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Spectroscopic and thermodynamic interaction studies of anti-diabetic drug with globular proteins

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ABSTRACT

Drug-protein interaction is a fundamental problem in estimating the serious side effects of the drug. Hence, the main objective of this study was to study the interaction of acarbose with three different globular proteins i.e.; bovine serum albumin (BSA), human serum albumin (HSA), and hemoglobin (Hb) via UV-Visible absorption spectroscopic analysis. We were determined physicochemical parameters, binding constant, distribution constant and thermodynamic parameters activation energy, enthalpy, entropy, and Gibbs free energy by using UV-visible data. These both properties of acarbose-protein complexes indicated that the hydrogen bonding and weak van der Waals force played a major role in the interaction for complexation. The binding of acarbose with different proteins leads to change in the structure of protein folding which confirms by physicochemical and thermodynamic analysis.

Keywords: *Binding constant, Distribution constant, Thermodynamic parameters, Anti-diabetic drug, Globular proteins.*

1. INTRODUCTION

Acarbose is a type of noninsulinotropic oral antidiabetic drug molecule, which mainly used to treat type two diabetes [1]. It is mainly used with a proper diet supplemented food and regular exercise to control the blood sugar in humans [2]. Acarbose also helps to prevent kidney damage, blindness, nerve cord problem, and other performance via controlling high blood sugar. Acarbose is also known as an α-glucosidase inhibitor that controls the postprandial blood glucose, which used as oral hypoglycaemic agents in Chinese medicines [3]. Acarbose mainly targeted the α-glucosidase in the small intestine, whereas the α-glucosidase non-absorbable carbohydrates complex converts into absorbable monosaccharides molecules. Hence, acarbose is a most competitive and reverse inhibitor for the brush border glucosidase of the small intestine which completely decreases the degradation of starch or sucrose for the slow absorption of glucose or fructose in the upper small intestine; ultimately this process helps the controlling of the blood sugar level in blood [4–6]. In recent years many more such drugs, particularly those having anti-diabetic activity, studied for their interaction with the biological membranes [7]. These compounds must be carried out to their site of action where these are properly delivered. This action can be achieved by the globular protein like BSA, HSA, and Hb, at which they are binds or attached with different interactions. Such protein and drug binding play a crucial role in the long circulation and distribution [8,9]. Drug-protein interactions are the most important mutual properties; most of the drugs are widely and reversibly binds with the proteins where these drugs were delivered via complex formation. The nature of drug-proteins interactions mainly affected the pharmacokinetic parameters of the drug and the binding parameters are mainly used for studying the protein–drug binding which regulates the absorption, distribution, metabolism, and excretion properties of drugs [10–13]. Earlier researchers have been reported on the non-covalent bonding through physicochemical and spectroscopic analysis [14-23].

Hence, our proposed study mainly focused on the acarbose and three globular proteins i.e., BSA, HSA and Hb interaction at the molecular level which provides the information about the optimization and preclinical formulation development of the acarbose for the pharmaceutical applications. Recently, various spectroscopic studies of proteins like serum albumins have been explained based on their UV-visible, fluorescence, absorption, FTIR, and CD spectroscopic methods [13,24-25]. After the successfully binding of the drug molecules with protein, the intramolecular forces significantly changed with secondary structure alteration. Hence, the application of such spectral methods could be revealed the chemical and biological activity at low dose concentration in the biological media and slandered physiological conditions. In this study, the structural interaction activities mechanism of acarbose with three different proteins has been studied via spectroscopic and thermodynamic properties.

2. MATERIALS AND METHODS

Acarbose, bovine serum albumins (BSA), human serum albumin (HSA), human hemoglobin (Hb) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Phosphate buffer saline (PBS) (10X PBS) was purchased from Sisco Research Laboratories Pvt. Ltd. (SRL) – India.

BSA, HSA and Hb stock solutions (10 μM) were prepared in 0.1M phosphate buffer (1X PBS) of pH 7.4. The acarbose concentrations were varied from 10-100 μM into 10 μM and used as a solute for 10 μM BSA, HSA, and Hb solution. All solutions were prepared at 298.15 K and atmospheric pressure (p = 0.1

MPa) using Milli-Q water of 0.71 μS·cm^{-1}, at pH 7. All prepared solutions were stored in the dark at 4 °C.

UV-Visible study.

UV-vis absorption spectra were recorded with a Spectro 2060 plus spectrophotometer over 220–500 nm in the cuvette of 1 cm path length. The reference blank was a phosphate buffer solution. For analysis, 10mM PBS solution (pH = 7.4) solutions of BSA, HSA, and Hb were used for UV absorbance studies.

Calculations.

