Influence of Curcumin in Human Serum Albumin at different Temperatures

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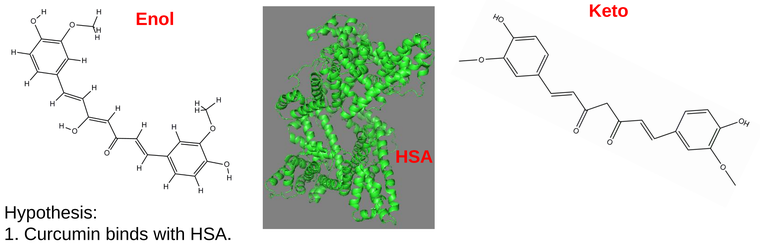
Abstract

Curcumin (diferuloylmethane), a polyphenol found in the rhizomes of the plant *Curcuma longa,* has been in the prominence due to its diverse pharmacological activities. Daily consumption of curcumin boosts immune power which is critical to fight against different microbial diseases including Covid-19 and other emerging diseases. Here, we report for the first time a study on the interactions of curcumin with the plasma protein human serum albumin (HSA), exploiting the intrinsic fluorescence emission properties of curcumin as a probe, along with the intrinsic chromophore tryptophan (Trp) of HSA. Moreover, far UV circular dicroism (CD) spectroscopy, combined with molecular modeling computations were performed to study the influence of curcumin on the protein secondary structure and the binding sites respectively. Specific interaction of the curcuminoid with HSA is confirmed from drastic increase and blue shift in curcumin fluorescence with increasing [HSA]. The emission spectra of Trp suggests occurrence of efficient Förster type resonance energy transfer (FRET) from the single tryptophan-214 residue of HSA to the curcumin. With increase in temperature, further blue shift and decrease in emission were observed. Both temperature dependent fluorescence measurements and molecular docking studies reveal that hydrogen bonding, van der Waals interactions, and electrostatic interactions play crucial role in curcumin-HSA interactions.

Introduction

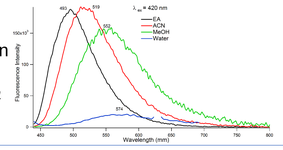
Curcumin exists in two tautomeric forms in solution:



Hypothesis:
1. Curcumin binds with HSA.
2. Curcumin helps to stabilize the structure of HSA.
3. Curcumin helps to prevent damage to HSA upon UV exposure.

Three Potential Channels of gaining Information on Protein-Curcumin Interaction

Curcumin is a polyphenol found in the rhizome of turmeric (*Curcuma Longa).*

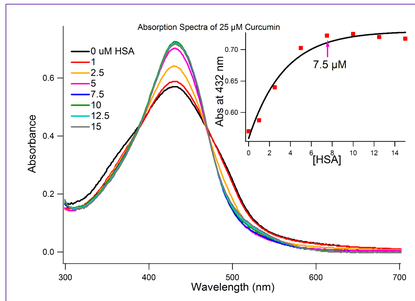


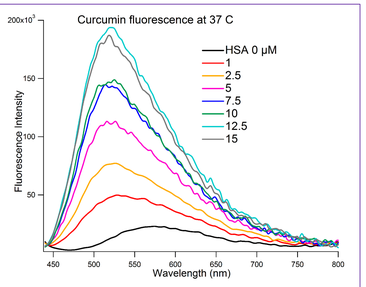
Fluorescence spectra of curcumin in solvents of varied polarity shows the sensitivity of its emission profiles.

Methods and Materials

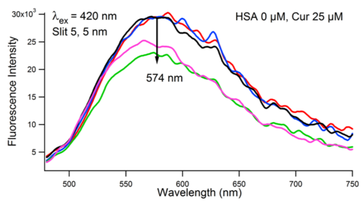
Aliquots of concentrated ethanolic solution of curcumin were added to 1 ml water to make the final concentration of 25 µM. The final concentrations of ethanol were kept <1% (by volume) in all samples. Stock solution of the protein (made in water) was titrated (0⟶15 µM) gradually in the curcumin solution and spectra was collected for each titrant at temperatures 20, 25, 30, 37, and 42 °C. Steady state absorption spectra were recorded with Shimadzu UV2550 spectrophotometer. Steady state fluorescence measurements were carried out with a Perkin Elmer 6500 (equipped with a temperature controlled accessory) spectrofluorometer. Quartz cuvettes of 0.4 cm path length were used for all experiments. Circular dichroism (CD) spectra were recorded on a Jasco J-815 spectropolarimeter. AutoDock4 was employed to dock curcumin with the receptor (HSA).

