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Mechanism of interaction of hypoglycemic agents glimepiride and glipizide with human serum albumin

Research Article

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Abstract: The mechanism of interaction of hypoglycemic drugs, glimepiride and glipizide with human serum albumin (HSA) has been studied using fluorescence spectroscopy. The results are discussed in terms of the binding parameters, thermodynamics of the binding process, nature of forces involved in the interaction, identification of drug binding site on serum albumin and the fluorescence quenching mechanism involved. The association constants were of the order of 10^5 and glipizide was found to have much higher affinity for HSA than glimepiride at all temperatures. Thermodynamic parameters for the binding suggested that hydrophobic interactions are primarily involved in the binding of these drugs to HSA. However, glimepiride and glipizide appear to cause temperature-dependent conformational changes in the albumin molecule and, therefore, the nature of interaction varied with temperature. Glimepiride and glipizide bind to both site I and site II on HSA, but the primary interaction occurs at site II. The binding region in site II is different for the two drugs. Stern-Volmer analysis of quenching data indicated that tryptophan residues of HSA are not fully accessible to the drugs and a predominantly dynamic quenching mechanism is involved in the binding. Results can provide useful insight into prediction of competitive displacement of these drugs by other co-administered drugs and excipients, resulting in serious fluctuations of the blood glucose levels in diabetic patients.

Keywords: *Binding • Fluorescence • Glimepiride • Glipizide • HSA*

1. Introduction

Binding affinity for human serum albumin is one of the important factors controlling the distribution, elimination, therapeutic activity and toxicity of drugs since only the free (unbound) drug is pharmacologically active. The effect is especially significant for drugs that are highly protein bound, have a narrow therapeutic index and a small volume of distribution. In such cases, a small alteration in the bound fraction can produce a profound change in the pharmacodynamically active free drug concentration. Hypoglycemic agents used in the present work fall in this category and, therefore, their binding characteristics are primary determinants of their pharmacokinetic properties. The mechanism of interaction of a wide range of drugs with human serum albumin has been reported in the literature [1-3]. Amongst antidiabetic drugs, such studies on some sulfonylureas and troglitazone are available [4-5].

Glimepiride (GMP) and glipzide (GLZ) are second generation sulfonylureas, widely used in the treatment of non-insulin dependent diabetes mellitus. Although these drugs are widely prescribed, the detailed mechanism of their interaction with plasma proteins has not been reported. Both the drugs are highly bound to serum albumin where a small change in the degree of protein binding can result in serious fluctuations in the plasma glucose concentration of diabetic patients. Thus, it is important to understand not only how much drug is bound to protein, but also the site-specificity and the nature of forces involved in the binding of these agents with serum albumin, the major drug binding protein in blood plasma. Fluorescence spectroscopy, due to its exceptional sensitivity, selectivity and sound theoretical foundation [6], is a convenient method for studying drug-protein interaction [7-8]. In the present work, the mechanism of interaction and detailed physico-chemical characterization of the binding of glimepiride and glipizide with human serum albumin

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has been studied using fluorescence spectroscopy. The results are discussed in terms of the binding parameters, thermodynamics of the binding process, nature of forces involved in the interaction, identification of drug binding site on serum albumin and the fluorescence quenching mechanism involved. Such studies form the basis of the displacement and hence the altered physiological activity of these drugs in the presence of other co-administered drugs and excipients.

2. Expermental Procedures

Pure glimepiride and glipizide samples were donated by M/s USV Pharmaceuticals Ltd., Solan, H.P., India and M/s Torrent Pharmaceuticals Ltd., Gujarat, India, respectively. All other reagents were analytical grade. Water was double distilled in an all glass apparatus. HSA solutions were prepared in 0.1 M phosphate buffer (pH 7.4), based on a molecular weight of 66,500 daltons. Due to the limited solubility of drugs used in aqueous pH 7.4 buffer, glimepiride was dissolved in DMSO and slowly diluted with phosphate buffer (pH 7.4) to obtain a stock solution containing 10% DMSO. The concentration of DMSO during the titration was less than 2.5%. The presence of 2.5% DMSO had negligible effect on the fluorescence spectrum of HSA. Glipizide stock solution was prepared in phosphate buffer (pH 8.0). However, change in the pH of the reaction mixture during titration was negligible. A Perkin Elmer fluorescence spectrophotometer equipped with a 150 W xenon lamp source was used.

