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Mendeleev Commun., 2024, **34**, 421–423

Mendeleev Communications

Detection of green fluorescence in serum albumin upon excitation with 375 nm light: revealing new fluorescent properties

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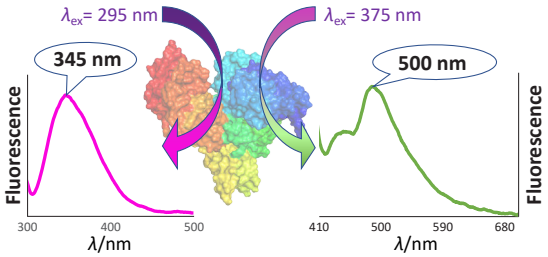
DOI: 10.1016/j.mencom.2024.04.035

The novel data on the fluorescence behavior of bovine serum albumin and human serum albumin under 375 nm excitation are presented, introducing a distinctive fluorescence phenomenon that holds promising implications for protein research. Extensive studies of these proteins are conducted to understand their intrinsic fluorescence properties, typically observed upon excitation with light in the range of 280–300 nm, resulting in fluorescence emission around 345 nm.



Keywords: fluorescence, albumin, protein, spectroscopy, aggregation, denaturation.

Serum albumins, including bovine serum albumin (BSA) and human serum albumin (HSA), being widely employed in various fields of research, possess inherent fluorescence properties attributed to the presence of aromatic amino acid residues within their structure. BSA has gained significant attention as a model protein in diverse fields due to its high structural similarity to human serum albumin, as well as its cost-effectiveness. BSA and HSA contain tryptophan, tyrosine and phenylalanine amino acid residues that are responsible for protein fluorescence.[1,2] Tryptophan residues fluoresce at 340–350 nm at an excitation wavelength of 295 nm, while phenylalanine and tyrosine residues fluoresce in the shorter wavelength region.[1–3]

Fluorescence spectroscopy has been widely employed in the field of biochemistry and biophysics to investigate the conformational changes of serum albumin, including its folding, unfolding, aggregation and denaturation.[4–6]

Fluorescence-based assays have also been developed to study the binding affinity and kinetics of serum albumin towards small molecules, drugs, and biomolecules.[7] The unique properties of serum albumin make it an ideal candidate for investigating drug–protein interactions, drug delivery systems and the transportation of various substances in the body.[8–10] By monitoring changes in fluorescence intensity or wavelength shifts researchers can determine binding constants, thermodynamic parameters, and assess the impact of different factors on these interactions.[11]

In the field of medical diagnostics, fluorescence-based techniques have been employed for the detection and quantification of serum albumin levels in biological samples.[12,13] These methods offer high sensitivity, specificity and rapid analysis, making them valuable tools for assessing serum albumin concentrations in clinical settings. Additionally, fluorescence imaging techniques, such as fluorescence microscopy and live-cell imaging, enable the visualization and tracking of serum albumin in biological systems, providing valuable information about its localization and behavior in complex environments.[14]

Fluorescence-based biosensors utilizing serum albumin as a recognition element have been developed for the detection of various analytes and biomarkers.[15–17] These biosensors exploit the specific interactions between serum albumin and the target analytes, resulting in changes in fluorescence signals that can be quantitatively measured. This approach offers a versatile and sensitive platform for the development of diagnostic devices and systems.[18,19] Fluorescent proteins have become valuable tools in molecular and cellular biology, allowing the visualization and control of cellular processes.[20] The green fluorescent protein from the jellyfish *Aequorea victoria* was the first fluorescent protein to be discovered and explored, widely known for its green fluorescence.[21] Since then, the discovery of other variants of fluorescent proteins with different emission wavelengths has further expanded the possibilities of fluorescence-based research. In this work we examined the fluorescent properties of BSA excited with light at a wavelength of 375 nm, compared to commonly used excitation wavelengths. Our experimental findings unequivocally demonstrate the presence of green fluorescence in BSA subjected to this higher excitation wavelength. This discovery challenges the prevailing understanding of BSA's fluorescence behavior and provides new insights into the photophysical properties of this protein. The green fluorescence of BSA and HSA is presented in Figure 1.

