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INTERACTION OF ANTI MERS-COV RIBAVIRIN WITH HUMAN SERUM ALBUMIN IN DISEASE MIMETIC CONDITIONS OF DIABETES AND UREMIA AN *IN-VITRO* STUDY

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Abstract:

The binding characteristics of ribavirin to human serum albumin (HSA) have been studied using fluorescence technique under in vitro disease mimetic conditions of uremia and diabetes. Ribavirin is found to bind moderately (K_b-10^4) with glycated and native HSA. There is decrease in binding both in glycated human serum albumin and with human serum albumin in presence of urea. Ribavirin binding leads to intrinsic fluorescence quenching of HSA, glycated albumin and HSA in presence of urea indicating ribavirin binding to transport protein. Binding constant values decreased with increasing temperatures suggesting the quenching mode operating in the binding interaction of ribavirin with HSA and GHSA is static. Binding process was found to be spontaneous and exothermic. Analysis of the quenching and thermodynamic parameters of protein ligand system suggested the change in intermolecular interactions between ribavirin and HSA upon protein modification by glycation and in presence of urea leading to loss in ligand binding.

Keywords: *Glycated human serum albumin; uremia; fluorescence spectroscopy; ligand binding; ribavirin*

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INTRODUCTION:

Ligand binding phenomenon is important as drug pharmacokinetics is essentially controlled by this process (Dahl & Akerud, 2013; Elfaki et al., 2011; Elfaki et al., 2013; Schuetz et al., 2018). Extent and affinity of binding of ligand to major transport proteins in blood may strongly influence the absorption, metabolism distribution and excretion of drugs (Ascoli et al., 2006; Ghuman et al., 2005; Neault & Tajmir-Riahi, 1998; Oravcova et al., 1996). Circulating lifetimes and bioavailability can be affected by affinity of the drug towards protein. Weak interaction of ligand with transport proteins results in shorter lifetimes while strong binders have decreased free fraction in plasma and stay for long time. Strongly bound ligand are released slowly over a period of time and thus remain in biological system for comparatively long durations. We need to standardize the drug dose for maintaining a balance between the free and bound form of drug molecules. Desired therapeutic doses need to be delivered to target tissues for achieving treatment targets. At the same time we can manage balance between the proper stay time and clearance of drug from the system. This can help to reduce toxic side effects of drugs on human system. These concerns make it imperative to study ligand binding behavior of therapeutic drugs. Human serum albumin (HSA) is a major transport protein in human blood. Albumin is known to serve some important functions in human body including maintenance of oncotic pressure, binding and transport of ligand, hormones and therapeutic drugs(Ghuman et al., 2005; Yue et al., 2018). HSA is single chain, non-glycosylated protein having 66.5Kda molecular weight. HSA is essential component of the interstitial body fluid. It consists of three homologous domains. HSA domains are arranged in heart shaped manner with 80X80X30 A^0 in dimension. Domain I, domain II and domain III, are present each domain has two sub domains designated as sub domains A and sub domains B. There are two principal ligand binding sites on HSA molecule (Mabuchi & Nakahashi, 1988; Sulkowska, 2002; Zaidi et al., 2013). These binding sites are located in sub domain II A and III B known as Sudlow site I and II respectively (Alam et al., 2018; Amroabadi et al., 2018). The important function of this protein is solute transport. It helps in solubilization and targeting ligand to cells to by binding to surface receptors on cells which recognize the specific ligand induced changes in conformation of HSA. Structural changes can arise due to disease conditions like uremia and hyperglycemia which can alter binding behavior of ligand (Florens et al., 2018; Jing et al., 2018; Sakai et al., 1995; Takamura et al., 1997). Hyperglycemia can cause glycation of proteins. Glycation is one of the main reasons for altered physiological function of protein owing to associated conformational alterations(Ahmed et al., 2005; Wolff et al., 1991). The present study is fluorescence spectroscopic insight in the binding behavior of ribavirin to HSA in *in vitro* disease mimetic conditions of diabetes and uremia. Uremia is caused by chronic kidney dysfunction (CKD). CKD affects many body fuctions (Boccardo et al., 2004; Kato et al., 2008; Owen Jr et al., 1993). Binding defect of uremic and diabetic plasma for ribavirin can demand for customized drug dosage as may be required for patients suffering from viral infections in which ribavirin serves as drug of choice in treatment plan. Ribavirin serves as an important antiviral drug used in treatment plan of Middle East Respiratory Syndrome Corona virus *(*MERS-CoV*)* infection (Falzarano et al., 2013; Shalhoub et al., 2015). For this disease many cases have been reported worldwide since the discovery of (MERS-CoV) in Saudi Arabia. Since then the virus has caused many deaths, with high fatality rate of about 50% (Assiri et al., 2013; Kilianski et al., 2013). The disease was initially treated with described therapies used for the related disease caused by severe acute respiratory syndrome (SARS) corona virus (Assiri et al., 2013; Zumla et al., 2015; Zumla et al., 2014). With advancements in clinical care and drug discovery, use of combination therapy of interferon and ribavirin was considered as an improved therapeutic option for treatment of disease. This disease is known to complicate when co morbid conditions of hyperglycemia and chronic kidney disease are present. The increased glycation and increased urea levels pertaining to diabetes and uremia respectively can affect Ribavirin in the binding characteristics. Treatment outcome in MERS-CoV infection have been strongly correlated to preexisting chronic conditions of diabetes and chronic kidney disease (Pietrement et al., 2013). In co-morbid disease environments, advanced age, smoking severity treatment outcome is affected in such infectious diseases (Chan et al., 2003; Esper et al., 2006; Golden et al., 1999). We evaluate the drug binding defects in ribavirin in normal and disease mimetic HSA solution using fluorescence spectroscopy. Fluorescence data can be used to elucidate the parameters of the drug complexation in presence of urea and glucose (Ajmal et al., 2016; Elfaki et al., 2018; Siddiqi et al., 2018; Siddiqi et al., 2017). The present study is an attempt to examine binding refashioning under these disease conditions and will help to understand the changes in drug behavior. This will help in dosage design so that appropriate dosage for achieving optimum treatment outcome can be given to the patients. This

