Journal of Molecular Liquids 256 (2018) 127–138

Contents lists available at ScienceDirect

Journal of Molecular Liquids

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

Probing the binding of lomefloxacin to a calf thymus DNA-histone H1 complex by multi-spectroscopic and molecular modeling techniques

Tahmineh Sohrabi [a], Maral Hosseinzadeh [a], Sima Beigoli [b], Mohammad Reza Saberi [c], Jamshidkhan Chamani [a,*]

[a] Department of Biology, Faculty of Sciences, Mashhad Branch, Islamic Azad University, Mashhad, Iran
[b] Endoscopic and Minimally Invasive Surgery Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[c] Medical Chemistry Department, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

ARTICLE INFO

Article history:
Received 29 October 2017
Received in revised form 23 January 2018
Accepted 6 February 2018
Available online 07 February 2018

Keywords:
LMF
Histone H_1
Acridine orange
Ethidium bromide
RLS
Viscosity

ABSTRACT

The interaction between lomefloxacin (LMF) and a ctDNA-H_1 complex was investigated by various spectroscopies, as well as viscometry and molecular modeling techniques. The RLS results pointed at different intensities of the ctDNA, H_1 and ctDNA-H_1 complex systems in the presence of LMF. The fluorescence intensity measurements of the ctDNA, H_1 and ctDNA-H_1 complex in the presence of LMF as binary and ternary systems at three temperatures exhibited regular quenching of the fluorescence emission curves signifying a static behavior of the binary and ternary systems. Stern-Volmer constant values of ctDNA-LMF complex formation were 7.94×10^7, 4.94×10^7 and 3.54×10^7 M^{-1} at 298, 303 and 308 K respectively. In the presence of H1, the Stern-Volmer constant values had been changed to 1.41×10^8, 0.84×10^8 and 0.21×10^8 M^{-1} at 298, 303 and 308 K respectively. The Stern-Volmer constant values of binary and ternary systems clearly revealed static quenching behavior of ctDNA upon interaction with LMF in absence and presence of H1. Thermodynamic parameters obtained from Van't Hoff plots revealed the different essence of interaction between LMF with ctDNA, H_1 and ctDNA complex systems. The interaction between LMF with ctDNA and the ctDNA-H_1 complex in the presence of ethidium bromide (EB) and acridine orange (AO) as intercalator probes showed that a competitive ness occurred between EB and AO with LMF, indicating that the LMF bound to ctDNA as an intercalator. Circular dichroism (CD) curves in far UV-CD were used to determine the enhancement of ellipticity in ctDNA upon interaction with LMF in the absence and presence of H_1. The specific viscosity of ctDNA in the absence and presence of H_1 increased with an enhancement of the LMF concentration which was another reason for LMF binding to ctDNA as an intercalator. Molecular modeling data confirmed the experimental results pertaining to the interaction behavior in the ctDNA-LMF complex in the absence and presence of H_1.

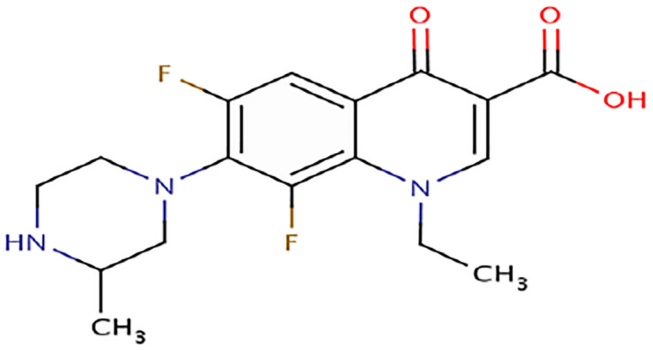
1. Introduction

The interaction of small molecules with DNA is a subject at the interface between chemistry and biology. Small molecules can bind to DNA by covalent or non-covalent interactions [1]. Nowadays, the methods used to evaluate the binding interaction of DNA with small molecules involve docking and experimental techniques [2–5]. Nucleic acids play a main role in biological systems since they carry significant genetic information. DNA is a good target for antiviral and antibiotic drugs. There are several types of sites in the DNA molecules where binding of ligand complexes can occur: between two base pairs (intercalation), in the minor or major grooves, and on the outside of the helix. The interaction between small molecules and DNA can cause DNA damage in cancer cells, thereby blocking the division of cancer cells and resulting in cell death [6–8].