2.1 Drug-albumin binding

Two milliliters of HSA solution was placed in a quartz cell and increasing amounts of drug stock solution added. The concentration of HSA was kept fixed at 10 µM and the drug concentration was varied from 5-70 µM. The dilution caused by the addition of the drug to the HSA solution was compensated by the addition of an equivalent volume of 20 µM HSA to the cell. Excitation wavelengths of 280 and 295 nm were used and fluorescence emission spectra of HSA were recorded in the range 300 - 400 nm. No correction for the inner filter effect was applied, since the absorbance at the excitation wavelength (295 nm) was very small; less than 0.01 in the case of glimepiride and less than 0.04 in the case of glipizide, even at the highest drug concentration. The absorbance was almost zero at the emission wavelength (340 nm). The stoichiometry of interaction was determined by the method of continuous variations [9].

2.2 Data analysis

The fractional occupancy of the total protein binding sites by drug was obtained from the ratio, $\theta = \Delta F/\Delta F_{max}$ [10-11], where $\Delta F = F_0 - F$. F_0 and F are the fluorescence intensities of serum albumin in the absence and presence of the drug, respectively. ΔF_{max} values were obtained from double reciprocal ($1/\Delta F$ *vs* $1/D_t$) plots, where D_t is the total amount of drug added.

The concentration of bound sites on protein, which is also equal to the concentration of bound drug (D_b), is given by $n\theta P_t$, where n is the total number of binding sites and P_t is the total protein concentration [12]. D_f, the number of moles of free drug, was obtained from the difference, $D_t - D_b$. The amount bound was expressed as moles of drug bound per mole protein, $r (= D_b / P_t)$. The association constant (K_a) for the binding was computed directly by fitting the experimental data (r and D_f values) to the following general (Scatchard) equation using an iterative non-linear least squares regression program developed for this purpose.

$$r = \sum_{i=1}^{i=m} n_i K_{ai} D_f / (1 + K_{ai} D_f) \quad (1)$$

Data has been reported as an average of three measurements. The standard error of mean (SEM), calculated using statistical software (SPSS for Windows), was less than ± 0.025 × 10^5 in all cases. Thermodynamic parameters (ΔG^0, ΔH^0, ΔS^0) for drug-protein interaction were determined for both the drugs at pH 7.4, from the experiments conducted at five different temperatures; 15, 20, 25, 30 and 37^{0}C, using equations (2) & (3).

$$\Delta G^0 = -RT \ln K_a \quad (2)$$

$$\ln K = -\Delta H^0/RT + \Delta S^0/R \quad (3)$$

The percentage of free drug ($\alpha = D_f/D_t \times 100$) was calculated from the dissociation constants for the drug-protein complex ($K_d = 1/K_a$) using equation (4) [13].

$$\alpha = [(K_d + D_f)/\{[P_t] + K_d + [D_f]\}] \times 100 \quad (4)$$

2. 3 Drug-HSA interaction in the presence of fluorescence probes

2.3.1 Hydrophobic probe.

ANS: In the first set of experiments, the interaction of drugs and ANS with HSA was studied under identical conditions. The HSA concentration was kept fixed at 10 µM, while the ANS/drug concentration was varied from 5 to 70 µM. Fluorescence of HSA was recorded

at 334 nm after excitation at 295 nm. In the second set of experiments, an increasing amount of drug (5-70 μM) was added to an equimolar HSA-ANS mixture (10 μM each) and the fluorescence of ANS was recorded at 470 nm after excitation at 370 nm. The concentration of the HSA-ANS mixture was kept fixed at 10 μM each by adding the same volume of albumin-ANS mixture (20 μM each) to the cell.

2.3.2 Site-selective probes

Fluorescence probe displacement experiments were also carried out using site I-selective probe dansylamide (DA), and site II-selective probe dansylsarcosine (DSS). Due to limited solubility of dansylamide in phosphate buffer (pH 7.4), a stock solution of DA was prepared using 10% DMSO as co-solvent. The fluorescence of the probe in each case was measured at 25ºC in the probe-HSA mixture (1:1, 5 μM each) before and after the addition of drug (5-70 μM). DA/DSS fluorescence was measured at 480 nm after excitation at 350 nm. The probe to HSA ratio was kept at 1:1, in order to keep the non-specific binding of the probe to a minimum.

3. Results and Discussion

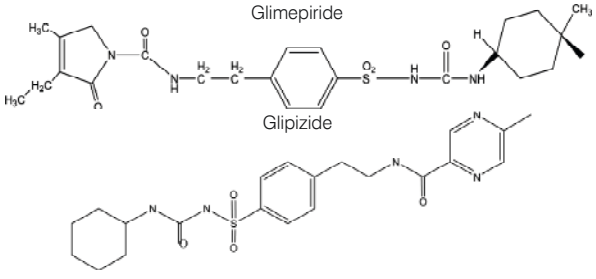

Figure 1. Structures of glimepiride and glipizide

The structures of glimepiride and glipizide are shown in Fig. 1. Some physico-chemical properties of the antidiabetic drugs used in the present work are given in Table 1. Sulfonylureas are weak acids due to the marked delocalization of the nitrogen electron pair by the sulfonyl group. pK values of glimepiride and glipizide are 6.3 and 5.9, respectively [14]. Both drugs therefore exist as anionic species at physiological pH. However, low aqueous and phosphate buffer (pH 7.4) solubility indicates that these drugs also possess sufficient lipophilicity. Octanol-water partition coefficients (log P) had large positive values for both drugs. However, octanol-buffer (pH 7.4) distribution coefficients (log D) were found to be positive for glimepiride and negative for glipizide, indicating that at physiological pH, glimepiride is more lipophilic than glipizide.

