Chemistry Research Journal, 2017, 2(2):84-89

Available online www.chemrj.org

Research Article

ISSN: 2455-8990
CODEN(USA): CRJHA5

An Insight on Cooperative Binding of Food Coloring Allura Red AC with Bio Macromolecule

Nasrin Sohrabi*, Nahid Rasouli, Zahra Arabpour

Department of Chemistry, Payame Noor University (PNU), 19395- 3697, Tehran, I.R. Iran

Abstract In this study, interaction of a food colouring such as Allura Red AC with Bovine Serum Albumin (BSA) biomacromolecule in 5 mM phosphate buffer, pH=7 was studied by absorption spectroscopy, competitive binding and thermodynamic study. The binding isotherm, binding capacity and Scat chard plots were plotted at various temperatures. The binding constant obtained from the absorption titration data gives a binding constant of 3.38 M^{-1} at 25° C. The thermodynamic parameters such as binding Gibbs free energy, enthalpy and entropy changes was calculated. The positive values of ΔH and ΔS indicated that the process is entropy-driven and suggest that the main driving forces are hydrophobic interaction. Also, the negative ΔG values for the interaction of Allura Red AC with the Bovine Serum Albumin indicate the spontaneity of the interaction.

Keywords binding analysis; ligand binding; food coloring; bovine serum albumin

1. Introduction

Recently, small molecule–biomacromolecule interactions are focused increasingly for many biochemists and thousands of new findings are reported every year. Understanding the interaction between organic ligand and biomacromolecule is always helpful for us to recognize the structure, function and activity of macromolecule as well as the toxicity of an organic toxicant, e.g. protein–protein [1], protein–DNA [2], protein–glycosaminoglycan [3] and protein–ligand [4] interactions. Though people have clarified the structure, conformation, activity and function of many proteins, the protein interaction with organic substance as well as conformational changes of protein is still in the extensive research so as to be realized the structure-activity relationships and particular biological roles of biomacromolecule. The structural stability keeps individual function and relative stability of species. Organic substance–protein interactions modulate the structure of protein and thus affect its biological functions. So, structural transformation that occurs in the process of protein folding and functioning is of great significance in biological organisms [5]. In the formation of complex, small organic compound may insert into the protein inner to regulate their structures and functions [6] through non-covalent union, e.g. hydrophobic bond, van der Waals force, dipole effect and hydrogen bond. The non-covalent binding is often weak and non-specific [7].In the present work, we undertook in an attempt to clarify the general principle involved in the protein–organic substance interaction, characterization of the formed complex and effect of organic substance on protein conformation. Serum albumins are the most extensively studied and applied proteins because of their availability, low cost, stability and unusual ligand binding properties. For this reason, a huge number of papers dealing with albumins have been reviewed so far [8]. Albumin is the most abundant protein in blood plasma and serves as a depot protein and transport protein for numerous endogenous and exogenous compounds. The aim of the research was to study binding of Allura Red AC with protein so as to provide a theoretical basis and methodology for estimation of target toxicity of an organic

toxicant. Allura Red AC (Fig. 1) belongs to the monoazo class of food colorants and chemically it is disodium 6-hydroxy-5-(2 meth- oxy-5-methyl-4-sulphophenylazo)-2-naphthalenesulphonate.AlluraRed AC is a highly used synthetic azo dye which is used in many food products such as soft drinks, juices, gelatins, puddings, snacks, dairy products, confections, decoration, coatings, condiments, medications, and cosmetics.

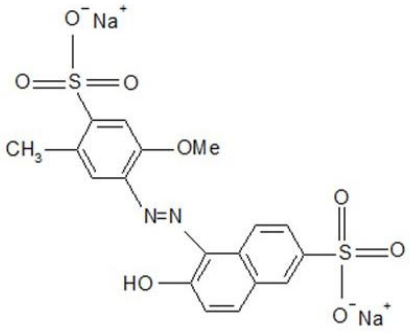

Figure 1: Chemical structure of Allura Red AC

In the present work we undertook in an attempt to clarify the general principle involved in the protein–organic substance interaction, characterization of the complex and effect of organic substance on protein conformation. The aim of this study is to determine the binding constant, thermodynamic parameters and the mechanism and the kind of binding by analyzing absorption data using a simple binding model.

