Journal of Physical and Theoretical Chemistry
of Islamic Azad University of Iran, 4 (4) 239-244**:** Winter 2008
(J.Phys.Theor.Chem.IAU Iran: Winter 2008)
ISSN: 1735-2126

Thermodynamic Studies on the Interaction of Phthalocyanine with Bovine serum albumin

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ABSTRACT

Using UV-Vis spectrophotometric method the interaction of water soluble phthalocyanine, Cobalt(II) 4,4′,4″,4‴- tetrasulfophthalocyanine(CoTSPc), with bovine serum albumin (BSA) to determine the formation constant and related thermodynamic functions. The measurements were considered in 1mM sodium phosphate buffer, pH 7.0 and at 5 different temperatures 20, 25, 30, 35 and 40ºC. The results showed that the best fitting corresponds to a 1:1 complex model between BSA and CoTSPc. The optical adsorption spectra of phthalocyanine was analyzed in order to obtain binding constants, K, using SQUAD software. By using the Van't Hoff equation, values of enthalpy, ΔH°,and entropy, ΔS°,changes associated to the (BSA+ CoTSPc) were determined, and the values of ΔG° were calculated by using $\Delta G^\circ = -RT\ln K$ at 5 different temperatures.

Keywords: BSA; Phthalocyanine; SQUAD; Formation constant

INTRODUCTION

Many proteins spontaneously take on their secondary, tertiary, and quaternary structures. Apparantly, all of the required information for the formation of the biologically active conformation of proteins(called native form) is encoded in their amino acid sequence. It is an important goal of biochemistry to better understand the rules that govern protein folding and its role in biological activity [1-4]. The interactions, which stabilize protein conformation includes hydrogenbonds, disulfide bridges, electrostatic interactions, complex formation with metal ions, and hydrophobic effects. Many soluble proteins in water fold so that the majority of the nonpolar amino acid side chains lie within the molecule, whereas the polar side chains face toward the solvent[5, 6].Serum albumin, one of the most available and extensively studied of all proteins, is the most abundant protein in plasma, accounting for about 60% of its total protein content and providing about 80% of the blood osmotic pressure. It plays an important role in drug transport and storage in vertebrates [7, 8].

Bovine Serum Albumin (BSA)(fig.1) consists of 583 amino acids in a single polypeptide chain.It posses a wide range of physiological functions involving the binding, transport and delivery of fatty acids, porphyrins, bilirubin, tryptophan, thyroxin and steroids. It contains three homologousα-helices domains(I,IIandIII),

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and each domain is further divided into two subdomains(IA, IB, etc.) [9]. Serum albumin is the most abundant protein in animal 's including human circulatory system. It is in charge of the transport of a variety of endogenous and exogenous substants in body and plays an important role in the distribution and deposition of these substances[10]. When drugs are absorbed, they enter into the circulatory system and extensively and reversibly bind to serum albumin[11]. An important aspect of a drug's biodisposition profile is the extent to which it bonds to plasma proteins[12]. Drug- protein interaction has signifigance in pharmacology. It can affect the biological activity [13, 14] and toxicity[15- 17] of drug. The binding parameters are helpful in the study of pharmacokinetics and the design of dosage forms[18,19].

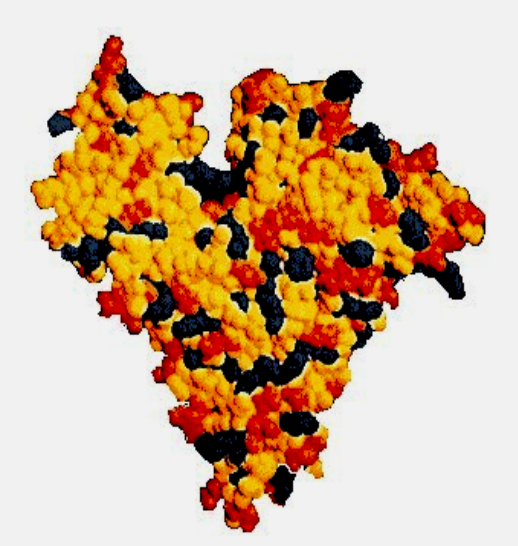


Fig.1. Space filling model of serum albumin.