Table 1. Some physico-chemical properties of glimepiride and glipizide.

Property	Drug	
	Glimepiride	Glipizide
pK_a[a]	6.30	5.90
log P[b]	3.81	2.31
Log D[c]	2.38	-1.32
log S_w[d]	-2.19	-1.99
log S_b[e]	-2.06	-1.18
TPSA[f]	124.67	130.15
Molar Volume[f]	445.90	393.90
H-bond donors[f]	3	3
H-bond acceptors[f]	9	9

[a] Literature values, [b] Octanol-water partition coefficient (Literature values), [c] Octanol-phosphate buffer (pH 7.4) distribution coefficient (Experimentally determined), [d] Log aqueous solubility (25ºC) (mg mL⁻¹) (Experimentally determined), [e] Phosphate buffer (pH 7.4) solubility (Experimentally determined), [f] Calculated using software molinspiration

Human serum albumin (HSA) is a single chain protein containing 585 amino acids and a single tryptophan residue (^{214}trp). HSA consists of three homologous domains (I, II, III) and each of these is comprised of two subdomains (A and B). Most drugs bind with high affinity to one of the two sites, called site I and site II, located in subdomains IIA and IIIA, respectively. Drugs can also bind non-specifically to hydrophobic surfaces on the protein molecule.

Both glimepiride and glipizide were found to quench the intrinsic fluorescence of HSA and the binding was accompanied by a small red shift (4-8 nm) in the emission wavelength. Wavelength shift is usually associated with change in the microenvironment of the tryptophan residue of HSA on drug binding. Red shift indicates that the tryptophan residues in protein are shifted to a more hydrophilic environment [15]. Since glimepiride and glipizide exist as predominantly ionized species at physiological pH, drug binding appears to decrease the hydrophobicity in the microenvironment of tryptophan residues [16]. The number of binding sites, n, determined by the method of continuous variations [9], was found to be 0.67 for glimepiride and 1.0 for glipizide. It may be mentioned that the values of n are not necessarily integral because they are average values and the protein may not be molecularly homogeneous with respect to its binding properties. The experimental data could be fitted into an equation for only one class of binding sites (m = 1). The association constant (K_a) was found to be much higher for glipizide as compared to glimepiride at all temperatures. However, the order of values was 10^5 in each case (Table 2). An association

constant of this order (10^5) has also been reported in the literature for some other antidiabetic drugs [17]. The association constant for the binding of glipizide to bovine serum albumin (BSA) has been reported [18] to be of the order of 10^4 as compared to the 10^5 reported herein. However, the values cannot be compared directly since, in addition to other differences, BSA has two tryptophan residues while HSA has only one. A comparison of the physical properties of the two drugs (Table 1) shows that both the drugs have the same number of hydrogen bond donors and acceptors. However, glimepiride has a relatively lower total polar surface area and higher partition coefficient, molecular weight and molar volume and is, therefore, relatively more hydrophobic and larger in size than glipizide. In spite of being more hydrophobic, glimepiride has smaller binding constants. It therefore appears that the relatively smaller glipizide molecule can be more easily accommodated in the binding cavity on the HSA molecule. The importance of molecular size in the structure-activity correlations has also been emphasized by other workers [11,19]. The percentage of free drug (α) at different drug:protein ratios, calculated from the association constant data using equation (4), is shown in Fig. 2 for both drugs at 37°C. It can be seen that only a small percentage of the added drug is free and pharmacologically active, especially at low drug:protein ratios, which are frequently encountered in the physiological system. For example, at a drug:protein ratio of 0.60, only about 55% and 31%, respectively of glimepiride and glipizide exist as free drug. Thus a large amount of the added drug is retained by serum albumin. Since the nature of interaction governs the release of drugs from the drug-protein complex, it is important to understand the forces involved in the interaction

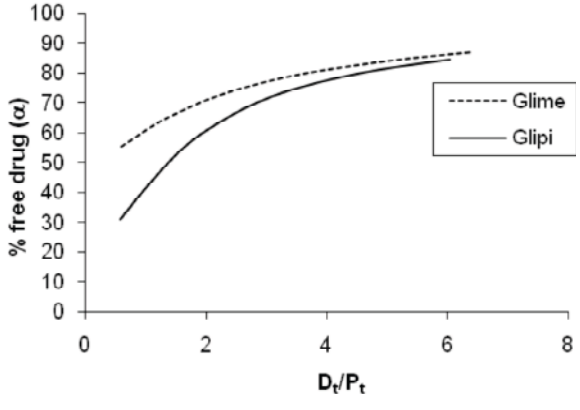

Figure 2. Percentage of free drug (37°C) at different drug:protein ratios.

