Spectroscopic Studies of the Interaction between Metformin Hydrochloride and Bovine Serum Albumin

Ahmad Tanwir[1], Rahat Jahan[2], Mohiuddin Abdul Quadir[1], Mohammad A. Kaisar[1] and Md. Khalid Hossain[1]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka 1000, Bangladesh
[2]Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Dhaka, Dhaka 1000, Bangladesh

ABSTRACT: The affinity of a drug to serum albumin has influence on the pharmacokinetics of a drug. In the present study, the mutual interaction of metformin hydrochloride (MET) with bovine serum albumin (BSA) was investigated using fluorescence spectroscopy under different conditions. It was observed that the fluorescence quenching of BSA by metformin hydrochloride is a result of the formation of metformin hydrochloride- BSA complex with probable involvement of tryptophan residue. Fluorescence quenching constants were determined using the Stern- Volmer equation and Van't Hoff equation to provide a measure of the thermodynamic parameters ΔG, ΔH, and ΔS at different temperatures indicating that the hydrogen bond and the hydrophobic forces play a major role for metformin hydrochloride- BSA association.

Key words: Metformin hydrochloride, bovine serum albumin, fluorescence quenching, thermodynamic parameters

INTRODUCTION

Metformin hydrochloride (MET) tablets are available in the market for the treatment of type-2 diabetes. Type-2 diabetes is a long- term metabolic disorder wherein the body becomes resistant to the effects of insulin, a hormone that regulates sugar absorption.[1]

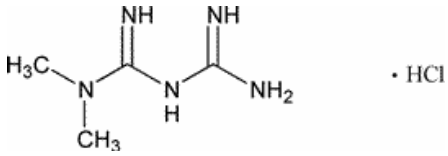

Figure 1. Molecular structure of metformin hydrochloride.

Plasma protein binding is an important factor to understand the pharmacokinetics and pharmaco-dynamics properties of drug candidates, as it strongly influences drug distribution and the free fraction, which is available to the target.[2] Serum albumins are the most abundant proteins in blood. They have many important physiological functions. For instance, they contribute to the osmotic blood pressure and are chiefly maintaining the pH of blood. But the most important physiological feature of the albumin involves the binding, transport and delivery of numerous ligands, such as fatty acids drugs and metal ions, in the bloodstream to their target organs. Therefore the investigation of such molecules with respect to albumin binding is of imperative and fundamental importance. Bovine and human serum albumins display approximately 76% homology and 3D structure of BSA is believed to be similar to that of HSA. In this work BSA is selected as our protein model because of its medical importance, low cost, ready availability, and the results of all studies are consistent with the fact that bovine and human serum albumins are homologous proteins.[3]

Spectral methods are powerful tool for the study of the reactivity of chemical and biological systems since it allows nonintrusive measurements of substance in low concentration under physiological conditions, and there are several studies of albumin

Correspondence to: Mohammad A. Kaisar
E-mail: kaisarpharma@gmail.com
Tel: +88029661900 (Ext-8153, 8131)

induced by drugs or other bioactive small molecules using spectral methods.[2]

In this work, we employed fluorescence spectroscopic method to obtain information related to the binding mechanism of metformin hydrochloride to BSA such as participating residues, quenching rate constant, binding modes, and binding constant.

MATERIALS AND METHODS

Materials. Metformin (MET) was obtained from Beximco Pharmaceuticals, Bangladesh (> 99.5%). BSA (fatty acid free, fraction V, 96-98%) was purchased from Sigma Chemical CO, USA. All other chemicals were of analytical reagent grade and doubly distilled water was used throughout.

Apparatus. Steady- state fluorescence measurements were carried out on a Hitachi F- 7000 Fluorescence spectrophotometer (Hitachi, Japan) equipped with 1.0 cm quartz cells. For different temperature a thermostat bath (Unitronic Orbital, P- Spectra, Spain) was used.

Spectroscopic measurement. The fluorescence spectra of were performed at different temperatures (298º K and 308º K). The widths of both entrance and exit slit were set to 5nm. The concentrations of BSA were stabilized 5.0 x 10^{-6} mol L^{-1}, and the concentration of aqueous MET varied from 2.5 to 27.5 x 10^{-6} mol L^{-1}. Most of the fluorescence emission spectra in the range of 320- 460 nm were recorded at excitation wavelength of 280 nm and 293 nm, respectively, in the same experimental conditions. MET has no fluorescence in the range of 320- 460 nm. The mixture solution of BSA and MET must be hatched at least 10 min before the spectrum measurements.

