Fluorescence Spectroscopic Insights on the Interaction between Mesalamine and Tyrosine

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Abstract

The interaction between L- tyrosine (Tyr) and mesalamine (MES) was explored by fluorescence spectroscopy under physiological conditions. The fluorescence of Tyr was quenched by MES via static quenching as analyzed by temperature study based on Stern-Volmer equation and is also supported by life time measurement. Thermodynamic parameters recommend that the hydrophobic forces and hydrogen bonding plays a key role in the binding of MES with Tyr. The distance 'r' between donor and acceptor was found to be 3.59 nm which is calculated according to Forster's non radiative energy transfer theory. Furthermore, the method can be successfully applied for the determination of MES in pharmaceutical samples directly.

Keywords: Tyrosine, Mesalamine, Static quenching, Fluorescence spectroscopy.

Introduction

In current years there has been considerable attention to the interaction of small molecule with proteinase that is related to chemistry, biology, food science, medicine and toxicology [1]. Twenty percent of human body is made up of protein which plays an essential role in almost all biological processes and amino acids are the building block of it. L-Tyrosine (Tyr) is one of the twenty two amino acids that are used by cells to synthesize proteins, plays an important role in biological systems, responsible for the conformation of almost all proteins in the human body and is the precursor of several neurotransmitters like L-dopa, dopamine, norepinephrine and epinephrine [2]. Chemical structure of Tyr is shown in fig. 1 a. Its side chain contains a hydrophilic hydroxyl group attached to benzene ring. Hydrophobic Tyr has phenolic OH group capable of H- bond donor or acceptor and involved in forming diverse network of H-bond playing crucial role in protein structure and function [3]. The photo physical assets of tyrosine and its derivatives are multidimensional and were broadly investigated [4-6]. The fluorescence of amino acids and their residues merged into peptide or protein is the subject of extensive studies [7].Tyrosine residue buried in protein have different photo physical properties than those of free tyrosine directly exposed to solvent.

Mesalamine (MES) chemically known as 5-Aminosalicylic acid (5-ASA) is an important non-steroid, anti-inflammatory drug used to treat bowel disease like ulcerative colitis [8-9], inflammation of digestive track, inflamed anus or rectum and provides protection against the development of colon cancer. This bowel specific drug metabolized in the gut and acts predominantly therefore having less side effects. It blocks formation of prostaglandins and leukotrienes, inhibits bacterial peptide induced neutrophil chemotaxis, trapping the free radicals, scavenging reactive oxygen metabolites etc. [10]. Chemical structure of MES is shown in fig. 1 b. Therapeutic importance of the drug has led to the development of analytical method for its determination.

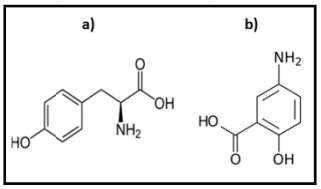

Figure 1 Chemical structure a) Tyrosine b) Mesalamine

A number of analytical methods has been developed for the analysis of MES in pharmaceutical dosage forms include voltammetry [11], spectrophotometry [12], spectrofluorometry [13], high-performance liquid chromatography (HPLC) [14], mass spectrometry (MS) [15] and electrochemical (EC) detections [16]. In literature many reports exists on the interaction of protein and drugs. Only few report exists on the use of Tyr as donar. A strong overlap between the emission spectrum of Tyr with excitation spectrum of MES has been observed and we expect the efficient energy transfer between Tyr (donor) and MES (acceptor). Attempts were made to evaluate the binding site, binding constant, binding distance and thermodynamic parameters. This study aims to develop simple, sensitive, fast, reliable, inexpensive method for the determination of MES in pharmaceutical tablets.

