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Absorption, Emission Spectroscopic and Molecular Docking Study of Glutamic Acid with Double-Stranded Calf Thymus DNA

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ABSTRACT

The interaction of the glutamic acid (Glu) with double-stranded (ds) calf thymus DNA (CT-DNA) has been studied by absorption and emission spectroscopy then further results are compared with molecular docking study. The calculated value of binding constant estimated by different techniques are very close to each other, which is around 3.54×10^3 ML^{-1}. Besides, the value of binding sites from fluorescence is 0.939, which suggests appropriate binding of Glu with CT-DNA. The result shows Glu binds to nitrogenous bases (adenine and guanine) of CT-DNA through the groove binding mode of interaction. The experimental results were compared successfully with docking results. The binding energy (ΔG) of the complex has been -4.76 kCal M^{-1} and -5.1 kCal M^{-1} from fluorescence and molecular docking studies, respectively. In our study a correlative intractability pattern for Glu with DNA has been identified.

Keywords: Glutamic Acid, Calf thymus DNA, Intercalating binding, Binding Constant, Molecular docking

INTRODUCTION

More than 300 amino acids (AA) exist in the nature, out of which only 20 α- (AA) are recognized building blocks of protein [1]. Recent studies have witnessed that AA are not only cell signaling molecule but also regulators of gene expression [2]. Among them, glutamic acid, which is a non-essential AA [4], commonly exists as glutamate, because conditions of the human body favors the loss of the hydrogen atom from glutamic acid [3,4]. Most often, L-glutamic acid (Glu) is used as flavor enhancer in fruits, seafood, meats, poultry, soups and snacks [5-8] besides, it also useful as stability enhancer of several proteins [9,10], protects intestinal wall against gastric attack [11], gut function, active brain nuclei related to appetite, memory, thermoregulation [12]. Nextly, it has a role in the nutrition diet of mal-nutritional patients [13-15].

DNA is the building block of cells and it contains genetic information which is obligatory for cellular functions. So the interaction between DNA and other molecules have unique consequences those are related to the replication, mutation and transcription [16]. More often, three types of binding modes are reported for interaction of AA to DNA, i.e., electrostatic interaction, intercalation of aromatic heterocyclic groups between the base pairs and Vander- Waal interaction [17-19]. Interaction between ligand Glu and its Cu and Ru complexes with DNA was studied and showed that these complexes may lead to analogues effect of pharmacologically active anticancer drugs [20].

In the present study, the interaction of Glu and DNA has been studied by UV-Visible and fluorescence spectroscopy and finally obtained results were correlated with docking study. The binding constant, binding sites and binding free energy of Glu-DNA complex were evaluated.

EXPERIMENTAL

Chemicals and reagents

Calf thymus DNA (CT-DNA) and L-Glutamic acid (Glu) were purchased from Sigma Aldrich, India and used without further purification. A stock solution of DNA was prepared by dissolving an appropriate amount of the DNA in deionized water and was stored at 4°C temperature for overnight duration. The concentrations of DNA solutions were determined by using UV-Vis spectrophotometry with the average extinction coefficient value of 6600 M^{-1} $cm^{-1}$for a single nucleotide at 260 nm [21]. L-Glutamic acid of 0.04 ML^{-1} solution was prepared by using deionized water and stored at 4°C temperature. Tris-buffer solution (0.04 ML^{-1}) of pH 7.0 was prepared by using Systronics μpH 361 digital analyzer. All

the other reagents and chemicals used were of analytical grade and were prepared by using deionized water as solution medium. Finally, all the experiments were carried out at room temperature.

UV-Visible Spectrophotometry

UV-Vis spectroscopy is one of the most utilized techniques to detect the binding strength and to predict the mode of binding exists in a molecular complex system [22]. If a small molecule interacts with CT-DNA, it leads to the alteration in the absorbance and the position of the signal obtained with only CT-DNA. UV-Vis spectra for Glu were recorded by Systronics Double Beam UV-Vis spectrophotometer, with the constant concentration of Glu (1 x 10^{-4} M) along with the variable concentration of CT-DNA (from 1x10^{-5} M to 1 x 10^{-4} M).