2. Experimental

2.1. Materials and Instruments

The chemical compounds were purchased from Sigma Chemical Company and was used without further purification. All experiments were performed in 5 mM phosphate buffer solution at pH 7.0 at 25 °C. The absorbance monitoring was performed on a Perkin-Elmer UV/Vis Lambada 2 equipped with thermostat cell compartment and lambda 2 software. Using differential UV/Vis absorption spectroscopy method the UV/Vis titration experiments were made by addition of Allura Red AC solution in to a 1.4mL curette containing the BSA solution of appropriate concentration with zero absorbance .The titration experiments were performed at various temperature with precision of ±1˚C.

2.2. Binding Analysis of interaction of BSA with Allura Red AC

The total absorbance of Allura Red AC- BSA complex at $\lambda_{max=}$ 505 nm is Eq. (1):

$$A_t^{505} = \varepsilon_b^{505} b[\text{Allura}]_b + \varepsilon_f^{505} b[\text{Allura}]_f \quad (1)$$

And the total concentration of Allura Red AC is:

$$[\text{Allura}]_t = [\text{Allura}]_b + [\text{Allura}]_f \quad (2)$$

Where $[\text{Allura}]_t$, $[\text{Allura}]_f$ and $[\text{Allura}]_b$ are total, free and binding complex concentrations and andε_b, ε_f are molar absorption coefficients for the free and bound forms of the Allura Red AC respectively. Then by dividing Eq. (1) to total concentration of $[BSA]_t$, we obtain:

$$\frac{A^{505}}{[\text{BSA}]_t} = \varepsilon_b \frac{[\text{Allur a}]_t}{[\text{BSA}]_t} + (\varepsilon_b - \varepsilon_f) \frac{[\text{Allura}]_b}{[\text{BSA}]_t} \quad (3)$$

Using Eq. 3, the bound and free molar absorbance coefficients, ε_b and ε_f respectively were calculated. Substituting in Eq.1 $[\text{Allura}]_f$ and $[\text{Allura}]_b$ are calculated. The parameter of ν is binding ligand concentration to total macromolecule concentration ($[\text{Allura}]_b/[\text{BSA}]_t$), then the binding isotherm or ν against ln $[\text{Allura}]_f$ was plotted in Fig. 4. We use scat chard plots Eq. 4 to calculate binding capacity, n_H and binding constant K_a that it show in Fig. 3. The results show that scat chard plots have positive slop (n_H>0) then the results show that the Allura-BSA has presents positive cooperatively and it is nonlinear therefore by using hill Eq.(5),the binding constant at each temperature was determined (Table. 1 & Fig.6) [10].

3. Results and Discussion

3.1. Effect of Concentration

In order to identify the solution properties of AlluraRed AC, we employed UV-Vis spectroscopy. The optical absorption spectrum of AlluraRed AC shows two bands in 314, 505 nm. In all calculations due to lack of absorption at a wavelength of 504 nm interferes with the absorption of this wavelength as appropriate wavelength of BSA were studied.

3.2. Effect of Temperature

The effect of temperature on the UV-Vis spectrum of AlluraRed AC (2.7×10^{-5} M) in phosphate buffer is shown in Fig.3. As shown in this figure, the wavelength of maximum absorption λ_{max} does not show considerable changes and no new band appears. The binding isotherm, binding capacity and scat chard plots Eq. (4) & (5) were plotted. The results show that the BSA has one binding site set and presents positive cooperatives in Fig. 2. The results show that scat chard plots [8] are nonlinear, therefore by using Hill plot due to Eq. (5)[9]:

$$\frac{\nu}{[L]} = K_a(n-\nu) \qquad (4)$$

$$\log(\tfrac{\nu}{n-\nu}) = \log k_a + n_H \log[L] \qquad (5)$$

Where n is the number of sites and n_H is Hill coefficient related to cooperatively. By increasing temperature, K_a is increased because of an endothermic interaction between BSA and AlluraRed AC, and n_H is decreased because of protein saturation.