Experimental Work

Material and Methods

L-Tyrosine (Tyr) was purchased from Himedia laboratories, Mumbai. Mesalamine (MES) was collected from Dr. Reddy's Laboratory, Hyderabad. Di Potassium hydrogen phosphate, Potassium dihydrogen phosphate were procured from Spectrochem. Ltd. Mumbai, India. These chemicals were used as received. Redistilled water was used for preparation of solution and dilution. Emission spectra was recorded on Jasco Spectrofluorimeter (FP 8300) equipped with 1 cm quartz cuvette. Both excitation and emission slit were set at 5 nm with resolution 1 and low sensitivity. The pH of solution was recorded with digital pH meter (Global Model –DPH-500).). Horiba's Jobin Yvon IBH time resolved fluorescence spectrometer (Model: Delta Flex TCSPC) was used for measurement of fluorescence decay.

Procedure

Stock solutions of Tyr and MES each with concentration (1×10^{-3}mol dm^{-3}) were prepared by dissolving suitable quantity in double distilled water, sonicated for 15 minutes and diluted with water. Suitable volume of Tyr ($0.5x10^{-3}$mol dm^{-3}) was kept constant while the MES was varied from 0.0 to 4.5 cm^3. To maintain the physiological pH (7.4) of solution 2 cm^3 of 0.1 mol dm^{-3} of phosphate buffer was added to each solution. Resulting combinations were diluted to 10 cm^3 by using double distilled water. Then fluorescence spectra of Tyr in absence and presence of varied concentrations of MES were recorded at three different temperatures (298 K, 308 K and 318 K) in the range of 280 to 700 nm using excitation wavelength of 275 nm.

Results and Discussions

Fluorescence Quenching of Tyr

Fluorescence quenching refers to the process which decreases the fluorescence intensity of fluorophore by the interaction with other molecule. Fig. 2 displays that fluorescence spectra of Tyr is sharp with maximum at 303 nm when excited at 275 nm and quenching of Tyr followed by the sensitization of MES with an isoemmisive point at 375 nm. It shows the interaction of Tyr with MES and specifies the existence of bound and free MES [17]. Blue shift at the maximum emission wavelength of Tyr from 303 nm to 297 nm was observed which indicates that the Tyr was placed in a more hydrophobic environment after addition of MES [18].

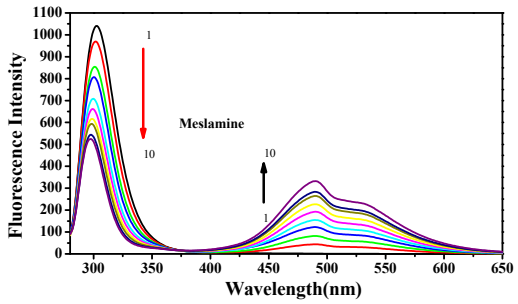

Figure 2 Fluorescence spectra of Tyrosine in presence of Mesalamine at 298 K and pH 7.4 .[Tyr]=5×10^{-5}mol dm^{-3}, [MES] = (0.0, 0.5× 10^{-4}, 1.0 × 10^{-4}, 1.5× 10^{-4}, 2× 10^{-4}, 2.5 × 10^{-4}, 3.0 × 10^{-4}, 3.5 × 10^{-4}, 4.0 × 10^{-4}, 4.5 × 10^{-4}) mol dm^{-3} for 1 to 10 respectively.

Molecular interactions like excited state reactions, energy transfer, molecular rearrangement, collisional quenching and ground state complex formation causes fluorescence quenching. The possible quenching mechanism includes static quenching, dynamic quenching or both [19]. Two types of fluorescence quenching mechanism can be differentiated by their dependence on temperature, viscosity and fluorescence life time. In dynamic quenching quencher diffuses through the solution and interacts with excited fluorophore. Diffusion coefficients increase with increasing temperature resulting the increase in bimolecular quenching constants in dynamic quenching. Static quenching refers to the fluorophore-quencher complex formation in the ground state. Stability of complexes decreases with increasing temperature ultimately lowers the value of static quenching constants [20]. To confirm the type of fluorescence quenching mechanism we carried out the fluorescence quenching study at different temperatures which obeys the Stern-Volmer plot fig.3. The fluorescence quenching mechanism is evaluated at different temperature by using Stern-Volmer equation [21].

$$\frac{F_0}{F} = 1 + K_q \tau_0 [Q] = 1 + K_{SV}[Q] \qquad (1)$$

Fluorescence intensity of the donor in absence of quencher is F_0 and in presence of quencher is F. K_q, K_{sv}, τ_0 and [Q] are the quenching rate constant, Stern-Volmer quenching constant, average fluorescence life time of fluorophore without quencher and concentration of quencher respectively.