The Δ*G*, Δ*H*, E^*, and ΔS are determined for studying the acarbose-protein interaction. The E^* value is calculated by the Arrhenius equation at *T* = 298.15 K and fitted as:

$$log(abs) = log A - \frac{E^*}{2.303RT} \quad (1)$$

$$= log A - \frac{E^*}{2.303R}\frac{1}{T} \quad (2)$$

Where *abs* is the absorbance, *T* is the temperature in Kelvin (K), *R* is the gas constant (8.314 J/mol·K), *A* is the frequency factor, and E^* is the activation energy (J/mol), Log (abs) versus 1/T.

Further, E^* data is used to calculate the Δ*H* for the pristine and annealed processes are given below.

$$\Delta H = E^* - 2.303RT \quad (3)$$

So, the ΔG and ΔS for stable systems are calculated by using equations given as:

$$\Delta S = \frac{(E^* - 2.303RT + 2.303RT\ log(abs))}{T} \quad (4)$$

$$\Delta S = \left(\frac{E^*}{T}\right) - 2.303R[1 - log(abs)] \quad (5)$$

$$\Delta G = -2.303RT\ log(abs) \quad (6)$$

3. RESULTS

Figure 1 depicted the absorption spectra of the pure protein and in the presence of acarbose, where these observed spectra suggested the acarbose binding mechanism or phenomena with different proteins. Generally, proteins have the interaction ability because of hydrophobic and hydrophilic domains, and also originating from the different amino acids present in the proteins, when these groups are binds with other molecules via non-covalent bonding, electrostatic, hydrogen bonding and Van der Waals forces with frequently changed with the type or concentration of the molecules [24, 26, 27]. In the current study, the concentration of proteins (HSA, BSA, and Hb) were fixed and the binder molecule i.e. acarbose concentration was varied from 10 to 100 μM. The UV-Visible absorption study of proteins is mainly depending on the high sensitivity of the protein functional groups and their environment [28, 29]. Thus, the change in absorption spectra of the proteins is responsible for the conformational transitions, association or dissociation of protein, molecular binding, and denaturation. Hence, the absorption study of the drug-protein can be providing useful information for the structural dynamics via protein association or dissociation reaction. A perusal of figure 1, the absorption spectra of HSA, BSA, and Hb were continuously decreased with a blue shift by increasing the concentration of acarbose from 10 to 100 μM. These observed results are suggested that the fluorescence chromophore groups of BSA, HSA, and Hb placed in the more hydrophilic environment with acarbose with the interaction effect via the formation of the non-fluorescent complex [30]. The decrease in absorption intensity may be due to the variety of intermolecular interactions, energy transfer, excitation state complex formation, and collision quenching mechanism. The observed quenching mechanism is also known as a dynamic and static mechanism, which could also be explained by the obtained thermodynamic parameters [31].

Further to confirm structural changes in the protein with acarbose, the UV-visible spectra were also measured with different concentrations. Figure 2 shows the UV-visible spectra of the different protein-drug complexes where the absorption intensity decreased with increasing concentration with a slight blue shift in the spectrum [1, 32]. These observed spectra revealed that the interaction between acarbose and proteins changed the structure of proteins. The observed results also suggested that with addition if acarbose concentration from 10 to 100 μM, the peptides junctions of BSA and HSA molecules more extended with increasing hydrophilic environment. In another, for the quenching mechanism elucidation, the observed results are analyzed by measuring the binding constant. Figure 1 showed that absorption of acarbose with different proteins decreased in the order of BSA>HSA>Hb, which suggested the interaction order of the used proteins [27, 32]. Hence, the observed results indicating that the quenching in between acarbose and different protein *via* complexes formations. In the case of acarbose with Hb, the binding constant values were higher at 405 nm than 280 nm. These observed results suggested that for the Hb-acarbose complexes, the absorption intensity by the present amino acids molecules is more effective as compared to BSA and HSA.

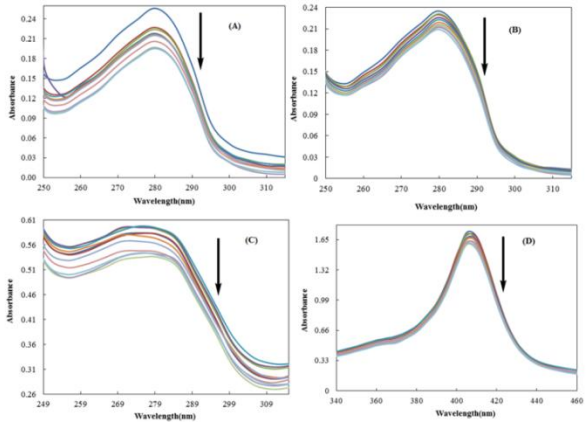

Figure 1. Absorption spectra of Acarbose in PBS buffer upon addition of HSA, BSA, and Hb. [Acarbose] = 10-100μM, (a) BSA. (b) HSA, (c and d) Hb. The arrow shows that the absorption intensities decrease upon increasing Acarbose concentration.

