Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Archives of Applied Science Research, 2011, 3 (2):174-179

(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-508X
CODEN (USA) AASRC9

Fluorometric Investigation on the Interaction of 5-Bromo and 5-Iodouracil with Bovine Serum Albumin

S. Bakkialakshmi* and D. Chandrakala

Department of Physics, Annamalai University Annamalai Nagar, Tamilnadu, India

---

ABSTRACT

The Interaction of 5-Bromouracil (5BU) and 5-Iodouracil (5IU) with bovine serum albumin (BSA) were studied by spectroscopic method. Quenching of fluorescence of serum albumin by these drugs was found to be a static quenching process. Based on the interaction studies carried out the number of binding sites for drug on protein have been evaluated. The binding constant (Ka) were 2.013 x 10^4 and 4.062 x 10^4 L mol^{-1} for 5BU and 5IU.

Keywords: Bovine serum albumin, 5-Bromouracil, 5-Iodouracil, quenching, binding constant.

INTRODUCTION

Fluorescence spectroscopy is a valuable tool in the study of proteins due to its great sensitivity. Fluorescent dyes are being increasingly used in clinical and medicinal applications[1]. Fluorescence serves as sensitive and convenient means indicating the alterations of the fluorophore environment, which can provide abundance of useful information. Alterations of proteins molecule can be followed via intrinsic (by emission of tryptophan residue) or extrinsic (fluorescent probe) emitters attached to the protein. The fluorescence quenching has been widely studied both as a fundamental phenomenon and also for the application of fluorescence to biochemical problems.

Uracil (U) and its derivaitives, constituents of the genetic materials, play a fundamental role in basic biological processes. The importance of uracil has been indicated by a considerable number of publications appeared in literature from the structure [2-7] and spectroscopic [7-18] point of view. The bioactivity of 5-substituted uracil also induces exponential interest in their bio-chemistry and pharmacology, and they are the most interesting studies. In the present paper the effect of BSA by uracil derivatives such as 5-Bromouracil (5BU) and 5-Iodouracil (5IU) have

been studied. It has been suggested that peroxides secreted by human phagocytes facilitate the formation of 5-Bromouracil (5BU) in the human inflammatory tissue [19,20]. The structure of 5-Bromo and 5-Iodouracils are shown in figure1. 5-Iodouracil (5IU) is also an important antimetabolite, being the most effective inhibitor of 4-aminobutyrate aminotransferase [21-23]. Some 5 IU based nucleoside analogues were found to be selective inhibitors of herpes simplex viruses[24,25] and some others (developed to track hepatis B infections) were shown to be toxic when incorporated into mitochondrial DNA [26].

Serum albumin are abundant with proteins in blood plasma, accounting for about 60% of the total protein. As a consequence, albumins have been used as model protein in several bio-physical, biochemical, and physicochemical studies for many years [27]. Bovine serum albumin (BSA) is a protein consisting of three intrinsic fluorophores: Tryptophan (trp), Tyrosine (tyr) and Phenylalanine (phy). BSA has two tryptophan residues [28]: Trp-134 in the first domain and Trp-212 in the second domain.Trp-134 is located at the surface of the molecule while Trp-212 is located within a hydrophobic binding pocket of the protein [29].The structure of BSA is shown in figure2.

MATERIALS AND METHODS

The steady state fluorescence spectra were recorded on Varian cary eclipse fluorescence spectrophotometer.

RESULTS AND DISCUSSION

3.1 Fluorescence quenching Mechanism

There are wide varieties of quenching process that includes excited-state complex formation and energy transfer intersystem crossing, singlet – to - triplet excitation and excited intersystem crossing, and singlet-to-triplet excitation. There are two basic types of quenching: static and dynamic. Both types require an interaction between the fluorophore and quencher. Fluorescence quenching process depends upon the nature of fluorophore and quencher molecule [30, 31].The fluorescence quenching of BSA by the quenchers 5BU and 5IU has been studied by steady state method at room temperature.