3.3. Investigation of thermodynamic interaction of BSA with AlluraRed AC

The thermodynamics of the interaction of BSA with AlluraRed AC was investigated in terms of the difference in$K_a$values determined at various temperatures, and the results are listed in Table 1.

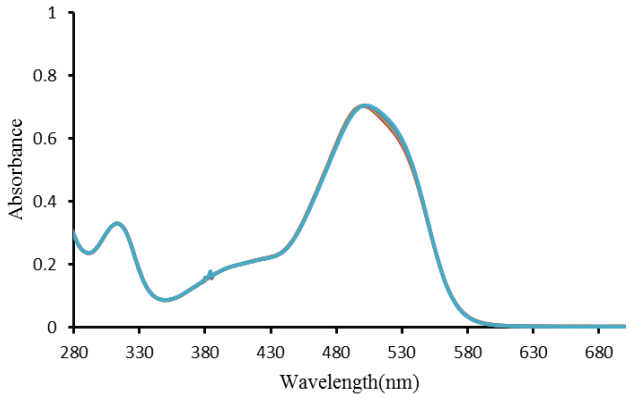

Figure 3: The effect of temperature change on the UV- Vis spectrum of AlluraRed AC solution 2.7×10^{-5}M in 5 mM phosphate buffer at temperatures 25-45 °C.

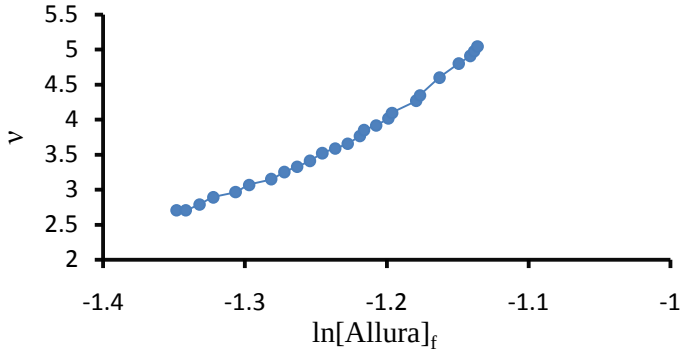

Figure 4: The binding isotherms of interaction Allura Red AC with BSA in 5 mM phosphate buffer solution, pH=7 at λ_{max}= 505 nm, t=25° C.

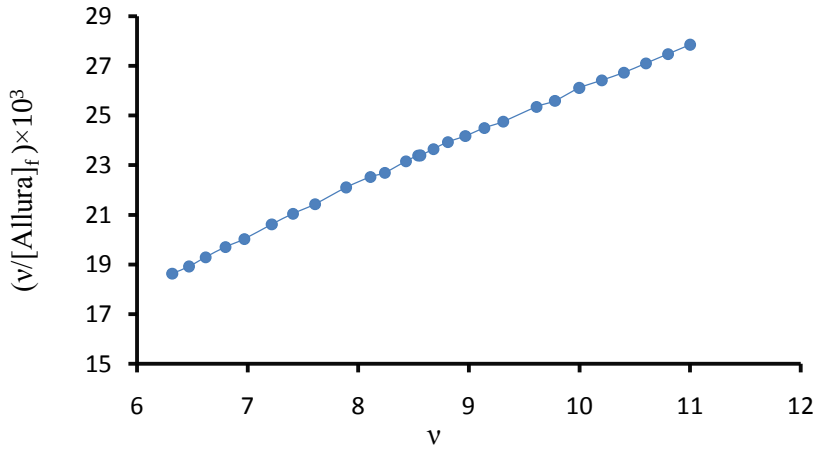

Figure 5: The Scat chard plots of interaction of Allura Red AC with BSA in 5 mM phosphate buffer solution, pH=7 at λ_{max}=505 nm, t=25 °C.