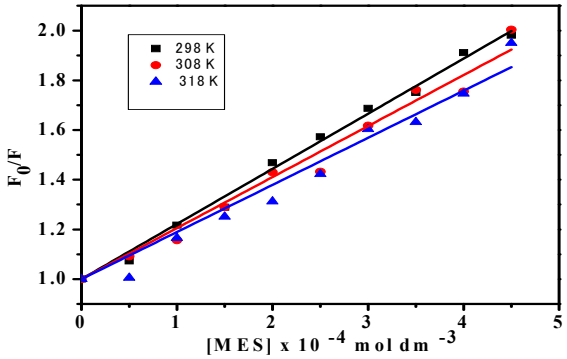

Figure 3 The Stern-Volmer plots of Tyr-MES system at different temperatures. Λ_{ex} = 275 nm, λ_{em}= 303 nm, pH= 7.4, [Tyr] = 5 × 10^{-5}mol dm^{-3}

The results are in good agreement with the Stern –Volmer equation within the concentration range selected for experiment. The plot F_0/F against concentration of MES at said temperatures are linear

with intercepts one [22] and it reveals the occurrence of single type of quenching. Equation (1) was applied to determine Ksv by linear regression of the plot F_0/F against [Q]. Ksv and K_q values along with correlation coefficient R^2 at different temperatures were obtained and tabulated in Table 1.

Table 1: Stern-Volmer Quenching Constants and Bimolecular Quenching Rate Constants for the Interaction of Tyr and MES at Various Temperatures

Temperature (K)	10^{-3} K_{sv} (dm^3 mol^{-1})	10^{-11} K_q (dm^3 $mol^{-1}s^{-1}$)	Correlation coefficient (R^2)
298	2.218	2.218	0 .9997
308	2.053	2.053	0.9988
318	1.896	1.896	0.9984

In the present case K_{sv} was negatively correlated with temperature. It indicates that quenching followed a static type of mechanism which is attributed by ground state complex formation between Tyr and MES. For dynamic quenching maximum scatter collision quenching rate constant Kq of various quenchers is 2.0×10^{10} dm^3 $mol^{-1}s^{-1}$. In this case the values of K_q have seen in the order of 10^{11} dm^3 $mol^{-1}s^{-1}$ which is greater than maximum collisional quenching constant of various biological molecules and it supports static type of mechanism rather than dynamic type.

Time resolved fluorescence studies

Quenchig mechanism of the interaction between Tyr and MES was further confirmed by life time measurement. Time resolved decay curves of Tyr and Tyr in presence of MES are presented in fig. 4. In case of static quenching life time of donor is not disturbed during complex formation [23]. In present case the type of quenching behavior was in good agreement with fluorescence lifetime measurement.

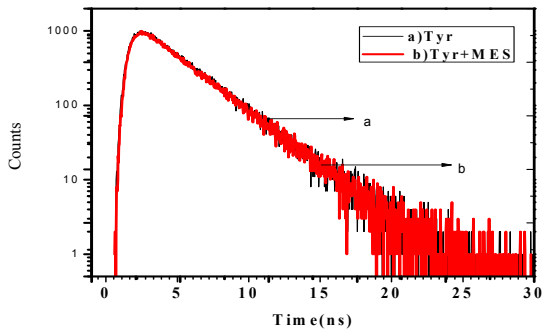

Figure 4 Fluorescence decay profile of a) Tyrosine and b) Tyrosine + Mesalamine

Analysis of binding mechanism

In case of bimolecular binding, equilibrium between free and bound molecule is given by the following modified Stern-Volmer equation [24].

$$\log_{10}\frac{F_0 - F}{F} = \log_{10} K + n\log_{10}[Q] \qquad (2)$$

K is the binding constant; n is the number of binding sites. K and n are determined by plotting the graph of log $(F_0\text{-}F)/F$ against log $_{10}$ [Q] shown in fig.5.

