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Investigation of Surface Tryptophan of Protein by Selective Excitation at 305 nm

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Abstract

Intrinsic fluorescence of tryptophan is a powerful tool that is used to investigate structure, dynamics, and folding-unfolding of proteins. Here, we have signified the importance of selective monitoring of "surface" tryptophans from the "buried" tryptophans in a protein via selective excitation of surface tryptophan using light of 305 nm wavelength. We have also enlightened the effect of pH and temperature on the conformation of protein by selective excitation of surface tryptophan of protein using 305 nm light. The result concludes that this novel approach could be used to investigate surface tryptophan of protein selectively at diverse conditions.

Keywords

Protein Fluorescence, Tryptophan Fluorescence, Fluorescence Spectroscopy

1. Introduction

Absorption of light promotes electron from ground state to excited state with the conversion of electron spin *i.e.* ground state into excited state. After radiationless deactivation like vibrational relaxation, internal conversion, external conversion and intersystem crossing, emission of photon from singlet excited state to singlet ground state result into fluorescence. Therefore florescence is the emitted light with higher wavelength than absorbed or excited light. Fluorescence is either intrinsic or extrinsic to biomolecule. The intrinsic fluorescence of the biomolecules is routinely used for the investigation of conformation of biomolecules. Proteins have intrinsic fluorescence due to the aromatic amino acid residues mainly tryptophan (Trp). Trp fluorescence is more sensitive to the environment as compared to Tyr and Phe due to its large redistribution of electron density in the asymmetric indole ring of the Trp residue after the excitation by the photons, while practically no redistribution occurs in Tyr and Phe symmetric rings [1]. It is principally useful to study protein conformation because we can follow

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the local conformational changes around Trp. Intrinsic fluorescence of Trp is a powerful tool for investigating the structure, dynamics, and folding-unfolding of proteins. This is due to high sensitivity of various parameters of the fluorescence of tryptophan residues (spectrum position, quantum yield, anisotropy, etc.) to their microenvironment and to the peculiarities of their location in the protein macromolecules. The dependence of intrinsic protein fluorescence on the unique location of tryptophan residues was elaborated by the study of the intrinsic fluorescence of model compounds and proteins in different structural states. The combined analysis of the characteristics of protein intrinsic fluorescence and protein three-dimensional structure with the peculiarities of the location of tryptophan residues in protein structure was performed for the first time on azurin [2]. The intrinsic fluorescence of total Trp of a protein is usually monitored by the excitation of Trp using light of 280 nm. In the present study, we have highlighted the significance of selective excitation of surface Trp of protein by 305 nm in the place of 280 nm that is used to monitor the total tryptophan.

A number of approaches have been used to develop a method for the selective monitoring of "surface" tryptophans from the "buried" tryptophans in a protein using selective excitation of surface tryptophan at 305 nm [3]-[7]. It was also observed that the quantum yield obtained at 305 nm excitation is about 10 times lower than that obtained by using 280 nm [4] [6] [7]. It is also noted that excitation of Trp using 280 nm showed fluorescence $\lambda_{max,em}$ at 332 nm while excitation at 305 nm showed the fluorescence $\lambda_{max,em}$ at 340 nm. Vashist *et al*. used excitation at 305 nm to monitor the surface Trp of a bacterial outer membrane protein, OmpAb [7]. They performed iodide quenching experiment using excitation of both 305 nm as well as 280 nm and revealed that there are more fluorescence quenching in the case of 305 excitation, which further confirm that fluorescence after excitation of 305 nm comes only from the surface Trp [7]. Spectroscopy result was further validated by N-Bromosuccinimide (NBS) oxidation methods that are used to calculate the number of Trp residue in the protein. NBS oxidation of OmpAb also suggests that about half of the total Trps are present on the molecular surface of OmpAb, which are available for oxidation by NBS [7]. Similar result was also obtained by the Rao *et al*. who have also explained the role of selective excitation of surface Trp of concanavalin-A using 305 nm [3]. They also monitored the change in the fluorescence of surface Trp in the presence of denaturant (8M urea). We have also confirms that excitation at 305 nm leads to the fluorescence of the "surface tryptophan" of β-lactamase [6]. Literatures show that experiments have been planed using excitation of 305 nm but its use in not very common. Therefore, in the present study, we have tried to show the significance of the excitation of 305 nm to monitor the surface tryptophan of model protein, OXA-51. We have also enlightened the effect of pH and temperate on the conformation of protein by selective excitation of surface tryptophan of protein using 305 nm light. The present study will be helpful in the study of the protein conformation by monitoring the surface tryptophan of protein.