Fluorescence emission spectroscopy

The fluorescence spectroscopic technique also plays an important role in the interaction study of molecular complex. The fluorescence emission measurements were carried out on RF-5301PC Spectro-fluorophotometer. The emission spectra in the fixed concentration of Glu (30 µM L^{-1}) in Tris-buffer solution (0.04 ML^{-1}) along with the aliquots of variable concentration of CT-DNA was recorded in the range of 270 to 400 nm at an excitation wavelength of 266 nm. The quenched fluorescence intensity was represented as F_0/F, where F and F_0 were fluorescence intensities of the system with and without DNA, respectively.

Molecular docking study

The structure of Glu was raised by Avogadro 1.2 and structure minimization calculation carried out using Gaussian 09W software by utilizing Becke's three parameter hybrid model with the Lee-Yang-Parr correlation functional (B3LYP) method. The 6-311G (d,p) basis set was employed to predict the molecular structure. The optimized geometry is shown in Figure 1.

Figure 1: Optimized structure of glutamic acid.

The molecular docking study of Glu with DNA was performed on Auto Dock Vina software with Lamarckian Genetic Algorithm (LGA). In the study DNA was used as a receptor molecule which was taken from Protein Data Bank (PDB CID: 5c51). Prior to use of DNA in docking interaction study, some expulsion step were carried out to remove the ligand, hetero atoms and water molecules. Polar hydrogen atoms and partial charges were added. The charges were calculated by the Geistenger method. The size of the grid box used during study was 50 Å x 60Å x 50Å in X x Y x Z directions with the spacing of 0.5 Å. The LGA was employed for docking calculations. All other parameters were used from default setting of the software. The binding mode which has lowest binding energy was selected for the further study. The result obtained from AutoDockVina was visualized with Biovia Discovery Studio Visualizer v16.1.0.

RESULTS AND DISCUSSION

UV-Vis spectroscopy

The interaction of Glu with CT-DNA was characterized by monitoring a titration using UV-Vis absorption in the Tris-buffer solution (pH 7.0).The absorption spectra recorded for the fixed concentration of Glu and the interaction was studied with varying concentration of CT-DNA. Glu shows two absorption peaks at 219 nm and 266 nm. The absorption peak at 266 nm got decreased with the addition of DNA as shown in Figure 2.

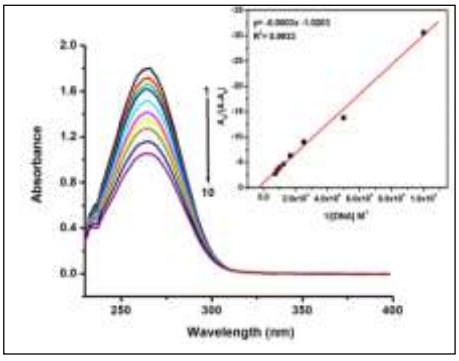

Figure 2: The UV spectra of Glu and Glu-DNA complex in tris-buffer solution (pH 7.0). From 1 to 10: (1) GLU (1.0 x 10^{-4}mol L^{-1})in absence of DNA and (2-10) with increasing concentration of DNA: (0.1 x 10^{-5}mol L^{-1}, 0.2 x 10^{-5}mol L^{-1}, 0.3 x 10^{-5}mol L^{-1}, 0.4 x 10^{-5}mol L^{-1}, 0.5 x 10^{-5}mol L^{-1}, 0.6 x 10^{-5}mol L^{-1}, 0.7 x 10^{-5}mol L^{-1}, 0.8 x 10^{-5}mol L^{-1}, 0.9 x 10^{-5}mol L^{-1}); Inset: The linear plot of $A_0/(A-A_0)$ *vs* 1/[DNA].

