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Review on Spectroscopic Studies of the Binding Interactions between Serum Albumins and Quercetin

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Abstract

Quercetin is known as a prevalent bioactive flavonoid derived from plants. It is recognised for its anti-tumour and anti-cancer properties, along with other important therapeutic effects. The interaction between serum albumins and quercetin was explored, and association constants were calculated by monitoring the absorption spectra of quercetin. The effect on the emission intensities of serum albumins with the addition of quercetin was examined. Time-resolved emission studies of serum albumins with the addition of quercetin indicate a reduction in average lifetime, which suggests quercetin may bind with tryptophan and the potential for energy transfer from tryptophan to quercetin in the excited state.

Keywords: *Energy Transfer; Serum Albumin; Tryptophan; Quercetin*

Introduction

Plants of higher genera contain flavonoids, a significant class of naturally occurring bioactive polyphenolics (Wu *et al.,* 2023). Flavonoids have recently attracted a lot of attention due to two main factors: Their biological and pertinent therapeutic uses, such as against cancer, tumours, and AIDS. Allergies, inflammation, etc., are the first intriguing features (Scalia *&* Mezzena, 2009). Numerous studies have examined flavonoids' antioxidative properties. Quercetin (3, 3′, 4′, 5, 7- Pentahydroxy flavone), one of the most prevalent naturally occurring flavonoids, has been shown to suppress the actions of DNA topoisomerases, phosphorylase kinase, tyrosine, and phosphatidylinositol 3-kinase (Russo *et al.*, 2014; Das, Majumder & Saha, 2017). Unusual fluorescence emission properties of quercetin, along with their dual emission nature, broad Stokes shifts, and reactiveness of different emission parameters towards the surrounding medium (like pH, temperature, hydrogen bond, polarity etc.), constitute their many intriguing features (Waychunas 2014; Ding, Peng & Peng, 2016; Munoz *et al*., 2016).

Often referred to as transport proteins, serum albumins are widely distributed in blood plasma (Steinhardt, Krijn & Leidy, 1971). They serve as carriers for a variety of endogenous and foreign substances and are repeatedly circulated throughout the body. HSA (Human serum albumin) and BSA (Bovine serum albumin) are the two most often researched albumins. Very strong conformational flexibility to a wide range of ligands is possessed by both HAS and BSA (Siddiqui *et al*., 2021). In ligand protein binding studies and the in vivo effects of drug and other metabolite binding to serum albumins, three primary methods have been used: absorption, fluorescence, and time-resolved emission studies (Kumar &

Buranaprapuk, 1999; Abaskharon & Gai, 2016; Mishra *et al*., 2005). These investigations have led to the molecular-level reporting of data on the binding mechanism of numerous exogenous ligands, including metals, amino acids, bilirubin, long-chain fatty acids etc (Bertozo *et al*., 2018; Chen *et al*., 2007). Moreover, it was suggested that this type of binding can make ligands more soluble and that certain ligands become less toxic when they connect to albumins (Sengupta & Sengupta, 2002).

Low tryptophan and high cystine levels are typical characteristics of albumins. Tryptophan content is the primary difference between HSA and BSA. Human serum albumin has one tryptophan group at position 214, whereas bovine serum albumin has two tryptophan groups at sites 134 and 212 (Steinhardt, Krijn & Leidy, 1971; Siddiqui *et al*., 2021). In this review, the binding constants for quercetin associated with HSA and BSA have been investigated utilising the energy transfer and fluorescence quenching experiments and determining the most likely location for quercetin binding with proteins.

Methodology

Steady State Absorption and Emission Studies

The steady-state absorption and emission spectra of free serum albumins, with varying concentrations of quercetin added, have been analysed (Rehman *et a*l., 2015). Using tryptophan as a reference, the quantum yields for both the HSA and BSA were calculated (Ameen *et al*., 2020). All protein solutions were excited at the wavelength 290 nm while keeping the absorbance nearly constant, and the steady-state emission spectra were drawn from the wavelength 300 nm to 500 nm.

Time Resolved Emission Studies

Fluorescence lifetime measurements have been conducted for both the albumins with and without the addition of quercetin (Berezin *et al*., 2008). The time-resolved emission decays were found to be multi-exponential, assessed using reduced χ^2 values and randomly distributed residuals among the various data channels.