3.1 Thermodynamic parameters for the binding

In the temperature range 15 to 25°C, association constants were found to decrease with increasing temperature in the case of glimepiride, whilst they increased with increasing temperature in the case of glipizide. However, when the temperature was increased from 25 to 37°C, the trend reversed for both drugs (Table 2). It appears that the presence of the drug causes temperature-dependent conformational changes in the albumin molecule and, therefore, the binding mode varies with temperature. Conformational changes induced by increasing temperature have also been suggested by Silva *et al.* [20] in chlorpromazine-serum albumin interaction.

Table 2. Association constants for the binding of glimepiride and glipizide to human serum albumin

Temperature (K)	Association Constant (K) × 10^5	
	Glimepiride	Glipizide
288.15	1.236	3.473
293.15	0.647	4.079
298.15	0.499	4.763
303.15	1.181	4.647
310.15	1.411	3.741

Thermodynamic parameters, ΔG^0, ΔH^0 and ΔS^0 for the interaction of the two antidiabetic drugs with HSA in the temperature range 15 - 25°C and 25 - 37°C, are given in Table 3. High negative ΔG^0 values in each case showed spontaneity of the binding process. The nature of the interaction can generally be predicted from the sign and magnitude of the standard enthalpy change (ΔH^0) and standard entropy change (ΔS^0) [20-23]. Positive entropy changes in all cases, except glimepiride in the lower temperature range, indicated that, in general, hydrophobic interactions are predominantly involved in the binding of these drugs to HSA [21]. A Hydrophobic interaction has also been reported to be the predominant intermolecular force stabilizing the complex for some other sulfonylureas [17]. Unfolding of the protein molecule during the binding process is also associated with positive enthalpy and entropy changes. In the case of glimepiride, in the lower temperature range both ΔH^0 and ΔS^0 were found to be negative, indicating hydrogen bonding and Van der Waal's interactions [22]. In the higher temperature range, small negative ΔH^0 and small positive ΔS^0 values for glipizide show that either hydrophobic, as well as hydrogen bonding interactions or electrostatic interactions, are involved [23]. Although

Table 3. Thermodynamic parameters for the binding of glimepiride and glipizide to HSA

Drug	Thermodynamic Parameter				
	ΔG^o (kJ mol^{-1})*	Temperature range 15 - 25°C		25 - 37°C	
		ΔH^o (kJ mol^{-1})	ΔS^o (J mol^{-1})	ΔH^o (kJ mol^{-1})	ΔS^o (J mol^{-1})
Glimepiride	-30.580	-64.920	- 128 316	+ 62.023	+ 299.289
Glipizide	-33.095	+ 22.569	+ 184.414	-16.113	+ 54.966

ΔG^0 values have been calculated at 37°C

at pH 7.4 the net charge on the protein is negative, the human serum albumin molecule has about 80 positively charged groups and, therefore, a possibility of electrostatic interactions between HSA and the anionic form of glipizide also exists.

3.2 Effect of excitation wavelength on fluorescence quenching

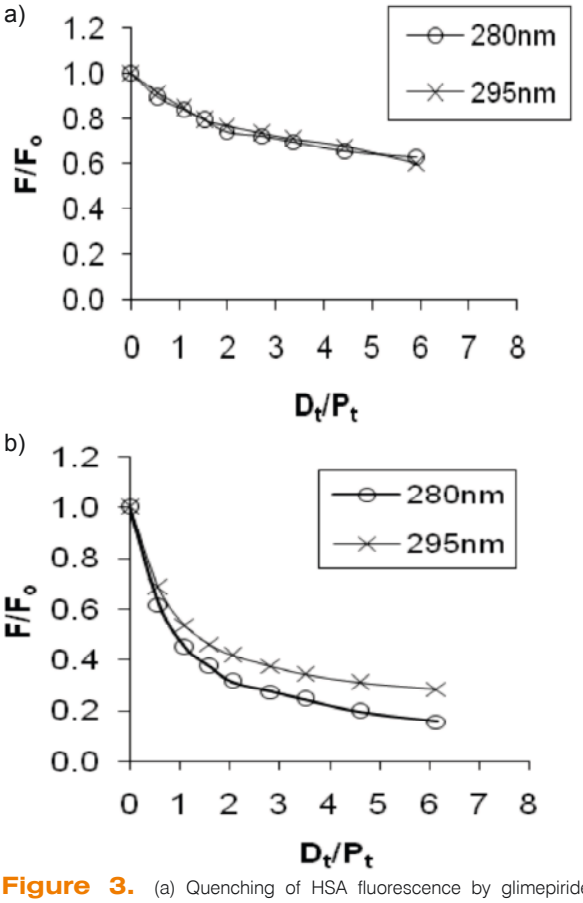

Figure 3. (a) Quenching of HSA fluorescence by glimepiride at excitation wavelengths of 280 and 295 nm. (b) Quenching of HSA fluorescence by glipizide at excitation wavelengths of 280 and 295 nm