Cobalt tetrasulfophthalocyanine (CoTSPc) (structure shown in fig.2) has however, not been explored industrially in large amonts as a catalyst for the oxidation of mercaptans in gasoline fraction[20-23]. Cobalt phthalocyanine(CoPc) and it's derivatives is an effective catalyst for the oxidation of sulfur- containing substrates[24] and are effective in the catalytic oxidation of mercaptans in oil fraction and in removing alkali sulfides from industrial waste water[25,26]; they can also serve as active elements in chemical sensors, especially for the detection of N2O[27,28].Application of the spectroscopic techniques can reveal the reactivity of chemical and biological systems in low concentration under physiological conditions, and there have been several studies on fluorescence quenching and secondary structure analysis of albumin induced by drugs or other bioactive small molecules[29-31].

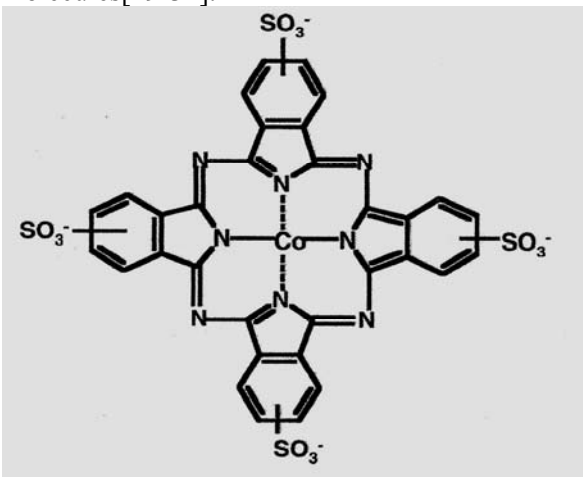


Fig.2. Molecular structure of cobalt tetrasulfophtalocyanine.

The aim of this study was to determine the affinity of CoTSPc to BSA and to investigate the thermodynamics of their interaction. In order to attain these objectives, we planned to carry out detailed investigation of CoTSPc-BSA association using UV-Vis absorption spectroscopy.

EXPERIMENTAL PROCEDURES

Bovine serum albumin, BSA(>98%, Roche) was purchased from Merk company and was used without further purification and its molecular weight was assumed to be 66,500. Highly purified phthalocyanine, CoTSPc, was prepared by chemistry department of Shahid Beheshti University of Iran. All considered solutions were prepared using double-distilled water. Sodium phosphate buffer(1mM pH 7.0), was used as buffer. All of the work solutions were made by dissolving the solid compounds in buffer solution. The phthalocyanine solutions were freshly prepared before spectra analysis and their concentration range were between(1.5×10^{-5}-7×10^{-5}M). BSA solutions(2×10^{-4} -3×10^{-4}M) were prepared with doubly distilled water and were kept in the dark at 4˚C. The titration of considered phthalocyanine solutions as a

function of BSA concentration was performed at 20, 25, 30, 35 and 40˚C.Spectrophotometric measurements were performed on a UV-Vis Shimadzu 2101 PC, using 1.00 cm quartz cuvettes in the spectral range 200-800 nm with thermostat cell compartment that controls the temperature around the cell within ±0.1˚C. The stoichiometry of complexes and its formation constant were determined by analyzing the optical absorption of considered phthalocyanine at various BSA concentrations by using SQUAD software.

RESULTS AND DISCUSSION

Phthalocyanine

The absoption spectrum of CoTSPc (fig.3) was recorded at 25˚C and shows that the band of CoTSPc consists of two components 631.5 and 670.0 nm. Figure 4 shows that the maximum band obeys beer's law over a concentration range between 1.5×10^{-5}-7×10^{-5}M.

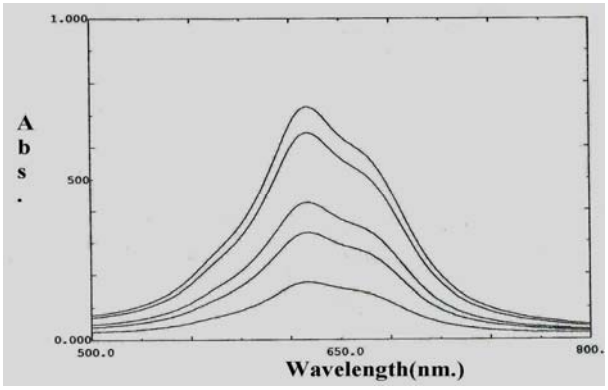


Fig.3. Absorption spectra of CoTSPc and their maximum band at 25˚C.