The fluorescence quenching spectra of BSA (λ_{ex}= 280 nm) in the presence of different concentrations of quenchers(5BU and 5IU) are shown in figure 3 and 4 respectively. The fluorescence intensity of BSA decreases regularly with increasing concentration of quenchers. The emission maxima(λ_{em}) is 344 nm and is slightly blue-shifted when in the presence of quenchers. For the calculation of Stern-Volmer constants, the following Stern-Volmer relation can be used.

$$F_0/F = 1+K_{sv}[Q] \qquad \text{----------(1)}$$

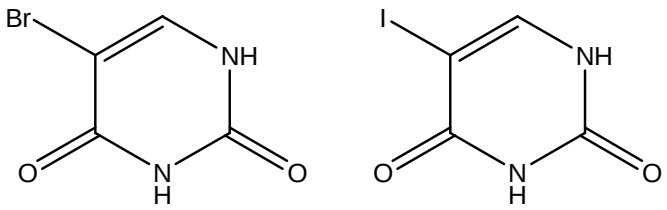
where F_0 and F are fluorescence intensities in the absence and presence of quenchers respectively. K_{sv} is the Stern-Volmer constant and [Q] is the quencher concentration. The Stern-Volmer plots of fluorescence quenching of BSA with Quenchers(5BU and 5IU) is shown in figure 5. Table 1. Summarises the calculated Stern-Volmer quenching constants and the regression co-efficients values.

3.2. Analysis of binding equilibria

For static quenching, the fluorescence intensity can also be used to analyze the apparent binding constant (K_a) and the number of binding sites (n) using Eqn.(2). When small molecules independently bind to set of equivalent sites on a macromolecule [33].

$$\log[(F_0/F)/F] = \log K_a + n \log[Q] \qquad \text{----------(2)}$$

where K_a is binding constant of 5BU and 5IU with BSA and n is the number of binding sites per albumin molecule, which can be determine by the slope and the intercept of double logaritham regression curve of *log[(F₀/F)/F]* versus *log[Q]* based on the Eqn.(2) (figure 6). Table 2 gives the result of K_a and n analyzed in this way for BSA. The correlation co-efficients are greater than 0.99, indicating that the assumption underlying the derivation Eqn(2) are satisfactory. The values of n at the experimental are approximately equal to 1, which indicates that there is one class of binding site to quenchers in BSA.