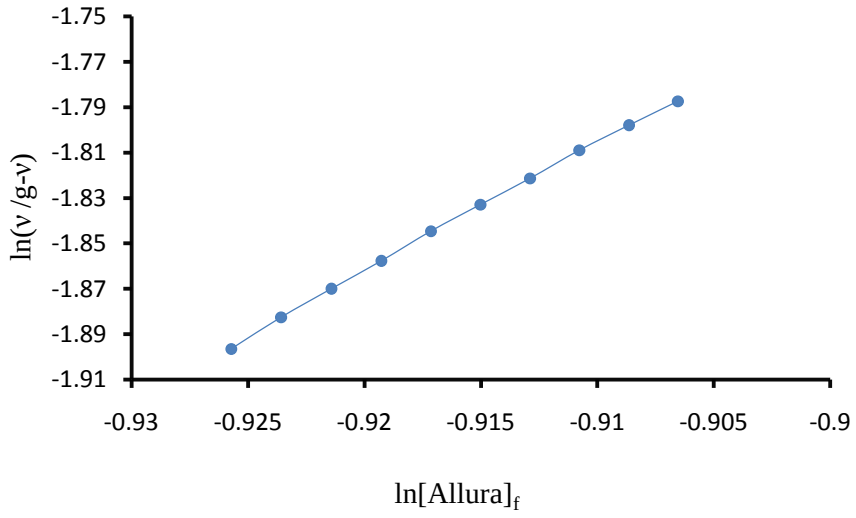

Figure 6: The Hill plot for interaction of AlluraRed AC with BSA in 5 mM phosphate buffer solution, pH=7 at λ_{max}=505 nm, t=25° C.

Table 1: The binding constants K_H, n_H for interaction of Allura Red AC with BSA at 5 mM phosphate buffer, pH=7at various temperatures.

K_H	g	n_H	T(K)
1.22	6.46	5.60	298
1.08	6.25	1.23	303
2.40	1.08	1.01	308
2.80	6.15	1.13	313
3.40	4.84	2.80	318

The binding and stage Gibbs free energy change of AlluraRed AC-BSA complex is usually calculated by the following Eq. (9) [11,12] as shown in Fig. 6, [L] is the free ligand concentration (Eq. 9).

$$\Delta G^{\circ}_{b,\nu} = -RTn_H \ln K_a + RT(1-n_H)\ln[L] \quad (9)$$

By analyzing the results of UV-Vis spectroscopy, the determined thermodynamic parameters ($\Delta H^o > 0$ and $\Delta S^o > 0$) showed that the interaction between BSA and Allura Red AC complex leads to the increasing enthalpy and entropy. The negative ΔG^o values for interaction of BSA with the AlluraRed AC indicate the spontaneity of the complexation. Therefore, the dominant force is entropy and the mode of this interaction is hydrophobic [13].

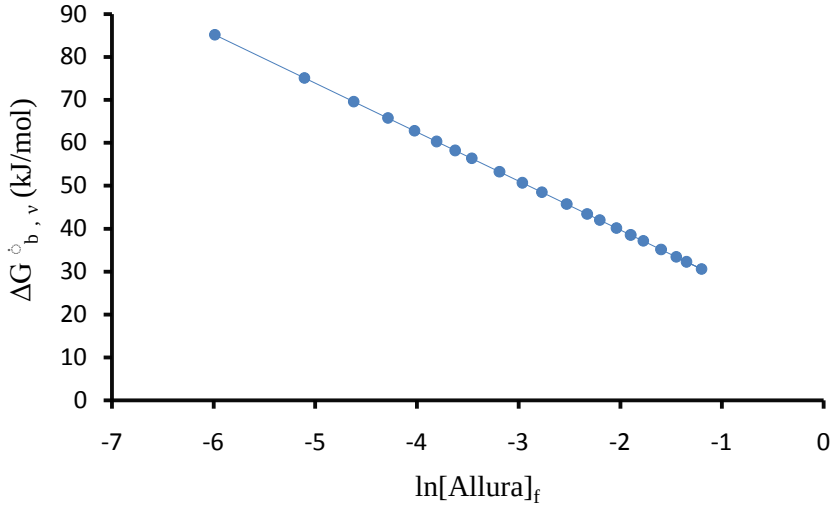

Figure 7: Gibbs free energy change BSA with Allura Red AC in 5 mM phosphate buffer solution, pH=7 at different temperatures