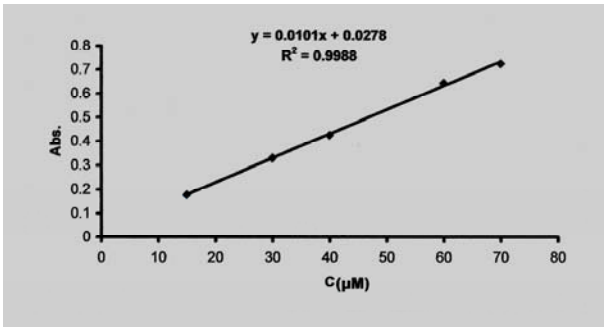
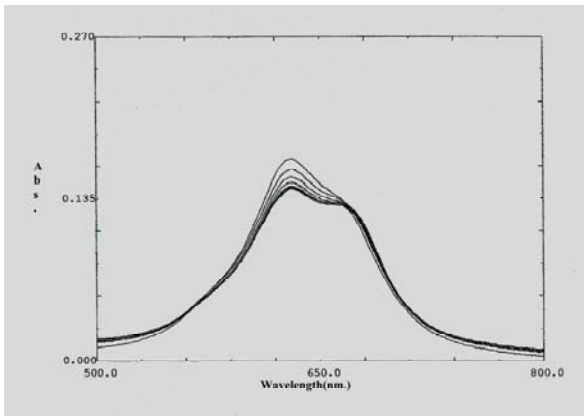


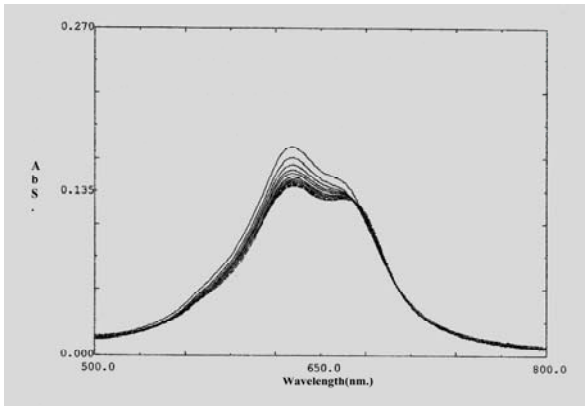
Fig.4. Absorbance as a function of concentration of CoTSPc at 25˚C.

Interaction of phthalocyanine with BSA

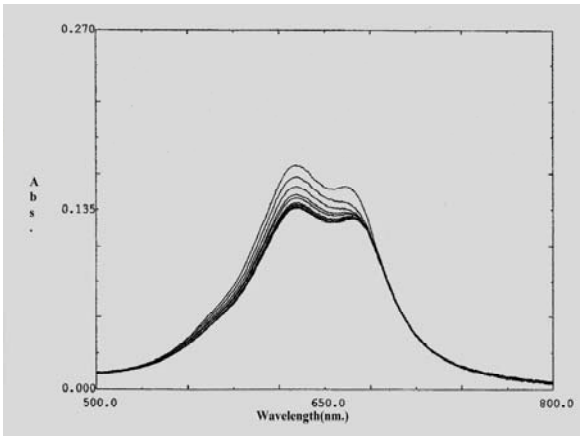
Spectrophotometric titrations were carried out by adding 40μl aliquots portions of an stock solution of the BSA into a quartz cell containing 2ml phthalocyanine. Every titration was continued until the absorbance of phthalocyanine solution in the UV-Vis range remained constant. The spectra were also corrected respect to dilution effect. The general features of CoTSPc spectra at various BSA concentration are shown in figure 5. The hypochromicity along with small red shift has been observed in soret band of phthalocyanine due to increasing of BSA concentration. This can be representing the outside-binding mode of phthalocyanine to groove of BSA.