information can help in development of rationalized ribavirin treatment plan for patients presented with diabetes and uremia coexisting with viral infection. Drug binding to human albumin plays a significant role in drug pharmacokinetics profile and can affect therapeutic and toxic effect of the drug on human system. Human serum albumin has versatile ability to bind diverse ligand including aromatic and heterocyclic compounds, endogenous and exogenous ligand, therapeutic molecules and many more. The binding process for most of the drugs is largely attributed to the two major binding regions in HSA molecule, namely Sudlow site I in sub domain IIA and Sudlow site II which is located in sub domain IIIA. These are specialized cavities lined by amino acid residues that supports fit of ligand into these cavities minimizing energy and helping in safe transport process. When there are protein modifications that may be induced by pathophysiological changes. Then native conformation of the protein can alter affecting binding capabilities of these major drug binding sites. Albumin is exposed to numerous structural modification spatially in the disease like diabetes where there is persistent increased blood glucose. Other such chronic condition is uremia where urea levels can increase up to thrice the levels found in healthy human adults, this is due to poor kidney function (De Groot et al., 2004; Kaysen, 2001). The unusual presence of urea and glucose can affect stability, activity, physical and chemical properties of proteins. These conditions expose proteins like transport protein HSA to different environment. Glycation is major modification concerning serum albumin. Glycation is non-enzymatic process that begins with the reaction between a reducing sugar and a free amine group on a protein molecule. Glycation results in covalent modification of polypeptide. Various reducing sugars including glucose, fructose, and galactose can also react with proteins and participates in glycation process. Metabolites which are primarily located intracellular can also participate in glycation reaction and sometimes glycation reaction with these molecules is much faster. Fructose is about eightfold reactive in glycation process; the contribution of fructose towards extracellular glycation is considerably less than glucose although it has high reactivity. This is because of the low plasma concentration of fructose which is about 35 µmol/L in contrast glucose (Ahmed et al., 2005; Morais et al., 2019; Qais et al., 2019; Ulrich & Cerami, 2001). Glucose is present in high concentration of about 5 mmol/L. Ribavirin (1-b-D-ribofuranosyl-1,2,4-triazole-3-carboxamide)(figure 5B) is a synthetic purine nucleoside. Ribavirin have antiviral activity against broad-spectrum of DNA and RNA viruses in vitro. Ribavirin has been used in management of many viral diseases like respiratory syncytial virus, influenza A and B virus, Lassa fever virus and chronic hepatitis C infections.(Espy et al., 2018; Reichard et al., 1991; Voet et al., 2018) Ribavirin in a combination of interferon-α2b has been indicated in the management of MERS-CoV infections (Chafekar & Fielding, 2018; Dandachi & Rodriguez-Barradas, 2018; Morra et al., 2018). Although no particular international recommendations or any specific therapy plan is available to treat the disease. The treatment line with ribavirin and combination therapy are chose treatment option.

MATERIALS AND METHODS:

Materials

Glycated human serum albumin (A8301) and human serum albumin (A1887) and ribavirin (R 9644) was purchased by Sigma Aldrich chemical company rest of the chemicals employed were of analytical grade

Preparation of HSA and ribavirin solutions

Ribavirin HSA and GHSA are dissolved in 20mM Sodium phosphate buffer of pH 7.4. HSA, GHSA were extensively dialyzed in buffer. Protein stock solutions (2.5mg/ml) were prepared. The concentration of HSA and GHSA was determined spectrophotometrically using $E^{1\%}_{280\,nm}$=5.3(Ajmal et al., 2017; Ishtikhar et al., 2018) in 20 mM phosphate buffer, Ribavirin solution was prepared by weight/volume (w/v) in distilled water.). The urea concentration used in preparation of UHSA and UGHSA solution is 0.5 M. Protein samples were incubated with urea for 2 hours prior to the titration with ribavirin.

