International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN: 0974-4290
Vol.7, No.2, pp 800-803, 2014-2015

ICONN 2015 [4th -6th Feb 2015]
International Conference on Nanoscience and Nanotechnology-2015
SRM University, Chennai, India

Quantification of BSA in Presence of Folic Acid

Rajat Gupta[1] and Sanat Mohanty[1]*

[1]Advance Materials and Nanoscience Laboratory, Department of Chemical Engineering, IIT Delhi, New Delhi, India-110016

Abstract : Folic acid is a biologically active molecule that self-assembles in water. There is an interest to capitalize folic acid self-assembly as a carrier to develop nanoparticles for protein delivery. In the present paper, Bovine Serum Albumin (BSA) is used as a model protein for characterization and quantification in presence of folic acid using HPLC. Molecular dynamics study is also used to explain the interaction between BSA and folic acid. HPLC is found to be successful in separation and quantification of both the components.
Keywords: Self-assembly; Folate; Bovine Serum Albumin; Tryptophan.

Introduction

Folic acid is a vitamin, biologically active chromonic molecule. Human body acid requires folic acid to prevent from several complications for example Alzheimer's disease, pregnancy complications, male infertility and cancer disease[1]. Human body cannot produce folic acid by itself, it provided through daily diet [2]. Therefore, to fulfill the need of folic acid in body, few researchers have developed protein nanoparticles by capitalizing the associative forces between protein and folic acid [2,3,4]. In another study, folic acid was also used as a carrier for protein delivery [5]. Researchers have also used this compound for different drug delivery applications[5–7].In all the application, separation and quantification of both folic acid and protein would be necessary. Apart from that, due to association between folic acid and BSA, it would be a challenge to measure BSA and folic acid concentration separately. Therefore, a separate study is required to develop a quantification method for BSA release study in presence of folic acid.

HPLC is a reliable technique to separate and quantify proteins. Due to potential to produce high reproducibility in the results, it is used to separate compounds in the pharmaceutical industry. In present paper, HPLC technique has been used for separation and quantification purpose. Previous literatures have shown that tryptophan forms complex and can be a responsible site for folic acid-BSA association[8,9]. To understand the mechanism of attachment of folic acid with tryptophan, a molecular dynamics study is also done in this paper.

Experimental

Material

Folic acid, sodium hydroxide and BSA were obtained from SRL chemicals. All the samples were prepared using Mili-Q water which is obtained Millipore water unit.

Sample preparation

First, 10 ml of deionized water was taken in a beaker and 0.01 gm of folic acid was added to obtain 0.1% (by weight) stock solution. Few drops of 1N sodium hydroxide solution were added into the folic acid solution till the solution becomes of shiny yellow color while ensuring the pH less than 7 (around 6.7). BSA stock solution was prepared separately in water. 0.1% (w/w) BSA stock solution was prepared by dissolving 0.01 gm BSA in 10 ml of water. Folic acid, BSA and folic acid-BSA mixture samples were prepared by diluting BSA and folic acid stock solution in an appropriate proportion.

Simulation study

This study was first carried out on MD Darshan software using graphics processor[10]. Folic acid and tryptophan molecules were kept in a cubic vessel of 30 Å under constant number, pressure and temperature conditions. Step size was kept at 0.001 ps and time after equilibrium was kept at 3000 ps.

HPLC Measurement

High Performance Liquid Chromatography was performed on UHPLC focused model name was ultimate 3000 (Make: Dionex). The column used in the study was C18 with 4.6×250 mm dimensions. Acetonitrile and water are used as a solvent in the mobile phase at 1 ml/min flow rate. HPLC was run in gradient mode and wavelength used UV detector was 215 nm.

Gradient run of mobile phase was performed for the sets of samples. In this case, for first 8 minutes ratio of acetonitrile to water was kept at 50:50 (V/V) and after 8 minutes, acetonitrile proportion was increased after each minute by 10% which results into 90:10 (V/V) in 12 minutes. After 12 minutes, we made this proportion again at 50:50 (V/V) and kept it at the same ratio till 14 minutes.

Results and Discussion

Study of Association through Molecular Dynamics (MD) Simulation

In the present study, this tool has been used to study interaction between folic acid molecules and tryptophan using MD Darshan software. Interaction of tryptophan molecules has been studied in presence of 10 folate ions and 500 molecules of water. In each simulation result, water molecules have been removed for closer view of folate ions and tryptophan molecules.