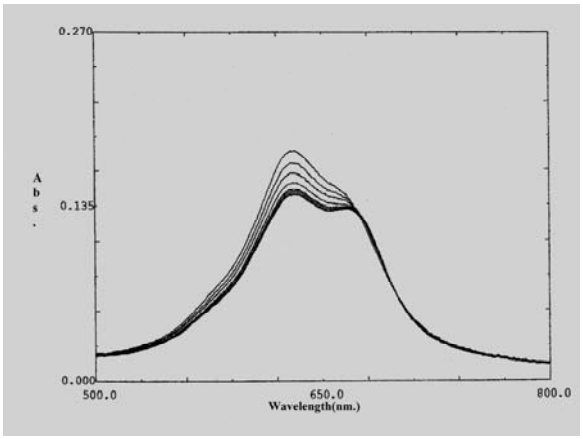
20˚C



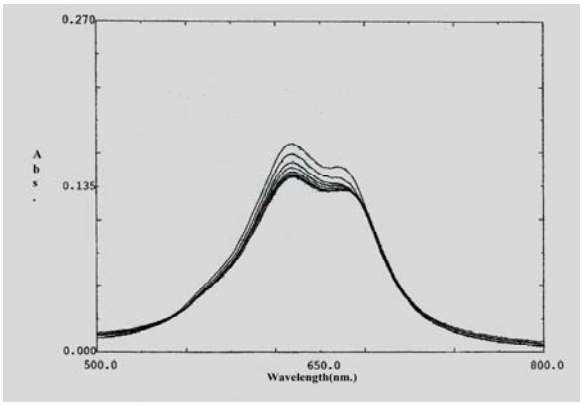
25˚C



30˚C



35˚C



40˚C

Fig.5. Absorption spectra of CoTSPc upon titration with BSA in phosphate buffer, pH 7.0 and different temperatures.

Thermodynamic functions of BSA interaction with CoTSPc can be characterized by standard Gibbs free energy change, ΔG°, enthalpy change, ΔH°, and entropy change, ΔS°. ΔG° can be calculated from the $\Delta G^{\circ} = -RT\ln K$ relationship where K is the association formation constant for the BSA + CoTSPc reaction , R and T referring to the gas constant and the Kelvin temperature, respectively.The Van't Hoff equation : $d\ln K/d(1/T) = -\Delta H^{\circ}/R$ gives a linear plot of lnK versus 1/T, if the heat capacity change for the reaction is negligible. The ΔH° can be calculated from the slope and the standard entropy change, ΔS°, from the equation: $\Delta S^{\circ} = (\Delta H^{\circ} - \Delta G^{\circ})/T$.The Van't Hoff plot for the interaction of " phthalocyanine+ BSA"; at different temperatures is shown in figure 6 and the thermodynamic functions are listed in table1.

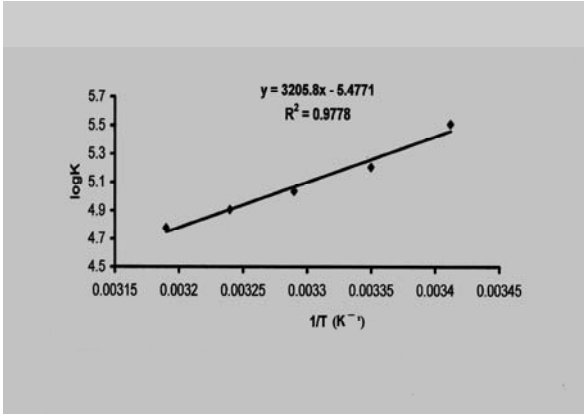


Fig.6. Plot of logK versus 1/T for binding of phthalocyanine to BSA in phosphate buffer, pH 7.0.

Table 1. Thermodynamic functions for binding of phthalocyanine to BSA in pH 7.0 and at various temperatures

Θ/ ˚C	$K\times10^{-4}$	ΔG°/ kJmol^{-1}	ΔH°/ kJmol^{-1}	ΔS°/ Jmol^{-1}K^{-}
20	38.02	-31.32	-61.382	-102.56
25	13.18	-29.22	-61.382	-107.86
30	10.96	-29.25	-61.382	-105.99
35	8.13	-28.96	-61.382	-105.21
40	6.02	-28.65	-61.382	-104.52

SQUAD program

Our input data for analysis of phthalocyanine-BSA system were absorbances of 50 different wavelength of 15 phthalocyanine solutions. The 50 wavelength showing suitable absorbance variations upon addition of BSA were selected from spectra of phthalocyanine. In order to calculate equilibrium formation constants using SQUAD software. This program is designed to calculate the best values for the stability constants of the proposed equilibrium model by employing a non-linear least square approach. The outputs are the logarithm of equilibrium formation constant,logK_{ij}, for the reaction(1)

$$iBSA+j\ phthalocyanine \leftrightarrow (BSA)_i\ (phthalocyanine)_j \quad (1)$$

$$K_{ij}= [\ (BSA)_i(\ phthalocyanine)_j\ ]\ /\ [BSA]^i\ [phthalocyanine\ ]^j \quad (2)$$

The results show that the best fitting corresponds to 1:1 complex model at various temperatures. These results are in good agreement with the existence of isobestic points that corresponds to a simple equilibrium between phthalocyanine and BSA. The estimated formation constants for the formation 1:1 complexes between BSA and CoTSPc are listed in table 1.