The λ_{max} was obtained remained constant but, the absorption intensity was changed due to the changes in conformation and structure of the Glu and DNA via their interaction. The hypochromic incorporated with bathochromic shift indicates the existence of intercalative binding modes [23]. But the absorption results of interaction between Glu-DNA shows hypochromic shift with minor change in bathochromic shift probably indicates the existence of groove binding mode of interaction [24]. The intrinsic binding constant, Kb, was determined from equation [25]:

$$\frac{A_0}{A-A_0} = \frac{\varepsilon_G}{\varepsilon_{H-G}-\varepsilon_G} + \frac{\varepsilon_G}{\varepsilon_{H-G}-\varepsilon_G} X \frac{1}{K_b[DNA]} \qquad \text{Eq. 1}$$

Where, A_0 and A are the absorbance of Glu and its complex with DNA, respectively. ƐG and ƐH-G are the absorption coefficients of Glu and Glu-DNA complex, respectively and Kb is the binding constant. The value of Kb was calculated from the intercept of linear plot (A0/A-A0) vs. 1/ [DNA] (Figure. 2, inset). The value of the binding constant (Kb) for Glu-DNA complex was obtained to be 3.39 x 10^3 M^{-1}.

Fluorescence spectroscopy

Fluorescence emission spectroscopy also gives the information for binding mode and sites of interactions between Glu and CT-DNA. Quenching of fluorescence provided valuable information about the interaction of quencher and fluorophore. The Glu solution in Tris buffer (pH 7.0) gives intrinsic fluorescence emission spectra when excited at 266 nm with emission maxima at wavelength 331 nm (Figure 3)**.**

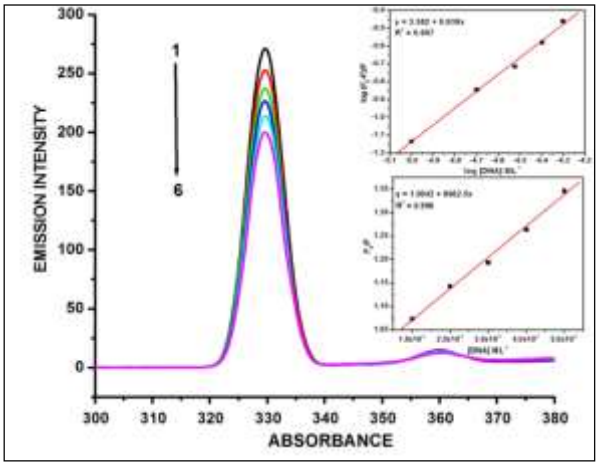

Figure 3:The Fluorescence spectra of Glu-DNA in tris-buffer (pH 7.0) from 1 to 5 : (1) Glu (1.0 x 10^{-4}mol L^{-1}) in absence of DNA and (2-5) with increasing concentration of DNA: (0.1 x 10^{-5}mol L^{-1}, 0.2 x 10^{-5}mol L^{-1}, 0.3 x 10^{-5}mol L^{-1}, 0.4 x 10^{-5}mol L^{-1}, 0.5 x 10^{-5}mol L^{-1}); Inset: Linear plot of F_0/F vs. concentration of DNA and the linear plot of log [(F_0 – F) / F] *vs* log C_{DNA}

The intensity of fluorescence spectra of 1.0 x 10^{-4} $molL^{-1}$ Glu got decreased successively with addition of CT-DNA. The decline in fluorescence intensity indicates the quenching of Glu during binding with DNA. Since the spectra shows that quenching of the Glu with DNA shifts the intensity but no shifts in the wavelength (λ) was observed, suggests that the Glu interacts with DNA through groove binding mode. The Stern–Volmer equation (eqn. 2) was used to study quenching process as well as to calculate the quenching constant [26]:

$$\frac{F_0}{F} = 1 + (K_D + K_S)[DNA] + K_S K_D [DNA]^2 \qquad \text{Eq.2}$$

where, KD and KS are the dynamic and static quenching constants, respectively. F0 and F are the fluorescence intensities in the absence and presence of quencher respectively. [DNA] is the molar concentration of DNA. In the case of combined static and dynamic quenching, plot between F_0/F vs. [DNA] should be non-linear. But Figure 3 inset (lower), clearly shows a linear plot with R_2 = 0.997 which indicate the quenching mechanism may be static or dynamic. The nature of quenching, static or dynamic can be determined by using linear classical Stern-Volmer equation [26].