It is known that an excitation wavelength of 295 nm is associated with only tryptophan residues in HSA, whereas an excitation wavelength of 280 nm involves both tryptophan and tyrosine residues [24]. The percentage quenching [($\Delta F/F_0$) × 100] versus drug:protein ratio (D_t/P_t) plots, for excitation at 280 and 295 nm, are shown in Fig. 3. In the case of glimepiride, the two curves almost overlap indicating that tyrosine residues are not involved in the binding. On the other hand, in the case of glipizide, higher percentage quenching at an excitation wavelength of 280 nm as compared to 295 nm, suggests that tyrosine residues are also involved in the binding of this drug to HSA.

3.3 Binding studies in the presence of fluorescence probes

3.3.1 Hydrophobic probe (ANS)

ANS (1-anilino-8-naphthalene sulfonate), a hydrophobic probe, is known to bind to non-polar (hydrophobic) surfaces of proteins and the binding is followed by an increase in ANS fluorescence intensity. It is used frequently for locating hydrophobic binding sites in proteins and synthetic polymers [25-26]. In order to further understand the nature of interaction of drugs with HSA, binding studies were carried out in the presence of ANS. When the quenching of HSA fluorescence by drugs and ANS was studied under identical conditions, it was found that the extent of quenching by drugs was much less as compared to ANS, in the case of both drugs. The relative fluorescence intensity, F/F_0 (where F and F_0 are the fluorescence intensity of HSA in the presence and absence of quencher, ANS/drug) versus quencher concentration plots are shown in Fig. 4. At a quencher concentration of 20 μM, ANS could quench about 83%, while glimepiride and glipizide could quench only about 20 and 43%, respectively, of HSA fluorescence. It thus appears that glimepiride and glipizide do not share common sites with ANS in human serum albumin. Since there are multiple sites for ANS on the albumin molecule [26], higher percentage quenching by ANS also suggests that the drugs bind only at specific sites. Moreover, ANS can have relatively easier accessibility to the binding sites on albumin, due to its smaller size as compared to the drugs used [19].

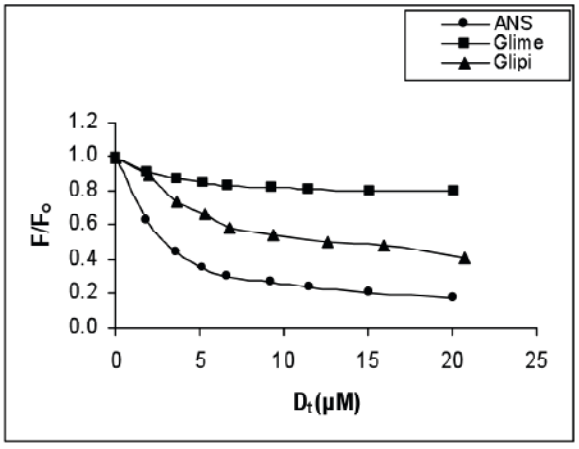

Figure 4. Quenching of HSA fluorescence by drugs and ANS under identical conditions

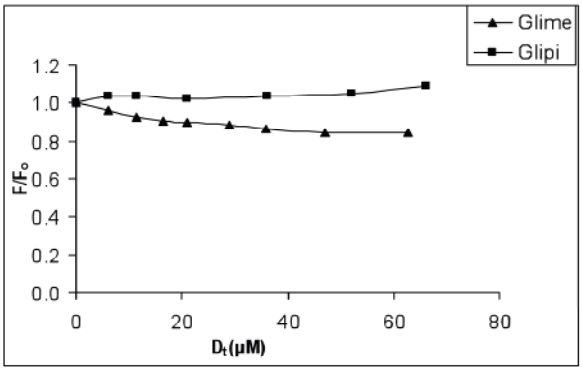

Figure 5. Relative fluorescence intensity of ANS in HSA-ANS complex as a function of drug concentration

