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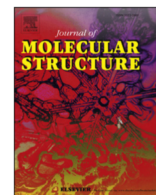

Spectroscopic and molecular modelling studies of binding mechanism of metformin with bovine serum albumin

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Metformin is a biguanide class of drug used for the treatment of diabetes mellitus. It is well known that serum protein-ligand binding interaction significantly influence the biodistribution of a drug. Current study was performed to characterize the binding mechanism of metformin with serum albumin. The binding interaction of the metformin with bovine serum albumin (BSA) was examined using UV–Vis absorption spectroscopy, fluorescence, circular dichroism, density functional theory and molecular docking studies. Absorption spectra and fluorescence emission spectra pointed out the weak binding of metformin with BSA as was apparent from the slight change in absorbance and fluorescence intensity of BSA in presence of metformin. Circular dichroism study implied the significant change in the conformation of BSA upon binding with metformin. Density functional theory calculations showed that metformin has non-planar geometry and has two energy states. The docking studies evidently signified that metformin could bind significantly to the three binding sites in BSA via hydrophobic, hydrogen bonding and electrostatic interactions. The data suggested the existence of non-covalent specific binding interaction in the complexation of metformin with BSA. The present study will certainly contribute to the development of metformin as a therapeutic molecule.

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1. Introduction

Metformin (N, N-dimethylimidodicarbonimidicdiamide) belongs to biguanide class of compound and currently used as US Food and Drug Administration approved drug for first line of treatment of type 2 diabetes [1,2]. The molecular structure of metformin is showed in electronic supplementary information (Fig. S1). Metformin inhibits the mitochondrial respiratory chain complex 1 and decreases the production of glucose in liver. It has been shown to have some therapeutic potential in conditions such as diabetic nephropathy, cardiovascular diseases, polycystic ovary syndrome and in the prevention and treatment of cancer. Also it lowers the basal and postprandial blood glucose elevation in patients with type 2 diabetes [3,4].

Metformin reduces the level of albuminuria in patients with diabetes mellitus and prevents renal failure by decreasing the ROS-mediated lipid peroxidation [3,5]. Metformin reduces the LDL cholesterol and triglycerides and increases the HDL cholesterol. Due to these effects, metformin-has been shown to have cardioprotective effects in murine models by increasing the tolerance for atherosclerosis, cardiac ischemia and reperfusion injury [1,3,6]. In women who are suffering from polycystic ovary syndrome (PCOS), treatment with metformin improves the menstrual cyclicity, increases the ovulation and pregnancy rates and reduces the levels of androgens in the blood stream [2,3,7]. In obese non-diabetic rat models, metformin improves the Nitrogen oxide (NO) signalling, decreases the superoxide production and hence it has antioxidant and vasculoprotective property [5,8,9]. The elevated levels of insulin has a growth promoting effects on various types of cells, which can lead to development of cancer of colon, breast, pancreas, prostate, ovary and lung specific cells. But treatment with

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metformin causes depletion of insulin from plasma and hence reduces the risk of these specific cancers [3,5].

Serum albumin is a major soluble protein component present in the blood [10,11]. It has various physiological functions such as transport of many exogenous and endogenous molecules like free fatty acids, nutrients, steroids and a number of drugs for the target organs [11–13].

Bovine serum albumin (BSA) was preferred as a model protein, because it has a structural homology with Human serum albumin (HSA). It is easily available and well characterized protein [14,15].

Tanwir et al. [16] showed the binding of metformin hydrochloride with BSA using fluorescence spectroscopy and concluded that the fluorescence quenching of BSA was a result of complex formation of metformin hydrochloride with BSA. Similarly, Nath et al. demonstrated decreased binding affinity of metformin hydrochloride with BSA in the presence of levofloxacin [17]. Therefore, the authors suggested a specific binding mechanism for metformin hydrochloride with albumin protein. However, there are studies available which suggested poor binding of metformin hydrochloride with plasma protein [18]. Hence, in literature there are contradicting views regarding the binding of metformin hydrochloride with plasma proteins.

The present study was carried out to develop a detailed mechanistic based approach to explore the binding affinity of metformin with plasma model protein (BSA) using UV–Vis absorption, fluorescence, circular dichroism (CD), and Density Functional Theory (DFT). Molecular docking studies were used to investigate binding mechanism of metformin with BSA.

The absorption spectra showed that there is no binding of metformin with the BSA in the region where tryptophan is present and the low quenching of tryptophan residues in the presence of metformin was confirmed by the fluorescence emission spectra. The binding of metformin with BSA was confirmed by CD and *in silico* studies that demonstrated the conformational change in the BSA structure in presence of metformin and also showed the groups involved in the complex formation between metformin and BSA.