Results and Discussion

There was a noticeable red shift in the quercetin absorption spectra when either HSA or BSA was present (Zsila, Bikádi & Simonyi, 2003). The spectroscopic changes suggest the forming of a complex between quercetin and albumins according to the given equation (Equation 1).

$$n\ \text{quercetin} + \text{SA} \rightleftharpoons \text{complex}. \quad (1)$$

The binding constant (K) is calculated using Equation 2.

$$K = \frac{[\text{complex}]}{[\text{SA}][\text{quercetin}]^n}. \quad (2)$$

Here, [SA] is the concentration of serum albumin, and n represents binding sites in the complex of quercetin and albumins. The changes in absorbance value (ΔA) were monitored at various quercetin strengths and plotted using the Scatchard Equation 3 at the wavelength where the absorbance of unbound quercetin and albumins is at its lowest and the absorbance changes the most upon binding. As a result, ΔA for HSA and BSA were measured at 425 nm, and 406 nm respectively.

$$\frac{1}{\Delta A} = \frac{1}{n\Delta\varepsilon[\mathrm{SA}]} + \frac{1}{n\Delta\varepsilon K[\mathrm{SA}][\mathrm{quercetin}]}. \quad (3)$$

Here, [SA] is the concentration of serum albumins, and Δε represents the molar extinction coefficient for quercetin forming complexes with HSA and BSA at 425nm and 406nm respectively.

Accordingly, the binding sites (n) and binding constant (K) for quercetin's binding to HSA were found to be 3.1 x 10^4 M^{-1}, and 1.1 respectively. Similar experiments were carried out for BSA also and the binding constant (K) was found to be 3.5 x 10^4 M^{-1} and n was 1.0. The extinction coefficients were obtained as 1.6 x 10^4 $M^{-1}cm^{-1}$ and 1.01 x 10^4 $M^{-1}cm^{-1}$ for quercetin bound HSA, and BSA at 406 nm respectively (Mishra *et al*., 2005). These values were utilised in all subsequent computations.

The fluorescence quantum yields were calculated to be 0.053 and 0.101 for HSA and BSA, respectively. As BSA has two tryptophan groups, its fluorescence quantum yield is almost doubled. The protein fluorescence was decreased in the presence of quercetin and the binding constant (K) was estimated using this fluorescence quenching. Solutions containing 2.5 to 20 mM quercetin and 30 mM serum albumin at pH 7.5 were stimulated at the wavelength 290 nm for these studies, and emission spectra were recorded in the range of 300-500 nm. It was predicted that a portion of light is directly absorbed by quercetin at concentrations above 20 mM because it has own absorption at around 290 nm. As a result, the excited states of the albumins were less populated, which decreases the fluorescence intensity of proteins. Therefore, measurement of binding constants did not use the data above 20 mM. The fluorescence measurement of Human serum albumin (HSA) with varying strengths of quercetin was carried out. The emission intensity of HSA at around 345 nm lowers when quercetin is added. Monitoring this quenching of fluorescence intensity at 345 nm, binding sites (n) and binding constant (K) were calculated using the following Equation 4 (Mohammadi *et al*., 2009).

$$\log\left(\frac{F_o - F}{F}\right) = \log K + n\log[\mathrm{quercetin}] \quad (4)$$

Here, F_0 and F are the fluorescence intensities of albumin, without and with the presence of varying strengths of quercetin, respectively. When log (Fo–F)/F vs. log(quercetin) was plotted, it was found to be linear in nature (He *et al*., 2008). Based on this, K and n were calculated for both the proteins using equation 4. For HSA the K value was 2.3 x 10^4 M^{-1}, and n was 1.10, whereas in the case of BSA the results changed to 4.9 x 10^4 M^{-1} and 1.19, respectively. According to this observation, quercetin attaches in a single location to both the HSA and BSA. Although they differ significantly from those for BSA, the binding constant

found above using both the absorption and fluorescence techniques matches closely for HSA.

This is because BSA contains two tryptophan moieties. When quercetin is attached to BSA, it quenches one of the two tryptophan's emission, and the other one remains undisturbed. This results in mistakes when estimating the binding constant for BSA using the fluorescence approach. Such errors are not anticipated in the case of HSA because there is only one tryptophan molecule present, and the fluorescence quenching is indicative of binding close to tryptophan alone. Further research was carried out on the potential for excited state energy transfer from tryptophan to quercetin (Sengupta & Sengupta, 2002).