CONCLUSION

In this work, the nature of interaction between phthalocyanine and BSA was investigated by UV-Vis spectrophotometric method upon using SQUAD program. The results of calculations showed the formation of 1:1 complex model for the "phthalocyanine-BSA" equilibrium and the predominant role of electrostatic forces in the interaction. The Association formation constant, K, is inversely correlated with temperature, which indicates that the probable quenching mechanism of the phthalocyanine- BSA bonding reaction is initiated by complex formation. Van't Hoff plot and related calculations indicate the spontaneity in phthalocyanine- BSA binding reaction and strongly through hydrogen bond and Vander Waals force.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge financial support of Science and Research Branch of Islamic Azad University.

REFERENCES

1. C. Mitchinson and J. A. Wells(1989). Biochemistry, 28,4807.
2. M. W. Pantoliano et al. (1989) . Biochemistry, 28, 7205.
3. L.Regan and W. F. Degrado (1988) . Science, 241,976.
4. C. N. Pace(1990). Trends Biochem. Sci.,15, 14.
5. M. N. Jones(1992). Chem. Soc. Rev., 21, 127.
6. M. N. Jones and A. Brass(1990). In: E. Dickinson(Ed.), Food polymers and colloids. Royal society of chemistry, special publication No. 82, 65- 80.
7. Carter D., Ho J. X., Adv. Protein chem.., 45, 153-203(1994).
8. Peters T., "All About Albumin. Biochemistry, Genetics and Medical Applications," Academic Press, San Diego, CA, 1996.
9. Peters T.,"All About Albumin, Biochemistry, Genetics and Medical Applications," Academic Press, San Diego, 1995.
10. K. Yamasaki, T. Maruyama, U. Kragh-Hansen, M. Otagiri, Biochim. Biophys. Acta 1295(1996) 146-157.
11. B. P. Kamat, J. Seetharamappa, J. Pharm. Biomed. Anal. 35(2004) 655- 664.
12. M. D. Reed, C. M. Myers, J. L. Blumer, Curr. Ther. Res. 8(2001) 558- 565.
13. N. Seedher, Ind. J. Pharm. Sci. 62 (2000) 16-20.
14. G. Zlotos, A. Bucker, M. Kinzig-Schippers, F. sorgel, U. Holzgrabe, J. Pharm. Sci. 87(1998) 215-219.
15. U. Kragh- Hansen, Pharm. Rev. 33(1981) 17- 53.
16. T. Cszerharti, E. Forgacs, J. Chromatogr. A 699(1995) 285- 290.
17. D.Silva, C. M. Cortez, J. Cunha- Bastos, S. R. W. Louro. Toxicol. Lett. 147(2004) 53-61.
18. Rieutord, P. Bourget, G. Torche, J. F. Zazzo, Int. J. Pharm. 119(1995) 57-62.
19. O. Borga, B. Borga, J. Biopharm. 25(1997) 63-77.
20. T. Buck, H. Bohlen, D. Wohrle, G. Schulz-Ekloff, A. Andrew, J. Mol. Catal. 80(1993) 253.
21. V. Iliev, A. Andreev, D. Wohrle, G. Schulz-Ekloff, J. Mol. Catal. 66(1991) 5.
22. V.Iliev, J.Mol. Catal. 85(1993) L 269.

23. J. Zwart, H. C. Vander Weide, N. Broker, C. Rummens, G. C. A. Schint, A. L. German, J. Mol. Catal. 3(1997) 151.
24. Leznoff CC, Lever ABP. Phthalocyanines properties and application. New York: VCH Publishers; 1993.
25. Almgren, Hagstram I. Water Res 1974; 8:395.
26. Fischer H, Schulz- Eklor G. Wohrle D. Chem Eng Technol 1997; 20: 624.
27. Collins RA, Mohammed KA. J. Phys. D 1988; 21: 142.
28. Hamann C, Heitschold M. Mrwa A, Mueller A, Starke M, Kilper R. Top. Mol. Organ. Eng. 1991: 129.
29. M. S. Baptista, G. L. Indig, J.Phys.Chem. B 102(1998) 4678- 4688.
30. J. Liv, J. Tian, Z. Hu, X. Chen, Biopolymers 73(2004) 443-450.
31. D. Wu, Q. Wei, Y. Li, B. Du, G. Xu, Int. J. Bio. Macromol. 37(2005) 69- 72.