2. Methods

Fluorescence spectra were recorded on a Carry Varian fluorometer with scanning rate of 50 nm/min and quartz cells of 1 cm path length. The excitation wavelength for the protein was chosen at 280 nm (excitation wavelength for total Trp) and at 305 nm (excitation wavelength for surface Trp). OXA-51 is a class D beta-lactamase and used as a model protein in the present study. Purified protein is available in our laboratory. Change in the fluorescence emission intensity and wavelength of protein (6 µM in cacodylate buffer 10 mM, pH7) was monitored by with increasing the temperature at the rate of 1˚C/min from 20˚C to 90˚C. Change in fluorescence emission intensity and wavelength of protein (6 µM) was also performed at different pH conditions. All the fluorescence spectra reported were an average of three scans.

3. Result and Discussion

The results of the comparison of the fluorescence of surface Trp and total Trp at different pH (**Figure 1(a)** and **Figure 1(b)**) as well as temperature (**Figure 1(c)** & **Figure 1(d)**) by selective excitation at 305 nm (plot 2) and 280 nm (plot 1) respectively, are shown in **Figure 1**. Similar profile of the fluorescence emission intensity as a function of pH was observed when the tryptophans were excited by 280 nm (plot 1, **Figure 1(a)**), and 305 nm (plot 2, **Figure 1(a)**). The result shows that pH below 4, the Asp and Glu residue get protonated that causes such characteristic fluorescence changes as seen in the **Figure 1(a)**. This result also suggests that the side chains of neighboring Asp and Glu residues influence the same tryptophan residues during excitation at 280 nm and 305 nm. Bioinformatics data highlighted that modeled OXA-51 have shown to have four (positions 102, 112, 218