5-Bromouracil **5-Iodouracil**

Fig.1 : Molecular Structure of quenchers

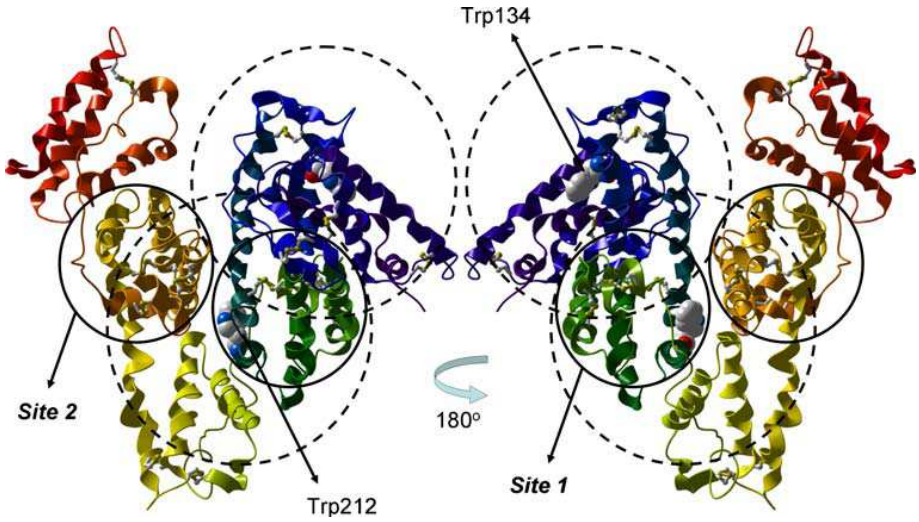


Fig.2 : Structure of Bovine Serum Albumin

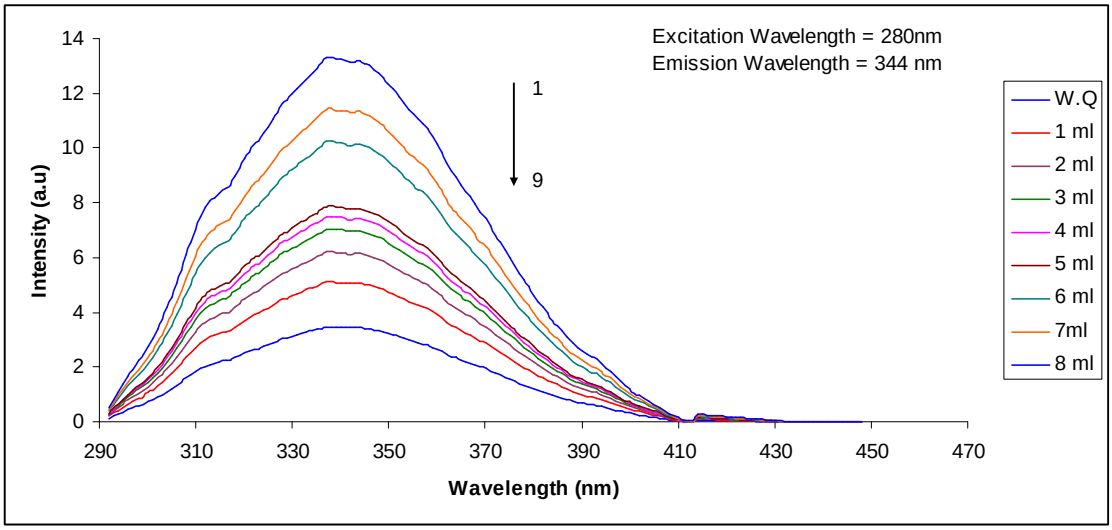


Fig. 3: Fluorescence quenching spectra of BSA without and with different concentrations of 5-Bromouracil (5IU) at 303K

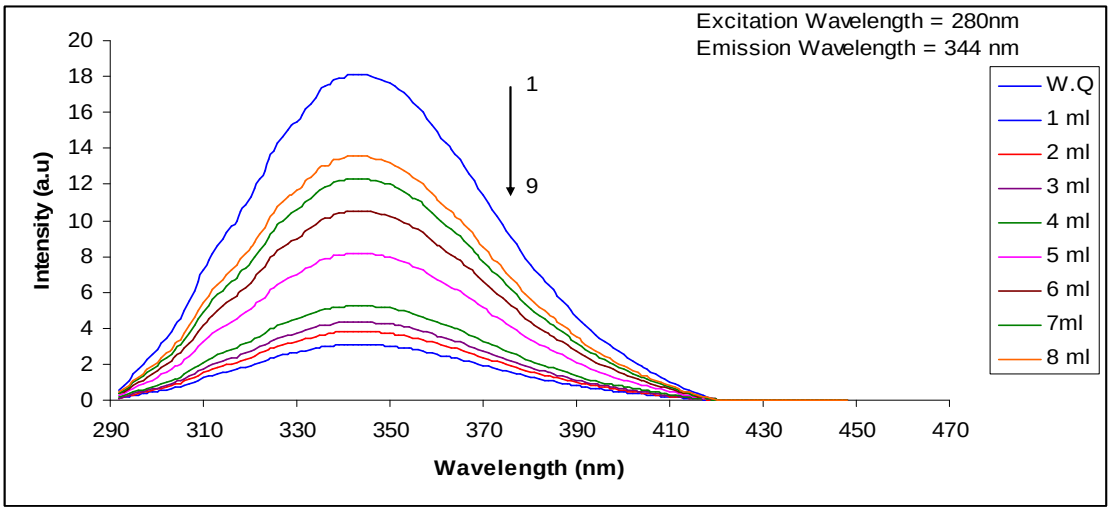


Table.1 Stern-Volmer quenching constants (K_{sv}) of the drug-BSA complexes

Quenchers	K_{sv} x 10^3 (L mol^{-1})	R[a]	S.D.[b]
5 BU	4.480	0.99	0.513
5 IU	5.200	0.99	0.662

[a] – *The correlation coefficient*
[b] – *The standard deviation for the K_{sv} values*

Table. 2 Binding constant(K_a) and binding site (n) of Drug-BSA complexes

Quenchers	K_a x 10^4 (L mol^{-1})	n
5 BU	2.013	1.16
5 IU	4.092	1.23

Fig. 4: Fluorescence quenching spectra of BSA without and with different concentrations of 5-Iodouracil (5IU) at 303K

