Colloids and Surfaces A: Physicochem. Eng. Aspects 469 (2015) 187–193

Contents lists available at ScienceDirect

Colloids and Surfaces A: Physicochemical and Engineering Aspects

journal homepage: www.elsevier.com/locate/colsurfa

Spectroscopic and molecular modeling study of cyanine dye interacting with human serum transferrin

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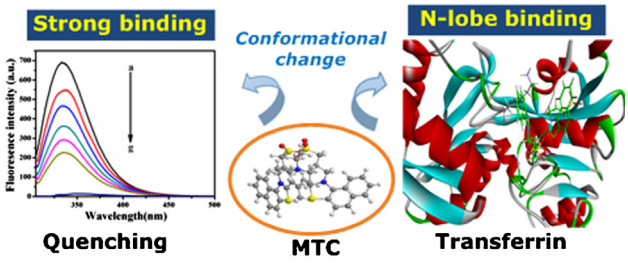
HIGHLIGHTS

- MTC binds strongly to Tf with the binding constant K_a, in the order of $10^9\ M^{-1}$.
- The fluorescence quenching of Tf by MTC was a static quenching process.
- MTC binds to Tf via hydrogen bonds and van der Waals forces.
- SFS and CD spectral studies of MTC–Tf revealed structural changes in protein.
- Docking study proved that the binding site of MTC to Tf is located in the N-lobe.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 22 September 2014
Received in revised form 8 January 2015
Accepted 9 January 2015
Available online 21 January 2015

Keywords:
Transferrin
Cyanine dye
Fluorescent quenching
Conformational transition
CD
Docking

ABSTRACT

The study of binding mechanisms and any associated conformational changes of human serum transferrin (Tf) on interaction with cyanine dye 3,3′-di(3-sulfopropyl)-4,5,4′,5′-dibenzo-9-methyl-thiacarbocyanine triethylam-monium salt (MTC) are of great importance in the process of Tf targeting dye delivery into cancer cells. It is possible to lay theoretical foundations for cyanine dye as a potential photosensitizer to achieve the photodynamic therapy (PDT). The mechanisms of interaction between MTC and Tf were portrayed by means of fluorescence spectra, UV–vis absorption spectra, synchronous fluorescence spectra, circular dichroism (CD) and molecular dynamic docking. The data of fluorescence spectra displayed that the formation of MTC–Tf complex is a static quenching process through van der Waals forces and hydrogen bonds with a high affinity of $10^9\ M^{-1}$. Binding distance between MTC and Tf substantiated that the non-radioactive energy transfer mechanism is also involved in the fluorescence quenching of protein. Furthermore, structural analysis indicated that MTC binding result in an increased of α-helix content and an increased hydrophobic around the tryptophan residues of Tf as well as a certain structural changes in Tf, which confirmed by the CD, synchronous fluorescence and UV–vis experiments. Additionally, the results of molecular dynamic docking elucidated that the dye was located in N-lobe of Tf.

1. Introduction

Cyanine dye, is the novel photographic materials with excellent optical properties including wide spectral range (400–700 nm), high fluorescence quantum yield and high sensitivity to environment [1,2]. Especially, it has been found to exhibit interesting

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http://dx.doi.org/10.1016/j.colsurfa.2015.01.023
0927-7757/

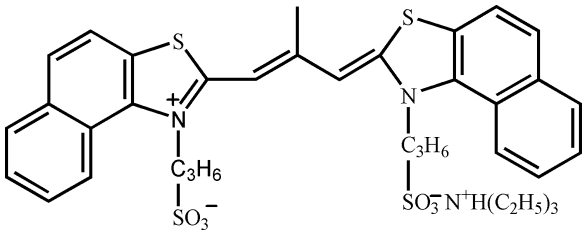

Fig. 1. Chemical structure of MTC.

biological properties, such as anticancer and phototoxic properties [3–6]. It is reported that cyanine dyes can discern a certain types of cell and pass into labeling cancer cells, or used as phototoxic agent to kill cancer cells [7]. Furthermore, cyanine dyes can also be directly used as a photosensitizer in photodynamic therapy, or used as a radiation sensitizing agent in the treatment of solid tumors [7]. Cyanine dyes as a candidate anticancer agent for cancer chemo-therapy have been gradually applied in the field of biology. However, most cyanine dyes do not have the ability to identify specific target organs and tissues, so this type of dye must be combined with biological activity carrier such as proteins or peptides, making the dye into a particular area of the body, so as to achieve the purposes of selective recognition tumor cell or kill tumor cell [8]. Therefore, based on the special properties of cyanine dye, we put up with a new idea to use transferrin as a potential dye carrier for targeted dye delivery into cancer cells.

