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Fluorescence Resonance Energy Transfer for Conformational Changes of Egg Albumin

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ABSTRACT

Drug-protein interactions are important since most of the administered drugs are extensively and reversibly bound to albumin and drug is transported mainly as a complex with protein. The interactions between quercetin and Egg albumin was analyzed by fluorescence resonance energy transfer theory. According to this, the average binding distance between quercetin and egg albumin was obtained.

Keywords: FRET, Egg albumin, quercetin, fluorescence.

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INTRODUCTION

Many synthetic and naturally occurring antioxidants are phenolic compounds for which the generic terms ArOH will be used since, by definition, they have at least one hydroxyl group attached to a benzene ring

The more popular natural antioxidants are flavonoids. Flavonoids are a group of polyphenolic compounds extensively distributed in the medical plants, vegetables, fruit juices and a variety of beverages like tea, coffee, wines and fruit drinks. Flavonoids, and particularly querceting, derivatives, have received more attention as dietary constituents during the last few years [1] Many studies showed that flavonoids have a wide range of biological activities, such as anticancer, antiviral, anti bacterial, antioxidants and anti-inflammatory effects [2-5]. Flavonoids are best known as radical scavengers, while these valuable effects generally are due to their abilities to accept free radicals, complexation properties with metal ions have also been recognized to contribute to the total biological activity [1].

MATERIALS AND METHOD

Egg albumin, SPAN 40, and quercetin were purchased from sigma Aldrich company, Banglore and wee used without further purification. UV/Vis., absorption spectra were taken using SHIMADZU 1650 P4 UV-Visible Spectrophotometer and fluorescence measurements were made by SCIMADZU RF 5301 PC Spectrofluorophotometer.

RESULTS AND DISCUSSION

Fluorescence resonance energy transfer occur when the emission spectrum of the donor overlaps the absorption spectrum of the acceptor. The dependence of the energy transfer rate on the interaction distance has been widely used to measure the distance between the donor and the acceptor.

Generally, the maximum distance is in the range of 1-10nm [6]. According to Forster non-radiation energy transfer theory (6,7), energy transfer is related not only to the distance between the acceptor and donor, but also to the critical energy transfer distance, (R_0)

$$E = \left(\frac{R_0^6}{R_0^6 + r^6} \right) \qquad (1)$$

Where R_0 is the critical transfer distance when the transfer efficiency is 50% and r, the mean distance between the centres of the donor and acceptor dipoles. The donor and acceptor here EA and quercetin respectively. E is the energy transfer efficiency calculated with equation (2)

$$E = \left(1 - \frac{I}{I_0}\right) \quad (2)$$

Where I and I_0 are the fluorescence intensity of EA with and without quercetin respectively. R_0 can be given by,

$$R_0^6 = 8.8 \times 10^{-25}\ K^2\ N^{-4}\ \phi\ J \quad (3)$$

where K^2 is the spatial orientation factor of the dipole, N, the refractive index of the medium, ϕ the fluorescence quantum of cold of the donor in the absence of the acceptors, J expresses the overlap integral of the fluorescence emission spectrum of the donor and the absorption spectrum of the acceptor. J is given by,

$$J = \frac{\Sigma F(\lambda)\ \varepsilon(\lambda)\lambda^4 \Delta\lambda}{\Sigma F(\lambda)\Delta\lambda} \quad (4)$$

where F (λ) is the fluorescence intensity of donor at wavelength range λ and Σ(λ), the molar absorption coefficient of the acceptor at wavelength λ with unit of $Cm^{-1}mol^{-1}$. In this work, J was obtained as 8.32×10^{-14} Cm^3 M^{-1} integrating the overlap of the UV absorption spectrum of quercetin and the fluorescence emission spectrum of EA.

The calculated values are compiled in Table 1 and the overlap spectrum is shown in Fig.1.

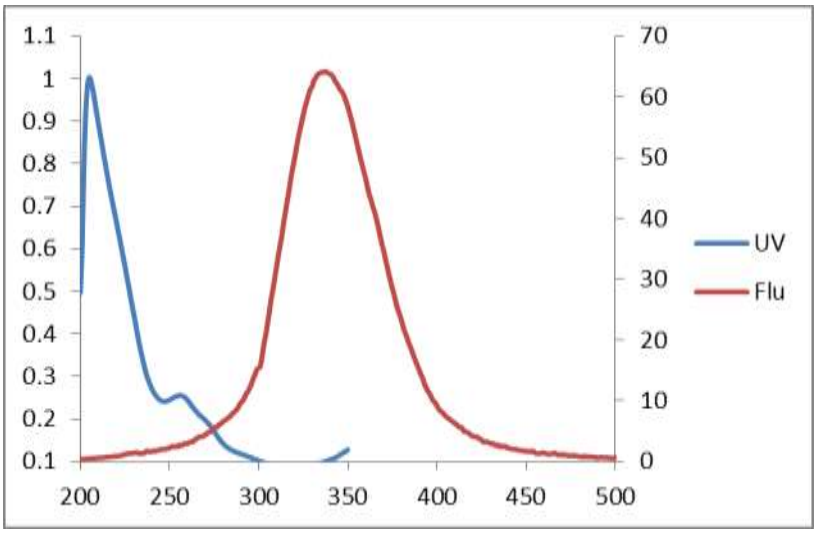

Figure 1: The overlap of UV absorption of Quercetin (solid line) with the fluorescence Emission Spectrum of EA (Dotted line) with SPAN 40

Table 1: Efficiency transfer energy (E), Critical energy transfer distance (R_o) of EA with Quercetin

Quencher	Energy (E)	R_o Å	J (cm^3M^{-1})	r(Å)
Quercetin	0.4566	38.94	8.32 X 10^{-40}	40.09

CONCLUSION

Flavonoids of plants are natural products that exhibit a various biological and pharmaceutical properties. In view of this broad incidence in nature are considerably low toxicities, prospective development and use of these compounds as efficient Pharmaceutical agents particularly as anticancer drugs is a matter of significant current interest. The result indicates that the energy transfer from EA to quercetin occurs with high probability, for r is in the slope of 2-8, it implies that the energy transfer from EA to drug with high possibilities.

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