Binding force via thermodynamic parameters

Thermodynamic properties are the responsible parameters for the drug-protein complexes formation. Hence, the thermodynamic parameters of the acarbose-protein complexes have been calculated in terms of change in free energy (ΔG), enthalpy changes (ΔH), entropy change (ΔS) and activation energy

changes (E^*). As reported previously, the working forces in between small biomolecules and macro biomolecules mainly worked in terms of hydrogen bonding, Van der Waals forces, electrostatic forces, hydrophobic-hydrophobic interaction, and hydrophobic-hydrophilic interaction [33,34]. These thermodynamic parameters have been calculated by the above mention eq. 1 to 6. Table 1 and 2 represent the thermodynamic properties of acarbose and different protein interactions.

Table 1. Thermodynamic parameters for acarbose with BSA and HSA interaction at λ_{max} = 280 nm.

Acarbose (μM)	Δ*S* (kJ·mol^{-1})	*E* (J·mol^{-1})	Δ*H* (kJ·mol^{-1})	Δ*G* (kJ·mol^{-1})
acarbose with BSA at λ_{max} 280 nm				
0	-0.02	3378.19	-2.33	3.38
10		3676.27	-2.03	3.68
20		3709.25	-2.00	3.71
30		3776.57	-1.93	3.78
40		3787.97	-1.92	3.79
50		3799.42	-1.91	3.80
60		3810.92	-1.90	3.81
70		3916.94	-1.79	3.92
80		4027.70	-1.68	4.03
90		4040.31	-1.67	4.04
100		4052.99	-1.66	4.05
acarbose with HSA at λ_{max} 280 nm				
0	-0.02	3590.40	-2.12	3.59
10		3643.72	-2.07	3.64
20		3632.96	-2.08	3.63
30		3687.21	-2.02	3.69
40		3720.35	-1.99	3.72
50		3765.22	-1.94	3.77
60		3799.42	-1.91	3.80
70		3834.09	-1.87	3.83
80		3810.92	-1.90	3.81
90		3845.76	-1.86	3.85
100		3881.09	-1.83	3.88

The values of Δ*G* shows the interaction between acarbose and protein is spontaneous or not [33, 35, 36]. In the current study, Δ*G* is observed less positive at 280 nm for BSA and HSA and with Hb at 275 nm. However, the Δ*G* values observed in negative for acarbose-Hb interaction at 405 nm. Hence, the acarbose-Hb binding is spontaneous at 405 nm. Similarly, it has been reported that the sign and magnitude of the thermodynamic properties explained a different kind of intermolecular interactions. For instance, Δ*H* > 0 and Δ*S* > 0 hydrophobic interactions, Δ*H* < 0 and Δ*S* < 0 Van der Waals' force and hydrogen bonding and Δ*H* < 0 and Δ*S* > 0 reveals the different electrostatic interaction. The negative Δ*H* and Δ*S* values indicate the binding of acarbose and proteins, enthalpy and entropy drove where the Δ*S* is indicating the decrease in the entropy after complex formation in between acarbose and proteins. In another, the *E** value of the acarbose observed positive except with Hb at 405 nm. These observed negative values suggested the spontaneity and stable complex formation. Thus, it can be concluded that intramolecular interactions play a major role in the stable complex formation.

Protein molecules have been assumed a distribution between solvent and molecular phase according to Nernstian law. To describe quantitatively, the partitioning of solute molecules between Acarbose molecules and organic solvent, the total absorption of solute (*A*) has been described as a contribution of the fraction of solute solubilized in molecules (x_m) and organic solvent ($1 - x_m$) and it follows that:

$$A = x_m A_m + (1 - x_m) A_o \quad (7)$$

where A_o and A_m are absorption values when a solute is totally solubilized in the pure solvent and Acarbose molecules, respectively.

Following the procedure developed by Magid et al. [37] to analyze UV-vis spectra, the total solute absorption as a function of Acarbose concentration is given by the equation:

$$A = A_o + \frac{(A_m - A_o)K'[\text{Acarbose}]}{1 + K'[\text{Acarbose}]} \quad (8)$$

where K' is a binding constant defined as $K' = n_m / n_o[\text{Acarbose}]$

The binding constant (K') and distribution constant (K) are related as follows: $K' = K^* M_{[\text{Acarbose}]}$, where $M_{[\text{Acarbose}]}$ is the Acarbose molecular weight.