Because the fluorescence spectra of tryptophan of human serum albumin significantly overlaps with absorption spectra of quercetin, the reduction in tryptophan emission might be attributed to either the inner filter effect or the static quenching of tryptophan's emission by quercetin. The possible energy transfer precisely from the tryptophan moiety to quercetin in the excited state could not be ruled out (dynamic quenching) (Siddiqui *et al*., 2019).

Time resolved fluorescence studies were performed to confirm this observation. Both HSA and BSA were subjected to time resolved fluorescence lifetime studies with and without quercetin. 30 mM protein solutions were prepared for this experiment. All the solutions were stimulated at the wavelength of 290 nm, and the emission intensities were measured at 345 nm. Time resolved emission decays for both the HSA and BSA were found to fit multi exponential function. HSA exhibits a biexponential fit with an average lifetime (τ_0) of 4.76 ns [6·33 ns (61.0%), and 2·34 ns (39.0%)], while BSA shows triple exponential decay with τ_0 value 5.95 ns [7·21 ns (65.0%), 3·84 ns (32·5%), and 0·13 ns (2·5%)].

The fluorescence lifetime (τ_0) for both the albumins was decreased after the addition of 30 mM quercetin. The average lifetime of HSA decreases to 3.745 ns [5.87 ns (44.6%), and 2.02 ns (55.4%)] after addition of quercetin (Mishra *et al*., 2005). It is evident that both the components decrease, and the decay stays biexponential. These results indicate that quercetin binds closely to the tryptophan and the potential for excited state energy transfer between tryptophan and quercetin.

Conclusion

The binding sites (n) and binding constant (K) for the interaction between quercetin and both the proteins were ascertained by means of absorption and fluorescence spectroscopy. For both the systems, single binding sites and binding constants in the order of 10^4 M^{-1} were determined. The elevated K values indicate that quercetin ties up with bioactive sites of proteins. For HSA, there is a good agreement between the results obtained from the absorption and fluorescence studies, but for BSA, as two tryptophan are there, the values diverge by an order of magnitude. In the presence of quercetin, the average lifetimes (τ_0) decrease, and the protein fluorescence exhibits multi exponential decay, according to the time resolved fluorescence lifetime experiments. Based on these findings, it is proposed that the single tryptophan is the most likely location for quercetin to bind in HSA.

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References

Abaskharon, R. M., & Gai, F. (2016). Direct measurement of the tryptophan-mediated photocleavage kinetics of a protein disulfide bond. *Physical Chemistry Chemical Physics, 18*(14), 9602-9607. https://doi.org/10.1039/C6CP00865H

Ameen, F., Siddiqui, S., Jahan, I., Nayeem, S. M., ur Rehman, S., & Tabish, M. (2020). A detailed insight into the interaction of memantine with bovine serum albumin: A spectroscopic and computational approach. *Journal of Molecular Liquids, 303*, 112671. https://doi.org/10.1016/j.molliq.2020.112671

Berezin, M. Y., Lee, H., Akers, W., Nikiforovich, G., & Achilefu, S. (2008, February). Fluorescent lifetime of near infrared dyes for structural analysis of serum albumin. In *Molecular Probes for Biomedical Applications II* (Vol. 6867, pp. 257-267). SPIE. https://doi.org/10.1117/12.769262

Bertozo, L. D. C., Tavares Neto, E., Oliveira, L. C. D., & Ximenes, V. F. (2018). Oxidative alteration of Trp-214 and Lys-199 in human serum albumin increases binding affinity with phenylbutazone: A combined experimental and computational investigation. *International Journal of Molecular Sciences, 19*(10), 2868. https://doi.org/10.3390/ijms19102868

Chen, J., Song, G., He, Y., & Yan, Q. (2007). Spectroscopic analysis of the interaction between bilirubin and bovine serum albumin. *Microchimica Acta, 159*, 79-85. https://doi.org/10.1007/s00604-007-0736-9

Das, A., Majumder, D., & Saha, C. (2017). Correlation of binding efficacies of DNA to flavonoids and their induced cellular damage. *Journal of Photochemistry and Photobiology B: Biology, 170*, 256-262. https://doi.org/10.1016/j.jphotobiol.2017.04.019

Ding, F., Peng, W., & Peng, Y. K. (2016). Biophysical exploration of protein–flavonol recognition: effects of molecular properties and conformational flexibility. *Physical Chemistry Chemical Physics, 18*(17), 11959-11971. https://doi.org/10.1039/C5CP07754K