To validate the robustness and significance of our findings, we performed a comprehensive analysis and compared the results with the previously published ones.

Remarkably, our results align with the old investigations conducted by Macías *et al.*,[22] who found the albumin fluorescence bands with maxima at 600 and 675 nm, which had a fluorescence intensity of ~ 0.4–2.7% of that of the band at 345 nm. Subsequently, it was found that these bands were associated with second-order diffraction.[23] In our case, this effect cannot be observed, because we use a LED rather than a diffraction monochromator as an excitation source. The spectral characteristics of the LED sources are shown in Figure 2. Several protein samples were used, and the presence of long-wavelength fluorescence in all of them confirms the obtained data. Thus, it can be concluded that the specified fluorescence refers directly to the protein.

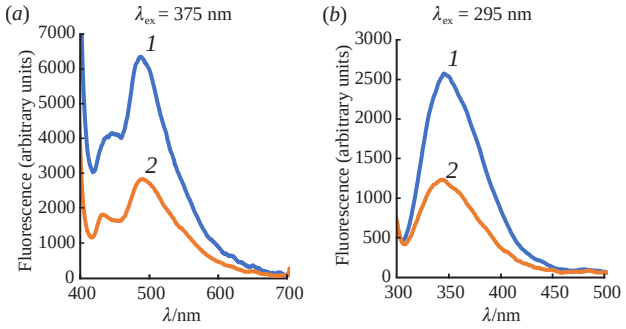


Figure 1 (*a*) Fluorescence spectra of (*1*) BSA and (*2*) HSA, 0.08 wt% in PBS, λ_{ex} = 375 nm. (*b*) Fluorescence spectra of (*1*) BSA and (*2*) HSA, 0.08 wt% in PBS, λ_{ex} = 295 nm. The absorbance of the solutions was the same and presented in Figure S1 (see Online Supplementary Materials).†

When bovine serum albumin is excited at 375 nm, the observed fluorescence could potentially arise from several sources. The possible options are as follows:

1. Tryptophan/tyrosine residues: BSA contains tryptophan and tyrosine residues, which are known to exhibit intrinsic fluorescence. Their fluorescence can be influenced by the local environment, solvent polarity, and interactions with neighboring amino acids or ligands.

2. Structural changes in other residues: it is possible that the excitation at 375 nm induces conformational changes in BSA, resulting in altered fluorescence properties. These structural changes may expose buried fluorophores to excitation light or alter the local environment around the existing fluorophores, leading to enhanced or modified fluorescence emission.

3. Aggregates or amyloid structures: BSA can undergo aggregation or amyloid formation under certain conditions. These aggregated or misfolded structures may exhibit distinct fluorescence properties under 375 nm excitation compared to the monomeric form of BSA.

The fact that the fluorescence intensity of HSA is approximately twice lower than that of BSA at the both excitation wavelengths (Figure 1) can indeed be explained by the difference in the number of tryptophan residues present in each protein. Tryptophan residues are known to be a significant source of intrinsic fluorescence in proteins. When excited by light, tryptophan residues can emit fluorescence due to the electronic transitions within their aromatic ring system. Since tryptophan residues contribute to the fluorescence of BSA and HSA, the difference in fluorescence intensity between the two proteins can be attributed to the various number of tryptophan residues. BSA has two tryptophan residues, whereas HSA has only one tryptophan residue. This difference in tryptophan content directly affects the fluorescence intensity, as the presence of more tryptophan residues provides additional opportunities for fluorescence emission. Therefore, it is expected that BSA, with its higher number of tryptophan residues, would exhibit a higher fluorescence intensity compared to HSA. Based on this understanding, it is logical to conclude that the observed green fluorescence, as well as the difference in fluorescence intensity between BSA and HSA at 345 nm, can be associated with the participation of tryptophan residues.

