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Absorption and steady state fluorescence study of interaction between Quercetin and Egg Albumin

KEYWORDS: Quercetin, Steady State Fluorescence, Egg Albumin

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ABSTRACT *The interaction between Quercetin and Egg albumin in buffer solution, at p^H 7.0 has been studied by means of absorption and emission spectroscopy. Applying the Scatchard model to the absorbance data a linear plot was obtained linear plot was observed in the fluorescence spectra also. Upon increasing the protein – drug ratio the intensity of quercetin emission band decreases to approximately 30% of its initial value. During this quenching, a small red shift was noticed.*

Introduction

Many biologically active compounds (drugs, natural products, etc.) in the body are correlated with their affinities toward serum albumins (i.e one of the most abundant carrier protein plays an important role in the transport of endogenous and exogenous ligands present in the blood) for their distribution and metabolism[1]. Consequently, the binding study of such molecules to albumin is of impetrative and fundamental importance and many compounds have been investigated successfully including many drugs[2-8].

The drug stability and toxicity during the chemotherapeutic process can be influenced by the effectiveness of drugs as pharmaceutical agents which depends on their binding ability. Furthermore, the drug-albumin complex may be considered as model for gaining general fundamental insights into the drug-protein binding. Thus, it is important in regard to improving our understanding of the bimolecular recognition and it forms an essential part of the rational drug-design process.

The analysis of binding capacity and structure of the complexes between Egg albumin and drug are of particular interest in order to elucidate the means by which ligand affinity is regulated and how the protein complexation is altered upon complexation. These factors play a key role in a vast range of important biochemical phenomena, for example, the reversible binding of oxygen not myoglobin[9].

MATERIALS AND METHODS

Quercetin and Egg Albumin were purchased from sigma Aldrich and used without further purification. All the experiments were performed in p^H -7.0 at temperature 33°C. Fluorescence measurements were performed on Shimadzu RFPC 5301 spectrofluorimeter equipped with a PC. Absorption measurements were performed on Shimadzu 1650 PC spectrophotometer.

RESULTS AND DISCUSSION

Binding of drug with Egg Albumin

Fluorescence measurements give information about the molecular environment in vicinity of the fluorophore molecules. Therefore, conformational changes of albumin were evaluated by the intrinsic fluorescence intensity before and after addition of drug. The binding of small molecule substances to protein, such as the binding mechanism, binding constants, binding media, binding sites and intermolecular distances, can be evaluated by the fluorescence measurements, for macro molecules. The ligand quenches the fluorescence emission spectrum of Egg albumin due to the changes in environment around tryptophan and / or tyrosine caused by interaction of the ligand with albumin. On titration of albumin with the drug solution, the fluorescence intensity decreased due to a variety of molecular interactions, viz., excited state reactions, molecular rearrangements, energy transfer, ground state complex formation and collisional quenching. Such decrease in fluorescence intensity in known as quenching .

Fluorescence quenching spectra of Egg Albumin with Quercetin is given in Fig – 1. Table – 1, gives the Stern – Volmer quenching constant (K_{SV}) and the regression coefficient (r) Stern -Volmer Plot is shown in Fig. 2.

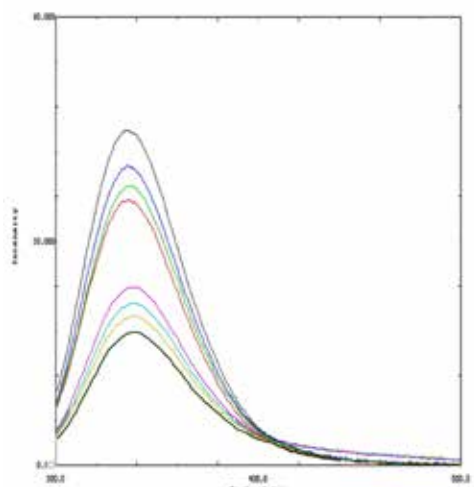

Fig. 1. Fluorescence quenching Spectra of the Egg Albumin with different concentrations of Quercetin mol dm^{-3} (1) 0, (2) 0.2 (3) 0.4, (4) 0.6, (5) 0.8, (6) 1.0, (7) 1.2, (8) 1.4

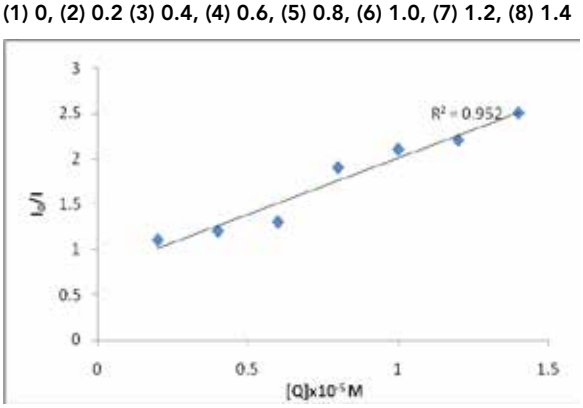

Fig. 2. Stern-Volmer plot of the Egg Albumin with different concentrations of Quercetin

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Table 1: Stern-Volmer Quenching Constant (K_{SV}), regression coefficient (r) ,quenching rate constant (k_q) and standard deviation

Quencher	K_{SV}	K_q	R^2	S.D
Quercetin	1.5	0.307	0.95	0.55

UV – Vis absorption studies

UV – Vis spectroscopy was used to study drug binding interactions [10]. Egg Albumin has a UV Absorption peak at 280 nm, at which particularly three amino acids like tryptophan, phenylalanine, and tyrosine absorb maximally. The formation of drug – albumin complexes is evident from UV – Vis adsorption spectral data. Absorbance decreases with the increase in drug concentration and the shift at 280 nm is not prominent while no shift was observed from the fluorescence data. This again confirms the change in polarity around the tryptophan residue and the change in peptide strand of albumin and hence the change in hydrophobicity. Fig – 3 gives the UV absorption spectra of Egg Albumin with Quercetin.

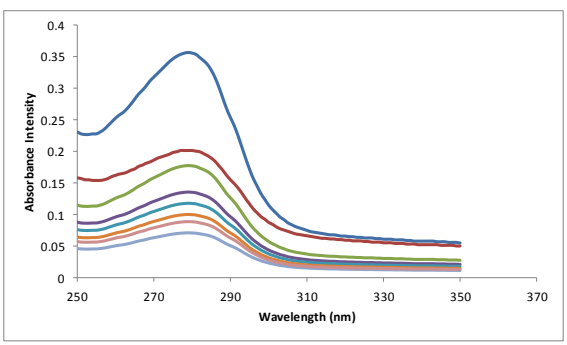

Fig. 3. UV – absorption spectra of Egg Albumin with Quercetin (1) 0, (2) 0.2 (3) 0.4, (4) 0.6, (5) 0.8, (6) 1.0, (7) 1.2, (8) 1.4

CONCLUSION

Herein, the interaction study of Quercetin with Egg Albumin were carried out using UV and fluorescence spectroscopic techniques under physiological conditions (i.e. pH 7.0). Fluorescence quenching method is sensitive and convenient to study binding behavior of Egg albumin by the drug molecules in the drug – Albumin complexes.

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