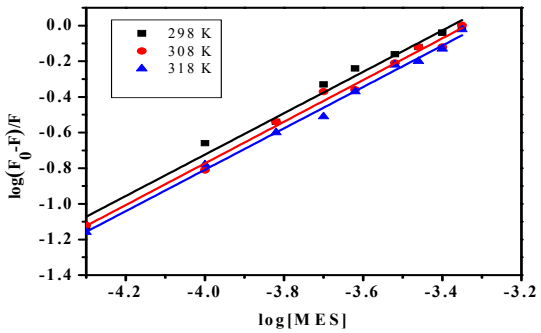

Figure 5 Plot of $\log_{10}(F_0\text{-}F)/F$ against log [MES] at three different temperatures

At three different temperatures values of K and n are determined from the intercept and slope respectively and results are shown in table 2. From these results it is observed that, the binding constant (K) decreases with rise in temperature due to the decomposition of Tyr-MES complex [25]. Moreover, at different temperature, values of n are found to be one which reveals that there is only one binding mode for MES on Tyr.

Table 2 : Binding Constants (K) and Number of Binding Sites (n) of interactions between Tyr and MES

Temperature (K)	10^{-3} K (dm^3 mol^{-1})	Binding sites (n)	Correlation Coefficient (R^2)
298	8.90	1.17	0 .9835
308	7.99	1.17	0.9907
318	6.86	1.16	0.9934

Thermodynamic Parameters and Nature of Binding mode

Generally there are four types of non-covalent interactions exists in the binding between drug and biomolecule. These are hydrogen bonding, van der waals forces, electrostatic and hydrophobic interactions. The forces responsible for binding are determined from the signs and magnitude of thermodynamic parameters which were determined by using van't Hoff equation.

$$\ln k = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \qquad (3)$$

K, ΔH, ΔS, R and T are the binding constant, enthalpy change, entropy change, gas constant and absolute temperature respectively. The enthalpy change (ΔH) and entropy change (ΔS) were calculated from the slope and intercept of the plot ln K against 1/T (fig.6). Gibb's free energy change (ΔG) is also calculated by following equation,

$$\Delta G = \Delta H - T\Delta S \qquad (4)$$

Table 3. Thermodynamic Parameters of Tyr-MES interaction at pH 7.4

Temperature (K)	ΔG (kJmol^{-1})	ΔH (kJ mol^{-1})	ΔS (J mol^{-1} K^{-1})	Correlation Coefficient (R^2)
298	-22.558			
308	-22.969	-10.323	41.059	0.9504
318	-23.379			

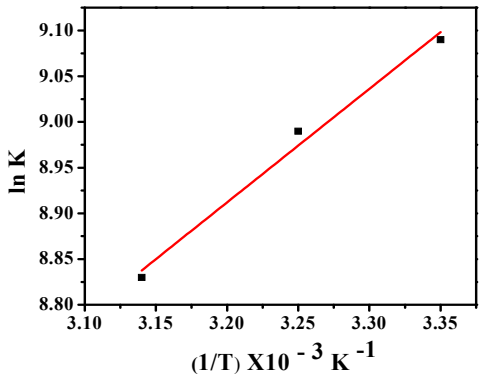

Figure 6 Van't Hoff plot for binding of Tyr to MES

The values of ΔH, ΔS and ΔG are listed in Table 3. The negative values of ΔG indicate that the binding process is spontaneous [26]. Positive value of ΔS and negative value of ΔH shows that hydrophobic interaction and hydrogen bonding plays major role in the binding process [27-28].

