ISSN 0973-3450 (Print)
JUC Vol. 14(6), 156-162 (2018). Periodicity 2-Monthly
(Online) ISSN 2319-8036

JOURNAL OF ULTRA CHEMISTRY
An International Open Free Access Peer Reviewed Research Journal of Chemical Sciences and Chemical Engineering
website:- www.journalofchemistry.org
Estd. 2005

"Drug Delivery and Binding Analysis of Isoflavones with Special Reference to Chicken Egg Lysozyme"

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http://dx.doi.org/10.22147/juc/140602

Acceptance Date 17th October, 2018, Online Publication Date 02nd November, 2018

Abstract

Drug delivery is the formulations, technologies and systems for transporting a pharmaceutical compound in the body as needed to safely achieve its desired therapeutic effect. Dietary polyphenols are very essential and are widely studied due to their various biological activities like anti-cancerous, antibacterial, anti inflammatory, anti diabetic etc. Lysozyme is an enzyme having antibacterial property which is found in various types of birds, mammals and insects. Lysozyme has the capacity to cure various diseases by binding with various drugs and realizing them in their target site. So their binding at molecular level is essential in the field of pharmaceutics. The binding of isoflavones with chicken egg lysozyme have been investigated using viscometer, UV-Visible spectrophotometer, circular dichroism, spectroflurometer and molecular docking technique. Drug delivery has been studied by using experimental animal, testing of anti cancerous activity and histopathological study. CD results indicate that the polyphenols are capable to increase the helical content of lysozyme during binding process. Molecular docking studies have been performed to substantiate the experimental facts. It is observed that both the ligands bind near to Trp 62, Trp 63 and Trp 108. Binding free energies are obtained from docking results and PEARLS will in good correlation with each other for the protein– ligand complexes. The binding of dietary polyphenols with lysozyme at the molecular level suggests a further insight into the success of the drugs in pharmaceutics.

Key word: Lysozyme, Molecular docking, Drug delivery.

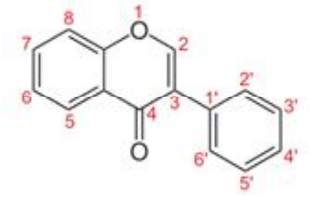
Introduction

A drug is a chemical substance which is consumed for temporary physiological change in the body[1]. It is also known as medicine which is used to cure, prevent or diagnose a disease and are used for limited duration on a regular basis for chronic disorders[2]. Drugs can be classified according to

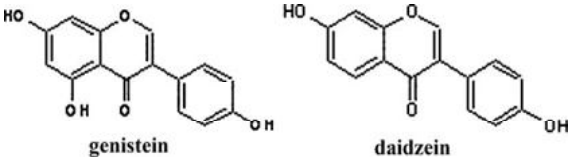
chemical structure, binding to same biological target etc. The process in which new medications are discovered is known as drug discovery. The modern drug discovery involves various processes. Those are identification of screening hits, medicinal chemistry and optimization of those hits to increase the affinity, selectivity, potency, metabolic stability & oral bioavailability. Once the compound fulfills all of these requirements then the compound got identified and then proceeds for drug development. After that proceeds through clinical trials. Then it proceeds through computer-aided drug designing. Discovering drug may be a commercial success or public health success involving a complex interaction between investor, industry, academia patent laws, regulatory exclusivity, marketing etc[3].

Flavonoids constitute the largest family of polyphenols. It is characterized by diphenylpropane skeleton ($C_6C_3C_6$), in which two aromatic rings are linked by three carbons by forming a heterocyclic ring [4].These compounds have radical scavenging, anti-inflammatory, anti-cancer, anti-tumor, anti-oxidative properties[5]. Flavonoids have two bands in UV visible spectra, one is at 240-280 due to benzoyl moiety and other is due to cinnamoyl moiety at 300-400 nm[5].

Isoflavonoids are 3-phenylchromen-4-one.



[3-phenylchromen-4-one]



These are naturally occurring iso flavonoids[6], which act as phytoestrogen in mammals. Isoflavones are sold as dietary supplements. Soybeans are the most common source of isoflavones in human food. Mainly genistein and diadzein are found in soybeans. The isoflavones have various biological properties like anti-inflammatory, anti- diabetic, anticancer, antibacterial, antitumor etc. Genistein (4', 5, 7-trihydroxyisoflavone) is a phytoestrogen having a wide variety of pharmacological effects in animal cells. Dietary genistein ingestion has been linked with a range of potential beneficial health effects; especially it has the effect on breast cancer attributed to its moderate binding affinities to estrogen on various tissues. Diadzein has the potential to protect against chemically induced mammary cancer. Genistein has the antitumor effects on tongue carcinoma cells [7]. Despite its low molecular weight and favorable absorption behavior, genistein struggles through its major concerns of low bioavailability and solubility inside human body, which in its turn affects its remedial potential[8].