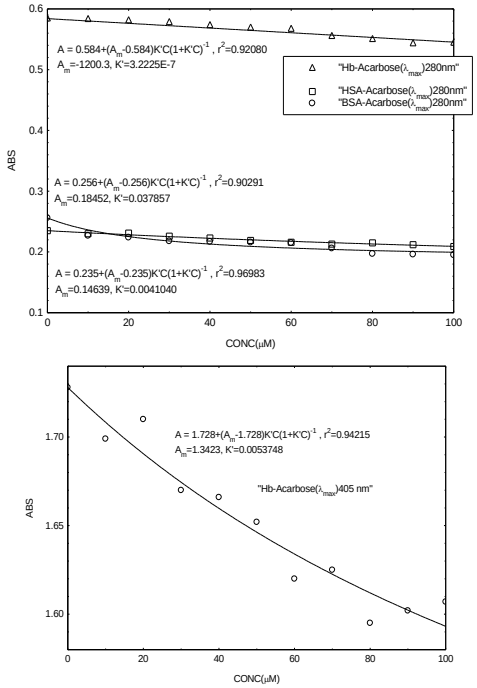

Figure 2. Variation of Abs with Concentration of Acarbose-Hb, Acarbose-HSA, Acarbose-BSA at 280nm and Acarbose-Hb at 405nm. Points – experimental data, solid lines are suitable fitting curves in NLREG.

Table 2. Thermodynamic parameters for acarbose with Hb interaction at λ_{max} = 275 and 405 nm.

Acarbose (μM)	Δ*S* (kJ·mol^{-1})	*E* (J·mol^{-1})	Δ*H* (kJ·mol^{-1})	Δ*G* (kJ·mol^{-1})
acarbose with Hb at 275 nm				
0	-0.02	1333.48	-4.38	1.33
10		1337.73	-4.37	1.34
20		1283.06	-4.43	1.28
30		1287.22	-4.42	1.29
40		1274.75	-4.43	1.27
50		1359.09	-4.35	1.36
60		1406.73	-4.30	1.41
70		1491.23	-4.22	1.49
80		1550.75	-4.16	1.55
90		1513.95	-4.19	1.51
100		1509.39	-4.20	1.51
acarbose with Hb at 405 nm				
0	-0.02	-1356.07	-7.06	-1.36
10		-1314.11	-7.02	-1.31
20		-1330.11	-7.04	-1.33
30		-1271.43	-6.98	-1.27
40		-1265.48	-6.97	-1.27
50		-1244.56	-6.95	-1.24
60		-1196.06	-6.90	-1.20
70		-1203.70	-6.91	-1.20
80		-1157.50	-6.87	-1.16
90		-1168.36	-6.88	-1.17
100		-1176.09	-6.88	-1.18

In equation (8) binding constant *K′* and solute absorption dissolved in molecular phase A_m were the fitting parameters in NLREG procedure in which experimental UV-vis solute absorbance data were analyzed as a function of the Acarbose concentration. Equations and numerical values of these parameters are given in Figure 2 and compared to experimental data for the suitable solute.

The variation of absorbance with a concentration of Acarbose -protein is shown in figure 2.

Thus from the calculation, the binding constant(*K′)* = 0.000000322 and distribution constant(*K)* = 0.000000000498761 for Hb-Acarbose at 280 nm, the binding constant(*K′)* = 0.037857 and distribution constant (*K)* = 0.00005864 for HSA-Acarbose at 280 nm, the binding constant(*K′)* = 0.004104 and distribution constant(*K)* = 0.000006357 for BSA-Acarbose at 280 nm and the binding constant(*K′)* = 0.0053748 and distribution constant(*K)* = 0.000008325 for Hb-Acarbose at 405 nm respectively. These consequences indicate that the Hb showing stronger interaction with acarbose as compared to the BSA and HSA. Such result also is supported by the thermodynamic properties.

4. CONCLUSIONS

The spectroscopic and thermodynamic interaction of acarbose with three different globular proteins studied for the complex formation. Acarbose significantly quenches the used proteins via absorption methods which conform by the binding constant and distribution rate constants were measured. The observed thermodynamic parameters of acarbose-proteins complexes suggested the hydrogen bonding or other intermolecular interaction plays in the interaction of acarbose and proteins with a negative value of Δ*G* infers the spontaneity of the acarbose-protein complexation. The observed spectra and thermodynamic parameters are useful to understand the biochemical process. Thus, the binding constant and distribution constant also indicate the interactions abilities among solute and solvent. However, Hb shows strong interaction with acarbose because of higher binding constant the BSA-acarbose and HAS-acarbose. From the biological application point of view, this study protein helps as a carrier molecule for multiple drugs. Hence, this study has great importance in pharmacology and clinical medicine trials. In another, the change in Δ*H*, *E*, and ΔS values suggested the phillic-phobic interaction in between acarbose and proteins.

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