Steady state fluorescence quenching measurements

Cary eclipse fluorescence spectrophotometer, Agilent technologies equipped with thermal controller water circulator, quantum north west was used to measure the fluorescence emission spectra of HSA, GHSA and in presence of urea, Emission spectra were taken from 292 nm- 498 nm with excitation width = 5nm and emission width = 5nm. 1µM HSA and GHSA were titrated manually using micropipettes with increasing ribavirin concentration into protein solution of fixed concentration. Drug concentration was increased gradually so as to obtain ribavirin to protein molar ratio d/p= 0 to d/p= 5. Experiments were performed at three temperatures 298K, 303 K and 310K. Emission data were recorded and plotted at 340 nm using origin 8 software, the decrement in fluorescence intensity at 340 nm was analyzed.

Stern–Volmer equation (Equation -1) is used to analyze the data.

$$Fo/F = Ksv\,[Q] + 1 = kq\tau o\,[Q] + 1$$

(1)

K_{sv} is the Stern–Volmer constant, k_q is the bimolecular rate constant of the quenching reaction and τ_0 the average integral fluorescence life time of tryptophan which is ~10^{-9} sec. Binding constants and number of binding sites were obtained using Equation- 2.

$$Log\,(Fo/F - 1) = log\,Kb + n\,log\,[Q]$$

(2)

Where F_o and F were the fluorescence intensities in absence and presence of ribavirin. K_b is the binding constant and n is the number of binding sites. Equation -3 was used for calculating the change in free energy while enthalpy and entropy change at different temperatures were analyzed from the Van't Hoff equation, Equation- 4

$$\Delta G° = -RTlnK_b$$

(3)

$$lnK_b = \frac{-\Delta H°}{RT} + \frac{\Delta S°}{R}$$

(4)

Where K_b is the binding constant at temperature T, ΔH° stands for the enthalpy change, whereas ΔG° is free energy change, ΔS° is entropy change, R is a gas constant (1.987 cal $mol^{-1}K^{-1}$) and T is the absolute temperature (K).

RESULTS AND DISCUSSION:

Fluorescence quenching of HSA and GHSA by ribavirin

Aromatic amino acid residues in proteins are internal fluorophores and show fluorescence. Photon emission upon excitation with light is fluorescence. Fluorescence is due to return of electron back to lower energy level from excited state. Amino acids tyrosine, tryptophan and phenylalanine are internal fluorophores in proteins. Contribution of tryptophan towards protein fluorescence is highest among three internal fluorophores of proteins. Fluorescence is sensitive to microenvironment around fluorophores. Many factors can affect this phenomenon of fluorescence; one of these factors is ligand binding near to fluorophores. The process of fluorescence quenching occurs when absorbed energy is transferred to bound ligand resulting in low emission yield of fluorophores. This quenching phenomenon can yield useful information about ligand protein interaction. Quenching has been observed to occur by two principal modes. Collision process results in dynamic quenching while complex formation between quencher and fluorophores leads to static quenching. The two modes of the fluorescence quenching can be identified by their dependence on temperature. Increase in temperature increase diffusion coefficients which favor dynamic quenching process, as more and more molecules will collide at a time due to increased mobility. While in static quenching process temperature increment lowers the stability of static complex between quencher and fluorophores therefore static quenching constant detrude. Quenching of intrinsic fluorescence of HSA and GHSA is measured with increasing molar concentrations of ribavirin. Upon excitation at 295, HSA and GHSA showed strong emission with peak at 340 nm which gradually decreased on successive addition of increased ribavirin concentrations. Protein concentration is kept fixed. Data was obtained at 298K, 303K and 310K. As shown in Figure-1and Figure 2.

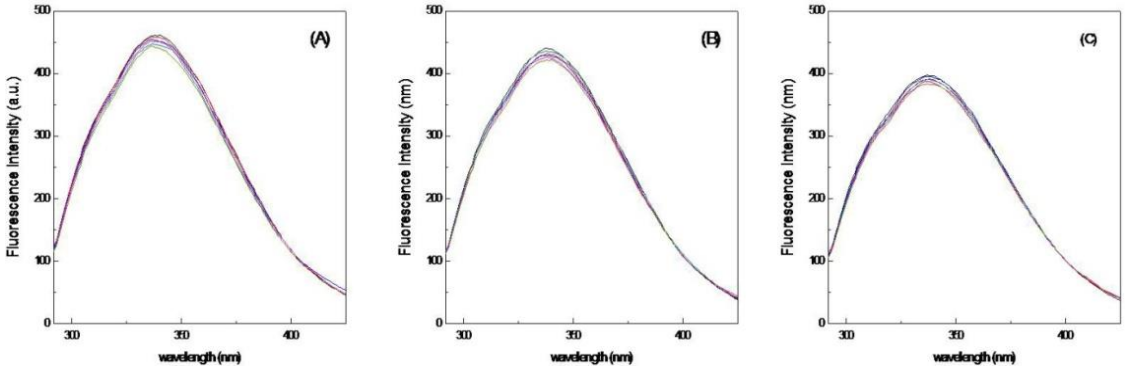

Figure 1. Fluorescence emission spectra of HSA titrated with increasing ribavirin concentrations at 293K (A), 298K (B) and 303K (C). The concentration of HSA was 1 µM and the concentration of ribavirin was varied from 0-5 µM.

The intrinsic fluorescence of the protein was measured in 20 mM sodium phosphate buffer, pH 7.4 upon excitation at 295 nm.

