Three dimensional fluorescence spectroscopy investigation of binary between human serum albumin and two nanoparticle drugs

M.Vahidzadeh[1*], M.Gharanfoli[3] and J. Chamani[2]

1. Department of Biology, Islamic Azad university–Central Tehran Branch, Young Researchers Club, Central Tehran Branch, Iran

2. Department of Biology, Faculty of Sciences, Islamic Azad university–Mashhad Branch, Mashhad, Iran

3. Department of Development of Biology University of Science and Culture, Tehran, Iran

Abstract:

The intraction between Colchicine (COL) and human serum albumin (HSA) in the presence of aspirin (ASA) as two nanoparticle drugs in physiological condition (pH=7.4) was investigated by fluorescence spectroscopy. The change on the protein conformation upon binding was followed as a function of added drug by Three-dimensional. Change in the environment of the aromatic residues are also obsorved. The results indicated that the structure of Trp and Tyr residues environments was altered and physiolosical function of drugs were affected by HSA. It was shown that the fluorescence of HSA have been quenched for reacting with drugs and forming a certain kind of new compound. Tree-dimensional Fluorescence spectroscopy shows that the secondary structure of HSA changed after drugs bound to HSA in binary and ternary systems.

Keywords:

HAS, COL, Three dimensional fluorescence spectroscopy binary and ternary systems

1. Introduction:

HSA is an important chemical substance in our life and the major target of many types of medicine in the body. COL is a naturally occurring alkaloid used in human and veterinary medicine. It has been used as a model antimitotic drug. ASA is used as analgesic against less intense pains; it is a soft and excellent antipyretic sedative.

The aim of this study is to analyze the interaction of COL with HSA in the presence of ASA and to evaluate the mechanism of competitive binding of COL and ASA to HSA.

2.

2.1. Materials and apparatus

COL, ASA and HSA was purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Three-dimensional spectra were obtained by measuring the emission spectra in the range from 250 to 700 nm repeatedly, at the excitation wavelengths from 220 to 500 nm by Fp-2600 fluorescence spectrophotometer (Hitachi, japan). Spectra were then concatenated into an excitation–emission matrix. The three-dimensional plots and contour maps were produced using Jasco w32 program. Each set of contour maps was plotted using the same scale range of fluorescence intensities and number of contours.

2.2. Method:

Three-dimensional fluorescence spectroscopy is a new analytical technique which is applied to investigate the conformational changes of proteins in recent years.

3. Results:

The excitation wavelength, the emission wavelength and the fluorescence intensity can be used as the axes making the investigation of the characteristic conformational changes of proteins be more scientific and credible [1]. The outstanding advantage of three-dimensional fluorescence spectra is that information regarding the fluorescence characteristics can be entirely acquired by changing excitation and emission wavelength simultaneously [2].

The three-dimensional fluorescence spectra and fluorescence contour map for HSA are shown in Fig. 1 A, 1B respectively. Also determined The three-dimensional fluorescence spectra and fluorescence contour map for HSA-ASA, HSA-COL and (HSA-ASA)COL. It is apparent from Fig. 1A and Fig. 1B that there are two peak in the three-dimensional fluorescence spectra for HSA, peak 1 mainly reveals the spectral behavior of Trp and Tyr residues. The reason is that when HSA is excited at 280 nm, it mainly reveals the intrinsic fluorescence of Trp and Tyr residues. Beside peak 1, there is another fluorescence peak 2 (λ_{ex} =280 nm, λ_{em} =340 nm) that mainly reflects the fluorescence spectral behavior of the polypeptide backbone structure of HSA [3]. We observed in three-dimensional fluorescence spectra, The fluorescence intensity of peak 1,2 decreased after the addition of drug, which means that the choromophor and peptide strands structure of HSA has been changed. The above phenomenon and analyzing of peak 1 and peak 2 revealed that the binding of drugs to HSA induced some microenvironment and conformational changes in HSA.

Also it can be seen that the presence of simultaneous two drug decreased fluorescence intencity of HSA more than binary system, it indicated that in the presence of COL, ASA more change conformation in HAS. Contour map spectra shown that fluorescence intensity of HSA quenched in intraction with drugs, explained that the interaction of ASA and COL with HSA in binary and ternary systems induced some conformational and microenvironmental changes HSA molecule.

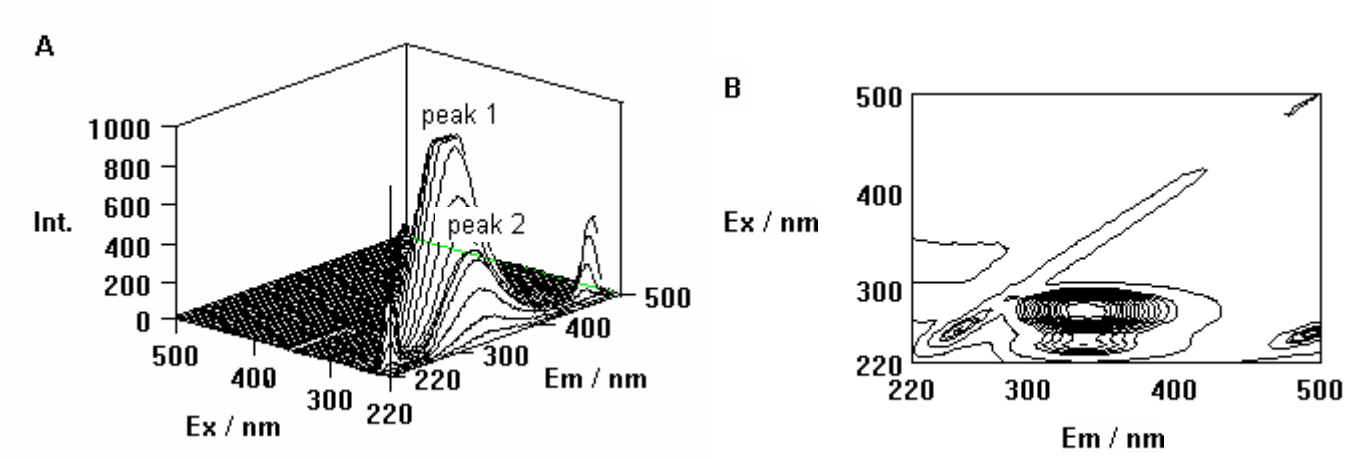

Fig.1. Three-dimensional fluorescence spectra (A) and Cantour spectra of HSA (B). T=298 K, pH=7.4.

4. Conclusion:

The intraction between HSA by ASA and COL has been investigated in this work using different spectroscopic techniques. The results of three-dimensional fluorescence spectroscopy show that secondary structure of HSA changes upon drugs binding.

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