvent molecules (which are provoking the decreasing of the excited state) even a fluorescence blue shift could be observed [6]. This phenomenon is known as an orientation constraint and put in evidence the ambiguity of interpreting the position of λ_{max} as a measure of solvent molecule polarity.

Another effect on the fluorescence parameters of different molecular species consists in fluorescence intensity changes; generally, the fluorescence intensity is increasing as far as the solvent polarity is decreasing.

The molecular microenvironment [4] is also affecting the quantum yield as well as the lifetime of the fluorescence.

FLUORESCENCE EMISSION OF TRP AND TYR

By the excitation at λ_{ex} = 280 nm, the fluorescence emission of the most proteins is dominated by the Trp fluorescence.

Trp fluorescence spectrum of the human serum albumin (HSA) is shifted to blue as compared with that of Trp in water, a very polar solvent. This blue shift is the result of Trp shielding by the chain of HSA molecules, against water molecules.

The low and even absence of Tyr fluorescence [10] in the case of the majority of proteins is dependent on their 3D native structure and, in general, by protein denaturation process, Tyr fluorescence is increasing.

In order to explain this very low fluorescence of Tyr, one can incriminate the energy transfer of the excited Tyr to Trp and/or Tyr fluorescence quenching by the presence, in its next proximity, of some chemical groups, situated on the protein chain [3].

Although Tyr fluorescence is not so important, it is desirable to avoid it, whenever necessary (e.g., when we are interested in Trp fluorescence), by exciting the proteins at λ_{ex} = 295 nm, where Tyr absorption is minimal.

MATERIALS AND METHODS

Spectrophotometric and spectrofluorimetric measurements were performed on biochemical samples obtained from mature Wistar rats with the weight of 200 – 250 g and bearing Walker tumours.

The tumors were induced by sub-dermal tumor cell injection in the right flank of the animal bodies. The first experimental determinations were performed

after seven days post inoculation. The rats were anaesthetized by chloroform and then sacrificed. Both tumor tissues as well as normal tissues from the hosts were harvested in order to extract proteins.

The tissues were broken and homogenized in a mortar, using quartz sand, then centrifuged and repeatedly washed with physiological saline solution.

The samples containing the total protein of the tumoral/normal tissue, but also glycoproteins and lipoproteins, were 100 times diluted prior to measurements.

The absorption spectra were recorded by a Perkin Elmer Lambda 2S spectrophotometer in the ultraviolet (UV) and visible (VIS) ranges, that is, in the domain between 200 nm and 700 nm.

The fluorescence excitation spectra were recorded by a Perkin Elmer MS 55 spectrofluorimeter, in the spectral range 200 – 320 nm, at λ_{em} = 348 nm, the Trp emission wavelength. The fluorescence emission spectra were recorded in the spectral range 300 – 500 nm, at λ_{ex} = 284 nm and λ_{ex} = 295 nm.

RESULTS AND DISCUSSIONS

The samples, suspended in physiological solution, at pH = 7, were coded, as follows: T1, proteins extracted from Walker tumor, after seven days from tumor transplantation; T2, proteins extracted from Walker tumor, after ten days from tumor transplantation; C1, proteins extracted from the brain; F2, proteins extracted from the liver; P4, proteins extracted from the lung; P5, proteins extracted from the skin; R4, proteins extracted from kidneys; S3, proteins extracted from suprarenal glands.

Fig. 1 shows the samples with the highest aromatic amino acid content and, implicitly, of proteins ($\lambda_a \approx 280$ nm): T2, F2, T1 and R4, and also the samples with the highest content of porphyrin (i.e., hemoglobin) absorbing at $\lambda_a \approx 410$ nm: P4 and F2.

At the same time, one can estimate the Tyr content. In the case of all measured samples, the content is greater than that of Trp, taking into account that the molar extinction coefficient of Tyr is four times smaller than that of Trp.

For all samples, the absorption spectra (Fig. 1), the fluorescence excitation spectra at λ_{em} = 348 nm (Fig. 2) and emission spectra (Fig. 3 and 4) for two excitation wavelengths (λ_{ex} = 284 nm and λ_{ex} = 295 nm) were recorded.

One can also notice that, in the case of Walker tumor (T_1) and (T_2), the absorption maximum is blue shifted, namely at $\lambda_a \approx 260$ nm, due to Tyr absorption.

Fig. 2 shows that the efficient excitation wavelength for Trp is situated in the spectral range 280 – 300 nm, as it was expected for the aromatic amino acids.