In two different simulation runs 2 and 10 molecules of tryptophan has been studied. Images obtained after the simulations has been show in Figure 1. In Figure 1(a) folate ions are in a well arranged. On the other hand, both the tryptophan molecules are found to be well aligned with in folate ion stacks. Figure 1(b) shows that folate ions are less arranged than in figure 1(a) but tryptophan molecules are still within the stacks of folic ions. This is an evidence of associative forces between folate ion and tryptophan molecules. Therefore, from this study it can be concluded that tryptophan can be the responsible site for BSA-folic acid complex formation.

HPLC Study for BSA Quantification

Running gradient mode is a very useful way for separating different compounds. Figure 2 below shows the signature peak of BSA at 1.647 minute and folic acid at 2.207 minute, separately. An increasing value of absorbance after 7.5 minutes in the figure is due to increasing concentration of acetonitrile. Two distinct peaks for both compounds in Figure 2 suggests that HPLC method is a useful technique to quantify BSA. Variation in the Peak position with concentration of BSA was not found, while variation in the area under BSA peak was found linear with BSA concentration (shown in Figure 3). This linear relationship can be used to quantify BSA in presence of folic acid. The linear variation also suggests that association between both the compounds is physical.

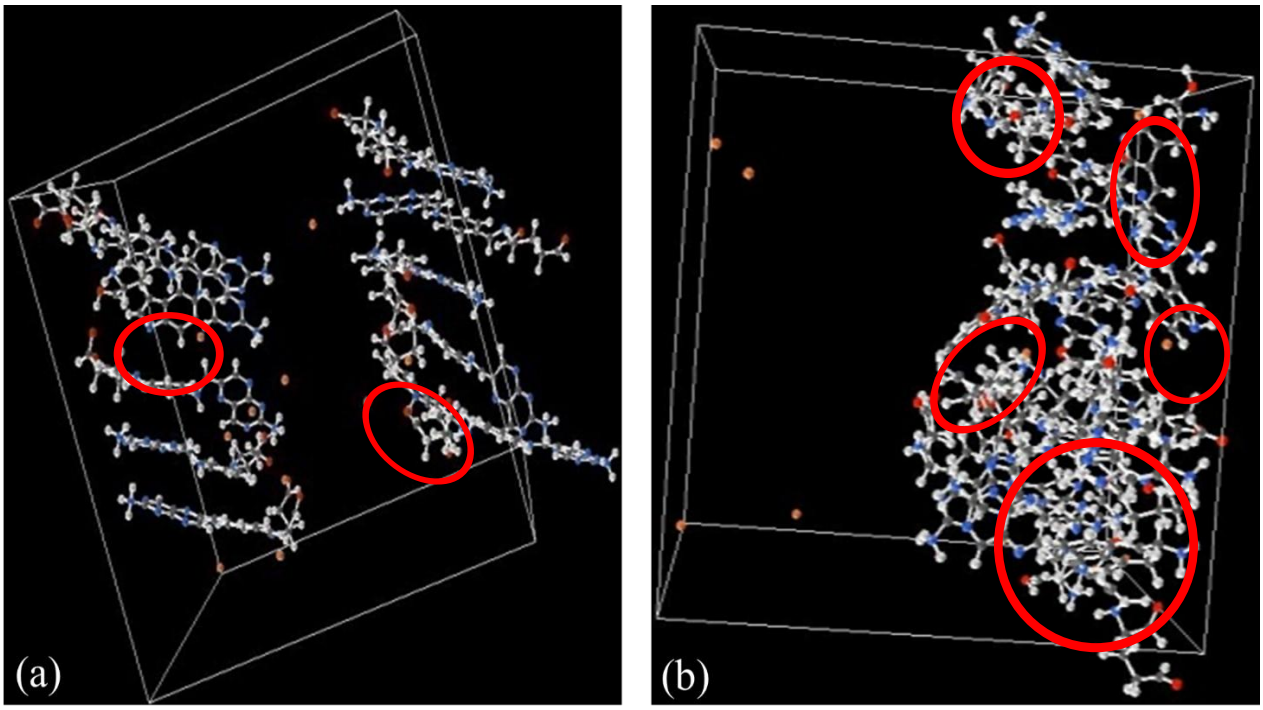

Figure 1. Image of mixture of 10 folate ions with (a) 2 tryptophan (b) 10 tryptophan molecules after simulation.