Lysozyme is an enzyme having antibacterial property which is found in various type of birds, mammals, insects. It is also known as muramidase[9]. It has various pharmaceutical and pharmacological properties like anti-tumor, anti-viral, anti histamic, anti-inflammatory, immune modulatory properties[10-14]. Lysozyme has the capacity to cure various diseases by binding with various drugs and realizing them in their target site. Lysozyme has the capacity to carry drug, so it has important role in medicinal point of view. Hence the study on lysozyme and ligand interaction is very important. Lysozyme is a globular protein which contains s 129 amino acids that is immensely important due to its biological doings[15-17] along with the knack of destruction of bacterial cell membrane. In the ligand binding site two tryptophan residues Trp 62 and 108 are present which are found to be the effective fluorophores among available six tryptophans in the protein structure.

Materials and Methords

Materials:

Chicken egg lysozyme (MB098) and genistein (RM5921) were taken from Hi-Media Laboratories, India. Diadzein (D7802) was taken from Merck and Sigma-Aldrich laboratories, India. The lysozyme was dissolved in 20 µM phosphate buffer (PB) of pH 7.2

containing 50 μM NaCl and the concentration was measured spectrophotometrically[15]. The polyphenols were dissolved in absolute grade ethanol. Experiments were executed containing less than 5 % alcohol.

Methods:

The work has been performed as follows:

Circular dichroism measurement :

Circular dichroism studies were executed on a Jasco-810 spectrophotometer in the region 190–240 nm at room temperature. Two sets of solutions were taken having protein: ligand ratios at 1:0 and 1:5 respectively for far UV CD. For far UV CD measurement the concentration of protein (lysozyme) was constant at 20 μM. The far-UV CD spectrum of the protein was collected using 0.1 cm cell with a scan speed of 50nm/ min. The mean residual ellipticity (MRE, in deg cm2 dmol-1) can be calculated by the following equation.

$$MRE = \frac{observedCD\ (m^{\circ})}{Cp \times n \times l \times 10}$$

Where CP is called molar concentration of lysozyme and l the path length of the cell. We can find out the a-helix content of native and complexed lysozyme from the following equation[18-5].

$$\alpha - helix(\%) = \frac{(-MRE_{208} - 4000)}{33000 - 4000} \times 100$$

Where MRE208 is the observed MRE values at 208 nm, MRE values of native α-helix content and that of a β-sheet and coil structure at 208 nm are 33,000 and 4,000 respectively.

Molecular docking technique :

Molecular modelling studies of targeted four compounds were carried out using Auto dock 4.2. Version software[19]. Docking of all compounds was carried out on PDB_ID 6lyz, chain A. This protein PDB was retrieved from protein data bank. During the docking we have used 1grid box parameters as centre: x = -0.709, y = 22.45, z = 19.252and grid box size: x=36, y=30, z=44. During docking 20 conformations were generated in each docking by using Lamarckian algorithm. Further parameters were kept as default settings. Input preparation carried out using MGL tools- 1.5.6 and final docking performed in Auto dock 4.2.

Result and Discussion

The CD spectra of lysozyme demonstrate two negative bands one around 208 nm (Π -Π * transition) and the other one at 222 nm (n – Π * transition) as presented in Fig. 1. It is a feature of a-helical pattern originated due to negative cotton effect[20,21]. The interface between genistein (or diadzein) and lysozyme leads to a slight increase in spectral intensity of the far UV-CD without any considerable shift of peak positions, indicating the increase of helical content after binding[5,22]. It has been found that the a-helix percentage of lysozyme enhanced from (31.84 ± 1.59; mean value ± se) to (37.81 ± 0.77) % and (37.45 ± 0.53) % at a molar ratio of 1:5 on binding with the genistein and diadzein respectively. The results indicate the presence of binding between ligands and protein, leading to a gain in helical stability of lysozyme.