The displacement of ANS from HSA-ANS complex by drugs was also studied. For this purpose, ANS fluorescence was measured in an HSA-ANS mixture in the absence and presence of increasing amounts of drug. The concentration of HSA-ANS mixture (10 μM each) was kept fixed while the drug concentration was varied from 5-70 μM in each case. The relative fluorescence intensity F/F_0 (where F and F_0 are the fluorescence intensity of ANS in HSA-ANS system, in the presence and absence of drug) has been plotted against the concentration of drug in Fig. 5. It is known that the hydrophobic probe, ANS, shows greatly increased fluorescence as a result of hydrophobic interaction with proteins and other macromolecules due to the transfer of the probe from an aqueous to a non-polar environment [26]. The drug, when added to the HSA-ANS system, can compete with ANS for hydrophobic sites on the surface. In that case it would inhibit the binding of ANS, *i.e.* displace ANS from its binding site, and the fluorescence intensity should decrease. In the case of glimepiride, a small decrease in the percentage displacement of the probe $[(F_0\text{-}F)/F_0 \times 100]$ (about 15%) was observed. In the case of glipizide there was practically no change in the fluorescence of ANS. Thus, although thermodynamic parameters show predominantly hydrophobic interaction, it appears that the drugs and ANS bind independently, and that they do not share common sites with ANS in the albumin molecule.

3.3.2 Site-specific probes

In order to identify the binding site for these drugs on human serum albumin, studies were also carried out using site-I specific probe, dansylamide (DA), and site-II specific probe, dansylsarcosine (DSS) [27-29]. Fluorescence probe displacement studies were carried out by measuring the fluorescence intensity (F) of the probe in HSA-probe system in the absence (F_0) and presence (F) of drug. Results have been expressed as relative fluorescence intensity (F/F_0) and percentage displacement of the probe $[(F_0\text{-}F)/F_0 \times 100]$ at different drug concentrations.

3.3.2.1 Site–I specific probe

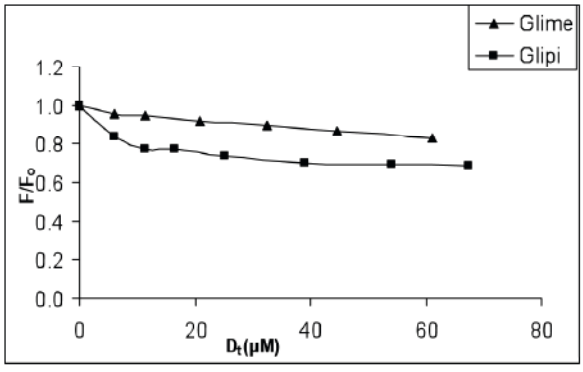

Figure 6. Relative fluorescence intensity of ANS in HSA-ANS complex as a function of drug concentration .

DA: The relative fluorescence intensity (F/F_0) of DA in HSA-DA mixture in the presence of increasing amounts of drugs is shown in Fig. 6. It is seen that the addition of drugs to HSA-DA mixture results in a small decrease in the fluorescence intensity of DA in the case of both the drugs. The percentage displacement of DA $[(F_0\text{-}F)/F_0 \times 100]$ at a drug concentration of 60 μM was 16 and 32%, for glimepiride and glipizide, respectively. The maximum displacement, calculated from the intercept of the linear double reciprocal (1/D versus 1/[Drug]) plots, was found to be 25 and 34%, respectively, in the case of glimepiride and glipizide.

3.3.2.2 Site–II specific probe

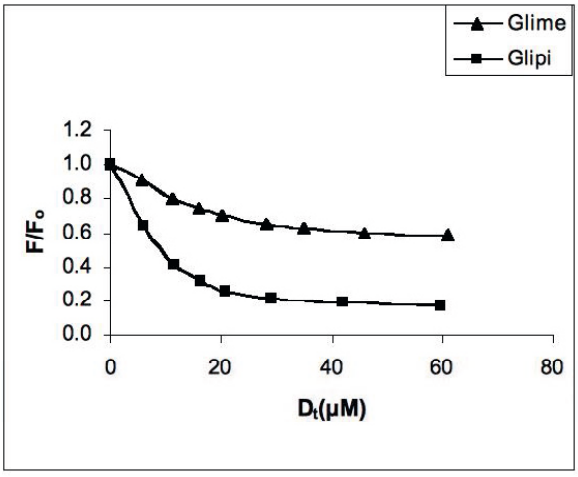

Figure 7. Relative fluorescence intensity of DSS in HSA-DSS complex as a function of drug concentration.