† Bovine serum albumin fraction V (>96%) was purchased from Acros Organics. Human serum albumin (>96%) lyophilized powder was purchased from Sigma–Aldrich. UV–VIS and fluorescence spectra were registered using an AvaSpec-2048 spectrophotometer with a qpod Temperature-Controlled Cuvette Holder (Avantes BV, The Netherlands). The monochromatic LEDs UVTOP-295 with λ_{max} = 295 nm (Sensor Electronic Technology, Inc., USA) and B5M-447-375S with λ_{max} = 375 nm (Roitner Lasertechnik Gmbh., Germany) were used as light sources for excitation.

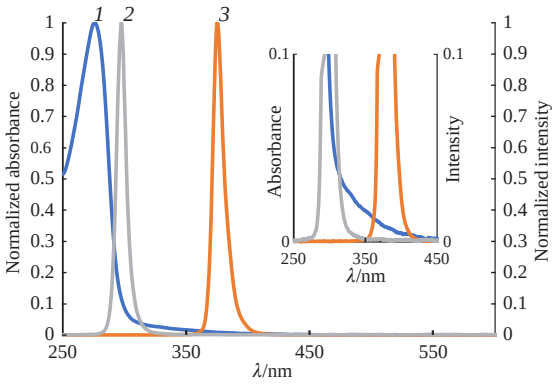


Figure 2 Normalized absorbance of (*1*) BSA in PBS; intensity spectra of (*2*) 295 nm and (*3*) 375 nm LED sources. The inset shows the spectra stretched along the intensity scale.

In the green fluorescent protein, the main fluorophore is the sequence Ser65, Tyr66, and Gly67.[24] It starts to fluoresce intensely being in the center of a stable barrel-shaped structure. In the hydrophobic environment within the core of the green fluorescent protein, a reaction occurs between the carboxyl carbon of Ser65 and the amino nitrogen of Gly67, leading to the formation of an imidazolin-5-one heterocyclic nitrogen ring system. Considering the above, we hypothesize that fluorescence may be associated with the interaction of multiple amino acid residues adjacent to tryptophan with the latter. Despite this, we were not able to detect patterns in the sequences of amino acid residues adjacent to tryptophane that would be performed for BSA and HSA (Figure 3).

It is likely that these interactions can occur in the globule between residues located in adjacent helices of the protein, or different mechanisms of fluorescence are realized for BSA and HSA. This assumption is supported by the different fluorescence profiles in the regions of 450 and 550 nm (Figure 4).

On the other hand, an increase in fluorescence of albumin in the region of 500 nm with time confirms the influence of aggregation. Thus, storage of a protein solution in a refrigerator for 1 month leads to a decrease in fluorescence at 345 nm by 20% and an increase in fluorescence at 500 nm by 500% (Figures S2 and S3). Moreover, heating the protein solution above the denaturation temperature leads to similar changes: an increase in fluorescence in the region of 450 and 550 nm (Figure S4). Comparing the fluorescence intensity ratios can become the basis for developing a method for assessing the state of albumin.

Figure 3 Amino acid residues of BSA and HSA located next to tryptophan.

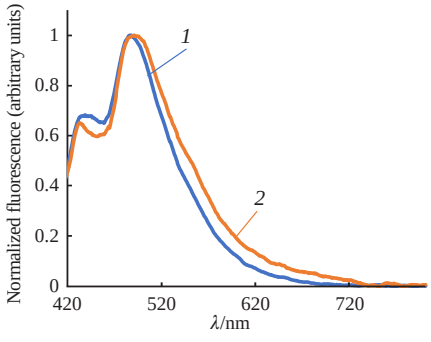


Figure 4 Normalized fluorescence spectra of (*1*) BSA and (*2*) HSA both 0.08 wt% in PBS. The absorbance of the solutions was the same and presented in Figure S1.

In conclusion, it should be noted that all hypotheses require further research. The detection of BSA green fluorescence upon excitation with 375 nm light represents a significant departure from the generally accepted understanding of its fluorescent properties. Our study contributes to the expansion of knowledge about the photophysical behavior of BSA and highlights the potential impact of this fluorescence phenomenon on various scientific disciplines.