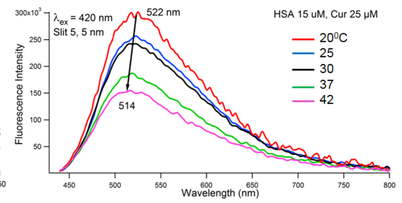
UV/Vis Absorption and Fluorescence Results





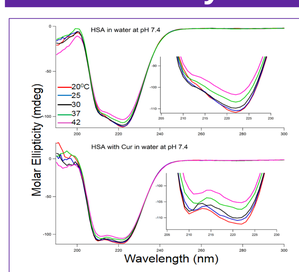
Observation
1. Increase in the absorbance of curcumin with the increase in [HSA].
2. No significant change in the absorption wavelength at 431 nm with [HSA].
3. The increase in absorption gradually slows down around [HSA] 10 µM, after which it saturates.
4. Around 8X Increase in the fluorescence of curcumin with the increase in [HSA].
5. Blue shift in λ_{em}^{max} from 574 nm in water to 514 nm in HSA 15 µM at 37°C.

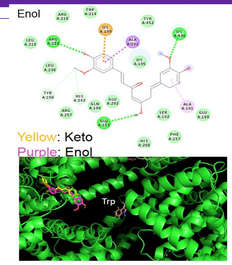


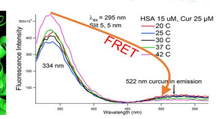


Observation
1. Around 10X Increase in the fluorescence of curcumin between that in water and with HSA.
2. Significant blue shift in the λ_{em}^{max} of curcumin with increase in temperature at 15 µM HSA..
3. Decrease in Curcumin fluorescence intensity with increase in temperature which is more in presence of protein.

Secondary Structure of HSA with Curcumin







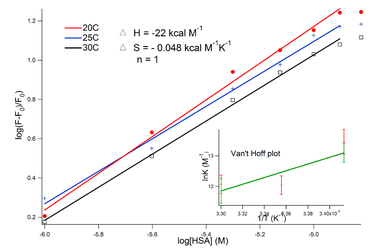
Observation
1. The α–helical structural changes were observed. With increase in temp, a 7.37% decrease at 222 nm, and a 2.63% decrease at 211 nm was observed.
2. In presence of curcumin, a 6% decrease at 222 nm, and a 5.16% decrease at 211 nm was observed.
3. The enol tautomeric form of curcumin makes 3 H-bonds with HSA compared to the keto tautomer which makes 2 H bonds.
4. Both form binds in the vicinity of Trp 214 which agrees with the FRET.

Thermodynamic Parameters

$Curcumin + nHSA \rightarrow Cur - HSA$ $\quad \log\left(\frac{F-F_0}{F_0}\right) = logK_b + n\log[\text{HSA}]$

$\frac{F-F_0}{F_0} \propto \frac{Cur-HSA}{Cur}$ F_0 and F denote the fluorescence intensity of curcumin in absence and presence of HSA, K_b is the binding constant and n is the number of binding site(s).

Binding Constant (K_b) and ΔG for Curcumin and HSA		
Temperature (°C)	K_b (x10^5 M^{-1})	ΔG *(kcal M^{-1})*
20	7.01	-7.89
25	1.7	-7.06
30	1.75	-7.07



The thermodynamic parameters are evaluated using the equations:

$\ln K_b = -\Delta H^0 / RT + \Delta S^0 / R,$ $\quad \Delta G^0 = -RT \ln K_b$

ΔG, ΔH and ΔS denote the change in Gibbs free energy, enthalpy and entropy upon curcumin binding with HSA

Conclusions

- Drastic changes in fluorescence of curcumin were observed at different temperatures.
- Curcumin influences the folding of the protein HSA to some extent.
- Computational studies indicate that the binding site of curcumin is close to Trp 214 which corroborates with our observation of energy transfer from Trp to curcumin.
- The high *–ve* ΔH and *-ve* ΔS suggest that the binding is enthalpy driven.
- The spontaneity of the binding is evident by the *–ve* ΔG.

FUTURE STUDIES

1. Compare the behavior of curcumin in human serum albumin with bovine serum albumin.
2. Compare curcumin with another flavone kaempferol in both proteins.
3. Investigate the role of curcumin in HSA under oxidative stress.
4. Study the folding of neuronal proteins in the presence of curcumin.

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