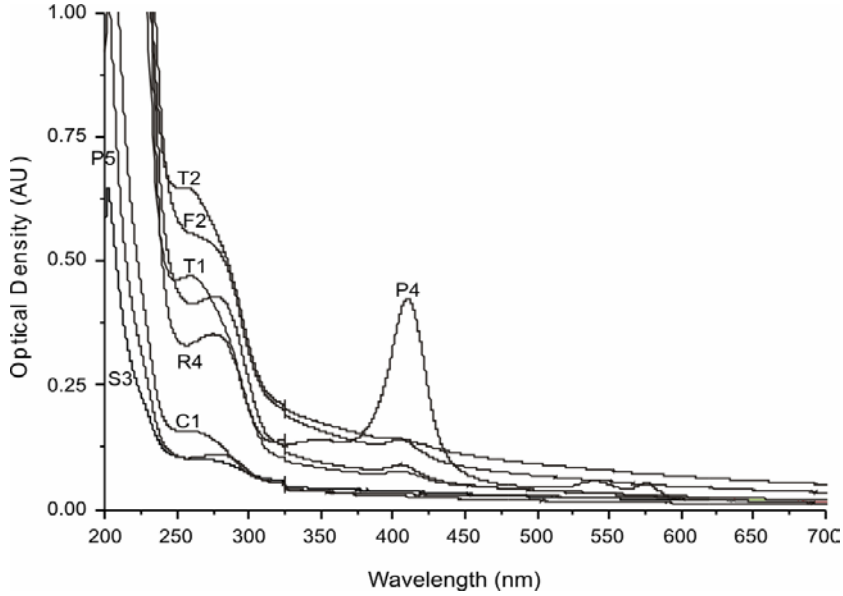

Fig. 1. The absorption spectra of the proteins extracted from various tumors. OD: optical density.

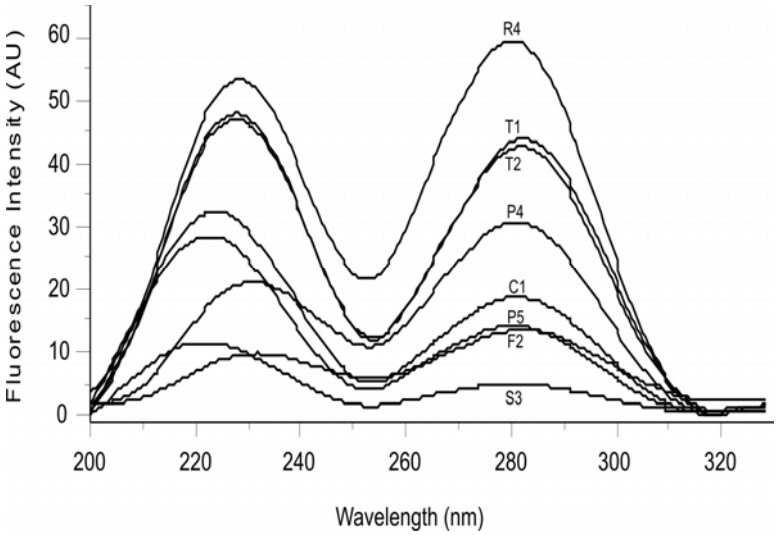

Fig. 2. The excitation spectra of the fluorescence for λ_{em} = 345 nm, in arbitrary units (AU).

Analyzing the evolution of the intensity of fluorescence, the excitation spectra show the different contents in aromatic amino acids of different samples and the manifestation of an energy transfer from Tyr to Trp. From Fig. 2 it results

that the highest content in aromatic amino acids is encountered in the proteins extracted from kidneys (R4) and Walker tumors (T1 and T2).

The fluorescence emission spectra, in the case of all samples, are presented in Fig. 3, for λ_{ex} = 284 nm (the characteristic excitation wavelength for Tyr) in the spectral range 300 – 500 nm.

One can notice from Fig. 3 that the fluorescence emission is due both to Trp and Tyr. The intensities of the fluorescence emission maxima are not correlated to those from the absorption spectra, because the energy transfer efficiency from Tyr to Trp is varying from sample to sample.

The most efficient energetic transfer from Tyr to Trp occurs in the case of R4 samples (extracted from the liver) when the fluorescence maxima are centered on λ_{em} = 345 nm.

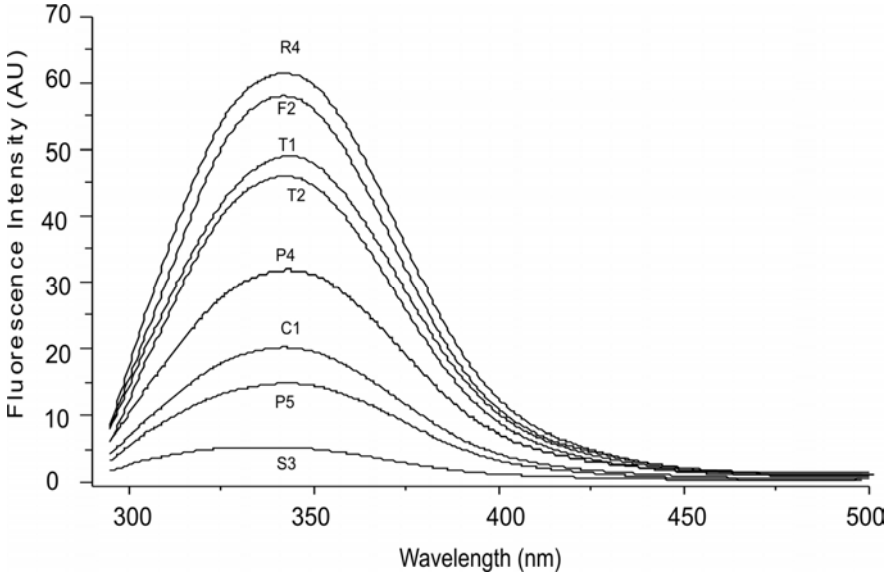

Fig. 3. Fluorescence emission spectra of the proteins induced by the excitation with λ_{ex} = 285 nm, in arbitrary units (AU).