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2024.04.035.

References

1 Y. Moriyama, D. Ohta, K. Hachiya, Y. Mitsui and K. Takeda, *J. Protein Chem.*, 1996, **15**, 265.
2 Y.-Z. Zhang, B. Zhou, Y.-X. Liu, C.-X. Zhou, X.-L. Ding and Y. Liu, *J. Fluoresc.*, 2008, **18**, 109.
3 K. Hirayama, S. Akashi, M. Furuya and K. Fukuhara, *Biochem. Biophys. Res. Commun.*, 1990, **173**, 639.
4 C. A. Royer, *Chem. Rev.*, 2006, **106**, 1769.
5 H. Chen and E. Rhoades, *Curr. Opin. Struct. Biol.*, 2008, **18**, 516.
6 N. Sh. Lebedeva, E. S. Yurina, Y. A. Gubarev, A. N. Kiselev and S. A. Syrbu, *Mendeleev Commun.*, 2020, **30**, 211.
7 E. Sijbesma, E. Visser, K. Plitzko, P. Thiel, L.-G. Milroy, M. Kaiser, L. Brunsveld and C. Ottmann, *Nat. Commun.*, 2020, **11**, 3954.
8 S. Reshma, S. K. Vaishanav, T. Yadav, S. Sinha, S. Tiwari, M. L. Satnami and K. K. Ghosh, *Heliyon*, 2019, **5**, e01631.
9 A. Shapira, G. Markman, Y. G. Assaraf and Y. D. Livney, *Nanomedicine*, 2010, **6**, 547.
10 N. Sh. Lebedeva, E. S. Yurina, Y. A. Gubarev and O. I. Koifman, *Mendeleev Commun.*, 2017, **27**, 47.
11 L. Wang, W. Zhang, Y. Shao, D. Zhang, G. Guo and X. Wang, *Anal. Chim. Acta*, 2022, **1219**, 340012.
12 B.-H. Jun, H. Kang, Y.-S. Lee and D. H. Jeong, *Molecules*, 2012, **17**, 2474.
13 H. Kobayashi, M. Ogawa, R. Alford, P. L. Choyke and Y. Urano, *Chem. Rev.*, 2010, **110**, 2620.
14 R. Yuste, *Nat. Methods*, 2005, **2**, 902.
15 A. Jahanban-Esfahlan, A. Ostadrahimi, R. Jahanban-Esfahlan, L. Roufegarinejad, M. Tabibiazar and R. Amarowicz, *Int. J. Biol. Macromol.*, 2019, **138**, 602.
16 A. V. Ambade, B. S. Sandanaraj, A. Klaikherd and S. Thayumanavan, *Polym. Int.*, 2007, **56**, 474.
17 N. Sh. Lebedeva, E. S. Yurina, Y. A. Gubarev, A. S. Semeikin and S. A. Syrbu, *Mendeleev Commun.*, 2022, **32**, 554.
18 G. Palestino, V. Agarwal, R. Aulombard, E. Pérez and C. Gergely, *Langmuir*, 2008, **24**, 13765.
19 Z. Pode, R. Peri-Naor, J. M. Georgeson, T. Ilani, V. Kiss, T. Unger, B. Markus, H. M. Barr, L. Motiei and D. Margulies, *Nat. Nanotechnol.*, 2017, **12**, 1161.
20 T. Misteli and D. L. Spector, *Nat. Biotechnol.*, 1997, **15**, 961.
21 J. Zhang, R. E. Campbell, A. Y. Ting and R. Y. Tsien, *Nat. Rev. Mol. Cell Biol.*, 2002, **3**, 906.
22 P. Macías, M. C. Pinto and C. Gutiérrez-Mérino, *Biochem. Int.*, 1987, **15**, 961.
23 C. M. Hutnik and A. G. Szabo, *Biochem. Int.*, 1988, **16**, 587.
24 D. P. Barondeau, J. A. Tainer and E. D. Getzoff, *J. Am. Chem. Soc.*, 2006, **128**, 3166.

Received: 17th January 2024; Com. 24/7372