Principle of fluorescence quenching. Fluorescence quenching is described by the well-known Stern- Volmer equation:

$$F_o/F = 1 + K_{SV}[Q] \quad (1)$$

Where F_o and F denotes the steady- state fluorescence intensities in the absence and presence of quencher (metformin hydrochloride), respective, K_{sv} is the Stern- Volmer quenching constant, and [Q] is the concentration of the quencher. Hence, eq 1 was applied to determine K_{sv} by linear regression of a plot of F_o/F against [Q].

RESULTS AND DISCUSSION

The interaction of MET with BSA. There are three types of fluorophores in BSA, namely, tryptophan residue, tyrosine residue and phenylalanine residue. When excited by appropriate wavelength of light all of them can emit fluorescence.[4] In order to determine whether both tryptophan and tyrosine residues are involved in the interaction with MET, the fluorescence of BSA excited at 280 nm and 293 nm in the presence of MET is compared. When 280 nm excitation wavelength is used, fluorescence of albumin comes from both tryptophan and tyrosine residues, whereas 293 nm wavelength only excites tryptophan residues.[4]

The plots F/F_o against [MET]/[BSA] at excitation wavelength 280 nm and 293 nm are measured to determine the interaction of drug and BSA. Here, F_o is the fluorescence intensity of BSA, F is the fluorescence intensity of BSA in presence of drug.

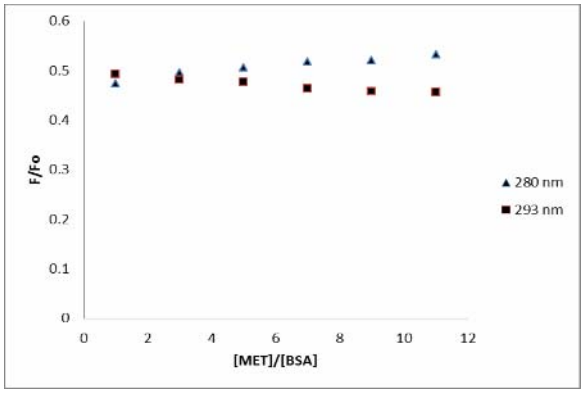

Figure 2. Fluorescence titration curve of BSA at the presence of MET at λ_{max} = 280 and 293 nm.

The plots F/F_o against [MET]/ [BSA] are shown in the Figure 2, which indicates that the fluorescence of BSA excited at 280 nm obviously differs from that excited at 293 nm in the presence of MET. This significant difference between quenching of serum

albumin fluorescence shows that the both tyrosine and tryptophan residues participate in the molecular interactions between BSA and MET.

Effect of MET on the fluorescence spectra of BSA. At the excitation wavelength of 280 nm, the fluorescence spectra of BSA with varying concentration are shown in the Figure 3.

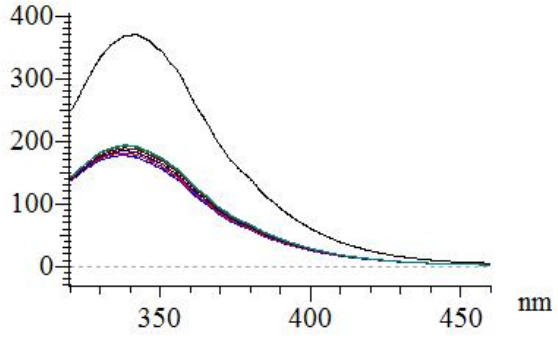

Figure 3. Fluorescence emission spectra of BSA in the presence of different concentration of MET (λ_{max} = 280 nm, T= 298ºK).

It shows the quenching of fluorescence of BSA. This indicates the strong interaction and energy transfer between MET and BSA.

Analysis of fluorescence quenching mechanism. A variety of molecular interctions can result in quenching, including excited- state reactions, molecular rearrangements, ground- state complex formation, and colloisional quenching, and so on. The different mechanisms of quenching are usually classified as either dynamic quenching or static quenching.[2] Static quenching refers to formation of complex between quencher and the fluorophore, while dynamic quenching refers to the collision of the quencher and fluorophore during the excitation.[5]

$$F_o/F = 1 + K_{SV}[Q]$$

Where F_o and F are the fluorescence intensities in the absence and presence of quencher, respectively. [Q] is the concentration of quencher, and K_{SV} is the Stern- Volmer quenching constant, which indicates the strength of the interaction between the drug and BSA. K_{SV} is the slope of the plot of F_o/F against [Drug] based on the fluorescence data at different temperatures (298ºK and 308ºK).