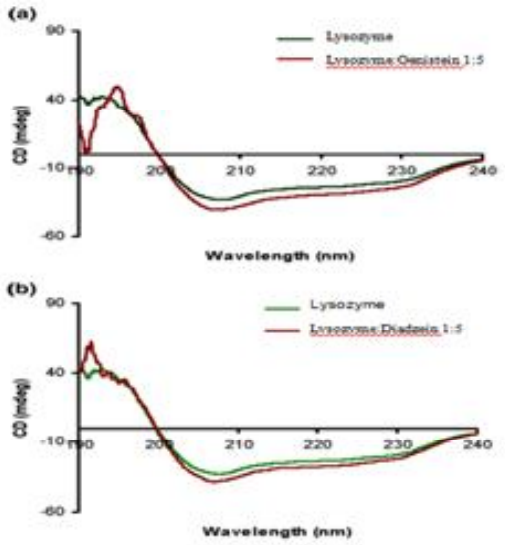
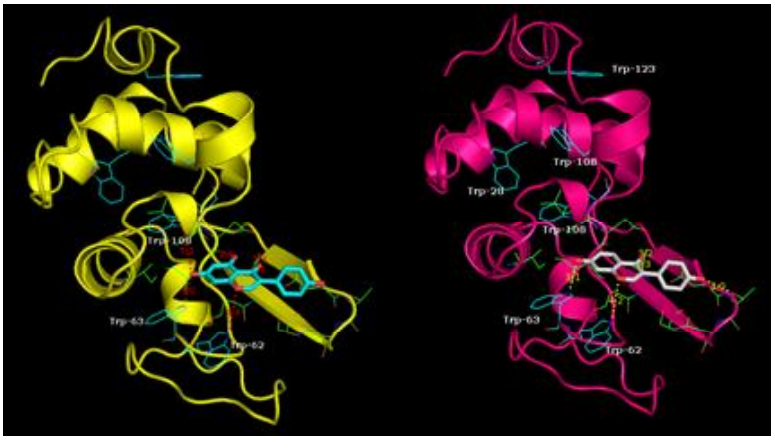


Fig.1 Far UV-CD spectra of lysozyme (green line) and its 1:5 complexes with a genistein and b diadzein in 20 μM PB of pH 7.2 containing 50 μM NaCl. [Protein] = 20 μM. (Color figure online)

Binding affinities of the lysozyme with molecules were calculated by using molecular docking studies, here

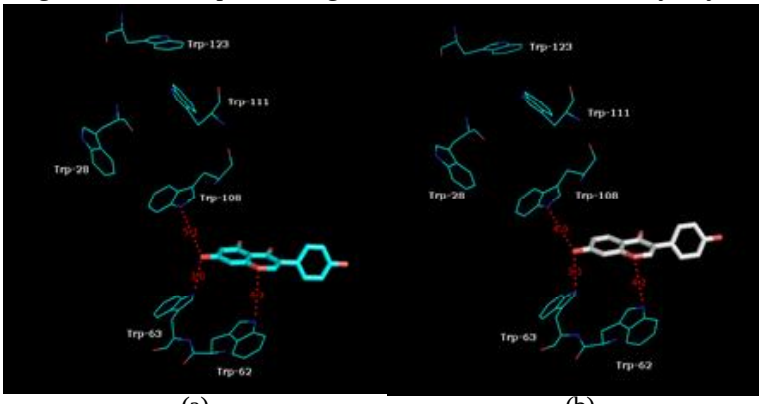
in we have carried out docking studies of two known molecules Diadzein and Genistein with lysozyme. These two molecules have more structural similarity and showed significant binding affinity at binding pocket of Lysozyme protein (PDBID: 6LYZ)[23]. In the present study we have chosen the total protein as the binding region to perform the molecular docking. The analysis of binding site interactions present in binding pocket amino acid residues showed good Hydrogen bond (HB) interactions with both molecules. We found the molecule Diadzein form hydrogen bonds with Trp63 (HB distance of 3.1 Å), It also forms hydrogen bonds with side chain carbonyl groups of ASP52 (HB distance of 3.2 Å) and with side chain OH of Thr47 (HB distance of 3.0 Å). The similar type of hydrogen bonds is formed by Genistein with Lysozyme. An additional hydrogen bond also observed due to the extra OH group on the bicyclic ring forming with main chain carbonyl group of Gln57 (HB distance of 2.9 Å).

These docking results reveal that both compounds Diadzein and Genistein showed good binding energy with -6.1 to-6.5 kcal/mol. These molecular docking results correlates very well with the results of biological evaluation analysis. Due to an extra hydrogen bond Genistein exhibited more binding energy than the Diadzein. Further, the tryptophan residues are also involving in binding if the molecules in the active site. The Trp62, Trp63 and Trp108 are closely bound to both the molecules. The other three tryptophan residues (Trp28, Trp111 and Trp123) are fallen at long range interaction distances. Thus, the docking procedure of Auto dock in reproducing the experimental binding affinity seems reliable, and therefore predicted as true positive.