DSS: Relative fluorescence intensity (F/F_0) of DSS in HSA-DSS mixture in the presence of increasing amounts of drugs is shown in Fig. 7. It is seen that the addition of drugs to HSA-DSS mixture results in a decrease of DSS fluorescence in the case of both the drugs indicating that the drugs displace DSS from its binding site. The percentage displacement (D) was about 41 and 83% at a drug concentration of 60 μM in the case of glimepiride and glipizide, respectively. The maximum displacement, calculated from the intercept of the linear double reciprocal (1/D versus 1/[Drug]) plots, was found to be nearly 100% in both cases. From the studies carried out in the presence of site-specific probes, it may thus be concluded that the antidiabetic drugs, glimepiride and glipizide, bind to both site I and site II on HSA. However, much higher percentage displacement of the site II specific probe, DSS, as compared to the site I-specific probe, DA, shows that the primary interaction occurs at site II. The drugs used have greater affinity for site II than site I. Antidiabetic agent SU-118 has also been reported to bind at both sites I and II [17]. A close look at the structures of glimepiride and glipizide (Fig. 1) shows that they are heterocyclic molecules with an ionisable group near the centre of the molecule and an extended configuration. They therefore possess structural features of both site I and site II drugs [1,17]. Moreover, based on the crystal structure of human serum albumin, He and Carter [30] have reported that ligands bound to domain III affect conformational changes, as well as the binding affinities in domain II, because the binding subdomains share a common interface and ^{214}Trp, in addition to playing an important structural role in the formation of binding pocket in subdomain IIA, also participates in the hydrophobic packing interaction at the interface between IIA and IIIA subdomains. The domains have a hydrophobic interior and polar exterior, with amino acids, ^{199}Lys, ^{411}Tyr and ^{410}Arg located close to bound ligands and therefore potentially involved in the binding. Amino acids, lysine, tyrosine and arginine can participate in the binding since glimepiride and glipizide exist as predominantly anionic species at physiological pH and contain a large number of hydrogen bond acceptors (Table 1). However, the comparison of fluorescence quenching data at excitation wavelengths of 280 and 295 nm, has shown the involvement of tyrosine residues in the binding of glipizide only. Thus, glimepiride and glipizide bind at different regions within site II. Site-specificity can be useful in predicting the competitive displacement of these drugs by other co-administered drugs, resulting in fluctuations of the blood glucose levels in diabetic patients. Both site I and site II drugs, co-administered with glimepiride and glipizide, can affect the binding of these drugs by a competitive or non-competitive mechanism, and hence also affect the free drug available for hypoglycemic effect.

3.4 Stern-Volmer Analysis

Stern-Volmer analysis is a useful tool for estimation of the accessibility of tryptophan residues in proteins to the drug (quencher) molecules, and understanding the queching mechanism involved. Fluorescence quenching data was also analysed by the Stern-Volmer law [31].

$$F_0/F = 1 + K_q D_t \quad (7)$$

where F_0 and F are the steady state fluorescence intensities at 334 nm in the absence and presence of quencher (drug), respectively, and K_q is the Stern-Volmer quenching constant. The Stern-Volmer plots (F_0/F versus D_t) were not linear. The plots showed downward curvature at all temperatures (Fig. 8), indicating the presence of buried residues, that is, the tryptophan residues are not fully accessible to the drug. Normally, a static component in the quenching mechanism leads to upward curvature in the Stern-Volmer plots [32]. Since static quenching does not require diffusion through the medium, the quenching is more efficient and the observed fluorescence intensity is lower. Downward curvature in the present case shows that dynamic quenching dominates over the static process.

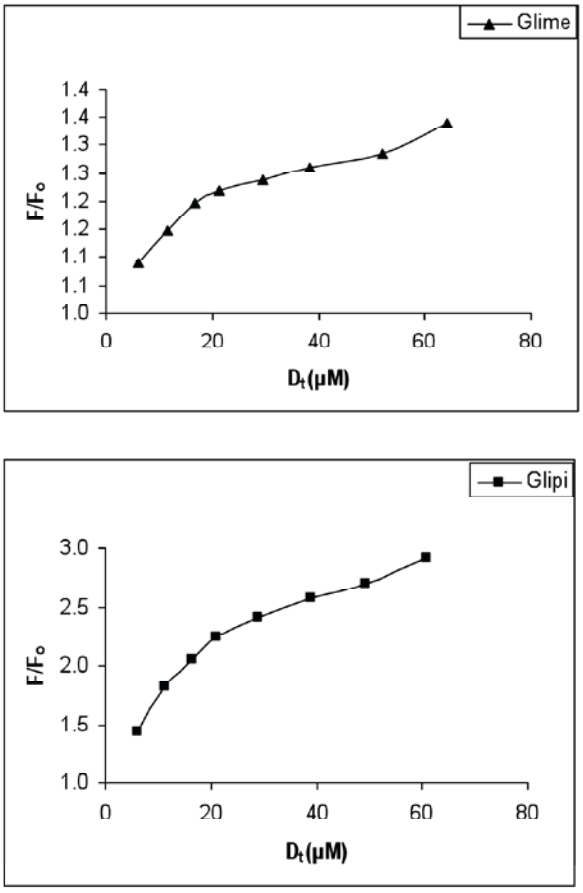

Figure 8. (a) Stern-Volmer plots for glimepiride at 37°C (b) Stern-Volmer plots for glipizide at 37°C

4. Conclusions

Glipizide was found to have higher binding affinity for serum albumin than glimepiride at all temperatures. Hydrophobic interactions were predominantly involved. However, drugs appear to cause temperature-dependent conformational changes in the albumin molecule and, therefore, the nature of interaction varied with temperature. Glimepiride and glipizide bind to both site I and site II on HSA, but they have greater affinity for site II than site I. Competitive/non-competitive interference of both site I and site II drugs can affect the availability of free glimepiride and glipizide for hypoglycemic effect. Stern-Volmer analysis of the quenching data showed that dynamic quenching dominates the static process in both cases.