$$\frac{F_0}{F} = 1 + kq\, \mathfrak{T}_0[DNA] = 1 + K_{SV}[DNA] \qquad \text{Eq.3}$$

where, kq and KSV are the bimolecular quenching constant and Stern-Volmer quenching constant, respectively. The values have been calculated from the linear Stern-Volmer plot between F_0/F versus concentration of CT-DNA as shown in Figure 3 inset (lower). The value of KSV was calculated from the slope of the curve, 6.662x103 M^{-1} L s^{-1}. The value of KSV is in the range of typical groove binders [27]. The equation (3), then was substituted with the values of KSV and fluorescence lifetime (τ_0) of biological macromolecules as 10^{-8} s, and solving that leads to the value of kq, i.e., 6.662 x 10^{11} M^{-1} L s^{-1}. It is larger than the limiting diffusion rate constant of biomolecule (2.0 x 10^{10} M^{-1} L s^{-1}) indicates the static quenching occurred in Glu quenching by DNA [28]. The binding constant 'Kb'and the binding sites 'n', calculated by using the following equation [29]:

$$log\frac{F_0-F}{F} = \log K_b + nlog[DNA] \qquad \text{Eq.4}$$

The intercept of the plot of log (F_0 – F) /F vs. log [DNA] gives the value of Kb and the n were evaluated from the slope value Figure 3, inset (top). The calculated binding sites were 0.939 and binding constant was 3.655 x 103 M^{-1}. The values are close to the values of binding parameters obtained through UV-Vis spectroscopy method. The binding Gibb's free energy (ΔGb*) for Glu-DNA was calculated from the following relation as - 4.86 $kCalM^{-1}$ [32]:

$$\Delta Gb^* = -RT \ln Kb \quad (5)$$

where, R represents the gas constant and T is the absolute room temperature.

Molecular Docking Study

Computer based docking study plays a significant role in drug design, interaction probability, most possible binding energy and elucidation of mechanisms of action [30]. It has a vital place for presumption of binding mode exists in complex. Prior to the experimental screening of molecules, molecular docking programs provides powerful computational filters and statistical measurements for existence and interaction of compounds which reduce labor and cost needed for the development of pharmacologically effective chemical moieties as drug [31]. In addition, they can assist the bioactive mechanisms in superior way [32]. The structure of Glu was drawn and subjected to energy optimization. The resulting Glu-DNA complex was used for calculating the energy parameters that substantiate the spectroscopic and voltammetric results [33]. Out of the 9 conformers, minimum energy conformer which has lowest binding energy (-5.1 kCal M^{-1}) was used for measurement of bonding parameters. Docking result shows that Glu interacts with DNA via inter molecular hydrogen bonding mode. Glu interacts with guanine site (DG8 and DG22) and adenine site (DA9) of DNA via minor groove binding mode (Figure 4a).

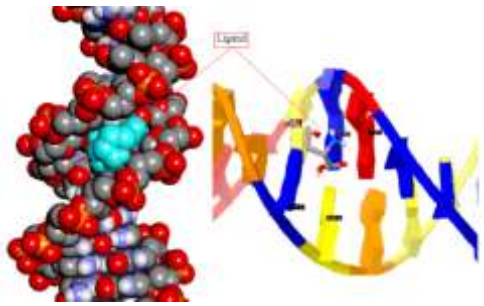

Figure 4 (a): Molecular docking study: surface and binding energy representation, showed that Glu interact with DNA with minorgroove binding

O13 and O8 atoms of Glu form a bond with guanine molecule of purine nucleotide [(DG8(H22) & DG22(H22); NH2], has bond length of 2.28Å and 1.98Å; O12 of Glu forms bond with adenine [DA9(H3)], has bond length of 2.11Å. O3′ of Phosphate group present in adenine forms hydrogen bonding with both the hydrogen present in NH2 group of Glu with bond length of 2.89Å and 3.05Å respectively. O12 and H3 of Glu also show intra molecular hydrogen bonding with BL 1.81Å. Figure 4b and Figure 4c shows all the possible interactions of Glu-DNA complex and surface of hydrogen bond donor and acceptor respectively.