(a) (b)

Fig. 2 The docked poses of a genistein and b diadzein with lysozyme



(a) (b)

Fig. 3 The H-bonding distances of (a) genistein and (b) diadzein with the residues of lysozyme

Table 3a. The changes in ASA of the free lysozyme and complexed lysozyme with isoflavones –genistein

Residue	ASA (Uncomplexed)	ASA (Complexed)	ASA ($A^{\circ 2}$)
ASN 46	56.458	26.825	29.633
THR 47	138.066	117.670	20.396
ASP 48	85.741	66.635	19.106
SER 50	1.341	0.340	01.001
ASP 52	26.246	9.698	16.548
LEU 56	1.248	1.248	00.000
GLN 57	12.953	3.246	09.707
ILE 58	3.405	0.000	03.405
ASN 59	30.830	0.000	30.830
TRP 62	121.846	102.173	19.673
TRP 63	44.965	31.317	13.648
ILE 98	10.066	5.299	04.767
ALA 107	54.169	24.252	29.917
TRP 108	10.130	0.000	10.130

Table 3b. The changes in ASA of the free lysozyme and complexed lysozyme with isoflavones –diadzein

Residue	ASA (Uncomplexed)	ASA (Complexed)	ASA ($A^{\circ 2}$)
ASN 46	56.458	26.319	30.139
THR 47	138.066	120.133	17.933
ASP 48	85.741	67.430	18.311
SER 50	1.341	0.340	01.001
ASP 52	26.246	11.007	15.239
LEU 56	1.248	1.248	00.000
GLN 57	12.953	4.608	08.345
ILE 58	3.405	0.000	03.405
ASN 59	30.830	0.000	30.830
TRP 62	121.846	102.360	19.486
TRP 63	44.965	31.524	13.441
ILE 98	10.066	5.299	04.767
ALA 107	54.169	24.673	29.496
TRP 108	10.130	0.849	09.281

Conclusion

The interactions of dietary isoflavones genistein and diadzein with chicken egg lysozyme have been executed using CD and molecular docking studies. The Kb value of genistein is found greater than diadzein towards lysozyme. The energy transfer parameters for the binding are calculated and it is observed that there is a chance of energy transfer from donor (lysozyme) to the acceptors (genistein and diadzein). CD results indicate that the polyphenols are capable to increase the helical content of lysozyme during binding process. Molecular docking study has been performed to substantiate the experimental facts. It has been observed that both the ligands bind near to Trp 62, Trp 63 and Trp 108, but the genistein binds closely than diadzein. Binding free energies obtained from docking results and PEARLS are in good correlation with each other for the protein– ligand complexes. The binding of dietary polyphenols with lysozyme at the molecular level suggests a further insight into the success of the drugs in pharmaceutics.

Scope of Future work :

Chicken egg lysozyme is almost similar to human lysozyme. The binding of dietary polyphenols with lysozyme at the molecular level suggests a further insight into the success of the drugs in pharmaceutics. If ionized drugs bind with lysozyme, so it can easily cross the membrane barrier. If the uncontrollable growth of cell inhibited by lysozyme- isoflavones, it can act as anticancer drug. However, in vivo studies are needed to further validate these results and develop a suitable delivery system that acts directly in situ to overcome the problems concerning the bioavailability.

Lysozyme is part of the innate immune system. Reduced lysozyme levels have been associated with bronchopulmonary dysplasia in newborns.[31] Piglets fed with human lysozyme milk can recover from diarrheal disease caused by *E. coli* faster. The concentration of lysozyme in human milk is 1,600 to 3,000 times greater than the concentration in livestock milk. Human lysozyme is more active than hen egg white lysozyme. A transgenic line of goats (with a founder named "Artemis") were developed to produce milk with human lysozyme to protect children from diarrhea if they can't get the benefits of human breastfeeding. In certain cancers (especially myelomonocytic leukemia) excessive production of lysozyme by cancer cells can lead to toxic levels of lysozyme in the blood. High lysozyme blood levels can lead to kidney failure and low blood potassium, conditions that may improve or resolve with treatment of the primary malignancy.

Serum lysozyme is much less specific for diagnosis of sarcoidosis than serum angiotensin converting enzyme; however, since it is more sensitive, it can be used as a marker of sarcoidosis disease activity and is suitable for disease monitoring in proven cases.

Acknowledgement

We would like to thanks to Prof. (Dr.) Byju John, Hon'ble Vice Chancellor & Shri Gokulananda Panda, Registrar of MATS University, Raipur(CG) for giving us permission to carry out research in the School of Biological and Chemical Sciences. Our deep gratitude towards the Director of Qstatix, Hyderabad & Hon'ble Director General, Chhattisgarh Council of Science and Technology, Raipur for their support and the positive response during few experimental researches. We are also greatly acknowledge the supportive nature to all the Staff members of School of Biological and Chemical Sciences, MATS University, Raipur(CG). Our sincere thanks to Prof.(Dr.) Kallol Kumar Ghosh, Professor, SOS in Chemistry, Pt. Ravishankar Shukla University, Raipur(CG) for his all time help, support and guidance towards this research work.

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