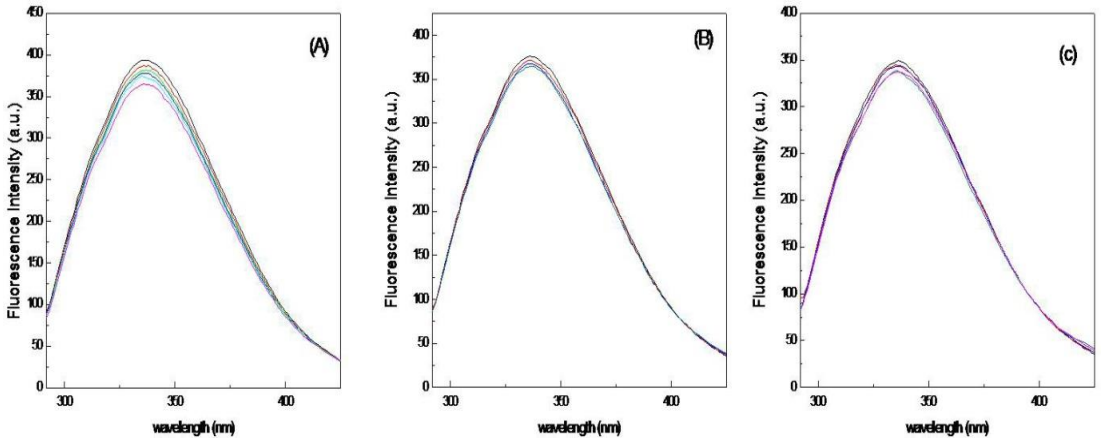

Figure 2. Fluorescence emission spectra of GHSA titrated with increasing ribavirin concentrations at 293K (A), 298K (B) and 303K (C). The concentration of HSA was 1 µM and the concentration of ribavirin was varied from 0-5 µM. The intrinsic fluorescence of the protein was measured in 20 mM sodium phosphate buffer, pH 7.4 upon excitation at 295 nm

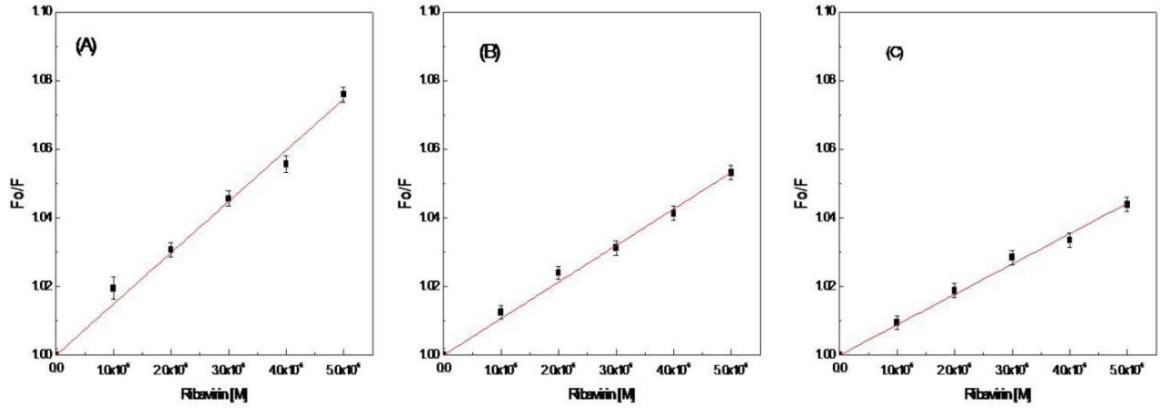

Figure 3. Stern-Volmer plots for HSA interactions with ribavirin at 293K (A), 298K (B) and 303K (C). The concentration of HSA was 1 µM and the concentrations of ribavirin were varied from 0-5 µM. The intrinsic fluorescence of the protein was measured in 20 mM sodium phosphate buffer, pH 7.4. Emission data is collected at 340 nm upon excitation at 295 nm.

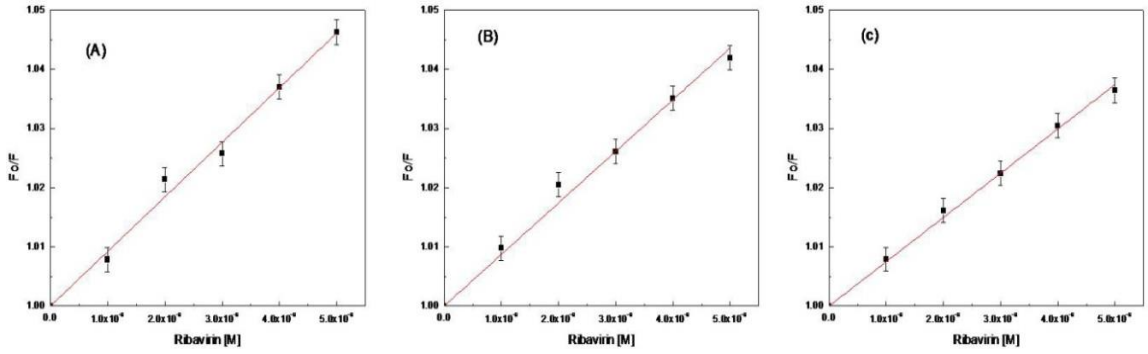

Figure 4. Stern-Volmer plots for GHSA interactions with ribavirin at 293K (A), 298K (B) and 303K (C). The concentration of GHSA was 1 µM and the concentrations of ribavirin were varied from 0-5 µM. The intrinsic fluorescence of the protein was measured in 20 mM sodium phosphate buffer, pH 7.4. Emission data is collected at 340 nm upon excitation at 295 nm.

