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The Investigation of the Interaction between Human Serum Transferring with Colchicine in the Presence of Pb^{+2} Ions: Synchronous Fluorescence Measurements

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Abstract: The interaction between Holo-Transferin (HTF) and Colchicine (COL) was investigated in the present of Pb^{+2} ions under physiological conditions by using synchronous fluorescence spectra. The synchronous fluorescence spectra show a slight change of tryptophan residue micro-environment. Synchronous fluorescence spectra show that the structure of the tyrosine residue environment was altered by interaction of the COL and Pb^{+2} ions with HTF. The fluorescence intensity of HTF decreased regularly beside a small blue shift with increasing concentrations of COL and Pb^{+2} ions. The intrinsic fluorescence of HTF was quenched in the presence of drug and ion. Interaction of drugs with HTF and HTF-Pb^{+2} can elucidate the properties of drug-protein and ion- protein complex, as it may provide useful information about the structural feature that determines the therapeutic effectiveness of ion and drugs. Therefore, it has become a significant research field in life science, chemistry, biotechnology and clinical medicine.

Keywords: Holo-Transferin, CHTF, Colchicine (COL), Nano- interaction, synchronous fluorescence spectra

INTRODUCTION

Synchronous flurescence technique was introduced by Ioyd in 1971, and it gives information about the molecular environment in a vicinity of the chromophore molecule (Lioyd and Evett, 1977). According to the theory of Miler, when the D-value ($\Delta\lambda$) between excitation and emission wavelength are stabilized at 15 or 60 nm, the synchronous fluorescence gives the characteristic information of Tyr or Trp residues (Katrahalli *et al*, 2010). Human transferrin is a single chain glycoprotein containing 679 amino acids with molecular mass of ~ 80 KD a glyco-protein composed of two homologous lobes. Transferrins are bilobal monomers, with each lobe containing one Fe^{3+} binding site. serotransferrin (STF), a key serum protein which serves as a shuttle for metabolic iron, ovotransferrin (OTF), found in avian egg white. And lactoferrin (LTF), present in milk and other secretory fluids (Katrahalli *et al*, 2010). Transferrin is the fourth most abundant protein in the serum and is not just restricted to blood. It is also found in places like cerebrospinal fluid, tears, saliva etc. which serve as alternative source of transferrin especially when they are situated on the other side of blood – brain barrier. Liver is the primary site of synthesis of serum transferrin. However organs like placenta, thymus also has transferrin synthesis taking place in them (M.LH. Metz- Boutigue *et al*., 1984). Colchicine (trade name ColBenemidR) has systematic IUPAC name: N-((7S)-5,6,7,9- tetrahydro-1,2,3,10-tetramethoxy-9-oxobenzo(a)heptalen-7-yl)-acetamide. Colchicine chemical formula is given by $C_{22}H_{25}NO_6$ and its molar mass is equal to 399.437 g/mol (Jonsson P., 2002). COL is a naturally occurring alkaloid used in human and veterinary medicine. It has been used as an antimitotic agent in cancer research involving cell cultures (Farce, A., 2004).

EXPERIMENTAL

Materials

Holo-transferrin, potassium phosphate, COL and (Lead) ion were purchased from Sigma chemical corporation, USA, and used without further purification. The HTF (1.265×10^{-3} mM) and COL (0.5 mM) solutions were prepared at room temperature as dissolutions in a 50-mM potassium phosphate buffer solution with pH =7.4. The Pb^{+2} ion (0.05 m M) solution was prepared by dissolution in double distilled water at (pH 7.4.)

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Instrumentation

The fluorescence spectra measurement of HTF and COL were performed using a Hitachi F-2500 spectrofluorometer (Hitachi, Tokyo, Japan) linked to a personal computer and equipped with a 150-W xenon arc lamp, gating excitation and emission monochromators, and a Hitachi recorder. Slit widths for both monochromators were set at 10 nm. A 1-cm quartz cell was used. Synchronous fluorescence spectra were obtained by simultaneously scanning the excitation and emission monochromators. Such synchronous fluorescence spectra only show the Tyr and Trp residues of hTf when the wavelength interval (Δλ) is 15 nm and 60 nm, respectively.