This study has explained the role of specific non-covalent (hydrophobic, hydrogen bonding and electrostatic) binding interactions in the complexation of metformin with BSA.

This study will help the other researchers to formulate the drug to increase its bioavailability and better distribution in the circulation.

2. Experimental section

2.1. Materials

Metformin was a kind gift from Ranbaxy Research Labs with purity ≤99% (New Delhi, India). Bovine serum albumin (BSA, Fraction V, approximately 99%; protease free and essentially γ-globulin free) and disodium hydrogen phosphate, sodium dihydrogen phosphate and sodium chloride of purity ≤ 99% were purchased from Himedia (India). The water used for the preparation of solutions was 18 Mega ohm MilliQ grade water derived from Millipore water system (model Elix 3, Millipore Corp USA).

2.2. Solution preparation

The protein stock solution (30 μM) was prepared by dissolving BSA powder in 10 mM phosphate buffer solution (pH 7.4) and protein solution was dialyzed overnight using dialysis cellulose membrane against the same PBS buffer at 277 K. The concentration of BSA was determined using UV–Vis absorption measurement, using the extinction coefficient of $\varepsilon^{(1\%)}_{280nm}$ 6.8. Stock solution of metformin (4000 μM) was prepared in the same phosphate buffer (pH 7.4) and the concentration was assessed using UV Vis absorption technique using the molar extinction coefficient of $\varepsilon_{232nm} = 26000\ M^{-1}\ cm^{-1}$.

2.3. Absorption measurements

Absorption spectrum was recorded using UV–Vis spectrophotometer (Cary 100 BIO, Varian, Australia) equipped with a Peltier thermostatic cell holder. BSA solution (15 μM) was kept in 3 ml capacity quartz cuvette of path length 1 cm. The sample was scanned from 200 nm to 400 nm. Subsequently, different volumes of metformin solution (1000 μM) were added to 3 ml solution of BSA at each titration step. The concentration of metformin was varied from 3.3 μM to 62.5 μM in the UV Vis absorption titration of BSA solution. All the measurements were measured at 298 K.

2.4. Fluorescence measurements

Fluorescence measurements were performed using a Fluorescence spectrophotometer (Cary Eclipse, Varian, Australia). The excitation source was xenon lamp (450 W) and sample chamber was equipped with Peltier accessory. Scanning parameters for all measurements were optimized with slit width 5 nm for excitation and emission, dwell time 0.2 s and wavelength step 0.5 nm. All fluorescence measurements were performed at 298 K. BSA solution of concentration 15 μM was kept in 3.5 ml quartz cuvette. The excitation wavelengths were set at 295 nm and 280 nm to selectively excite the tryptophan residues. The emission spectra of BSA were recorded in the wavelength range of 310–450 nm at a scan rate of 100 nm min^{-1} in the absence and presence of metformin. Metformin concentration was varied from 6.6 μM to 62.5 μM for titration.

2.5. Circular dichroism measurements

The alterations in the secondary and tertiary structure of the protein in the presence of metformin were recorded on spectropolarimeter (J815, Jasco, Japan) equipped with Peltier accessory. For CD experiments, the protein concentration and path lengths used were 1 μM and 0.1 cm, respectively. The spectropolarimeter was sufficiently purged with 99.9% dry nitrogen before measurement. The spectra were collected at a scan rate speed of 50 nm min^{-1}, and a response time of 1 s. Each spectrum was baseline corrected, and the final plot was taken as an average of three accumulated plots in the range of 200–250 nm. The molar ellipticity $[\Theta]$ was calculated from the observed ellipticity θ as given in Eq. (1)

$$[\Theta] = 100(\theta / c \times l) \quad (1)$$

where c is the concentration of the protein in 2×10^{-6} mol $dm^{-3}$and l is the path length of the cell (cm). These experiments were performed for the $\frac{[BSA]}{[Metformin]}$ molar ratio in 1:1, 1:2, 1:4, 1:8, 1:16 and 1:32. Changes in $[\Theta]$ of each protein sample were measured in the temperature range of 298 K.

2.6. Density functional theory (DFT)

All the studies were carried out at DFT level with Gauss View 5.0 molecular visualization program and Gaussian 09 program package. The molecular structure of metformin in the ground state was optimized by using Becke's three parameter exchange functional (B3) which is a linear combination of Hartree – Fock, local and gradient corrected exchange terms. The B3 hybrid functional was

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