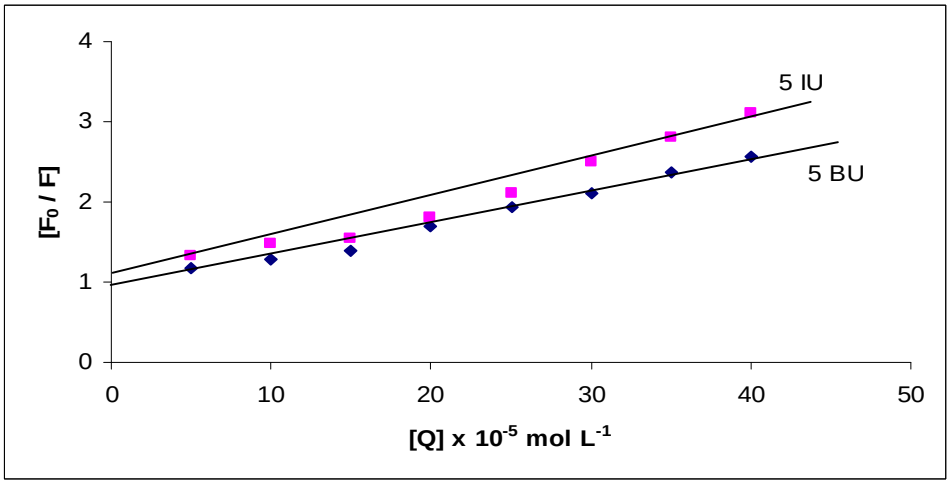


Fig. 5: The Stern-Volmer plot of fluorescence quenching of BSA by 5BU & 5IU

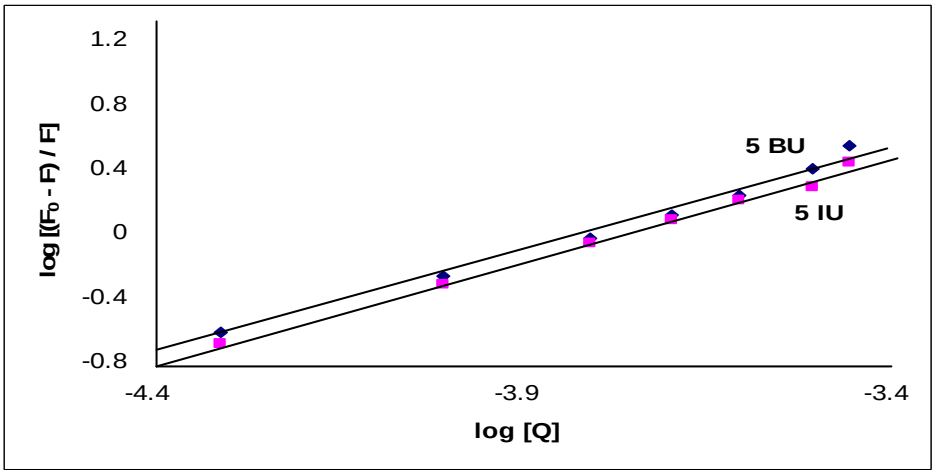


Fig. 6: Double-log plot of fluorescence quenching of BSA by 5BU & 5IU

CONCLUSION

This paper provides an approach for studying the binding of protein with 5BU and 5IU by using fluorescence technique. The experimental results indicate that complexes are formed between drugs and BSA through the static quenching procedure.

Acknowledgment

The authors gratefully acknowledges for the financial support from the University Grants Commission, New Delhi and Prof AN. Kanappan, Department of Physics, Annamalai University for his constant support thoughout the research.