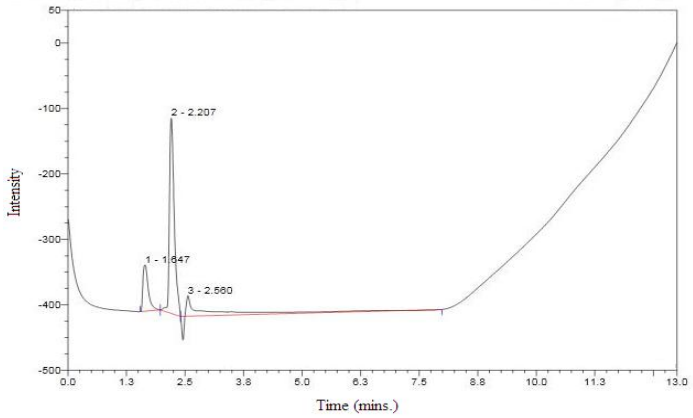

Figure 2. Chromatogram of BSA and folic acid mixture

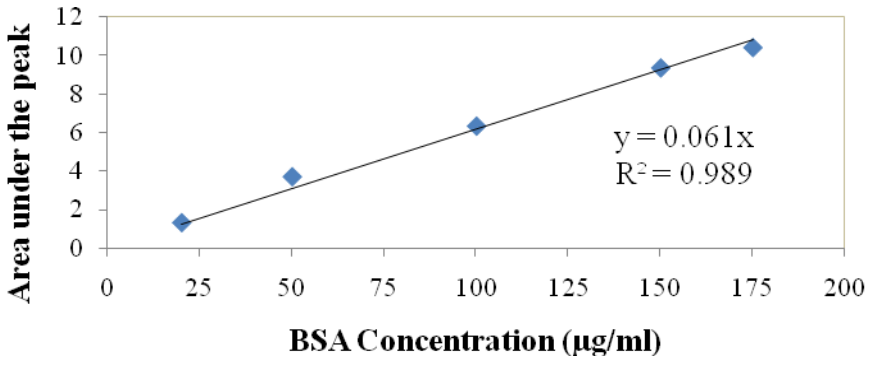

Figure 3. Graph shows variation in the area under the BSA peak with concentration of BSA at 100 µg/ml folic acid.

Conclusions

Association between folic acid and BSA has been shown through MD simulation studies. Tryptophan can be the binding site in BSA structure responsible for associations. In spite of these associative interactions between folic acid and BSA, HPLC was able to separate and quantify BSA successfully.

References

1. Off, M. K.; Steindal, A. E.; Porojnicu, A. C.; Juzeniene, A.; Vorobey, A.; Johnsson, A.; Moan, J. Ultraviolet Photodegradation of Folic Acid. *J. Photochem. Photobiol. B.* 2005, *80*, 47–55.
2. Ding, X.; Yao, P. Soy Protein/Soy Polysaccharide Complex Nanogels: Folic Acid Loading, Protection, and Controlled Delivery. 2013.
3. Jha, N. S.; Kishore, N. Thermodynamic Studies on the Interaction of Folic Acid with Bovine Serum Albumin. *J. Chem. Thermodyn.* 2011, *43*, 814–821.
4. Bourassa, P.; Hasni, I.; Tajmir-Riahi, H. A. Folic Acid Complexes with Human and Bovine Serum Albumins. *Food Chem.* 2011, *129*, 1148–1155.
5. Misra, R.; Mohanty, S. Sustained Release of Methotrexate through Liquid-Crystalline Folate Nanoparticles. *J. Mater. Sci. Mater. Med.* 2014, *25*, 2095–2109.
6. Le Gourrierec, L.; Di Giorgio, C.; Greiner, J.; Vierling, P. Formulation of PEG-Folic Acid Coated Nanometric DNA Particles from Perfluoroalkylated Cationic Dimerizable Detergents and in Vitro Folate-Targeted Intracellular Delivery. *New J. Chem.* 2008, *32*, 2027–2042.
7. Scrase, T. G.; Page, S. M.; Barker, P. D.; Boss, S. R. Folates Are Potential Ligands for Ruthenium Compounds in Vivo. *Dalt. Trans.* 2014, *43*, 8158–8161.
8. Bourassa, P.; Hasni, I.; Tajmir-Riahi, H. a. Folic Acid Complexes with Human and Bovine Serum Albumins. *Food Chem.* 2011, *129*, 1148–1155.
9. Fujimori, E. Interaction between Pteridines and Tryptophan. *Proc. Natl. Acad. Sci. U. S. A.* 1959, *45*, 133.
10. https://github.com/okpatil4u/MD-Darshan.

*****