There is decrease in fluorescence emission peak upon the titration of HSA and GHSA with ribavirin suggesting the binding of ribavirin to both proteins at all the three studied temperatures. In order to look into the type of quenching mechanism involved in the binding process, fluorescence emission data at three temperatures was analyzed using Stern Volmer equations, for HSA Figure-3 and GHSA Figure-4.

Table 1. Binding Parameters for the interaction of ribavirin with HSA in 20 mM Phosphate buffer of pH 7.4 at different temperatures obtained and calculated from fluorescence quenching experiments.

T (K)	K_{SV} ($\times 10^3\ M^{-1}$)	R^2	K_q ($\times 10^{12} M^{-1}\ S^{-1}$)	K_b ($\times 10^3\ M^{-1}$)	R^2	n	ΔG (kcal.mol^{-1})	ΔH (kcal.mol^{-1})	ΔS (cal. mol^{-1}.K^{-1})
293	14.92	0.99	14.92	10.28	0.99	0.96	-5.47	-4.69	2.66
298	10.64	0.99	10.64	9.69	0.99	0.98	-5.43		
303	8.84	0.99	8.84	7.65	0.99	0.98	-5.38		

Table 2. Binding Parameters for the interaction of ribavirin with GHSA in 20 mM Phosphate buffer of pH 7.4 at different temperatures calculated from fluorescence quenching experiments.

T (K)	K_{SV} ($\times 10^3\ M^{-1}$)	R^2	K_q ($\times 10^{12} M^{-1}\ S^{-1}$)	K_b ($\times 10^3\ M^{-1}$)	R^2	n	ΔG (kcal.mol^{-1})	ΔH (kcal.mol^{-1})	ΔS (cal. mol^{-1}.K^{-1})
293	9.22	0.99	9.22	9.03	0.99	0.98	-5.30	-3.30	6.99
298	8.71	0.99	8.71	7.90	0.99	0.99	-5.31		
303	7.48	0.99	7.48	7.24	0.99	0.99	-5.35		

Calculated K_{sv} values are listed in table 1 and table 2. The K_{sv} values obtained from fluorescence emission data decrease with increase in temperature leading to the idea that static mode of quenching is operative in ribavirin binding to HSA and GHSA. The values for K_q obtained for ribavirin interaction with HSA and GHSA are far larger than that of maximum scatter collision quenching constant of various kinds of biomolecules which is $2x10^{10}$ suggesting the static quenching mechanism is operative in binding of ribavirin with HSA and GHSA.

Albumin, the major circulating protein in blood, can undergo increased glycation in diabetes leading to structural and functional alterations. In this case interaction of ribavirin was studied with GHSA and it was found that K_{sv} values for normal HSA were about 1.6 times higher as compared to those obtained with GHSA. It is evident that protein glycation has important implications for protein functionality. The fluorescence emission is found to decrease on glycation, Figure-5 suggesting structural perturbations in HSA upon glycation.

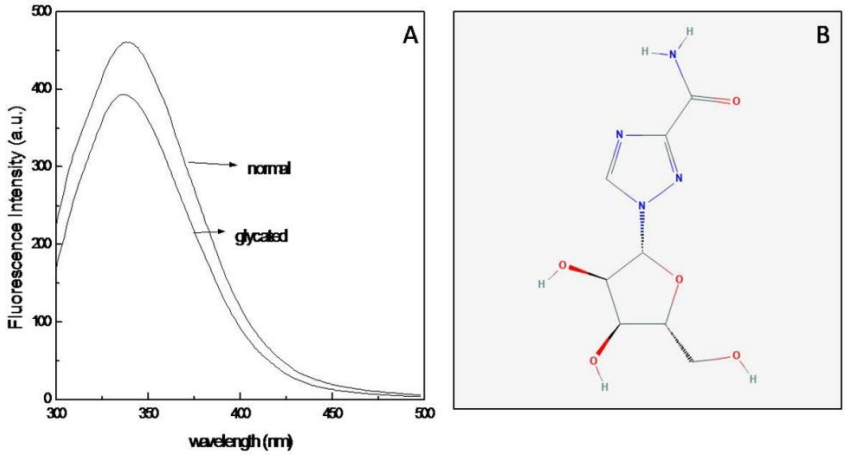

Figure 5. Fluorescence emission spectra of native HSA and glycated HSA (indicated by arrows). The intrinsic fluorescence of the protein (1µM) was measured in 20 mM sodium phosphate buffer, pH 7.4. Emission data is collected at 340 nm upon excitation at 295 nm.