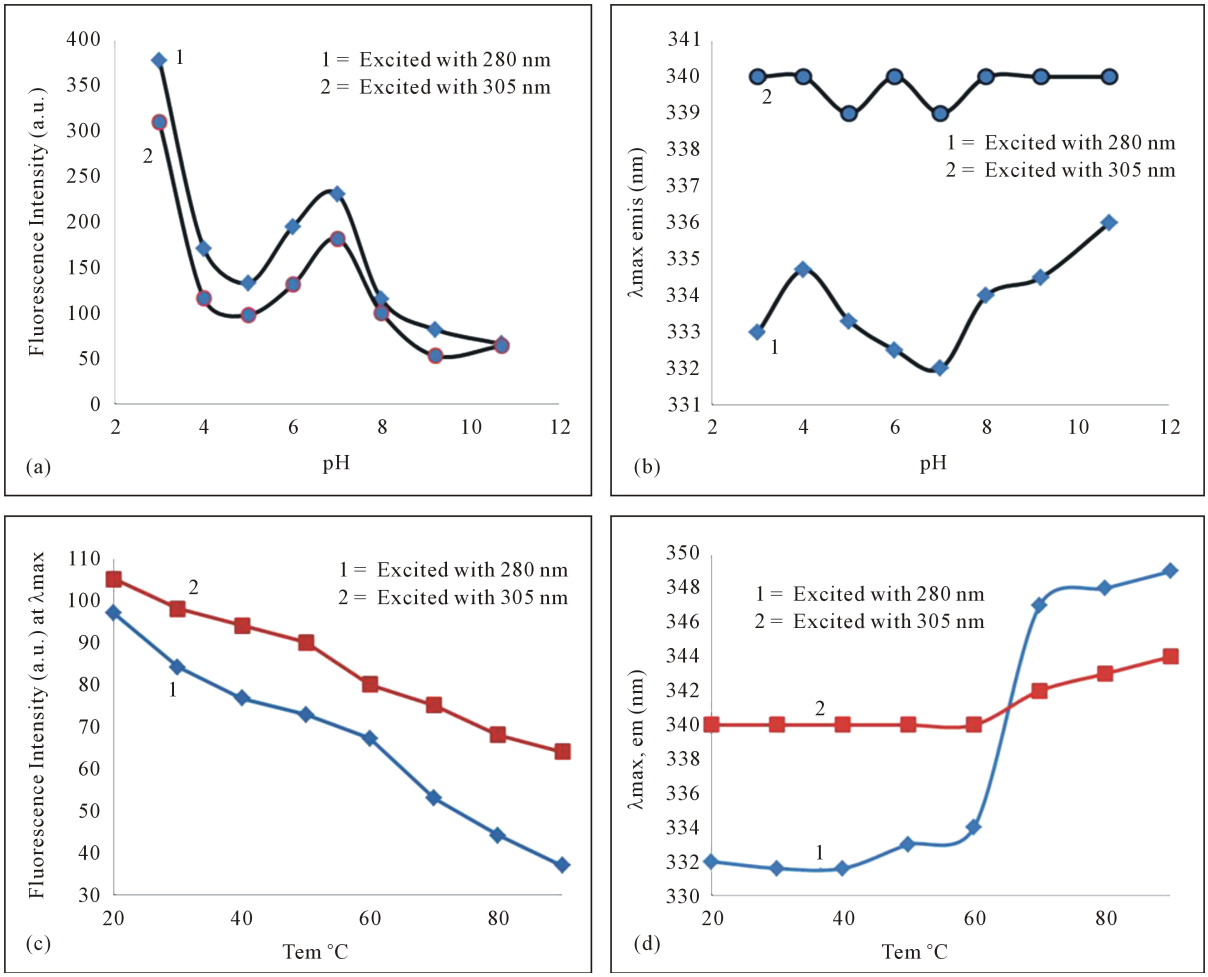

Figure 1. Comparative display of the change in fluorescence intensity and fluorescence wavelength of the surface and total tryptophan by selective excitation at 305 nm and 280 nm respectively at different pH ((a) and (b)) and temperature ((c) & (d)).

and 220) out of seven Trps on the surface of protein [6] that appear to emit fluorescence when excited at 305 nm. Therefore, it can be concluded that it is only surface Trp that is responsible for change in fluorescence at different pHs. Interestingly, when excited by 305 nm, Trp fluorescence emission maximum $\lambda_{max,em}$ (340 nm) slightly change throughout the pH (plot 2 **Figure 1(b)**) while when excited at 280 nm it showed a large shift (plot 1 **Figure 1(b)**). This explains that some interior Trps move from core to the surface of protein that causes an increase in $\lambda_{max,em}$ during excitation at 280 nm. These results again support the fact that the fluorescence after excitation at 305 nm comes from surface Trps in the protein. Surface Trp residues were also monitored at different temperature after excitation at 305 nm and 280 nm (**Figure 1(c)** and **Figure 1(d)**). It is found that $\lambda_{max,emis}$ at 340 nm is marginally influenced with the increase in the temperature till 60˚C (plot 2, **Figure 1(d)**) while influence of $\lambda_{max,emis}$ at 332 nm starts above 40˚C (plot 1, **Figure 1(d)**). There are increase (3 nm) in the $\lambda_{max,em}$ at 340 nm above 60˚C and decrease in the intensity of fluorescence above 60˚C (plot 2, **Figure 1(c)**). This is due to the movement of interior tryptophan to the surface and their contribution in the fluorescence of surface Trp.

4. Conclusion and Future Prospect

Based on the literature survey and our experimental results, it can be concluded that selective excitation of Trp at 305 nm may be emerged as important approach to explore the effect of surrounding environmental such as pH, ionic strength on the fluorescence of surface Trp which will significantly improve the study of bio-molecular structure and dynamics of the protein. Although limited information is available regarding selective excitation of

surface Trp of protein using light with 305 nm wavelength, this article initiates further research in this area.

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