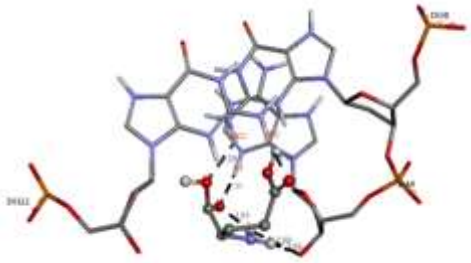

Figure 4 (b): Molecular docking study: bond length with different DNA nucleotides

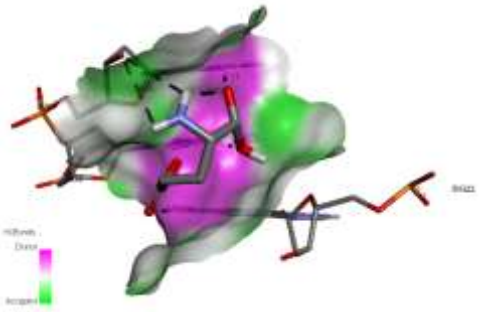

Figure 4 (c): Molecular docking study: figure showing H-bond donor and acceptor surface

From docking results, the free binding energy of Glu-DNA complex was obtained to be -5.1 kCal M^{-1}, which is near to binding energy obtained from fluorescence (-4.8 kCal M^{-1}). Now, it can be concluded that statistics of Glu-DNA docked model is in approximate correlation with our experimental results. The slight difference in the value of binding energy may cause due to the exclusion of the solvent system from the receptor binding site which directly contributed to the molecular intractability in in-vitro conditions with respect to the universal virtual condition applicable in molecular docking process or rigidity of some part of the receptor DNA in the molecular docking studies. Furthermore, the binding constant obtained from UV-Vis and fluorescence spectroscopy was correlated with values obtained from docking method. The value of binding constant was calculated with the help of binding energy using following equation [33].

$$\Delta G = - RT \ln K \qquad (6)$$

where, ΔG and K represent the binding free energy and binding constant, respectively, R represent gas constant and T indicates absolute temperature. The binding constant was calculated to be 5.47 x 10^3 M^{-1} from docked Glu-DNA model which is close to the value of binding constant obtained from fluorescence and UV-Vis techniques.

Table 1: The calculated value of binding constant obtained from spectroscopic and molecular docking study

Techniques	UV-Vis Spectroscopy	Fluorescence Spectroscopy	Molecular Docking
Binding Constant (K_b)	3.39 x 10^3 M^{-1}	3.65 x 10^3 M^{-1}	5.47 x 10^3 M^{-1}

CONCLUSION

The in-vitro interaction of Glu with CT-DNA was studied by performing UV-Vis and fluorescence spectroscopic techniques and the results obtained from the detailed experiment has been further correlated with the molecular docking approach to identify and explore its interaction pattern not only through the spectroscopic approach, also to boost it up violating the contribution of statistical evaluation parameters used in virtual conditions of the all known computational world. All the results of the detailed study performed in the instrumental laboratory, computational laboratory along with the statistical analysis of the data shows that the groove binding mode of interaction has been seemed to be predominant one which obviously contributed by the molecular hydrogen bond formation between the amino acid and the spatial interactive cavity of DNA molecule which plays the role of receptor here. Spectroscopic analysis shows relativity with the binding constant and mode of interaction obtained from the molecular docking study (5.47 x 10^3 M^{-1}). The values of binding sites and binding free energy obtained by performing fluorescence was very close to molecular docking results. The combination of the spectroscopic and molecular docking methods in corresponds to the statistical evaluation shows potential importance in understanding the mechanism and mode of action of this important class of amino acid with DNA which can contribute to the further exploitation of the interaction for understanding the basics to use the pattern in different field of DNA study, besides these, can be used for the generation of drug molecule having prior mechanism of action in DNA, in different aspect of disease related to DNA molecule interaction or DNA mutation.

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