References

[1] U. Kragh-Hansen, V.T.G. Chuang, M. Otagiri, Biol. Pharm. Bull. 25, 695 (2002)
[2] C. Bertucci, E. Domenici, Curr. Med. Chem. 9, 1463 (2002)
[3] U. Kragh-Hansen, Pharmacol. Rev. 33, 17 (1981)
[4] P. Hsu, J.K.H. Ma, L.A. Luzzi, J. Pharm. Sci. 63, 570 (1974)
[5] T. Izumi, Biopharm. Drug Disp. 18, 241 (1997)
[6] H.Gao, L.D.Lei, J.Q.Liu, Q.Kong, X.G.Chen, Z.D.Hu, J. Photochem. Photobiol. A: Chem. 167, 213 (2004)
[7] N. Zhou, Y. Liang, P. Wang, J. Mol. Struc. 872, 190 (2008)
[8] A. Sulkowska, M. Maciazek-Jurczyk, B. Bojko, J. Rownicka, I. Zubik-Skupien, E. Temba, D. Pentak, W.W. Sulkowski, J. Mol. Struc. 881, 97 (2008)
[9] M.H. Rahman, T. Maruyama, T. Okada, K. Yamasaki, M. Otagiri, Biochem Pharmacol 46, 1721 (1993)
[10] G. Weber, L.B. Young, J Biol. Chem. 239, 1415 (1964)
[11] T. Maruyama, M. Otagiri, S.G. Schulman, Int. J. Pharm. 59, 137 (1990)
[12] L.D. Ward, Methods Enzymol. 117, 400 (1985)
[13] B.K. Martin, Nature 207, 274 (1965)
[14] S. Jamzad, R. Fassihi, AAPS PharmSciTech. 7, E17 (2006)
[15] J. Kang, Y. Liu, M. Xie, S. Li, M. Jiang, Y. Wang, Biochim. Biophys. Acta 1674, 205 (2004)
[16] B. Kljnert, L. Stainislawska, M. Bryszewska, B. Palecz, Biochim. Biophys. Acta 1648, 115 (2003)
[17] W. Zhong, Y. Wang, J. Yu, Y. Liang, K. Ni, S. Tu, J. Pharm. Sci. 93, 1039 (2004)
[18] H. Zia, J.C. Price, J. Pharm. Sci. 64, 1177 (1975)
[19] E.J. Lien, P.H. Wang, J. Pharm. Sci. 69, 648 (1980)
[20] D. Silva, C.M. Cortez, R.W. Louro, Spectrochimica Acta Part A 60, 1215 (2004)
[21] Y.H. Pang, L.L. Yang, S.M. Shuang, C. Dong, M. Thompson, J. Photochem. Photobiol. B: Biology 80, 139 (2005)
[22] W. He, Y. Li, C. Xue, Z. Hu, X. Chen, F. Sheng, Bioorg. Med. Chem. 13, 1837 (2005)
[23] P.D. Ross, S. Subramanian, Biochemistry 20, 3096 (1981)
[24] A. Sulkowska, B. Bojko, J. Rownicka, W.W. Sulkowski, J. Mol. Struc. 792, 249 (2006)
[25] H.W. Jun, P.C. Ruenitz, J. Pharm. Sci. 67, 861 (1978)
[26] A.E.A. Thumser, A.G. Buckland, D.C. Wilton, J. Lipid Res. 39, 1033 (1998)

[27] G. Sudlow, D. Birkett, D. Wade, Mol. Pharmacol. 11, 824 (1975)
[28] B. Nerli, D. Romanini, G. Pico, Chemico-Biological Interact. 104, 179 (1997)
[29] S. Sugio, A. Kashima, S. Mochizuki, M. Noda, K. Kobayashi, Protein Eng. 12, 439 (1999)
[30] X.M. He, D.C. Carter, Nature 358, 209 (1992)
[31] M.R. Eftink, C.A. Ghiron, Anal. Biochem. 114, 199 (1981)
[32] P. Midoux, P. Wahl, J. Auchet, M. Monsigny, Biochim. Biophys. Acta 801, 16 (1984)