One way to distinguish the static quenching from dynamic quenching is to examine their differing dependence of temperature.[6] Dynamic quenching depends upon diffusion: higher temperatures result in larger diffusion coefficients. As a result, the bio-molecular quenching constants are expected to increase with increasing temperature. In contrast, increased temperature is likely to result in decreasing stability of complexes, and thus lower value of static quenching constants.[1]

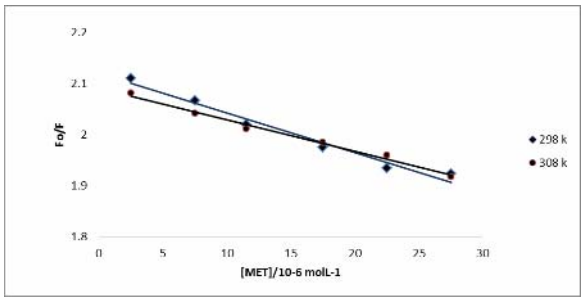

Figure 4. The Stern-Volmer plots for the quenching of BSA by MET at two different temperatures.

Table 1. Stern-Volmer quenching constant K_{SV} of the system of MET- BSA (R= Correlation co-efficient).

T (ºK)	K_{SV} (X 10^{-3}L mol^{-1})	R
298	7.79	0.9867
308	6.16	0.9947

Figure 4 displays the Stern- Volmer plots of the quenching of BSA fluorescence by MET at different temperatures. The plot shows that within the experimental concentrations, the results are in good agreement with the Stern- Volmer equation. The plots are linear and Stern- Volmer quenching constants are obtained from the slopes at various temperatures; these are listed in Table 1. The Stern-Volmer quenching constant decreases with increasing temperature for static quenching while for dynamic quenching the reverse effect is observed.[7]. From Table 1, it is clear that the probable quenching mechanism of the MET- BSA binding reaction is due to static quenching.

Thermodynamic parameters and nature of the binding forces. The interaction forces between quencher and bio- molecules may include hydrophobic force, electrostatic interactions, Van der Waals interactions, hydrogen bonds, etc.[8] The thermodynamic parameters are calculated in order to elucidate the interaction between the drug and BSA. The thermodynamic parameters can be determined from the Van't Hoff equation:

$$\ln K_a = -(\Delta H/\mathrm{RT}) + (\Delta S/\mathrm{R})$$

Where ΔS is the entropy change; constants K_a are analogous to the Stern- Volmer quenching constants K_{SV} at the corresponding temperature,[9] R is the gas constant. The enthalpy change (ΔH) and the entropy change (ΔS) can be determined from the slope and intercept of the fitted curve of ln K_{SV} against $1/T$ respectively.

The free energy (ΔG) can be estimated from the following relationship (3):

$$\Delta G = \Delta H - T\Delta S$$

For reversible drug- protein binding, binding forces like hydrogen bonds, Vander Waals forces, hydrophobic forces and electrostatic forces are involved.[10]

Table 2. Thermodynamic parameters of the system of MET- BSA.

T (K)	ΔH (KJ mol^{-1})	ΔS (Jmol-1K-$^{1)}$	ΔG (KJ mol^{-1})
298	-19.75	10.02	-22.74
308			-22.83

As shown in Table 2, the free energy change (ΔG) and the enthalpy change (ΔH) are negative and the entropy (ΔS) is positive. The positive ΔS value is considered evidence for hydrophobic interaction from the point of H_2O structure while negative ΔH implies the possibility of hydrogen bonds.[11] The negative sign for ΔG means that the binding process is spontaneous.[11] Thus both hydrogen bonding and hydrophobic interactions are present in the MET- BSA binding.

Binding constant. When small molecule bind independently to a set of equivalent sites on a macromolecule, the equilibrium between free and bound molecule is given by the following equation

$$\log \{(F_o - F)/ F\} = \log K + n \log [Q]$$

Where, K is the binding constant to a site. The values of K is calculated from the values of the plot of log $\{(F_o - F)/ F\}$ versus log [MET] (12).