He, Y., Wang, Y., Tang, L., Liu, H., Chen, W., Zheng, Z., & Zou, G. (2008). Binding of puerarin to human serum albumin: a spectroscopic analysis and molecular docking. *Journal of Fluorescence, 18*(2), 433-442. https://doi.org/10.1007/s10895-007-0283-0

Kumar, C. V., & Buranaprapuk, A. (1999). Tuning the selectivity of protein photocleavage: spectroscopic and photochemical studies. *Journal of the American Chemical Society, 121*(17), 4262-4270. https://pubs.acs.org/doi/abs/10.1021/ja9844377\

Mishra, B., Barik, A., Priyadarsini, K. I., & Mohan, H. (2005). Fluorescence spectroscopic studies on binding of a flavonoid antioxidant quercetin to serum albumins. *Journal of Chemical Sciences, 117*, 641-647. https://doi.org/10.1007/BF02708293

Mohammadi, F., Bordbar, A. K., Divsalar, A., Mohammadi, K., & Saboury, A. A. (2009). Analysis of binding interaction of curcumin and diacetylcurcumin with human and bovine serum albumin using fluorescence and circular dichroism spectroscopy. *The Protein Journal, 28*, 189-196. https://doi.org/10.1007/s10930-009-9184-1

Munoz, V. A., Ferrari, G. V., Sancho, M. I., & Montaña, M. P. (2016). Spectroscopic and thermodynamic study of chrysin and quercetin complexes with Cu (II). *Journal of Chemical & Engineering Data, 61*(2), 987-995. https://doi.org/10.1021/acs.jced.5b00837

Rehman, S. U., Sarwar, T., Husain, M. A., Ishqi, H. M., & Tabish, M. (2015). Studying non-covalent drug–DNA interactions. *Archives of Biochemistry and Biophysics, 576*, 49-60. https://doi.org/10.1016/j.abb.2015.03.024

Russo, G. L., Russo, M., Spagnuolo, C., Tedesco, I., Bilotto, S., Iannitti, R., & Palumbo, R. (2014). Quercetin: a pleiotropic kinase inhibitor against cancer. *Advances in Nutrition and Cancer*, 185-205. https://doi.org/10.1007/978-3-642-38007-5_11

Scalia, S., & Mezzena, M. (2009). Incorporation of quercetin in lipid microparticles: Effect on photo- and chemical-stability. *Journal of Pharmaceutical and Biomedical Analysis, 49*(1), 90-94. https://doi.org/10.1016/j.jpba.2008.10.011

Sengupta, B., & Sengupta, P. K. (2002). The interaction of quercetin with human serum albumin: a fluorescence spectroscopic study. *Biochemical and Biophysical Research Communications, 299*(3), 400-403. https://doi.org/10.1016/S0006-291X(02)02667-0

Siddiqui, S., Ameen, F., Jahan, I., Nayeem, S. M., & Tabish, M. (2019). A comprehensive spectroscopic and computational investigation on the binding of the anti-asthmatic drug triamcinolone with serum albumin. *New Journal of Chemistry, 43*(10), 4137-4151. https://doi.org/10.1039/C8NJ05486J

Siddiqui, S., Ameen, F., ur Rehman, S., Sarwar, T., & Tabish, M. (2021). Studying the interaction of drug/ligand with serum albumin. *Journal of Molecular Liquids, 336*, 116200. https://doi.org/10.1016/j.molliq.2021.116200

Steinhardt, J., Krijn, J., & Leidy, J. G. (1971). Differences between bovine and human serum albumins. Binding isotherms, optical rotatory dispersion, viscosity, hydrogen ion titration, and fluorescence effects. *Biochemistry, 10*(22), 4005-4015. https://pubs.acs.org/doi/pdf/10.1021/bi00798a001

Waychunas, G. A. (2014). Luminescence spectroscopy. *Reviews in Mineralogy and Geochemistry, 78*(1), 175-217. https://doi.org/10.2138/rmg.2014.78.5

Wu, J., Lv, S., Zhao, L., Gao, T., Yu, C., Hu, J., & Ma, F. (2023). Advances in the study of the function and mechanism of the action of flavonoids in plants under environmental stresses. *Planta, 257*(6), 108. https://doi.org/10.1007/s00425-023-04136-w

Zsila, F., Bikádi, Z., & Simonyi, M. (2003). Probing the binding of the flavonoid, quercetin to human serum albumin by circular dichroism, electronic absorption spectroscopy and molecular modelling methods. *Biochemical Pharmacology, 65*(3), 447-456. https://doi.org/10.1016/S0006-2952(02)01521-6