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Binding and Stability of Curcumin in Presence of Bovine Serum Albumin

SANKAR P. MITRA*

Integrated Pharmaceuticals Inc. 310 Authority Drive. Fitchberg, MA 01420, USA.

Abstract -Using equilibrium dialysis and visible absorption spectroscopy, the interaction between polyphenolic antioxidant, curcumin [1,7-bis (4-hydroxy-3-methoxy phenyl)-1, 6 heptadiene-3, 5-dione; diferuloylmethane] and Bovine Serum Albumin (BSA) was studied in detail. Curcumin bound to the protein with considerably high affinity ($K_a \sim 6.3 \times 10^6 \text{ M} / \text{L}$) and moderate capacity ($n \sim 0.30 \text{ Mole} / \text{Mole of BSA}$). Analysis by Hill plot indicated lack of cooperativity (Hill Coefficient, $h \sim 1.0$) in the overall binding process. The binding complex formed offered significant protection to the dye molecule from deterioration either by alkaline pH or UV irradiation. The binding was seen to be pH sensitive (n at $\text{pH } 7.0 > \text{pH } 6.0$) without significantly altering the affinity. (K_a) The temperature sensitivity (n at $37^\circ\text{C} > 22^\circ\text{C} > 10^\circ\text{C}$) and the standard free energy (ΔG°) calculation by van't Hoff's equation supported a prominent role of hydrophobicity besides proven electrostatic interaction in the binding process. The interaction with BSA induced red shift to the curcumin spectra ($\lambda_{\text{max}} = 440 \text{ nm}$) with simultaneous increase (~ 3 fold) in optical density, which was also noticeable in case of Do-decyl amine (DDA). As for further interest, the binding to BSA considerably increased the curcumin solubility in aqueous buffer media, e.g., the presence of 0.2% BSA at pH 7.4 raised the solubility level to ~ 2.5 fold in comparison to curcumin alone in aqueous medium. The increment in solubility in the presence of BSA was pH dependent and larger with increasing pH. At alkaline pH (~ 10.5) the curcumin solubility in presence of 0.2 % BSA was increased ~ 10 fold but the dye molecule lost the characteristic absorption maxima (λ_{max}) at visible region. Additionally, the presence of BSA protects the dye molecule from alkaline damage as shown by HPLC analysis. The dye alone underwent a red shift at pH over 7.0. In the presence of BSA the red shift was larger and almost 10 fold at pH near 9.0-10.0. Apart from high pH, the UV irradiation ($\lambda = 365-240 \text{ nm}$) for different length of time (1 to 4 hrs) reduced only ~ 20 % of the optical density in presence of BSA. All these events logically propose that BSA offered protection to the curcumin through binding, thereby preventing the damage of the dye molecule.

Keywords : Curcumin, BSA, Binding, UV stability, Spectral Shift and Solubility.

*E-mail:smitra@intepharm.com