These observations indicate that glycation is affecting protein in such a way that environment around tryptophan residues change, these conformational changes can affect the binding sites of albumin. The process of ligand binding can be nonspecific to very specific. Proteins are so effective in identification and sorting of molecule that the can identify and preferentially bind particular stereoisomer of compound. Such high sensitive structures can definitely be affected by changes incurred in glycation process so it is interesting to study the impact of glycation on binding behavior of important antiviral drug ribavirin with major transport protein HSA.

Binding parameters and mechanism

Binding of ligand to protein essentially controls their stay in human system and their clearance from the body. It also affects metabolism and bioavailability of drugs in human system. Binding constant of drug molecule (K_b) is pharmacologically significant term. Binding constant dictates the distribution of the drug and is deciding factor for drug bioavailability. Higher K_b values indicate the drug is bound to protein tightly so less concentration of free fraction of drug in plasma is available. While in other case where drug s bound weakly, they have faster clearance rate as more and more drug is free in system and easily flushed out. Such weakly bound drugs have shorter stay in the body and thus repeated doses are given so as to obtain desired therapeutic effect. For the determination of the binding constant and number of binding sites Equation-2 is used, from the slopes and intercepts of modified Stern Volmer plots, for HSA Figure-6 and GHSA Figure-7 we obtained the number of binding sites (n) and the value of binding constant K_b, values are listed in Table-1 for HSA-ribavirin system and Table-2 for GHSA-ribavirin system.

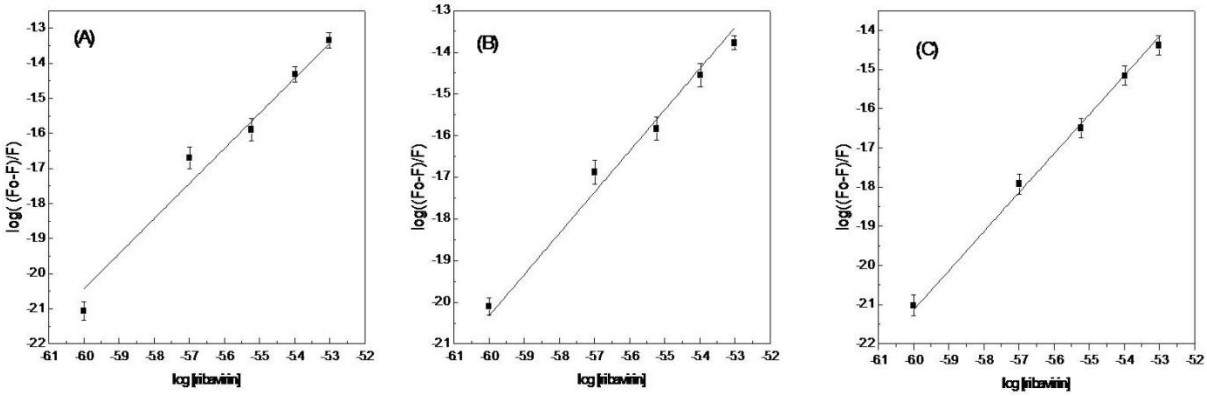

Figure 6. Modified Stern-Volmer plots for HSA interactions with ribavirin at 293K (A), 298K (B) and 303K (C). The concentration of HSA was 1µM and the concentrations of ribavirin were varied from 0-5 µM. The intrinsic

fluorescence of the protein was measured in 20 mM sodium phosphate buffer, pH 7.4. Emission data is collected at 340 nm upon excitation at 295 nm.

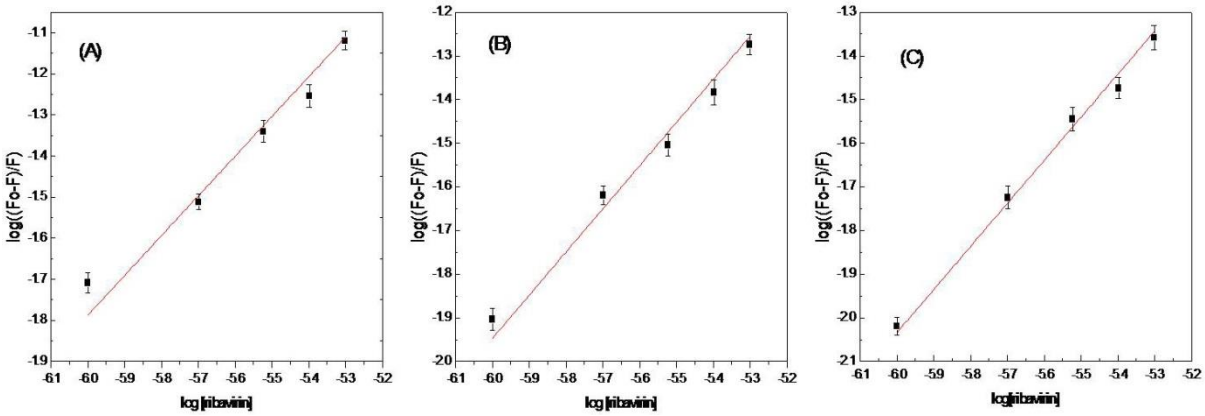

Figure 7. Modified Stern-Volmer plots for GHSA interactions with ribavirin at 293K (A), 298K (B) and 303K (C). The concentration of GHSA was 1μM and the concentrations of ribavirin were varied from 0-5 μM. The intrinsic fluorescence of the protein was measured in 20 mM sodium phosphate buffer, pH 7.4. Emission data is collected at 340 nm upon excitation at 295 nm.