Transferrin is a plasma protein which has the ability to transport multitude ligands such as metal ions and drugs, because its receptor that is over expressed on the surfaces of many cancer cells [9,10]. It has a molecular weight of ~80 kDa, and contains 679 amino acid residues which are arranged in two similar lobes that designated the N-lobe and C-lobe, connected by a short bridge. Transferrin is made up of two tyrosines, one aspartic acid and one histidine for the binding site in both the N- and C-terminal lobes, respectively [11]. It is well known that numerous investigations have been performed using transferrin as a drug carrier for targeted drug delivery into cancer cells. We found that the drug can bind to transferrin via covalent conjugation or noncovalent binding. Now, however, much attention has been paid to using transferrin as a carrier for cancer cell-targeted delivery of therapeutic agents loaded via noncovalent binding, the purpose is to fully develop the utility of the transferrin receptor system for targeted therapeutic agent delivery. Accordingly, in order to discuss whether cyanine dyes may interact with transferrin, we choose the dye MTC (structure shown in Fig. 1) and to investigate the interaction mechanism between MTC and transferrin with fluorescence spectra, UV–vis absorption spectra, synchronous fluorescence spectra and circular dichroism (CD). Furthermore, in order to obtain a clearer insight into the residues involved in the interaction and to substantiate our experimental studies, the molecular dynamic docking studies were performed. This study may provide valuable answers to the binding mechanism of MTC to transferrin at molecular level. It may be also helpful for the design of new method to effectively deliver anticancer agent via transferrin to target tissues and cells.

2. Materials and methods

2.1. Materials

Human serum transferrin (abbreviated as Tf, Catalog No. T3309, 98% purity) used in this experiment was purchased from Sigma and without further purification. The cyanine dye, 3, 3′-di(3-sulfopropyl)-4,5,4′,5′-dibenzo-9-methyl-thiacarbocyanine triethylam-monium salt (MTC) was synthesized according to Hamer [12] and Ficken's [13] methods, and the purity was verified by mass spectrometry and nuclear magnetic resonance (NMR). To prepare the phosphate buffered solution (PBS, pH 7.4), analytical grade $NaH_2PO_4{\cdot}2H_2O$, $Na_2HPO_4{\cdot}12H_2O$ and NaCl from Tianjin Chemical Plant (>99%, Tianjin, China) were used directly and double distilled water was used as solvents throughout the experiments.

The fresh stock solutions of MTC (2×10^{-3} M) were prepared by dissolving into PBS and containing 10% of dimethyl sulfoxide. The concentration of Tf (5×10^{-5} M) was prepared by dissolving with PBS. The measured sample was prepared by mixing a quantity of MTC with Tf solution, and then diluted by PBS for the fluorescence, UV–vis absorption and CD experiments. The sample solutions were kept at temperature (288 K, 293 K and 310 K) for more than 12-h before experiments.

2.2. Fluorescence, UV–vis absorption and circular dichroism (CD) spectroscopy

The fluorescence was performed on a Hitachi F-7000 spectrofluorimeter equipped with 1-cm path-length quartz cell. An excitation wavelength of 295 nm and different temperatures (288 K, 293 K and 310 K) were chosen in the experiment. Slit widths (5 nm), scan speed (12,000 nm/min) and excitation voltage (400 V) were kept constant within each data set. Synchronous fluorescence spectroscopy was carried out by simultaneously scanning the excitation and emission monochromators at 293 K and pH 7.4, and was recorded from 220 to 320 nm at $\Delta\lambda = 15$ nm and $\Delta\lambda = 60$ nm, respectively. UV–vis absorption spectra were recorded on a TU-1901 spectrophotometer at 293 K and pH 7.4 with 1-cm path-length quartz cell.

Circular dichroism (CD) spectra were recorded on a Jasco-815 automatic recording spectropolarimeter using quartz cells with a 1-mm optical path at 293 K. The spectra were collected with a scan speed of 500 nm/min and response time of 1 s. Each spectrum was the average of four scans and corrected by the PBS solution. The induced ellipticity, given in degrees, was obtained by the ellipticity of the ligand–protein mixture after subtraction of the ellipticity of the ligand at the same wavelength. CD data were expressed as the mean residue ellipticity $[\theta]$, which is defined as $[\theta] = 100 \times \theta_{obsd}/(LC)$ where θ_{obsd} is the observed ellipticity in degrees, C is the concentration in residue mol cm^{-3}, and L is the length of the light path in cm. Solution pH values were monitored with a PHS-3C pH meter.

2.3. Molecular docking

Molecular modeling method was obtained from a ThinkCenter M6300T workstation using LigandFit docking module in Discovery Studio 3.1 (Accelrys, San Diego, CA), with builder, biopolymer, binding site analysis, docking, and affinity modules, where the pH value is set to 7.4. The CHARMm forcefield was used for charge assignment for both the protein and ligands. The binding site was searched and defined by the binding cavity search tool. During docking, the residues inside the binding site are rigid. Six scoring functions, LigScore1, LigScore2, PLP1, PLP2, Jain, and PMF were used to score each ligand pose for a consensus scoring purpose. Then the top ranked ligand conformations were saved according to the combined scores given by consensus scoring system, followed by a 2000 steps steepest descent minimization and a 5000 steps conjugated gradient minimization for the binding site-ligand complex.

3. Results and discussion

3.1. Interaction between MTC and Tf

The intrinsic fluorescence quenching is an effective method utilized for studies on the interaction of ligand and protein,