RESULT AND DISCUSSION

The main aim of this research was to study the binding properties between human serum transferring with colchicine in the presence of Pb^{+2} ion due to importance of the binding in pharmacology, biochemistry and environment. The interaction of col and human serum transferring in the presence of Pb^{+2} ions was studied by synchronous fluorescence. According to the spectroscopic studies, the fluorescence intensity of transferring and col in the presence of Pb^{+2} ion decreased regularly and slight blue shift were observed for the emission wavelength with increasing of col and Pb^{+2} concentrations and it suggesting that col and ion could interact with HTF and quench intrinsic fluorescence. The blue shift in maximum wavelength and decreased the fluorescence intensity demonstrated that the decreased polarity of tryptophan and tyrosine residues microenvironments. It is reported in the literature that the binding of col to HTF and could in the presence of Pb^{+2} ion induce the conformational changes in HTF, because the intermolecular forces involved maintaining the secondary structure could be altered and resulted in conformational changes of protein. synchronous fluorescence spectra of (HTF -COL) Lead in the various concentration of Pb^{+2} at room temperature and phosphate buffer solution and pH=7.4 were recorded in Δλ =60 nm and Δλ=15 nm in figure 1.(A&B), its show that, the synchronous fluorescence of complex between HTF -col in the presence of Pb^{+2} ion. The maximum emission wavelength of the tryptophan with Δλ=15 nm and Δλ =60 nm, has no change, which suggest that the interaction of col and Pb^{+2} with HTF did not affect the conformation of the region around the Tyr and Trp residues.

Moreover, these results show that COL and Pb^{+2} could bind to HTF. Finally, using the synchronous fluorescence method we studied the affect of an additional drug and ion changes in the affinity of protein transport. Therefore, it has become a significant research field in life sciences, chemistry, biotechnology and clinical medicine.

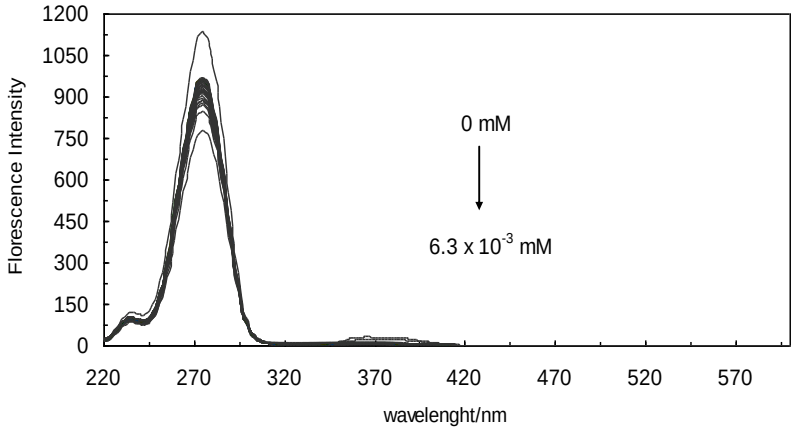

Fig. 1. (**A**) The synchronous fluorescence emission spectra of HTF at varying concentrations of col and Pb^{+2} ion at Δλ= 60 nm in phosphate buffer solution, pH=7.4.

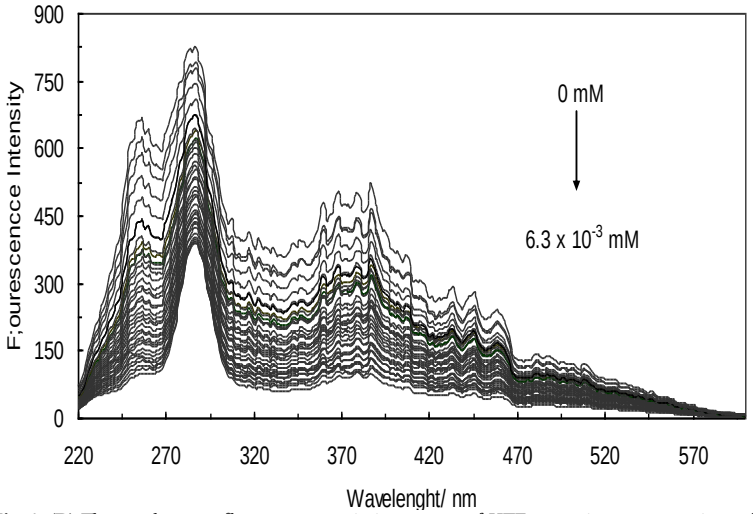

Fig. 1. (**B**) The synchronous fluorescence emission spectra of HTF at varying concentrations of col and Pb^{+2} ion at Δλ= 15 nm in phosphate buffer solution, pH=7.4.

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