Energy transfer from Tyr to MES

Fluorescence resonance energy transfer is an important nondestructive method which can be used extensively to calculate molecular distances and to investigate the structure and spatial distribution of protein complexes [29]. Basically efficiency of energy transfer depends on three parameters. 1) Proper orientation of transition dipole of the donor and acceptor, 2) fluorescence emission spectra of donor must overlap with an absorption spectrum of acceptor, 3) Distance between donor and acceptor must be between specified distance 2-8 nm [30]. Overlap is seen in fig. 7 having a) is the emission spectrum of Tyr and b) is excitation spectrum of MES. Good spectral overlap exists between donor and acceptor. The distance between donor and acceptor can be estimated according to the Forster's non –radiative energy transfer theory and the efficiency of energy transfer is calculated by using following equation

$$E = 1 - \frac{F}{F_0} = \frac{R_0^6}{R_0^6 + r^6} \qquad (5)$$

Where 'r' is the distance between donor (D) and acceptor (A), R_0 is the critical distance between them when their transfer efficiency is 50 %. R_0 can be calculated by using the equation

$$R_0^6 = 8.8 \times 10^{-25} k^2 n^{-4} \Phi j \qquad (6)$$

K^2 is the spatial factor of orientation (2/3), n is the refractive index of the medium (1.33), ϕ is the fluorescence quantum yield of the donor (0.13) and J is the overlap integral between donor emission and acceptor excitation spectra and it is calculated by using equation (7)

$$j = \frac{\sum F(\lambda)\varepsilon(\lambda)\lambda^4 \Delta\lambda}{\sum F(\lambda)\Delta\lambda} \qquad (7)$$

F (λ) is the fluorescence intensity of fluorescent donor at wavelength λ and ε (λ) is the molar absorption coefficient of acceptor at wavelength λ. Under the experimental conditions by substituting the values in equation 6, the value of R_0 = 3.29 nm and from Eq.5, value of r = 3.59 nm. Thus the average distance between donor and acceptor in the present study was less than 8 nm and 0.5 R_0 < r < 2.0 R_0. This indicates that energy transfer from Tyr to Mes takes place with high probability [31].

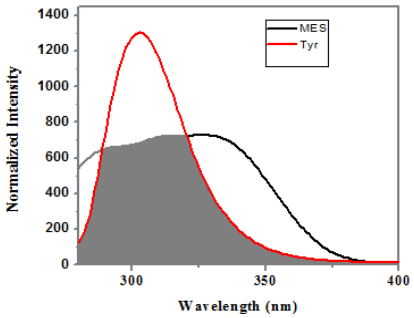

Figure 7 Spectral overlap a) emission spectra of Tyr b) excitation spectra of MES.
A) [Tyr] = 5×10^{-5}mol dm^{-3} b) [MES] = 2.5×10^{-4} $moldm^{-3}$

Analysis of Mesalamine from Pharmaceutical Samples

The proposed strategy was employed for the analysis of MES in pharmaceutical tablets and results are shown in table 4. Obtained results are in good agreement with the certified values. Proposed fluorescence quenching method was successfully applied to determine the amount of MES in pharmaceutical samples.

Table 4: Results of determination of MES from pharmaceutical samples

Sample	Composition	Amount of Mesalamine	
		Certified value	Found
Mesacol	Mesalamine USP Excipient q.s. Color red oxide Iron	400 mg	391.38 mg
Rowasa	Pellets 1 g	1.0 g	0.974 g

Conclusion

An approach is made to investigate the interaction between Tyr and MES using Fluorescence spectroscopy, the experimental results of fluorescence shows that quenching of Tyr fluorescence by MES is the result of complex formation between Tyr-MES in the ground state i.e. Static quenching. Thermodynamic parameters confirm that hydrogen bonding and hydrophobic interaction are responsible for binding between Tyr and MES. Binding reaction is spontaneous. Energy transfer from Tyr to MES occurs with high possibility. The proposed FRET strategy was successfully applied for the determination of MES directly from pharmaceutical samples.

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