Table 3. Binding constant and binding points of the system of MET- BSA (R= Correlation co-efficient).

T (K)	K (X 10^{-2} L mol^{-1})	R
298	8.62	0.9979
308	6.59	0.9926

Table 3 contains the values of K at different temperature, which obtained from the intercept. It was observed that the binding constant decrease with the increase in temperature, resulting in the reduction stability of the MET- BSA complex

CONCLUSION

In this paper, the interactions of MET with BSA have been investigated by fluorescence spectroscopy. The experiment result indicates that tryptophan and tyrosine residues participate in the interactions between MET with BSA at 280 nm excitation wavelength. The probable main quenching mechanism of fluorescence of BSA initiated by MET is static quenching process result of MET- BSA complex formation. The thermodynamic parameters such as enthalpy change (ΔH) and entropy change (ΔG) for MET- BSA, was calculated according to the Van't Hoff equation. The study of thermodynamic parameters proved the existence of hydrogen bonding and hydrophobic forces in MET- BSA complex. The decreasing binding constant for MET and BSA complex with increasing temperature indicates the decomposition of the complexes by increasing temperature.

ACKNOWLEDGEMENTS

The authors are thankful to Beximco Pharmaceuticals for a gift sample of metformin hydrochloride. They also thank Centre for Advanced

Research in Science (CARS), University of Dhaka, Bangladesh for Fluorescence spectrophotometer support.

REFERENCES

1. Kaushelendra, M., Himesh, S., Govind , N., Sita, S. P. and Singhai, A.K. 2011. Method Development and Validation of Metformin Hydrochloride in Tablet Dosage Form. *E. J. Chem.* **8**, 1309-1313.
2. Yan, J. H., Yi, L. and Xiao, H.X. 2009. Investigation of the Interaction between Berberine and Human Serum Albumin. *Biomacromolecules.* **10**, 517-521.
3. Xing, J.G., Xiu, D.S. and Shu, K.X. 2009. Spectroscopic investigation of the interaction between riboflavin and bovine serum albumin. *J. Mol. Structure.* **931**, 55-59.
4. Steinhardt, J., Krijn, J. and Leidy, J. G. 1971. Differences between bovine and human serum albumins: binding isotherms, optical rotatory dispersion, viscosity, hydrogen ion titration, and fluorescence effects. *Biochemistry*. **10**, 4005-4015.
5. Rasoulzadeh, F., Asgari, D., Naseri, A. and Rashidi, M.R. Spectroscopic studies on the interaction between erlotinib hydrochloride and bovine serum albumin. DARU *J. Pharm. Sci.* **18**, 179-184
6. Lakowicz, J.R. 1999. Principles of Fluorescence Spctroscopy. Second ed., Plenum Press, New York, pp. 237- 265.
7. Kandagal, P.B., Seetharamappa, J., Shaikh, S.M.T. and Manjunatha, D.H. 2007. Binding of trazodone hydrochloride with human serum albumin: A spectroscopic study. *J. Photochem. Photobiol. A, Chem*. **185**, 239-244
8. Papadopoulou, A., Green, R.J., Frazier, R.A. 2005. Interaction of flavonoids with bovine serum albumin: a fluorescence quenching study. *J. Agric. Food Chem.* **53**, 158-163
9. Shao-Fa Sun, Bo Zhou, Han-Na Hou, Yi Liu and Guang-Ya Xiang. 2006. Studies on the interaction between Oxaprozin-E and bovine serum albumin by spectroscopic methods. *Int. J. Biol. Macromol*. **39**, 197-200
10. Timaseff, S.N. 1972. Thermodynamics of protein interactions. In Proteins of Biological Fluids; Peeters H., Ed.; Pergamon Press: Oxford.
11. Ross, P.D. amd Subramanian, S. 1981. Thermodynamics of protein association reaction: forces contribution to stability. *Biochemistry* **20**, 3096-3102
12.Hu, Y.J., Liu, Y., Hou, A.X., Zhao, R.M., Qu, X.S. and Qu, S.S. 2004.Studies on the interaction between rare-earth salts of heteropoly EuHSiMo10-W2O40·25H2O and bovine serum albumin (in Chinese). *Acta. Chimica. Sinica.* **62**, 1519-152