It is observed that the binding constant values that binding stoichiometry is close to one in both cases. Ribavirin is moderately bound to HSA and weakly bound to GHSA whereas in both cases binding constant values were found to decrease with increase in temperature leading to the nation that static mode of fluorescence quenching is operative in ribavirin HSA and ribavirin GHSA systems.

Thermodynamics of binding interaction

There are specific regions on protein molecule that can preferentially fit particular ligands with high complementarity and compatibility. The ultimate aim is to minimize energy and attain stability. HSA has hydrophobic cavities in sub domain II and III which are the two main ligand binding sites Hydrophobic interactions and hydrogen bonding are the main forces for ribavirin interaction with HSA, interaction parameters and binding energy obtained from fluorescence quenching measurements are listed in Table-1. The results for dependency of binding constant studied using equations-3 and 4 on temperature indicated complex formation, thermodynamic parameters – Gibbs free energy change, enthalpy change and entropy change are elucidated using linear second law of thermodynamics plot. Vant Hoff plot is used for calculation of enthalpy and entropy changes figure-8, For HSA figure 8A and GHSA figure 8B.

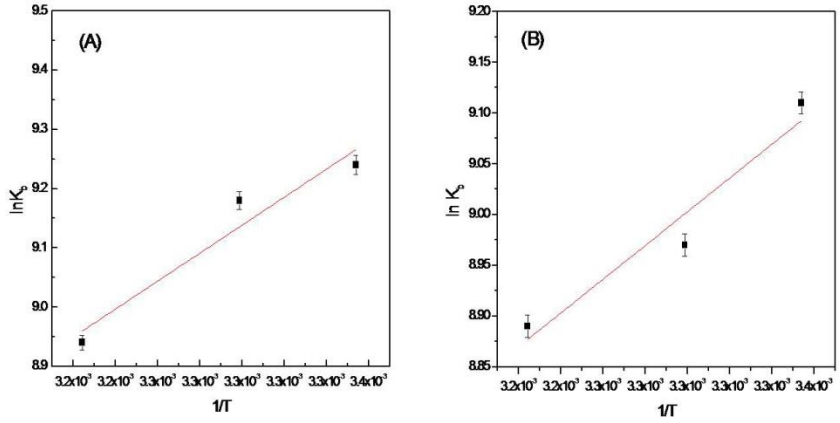

Figure 8. Van't Hoff plot for temperature dependence of K_b obtained from fluorescence quenching of HSA (A) and GHSA (B) by ribavirin at 293K, 298Kand 303K. Excitation wavelength was 295 nm.

The calculated values are listed in table 1 for HSA and Table 2 for GHSA. Negative values of free energy and enthalpy change signify the ligand binding process to be spontaneous and exothermic. The positive value of entropy change is evident of disruption in water structure. Entry of ligand into the binding area caused the changes in water structure in drug binding site to fit in the binding pocket.

Ribavirin binding in uremia mimetic conditions

There are many covalent and non covalent forces operative in ligand binding process although non covalent interactions are weak but cumulatively they can be very effective in essentially controlling the process, owing to their strength in numbers. For example water structure is essentially controlled by intermolecular hydrogen bonds leading to three physical states of water each with its own peculiarity and significance, same happens when ligand interacts with a protein in solution water structure is altered in the binding site and sometimes the rearrangement is far reaching and can involve far away amino acid residues or whole protein structure as such can be affected resulting in physical changes, like what we see in myosin. Molecules interact with each other by an invisible attractive or repulsive force. These forces include Vander Waal force, hydrogen bonding, hydrophobic interactions, dipole-dipole interactions and electrostatic interactions. Ligand binding to HSA is influenced by many factors one of them is presence of solutes and other competitive ligand that share the same site or part of it they can influence the binding of each other. Here we studied binding of ribavirin to human serum albumin in presence of ure UHSA, Figure-9 and glycated human serum albumin in presence of urea UGHSA, Figure-10.