Fig. 4 presents fluorescence emission spectra, for all the samples, in the spectral range, 300 – 500 nm, using the characteristic excitation wavelength for Trp, λ_{ex} = 295 nm. For all samples, the fluorescence emission maxima are centered on λ_{em} = 345 nm, the emission characteristic wavelength for Trp. Taking into account that at λ_{ex} = 295 nm, only Trp is excited, one can deduce that the greatest Trp content occurs in the following protein samples: F2, R4, T2 and T1.

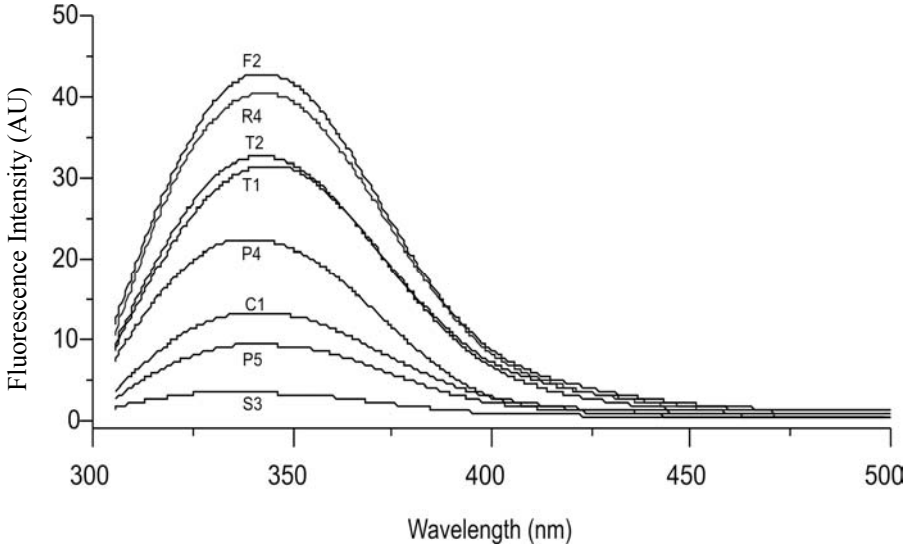

Fig. 4. The emission spectra of the fluorescence for λ_{ex} = 295 nm, in arbitrary units (AU).

From these experiments, it results that the proteins extracted from Walker tumor tissues seem to present a greater proportion of aromatic amino acids as compared with the normal tissue, excepting the samples extracted from the liver and the kidney/or that their intrinsic fluorophores are in a modified microenvironment more favorable to fluorescence emission than in the case of native proteins from normal tissues.

These experimental data confirm the findings that the tumor tissues have an increased metabolic activity [5, 9] as compared to the normal tissues. Therefore, the protein synthesis is more intense, but also the quality of proteins is different both in amino acid composition and, implicitly, the 3D folding.

We have chosen protein extracts from the liver and kidneys because the liver is a tissue deeply involved in protein synthesis, while the kidney is suffering both quantitative and qualitative modifications provoked by protein catabolism.

CONCLUSIONS AND PERSPECTIVES

The modification of the fluorescence intensity and the wavelength shift of the protein fluorescence maxima induced by the tumoral status of the tissues were revealed by absorption and fluorescence measurements.

The proteins with the highest aromatic amino acid contents are those extracted from the liver, kidneys and Walker tumors. The protein with the highest content of porphyrin (i.e., hemoglobin) absorbing at $\lambda_a \approx 410$ nm, are those from the lung and liver.

The fluorescence emission maxima of the proteins are situated very close to that of the free molecules of Trp in water, this meaning that, the side chains of Trp are not efficiently shielded by the matrix of the proteins extracted from tumour tissues, this suggesting a slight denaturation of tumour proteins.

The emission of Trp at λ_{em} = 345 nm is mainly the result of the energy transfer from excited state of Tyr to Trp, because only Tyr was excited by this wavelength.

The proteins extracted from Walker tumor tissues seem to present either a greater proportion of aromatic amino acids as compared with the normal tissue, excepting the cases of liver and kidney, or that their intrinsic fluorophores are situated in a microenvironment more favorable to fluorescence emission than in the case of native proteins.

The recognition of subtypes of breast carcinomas based on molecular features has brought new perspectives in cancer research. For this reason, the analysis of classes of proteins, instead of the protein pool and, finally of those of tumor specific proteins is imposed to be performed. In this way, in the future we intend to study some oncoproteins or other molecules involved in tumor angiogenesis (e.g., vascular endothelial growing factor, endostatin).

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