Comment on "The Interaction of 5-(Alkoxy)naphthalen-1-amine with Bovine Serum Albumin and Its Effect on the Conformation of Protein"

ARTICLE *in* THE JOURNAL OF PHYSICAL CHEMISTRY B · MAY 2011

Impact Factor: 3.3 · DOI: 10.1021/jp111012w · Source: PubMed

3 AUTHORS, INCLUDING:

Anasuya Mishra
Indian Institute of Technology Guwahati
9 PUBLICATIONS **80** CITATIONS

SEE PROFILE

THE JOURNAL OF PHYSICAL CHEMISTRY B

COMMENT

pubs.acs.org/JPCB

Comment on "The Interaction of 5-(Alkoxy)naphthalen-1-amine with Bovine Serum Albumin and Its Effect on the Conformation of Protein"

Nihar Dash, Anasuya Mishra, and G. Krishnamoorthy*

Department of Chemistry, Indian Institute of Technology Guwahati, Guwahati 781039, India

Recently the interactions of neutral amphiphilic ligands, 5-(alkoxy)naphthalen-1-amines, with different alkyl chain lengths on bovine serum albumin (BSA) were studied by Ohja and Das.[1] They found an interesting observation that these amphiphilic ligands on interaction with BSA induce conformational change in protein and the denaturation of protein depends on the alkyl chain length. The fluorescence spectrum of BSA was red-shifted with reduction in intensity and the fluorescence anisotropy increased upon interaction of ligands with BSA, and it was predicted that the ligands induced conformational change in BSA. The changes in the fluorescence characteristics of BSA were consistent with chemical unfolding of BSA.[2,3] The bathochromic shift and the decrease in the intensity of BSA fluorescence spectrum suggested the unfolding of BSA and the increased exposure of tryptophans to aqueous medium, as concluded by Ohja and Das.[1] They also observed that the decrease in the fluorescence intensity of BSA is more with the ligands where the spectral overlap between BSA fluorescence and ligand absorption is higher. On that basis, they have hypothesized that the quenching of BSA fluorescence is due to energy transfer from the tryptophan residues to the probe in each case. But it contradicts their own conclusion that the changes in the fluorescence spectra of BSA are due to changes in environment.

Our interpretation of their data is that the decrease in the fluorescence of BSA is not due to energy transfer. In resonance energy transfer the excited donor transfers the energy to the acceptor; as a result the acceptor is promoted to the excited state. Therefore, in energy transfer, the reduction in the fluorescence intensity of the donor is often accompanied by the increase in the fluorescence intensity of acceptor.[4,5] But upon excitation of BSA no fluorescence from ligands was observed in ref 1. However, the same authors reported strong fluorescence of 5-(alkoxy)naphthalen-1-amines upon excitation of ligands at ~400 nm in the presence of BSA.[6] Therefore, the absence of ligand fluorescence upon excitation of tryptophan clearly suggests that there is no resonance energy transfer from tryptophan to the ligands. The quenching of tryptophan fluorescence upon binding of the ligands to BSA is due to change in the environment rather than due to energy transfer. Therefore, the energy transfer efficiency and subsequent calculation of the distance between the tryptophan and ligands are invalid. One could argue that the increase in the fluorescence of the ligands is not significant and is buried under the strong fluorescence of BSA. The spectral changes did not signify such a possibility. Even if we assume the ligands are nonfluorescent, the quenching of tryptophan(s) fluorescence is not only due to energy transfer but also due to changes in environment. Therefore, it is not possible to calculate the energy transfer from the expression

$$E = 1 - (F_{DA}/F_D) \quad (1)$$

where F_{DA} and F_D are the fluorescence intensity of the donor in the presence and absence of the acceptor.[7] In addition, predictions about the distance of the ligands from tryptophan using resonance energy transfer in BSA are not simple; the presence of two tryptophan (Try-212 and Try-134) residues complicates it.[8] But the authors considered only Try-134.[1] It is also not clear in the article if the emission spectra are corrected for the inner-filter effect or not. The correction of the emission spectra for the inner-filter effect is important for any calculation of fluorescence intensity, as the absorption spectra of the ligands overlap with the fluorescence spectrum of BSA and the inner-filter effect is excepted to be significant at higher ligand concentrations.

Ohja and Das also calculated the association constant and number of binding sites using the equation

$$\log[(F_o - F)/F] = \log K_A + n \log[Q] \quad (2)$$

where F_o and F are the relative fluorescence intensities in the absence and presence of quencher, respectively, and [Q] is the concentration of the quencher. The expression was derived on the basis of the assumption that the quenching is static in nature.[9] The quenching of BSA fluorescence by 5-(alkoxy)naphthalen-1-amines was reported as both dynamic and static in nature.[1] In addition, as mentioned earlier, water also quenches the fluorescence of tryptophan. Thus, eq 2 may not be applicable. Lastly, the following expression was used to study the quenching of BSA fluorescence by 5-(alkoxy)naphthalen-1-amines

$$F_o/F = (1 + K_D[Q])(1 + K_S[Q]) \quad (3)$$

by completely ignoring the fact that BSA fluorescence has a contribution from two tryptophans and eq 3 is applicable only for a fluorophore in one environment. In addition, here one should also consider the change in environments of tryptophans.

However, Das et al. investigation on the interactions of 5-(alkoxy)naphthalen-1-amines with BSA and their main conclusion that the interaction of BSA with ligand decreases with increasing hydrocarbon chain length are quite interesting. The decrease in the interaction with increasing hydrocarbon chain length explains the dependence of quenching on the alkoxy chain length.

AUTHOR INFORMATION

Corresponding Author

*E-mail: gkrishna@iitg.ernet.in.

Received: November 18, 2010
Revised: February 21, 2011
Published: May 03, 2011

REFERENCES

(1) Ohja, B.; Das, G. *J. Phys. Chem. B* **2010**, *114*, 3979.
(2) Abou-Zied, O. K.; Al-Shihi, O. I. K. *J. Am. Chem. Soc.* **2008**, *130*, 10793.
(3) Eftink, M. R. *Methods Biochem. Anal.* **1990**, *35*, 117.
(4) Paul, B. K.; Samanta, A.; Guchhait, N. *J. Phys. Chem. B* **2010**, *114*, 6183.
(5) Wu, F. −Y.; Zhang, L. −N.; Ji, Z. −J.; Wan, X. −F. *J. Lumin.* **2010**, *130*, 1280.
(6) Ohja, B.; Das, G. *Chem. Commun.* **2010**, 2079.
(7) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*, 3rd ed.; Springer Science+Business Media: New York, 2006.
(8) Sun, C.; Yang, J. *Biophys. J.* **2005**, *88*, 3518.
(9) Bi, S.; Ding, L.; Tian, Y.; Song, D.; Zhou, X.; Liu, X.; Zhang, H. *J. Mol. Struct.* **2005**, *703*, 37.