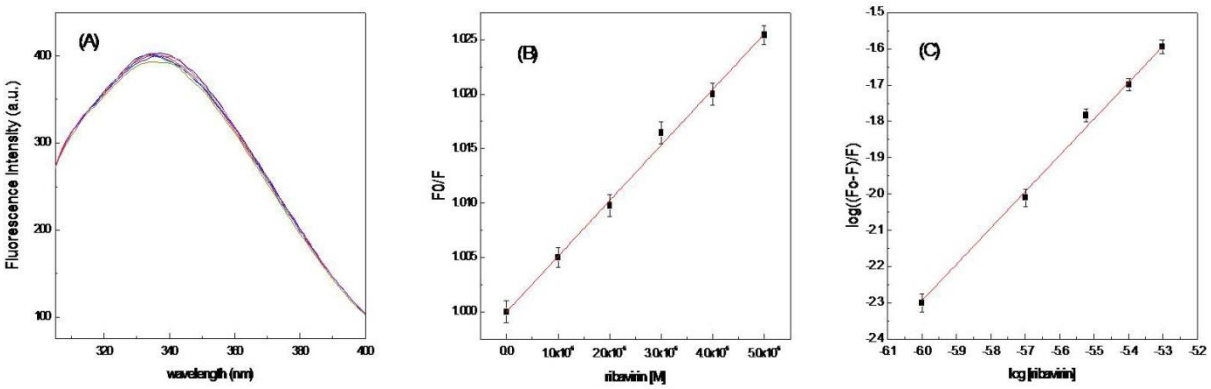

Figure 9. Fluorescence emission spectra of UHSA titrated with increasing concentration of ribavirin (A). The Stern–Volmer plots for the binding of ribavirin with UHSA (B) and modified Stern-Volmer plots for HSA interactions with ribavirin (C) The intrinsic fluorescence of the protein (1µM) was measured in 20 mM sodium phosphate buffer, pH 7.4. Emission data is collected at 340 nm upon excitation at 295 nm.

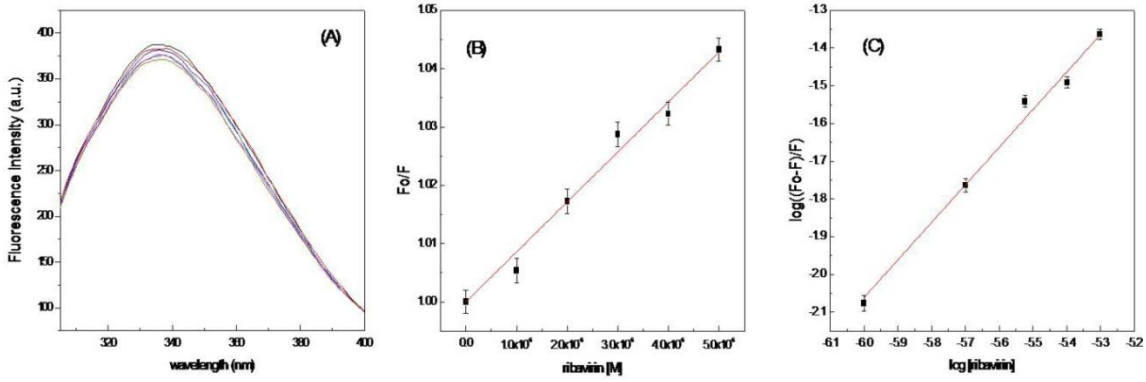

Figure 10. Fluorescence emission spectra of UGHSA titrated with increasing concentration of ribavirin(A). The Stern–Volmer plots for the binding of ribavirin with UGHSA (B) and modified Stern-Volmer plots for UGHSA interactions with ribavirin (C) The intrinsic fluorescence of the protein (1µM) was measured in 20 mM sodium phosphate buffer, pH 7.4. Emission data is collected at 340 nm upon excitation at 295 nm.

We found that the binding of ribavirin is affected by urea and the binding constant is found to decrease in presence of urea for both HSA and GHSA (Table-3).

Table 3. Binding Parameters for the interaction of ribavirin with HSA in presence of urea (0.5M) in 20 mM Phosphate buffer of pH 7.4 at 298 K

	K_{SV} ($\times 10^3 M^{-1}$)	R^2	K_q ($\times 10^{12} M^{-1} S^{-1}$)	K_b ($\times 10^3$ M^{-1})	R^2	n
HSA	8.57	0.99	8.57	8.165	0.99	.99
GHSA	5.11	0.99	5.11	5.140	0.99	.98

These observations are suggestive of decreased ribavirin binding to HSA and GHSA under uremia conditions

CONCLUSION:

Binding of ligand is important phenomenon the present study is about the binding of ribavirin with HSA using fluorescence spectroscopy technique. Ribavirin interacts with HSA with hydrophobic interactions and hydrogen bonding. Decrease in fluorescence emission in glycated albumin indicates microenvironment change around intrinsic fluorophores of protein upon glycation. HSA and GHSA differ in their binding ability for ribavirin drug, meaning conformational alterations induced by glycation resulted in remodeled binding of ribavirin to native and glycated HSA. Binding constant values are found to decrease in case of glycated HSA which suggest increase free drug in plasma of diabetic patients as compared to non diabetic patients treated with same concentration of ribavirin. Also, we found the decrease in binding when urea is present in system. These observations lead to conclusion that when co morbid states of diabetes and urea are present ribavirin binding to HSA is reduced to one third of the value in normal conditions. In this case with glycated HSA and in presence of urea more drug molecules are free in solution and less drug is bound to transport proteins. It would be better to give frequent doses in diabetics and uremic patients to obtain optimal therapeutic effect. Considering that many times diabetes is complicated with chronic kidney disease can influence ligand carrying function of HSA. This study provides insight into the effect of glycation and urea on binding of important drug ribavirin a compound currently in use for therapy when viral infection is complicated by co morbid conditions spatially in acute infections like MES-CoV infections. These findings are important in the context when it comes to the use of medications to treat particular condition which is complicated with co morbid conditions. Then we have to take care of these aspects, ligand binding studies are important since drug binding alterations in disease conditions can affect bioavailability of drug compounds and can significantly affect treatment outcome these considerations become important for efficacious treatment and positive outcome.

COMPETING INTERESTS:

The authors declare no competing interests

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