Table 2: Thermodynamic parameters for Binding of AlluraRed AC to BSA at 5 mM Phosphate Buffer, pH 7.0 at various temperatures

T(K)	G^0_b(KJ/mol)
298	-0.3635
303	-0.6450
308	-0.7392
313	-0.8764
318	-1.0800

4. Conclusion

The binding isotherm, binding capacity and Scat chard plots were plotted at various temperatures. The binding constant obtained from the absorption titration data gives a binding constant of 3.38 M^{-1} at 25° C. The thermodynamic parameters such as binding free energy, binding enthalpy and binding enthalpy changes was calculated. The positive value of ΔH, positive value of ΔS and the negative value of ΔG indicated that in interaction of Allura Red AC with BSA the driving force agent is entropy and this interaction is a hydrophobic interaction.

References

1. Nelson, T.J., Backlund, P.S., Yergey, A.L., & Alkon, D.L. (2002). Isolation of protein subpopulations undergoing protein-protein interactions. *Molecular & Cellular Proteomics*, 1: 253-259.
2. Hasche, A.A., & Voss, C. (2005). Immobilisation of a repressor protein for binding of plasmid DNA. *Journal of Chromatography* A, 1080: 76-82.
3. Raman, R., Sasisekharan, V., & Sasisekharan, R. (2005).Structural insights into biological roles of protein-glycosaminoglycan interactions. *Chemistry & Biology*, 12: 267-277.
4. Caligiuri, M., Molz, L., Liu, Q., Kaplan, F., Xu, J.P., Majeti, J.Z., Ramos Kelsey, R., Murthi, K., Lievens, S., Tavernier, J., & Kley, N. (2006). MASPIT: three-hybrid trap for quantitative proteome fingerprinting of small molecule-protein interactions in mammalian cells. *Chemistry & Biology*, 13: 711-722.

5. Stefani, M. (2004).Protein misfolding and aggregation: new examples in medicine and biology of the dark side of the protein world. *Biochimicaet Biophysica Acta*, 1739: 5-25.
6. Xie, M. X., Xu, X.Y.,& Wang, Y.D. (2005). Interaction between hesperet in and human serum albumin revealed by spectroscopic methods. *Biochimicaet Biophysica Acta*, 1724:215-224.
7. Piekarska, B., Skowronek, M., Rybarska, J., Stopa, B., Roterman, I., & Konieczny, L. (1996). Congo red-stabilized intermediates in the lambda light chain transition from native to molten state. *Biochimie*, 78:183-189.
8. Spahr, P.F., & Edsall, J.T. (1964). Amino Acid Composition of Human and Bovine Serum Mercaptalbumins. *Journal of Biological Chemistry*, 239: 850-854.
9. Masamitsu, H. (2015). Evaluation of the *in vivo* genotoxicity of Allura Red AC. *Food and Chemical Toxicology*, 84: 270-275.
10. Moosavi-Movahedi, A. A., & Nazari, K. (1995). Denaturation of horseradish peroxidase with urea and guanidine hydrochloride. *International Journal of Biological Macromolecules*, 17:43-47.
11. Housaindokht, M. R., & Moosavi-Movahedi, A. A. (1994). Determination of binding affinities of glucose oxidase for sodium n-dodecyl sulfate. *International Journal of Biological Macromolecules*, 16:77-80.
12. Ross, P. D., & Sabramanian, S. (1981). Thermodynamics of protein association reactions: Forces contributing to stability, *Biochemistry*, 20: 3096-3102.