REFERENCES

[1] J. Slavik (1994) Fluorescent probes in cell biology. CRC Press, Boca Raton.
[2] F. Stewart, L. H. Jensen. *Acta Crystallogr*. 23 **(1967)** 1102.
[3] Ozeki, N. Sakabe. J. Tanaka, *Acta Crystallogr*. Sect. B 25 **(1969)** 1038.
[4] Harsanyi, A. Csaszar, P. Csaszar, *J. Mol. Struct*. (Thermochem) 137 **(1987)** 207.
[5] Ferency, L. Harsanyi, B. Rozsondai, I. Hargittai, *J. Mol. Struct*. 140 **(1986)** 71.
[6] Portantone,L.Bencivenni,M.Colapietro,A.Pieretti,F.Ramondo,*Acta chem..scand*.53**(1999)**57.
[7] M. Alcolea palafox, N. Iza, M. Gil, *J. Mol. Struct*. (Thermochem) 585 **(2002)** 69.
[8] M. Alcolea Palafox, V.K. Rastogi, *spectrochim.acta* 58A **(2002)** 411.
[9] Wang, F. Zhang, X. Zeng, H. Zhou, B. Gu, W. Cheng, *Chin. Sci. Bull*, 51 **(2006)** 1804.
[10] Blicharska, T. Kupka, *J. Mol. Struct*. 613 **(2002)** 153.
[11] K. Szczepaniak, M. Szczesniak, M. Nowak, I. Scott, W. B. Person, *Int. J. Quant. chem. Quant. chem. symp*. 18 **(1984)** 547.
[12] M.Szczesniak, M.J.Nowak, H.Rostkowska, K.Szczepaniak, W.B.Person, D. Shugar, J. Am. Chem. soc. 105 **(1983)** 5969.
[13] S.Chin, V.Scott, K.Szczepaniak, W.B.Person, *J.Am.C.hem.Soc*.106**(1984)3**415.
[14] M.Maltese,S.Passerini,S.Nunziate- cesaro,S.Dobos,L.Harsanyi,*J.Mol.Struct*.116**(1984)**49.
[15] E.D.Radchenko, A.M.Plokhotnichenko, G.G.Sheina, Y.P.Blagoi, *Biofizika* 28 **(1983)**923.
[16] A. J. Barnes, M. A. Stuckey, L. L. Gall, *Spectrochim. Acta* 40A **(1984)** 419.
[17] M. Rozenberg, G. Shoham, I. Reva, R. Fausto, *Spectrochim. Acta* 60A **(2004)** 2323.
[18] S.Yarasi, B. E. Billinghurst, G.R. Loppnow, *J. Raman Spectosc*. 38 **(2007)** 1117.
[19] J. P. Henderson, J. Byun, J.Takeshita, J. W. Henecke, *J. Biol. Chem*. 278 **(2003)** 23522.
[20] J. P. Henderson, J. Byun, D. M. Muller, J. W. Heinecke, *Biochemistry* 40 **(2001)** 2052.
[21] H. Ogino, M. Fuiji, W. Satou, T. Suzuki, E. Michishita, D. Ayusawa, *DNA Res.* 9 **(2002)** 25.
[22] C. A. Presant, W. Wolf, V. Waluch, C. Wiseman, P. Kennedy, D. Blayney, R. R. Brechner, *Lancet* 343 **(1994)** 1184.
[23] T. Nakajima, *World J. Surg*. 19 **(1995)** 570.
[24] I. Verheggen, A. VanAerschot, L. VanMeervelt, J. Rozenski, L.Wiebe, R. Snoeck, G.Andrei, J. Balzarini, P. Claes, E. De clercq, et al., *J. Med. Chem*. 38 **(1995)** 826.
[25] I. Verheggen, A.VanAerschot, S.Toppet, R. Snoeck, G. Janssen, J. Balzarini, E.De clercq, P. Herdewijn, *J. Med. Chem*. 36 **(1993)** 2033.
[26] A. A. Johnson, A. S. Ray, J. Hanes, Z. Suo, J. M. Colacino, K. S. Anderson, K. A. Johnson, *J. Biol. Chem.* 276 **(2001)** 40847.
[27] E. L. Gelamo, C. H. T. P. Solva, H. Imasato, M. Tabak, *Biochim. Biophys. Acta* 1594 **(2002)** 84.
[28] T. Peter, serum albumin, *Adv. Protein Chem*. 37 **(1985)** 161.
[29] P.B. Kandagal, S. Ashoka, J. Seetharamappa, *J. Pharm. Biomed. Anal*. 41 **(2006)** 393.
[30] A. Samanta, W. E. Richard, *J. Phys. ChemA*. 104 **(2000)** 8972.
[31] K. K. Rothagi-Mukherjee, Fundamental of Photochemstry, **(1986)** Wiley Eastern Ltd, New Delhi.
[32] L. Streger, *J. Mol. Biol*. 13 **(1965)** 482.