Histones are alkaline proteins found in eukaryotic cell nuclei that package and order the DNA into structural units called nucleosomes. These are the main protein components of chromatin, acting as spools around which DNA winds and they also play a role in gene regulation [9–13] .The core of the nucleosomes is formed of two H2A-H2B dimers and a H3–H4 tetramer. The nucleosome core particle is composed of 147 bp of DNA wrapped around an octamer of two molecules each of the core histones H2A, H2B, H3 and H4. Five major families of histones exist: H1, H5, H2A, H2B, H3 and H4. Histones H2A, H2B, H3 and H4 are known as the core histones, while histones H_1 and H5 are known as the linker histones [14–17]. The H_1 histone family is the most divergent and heterogeneous group of histones and is generally a transcriptional repressor. The linker histone H_1 is an important structural component of chromatin and provides its functional flexibility. Histone H_1 binds the DNA entering and exiting the nucleosome, finishing two

* Corresponding author.
E-mail address: Chamani.J@ut.ac.ir (J. Chamani).

https://doi.org/10.1016/j.molliq.2018.02.031
0167-7322/



Scheme 1. The chemical structure of Lomefloxacin.

turns of DNA around the histone octamers. A typical H_1 structure includes a central globular domain flanked by unstructured N and C-terminal tails. The C-terminal tails are required for H_1-linker DNA binding and chromatin stabilization.

Lomefloxacin (LMF, Scheme 1) is an antibiotic in a class of drugs called fluoroquinolones with an extensive spectrum of activity against different bacterial infections, such as bronchitis and urinary tract infections [18,19], involving gram-positive and -negative microorganisms. The medical functions have been extensively studied [20], and proven to prevent bacterial DNA biosynthesis by inhibiting the bacterial enzyme DNA gyrase [21,22]. LMF is almost completely absorbed when taken orally and is slowly eliminated, with its half-life of seven to 8 h. As a third-generation quinolone, it also has the advantage of being effective against some anaerobic bacteria. Here in, we studied the interaction between LMF and calf thymus DNA (ct DNA) in the absence and presence of $H_1$and found a different affinity of LMF to ctDNA with different sides in ctDNA. Moreover, H_1 plays an important role in different binding types of LMF to ctDNA. This subject is of great interest for gene transcription and translation.

2. Materials and methods

2.1. Chemicals and reagents

Histone H1, lomefloxacin, ctDNA, ethidium bromide (EB) and acridine orange (AO) were obtained from Sigma Chemical Co. (St. Louis, MO. USA), Ltd. and were used without purification. Tris-HCl was obtained from Merck chemical Co. The protein and ctDNA (1.37×10^{-4} M) were dissolved in 10 mM Tris buffer solutions at pH = 6.8. The LMF solution (0.01 mM) was provided by dissolution in Tris buffer. EB (3.2×10^{-4} M) and AO (2×10^{-7} M) were dissolved in 10-mM Tris buffer solutions at pH = 6.8. The ct-DNA solution gave an absorbance ratio (A_{260}/A_{280}) of ~1.8, demonstrating that ctDNA was free from protein [23–26]. The ctDNA solution concentration per nucleotide was assigned from the absorption at 260 nm. The molar extinction coefficient and concentration of ct DNA were 6600 M^{-1} cm^{-1} and 1.37×10^{-4} M, respectively [27,28].

2.2. Methods

2.2.1. Spectrophotometric measurements

Fluorescence measurements were performed at room temperature using a spectrofluorometer model F-2500 (Hitachi-Japan). The excitation wavelengths were set at 280 nm and 295 nm, and the emission wavelength was recorded between 300 nm and 600 nm. The changes in fluorescence intensity were observed by titrating the fixed amount of LMF with varying concentration of ct DNA and histone H1 at different temperatures (298 K, 303 K, 308 K). The competitive interaction between LMF, EB and AO as intercalator probes with ctDNA was performed as follows: fixed amounts of EB, AO and ctDNA were titrated with increasing amounts of LMF solution. The EB and AO were excited at 440 nm and 490 nm and emission spectra were recorded between 450 nm and 800 nm for EB, and between 500 nm and 700 nm for AO.

Resonance light scattering (RLS) spectra were recorded by scanning both the excitation and emission monochromators of the spectrofluorometer with $\Delta\lambda = 0$. A resonance light scattering can be developed, and has proven to determine the aggregation of small molecules and the long-range assembly of drugs on biological templates. Suitable amounts of ctDNA and LMF were added to a cuvette. The spectrum was obtained by scanning from 220 nm to 600 nm.

UV–visible absorption spectra were obtained with a Jasco V-630 spectrophotometer. The UV spectra of ctDNA and ct DNA-histone H1 with LMF were recorded in the wavelength range from 200 nm to 800 nm. The experiment was performed in the presence of a fixed concentration of LMF. The base-line concentration was obtained using a blank solution containing 6.8×10^{-5} mM of ctDNA and H_1 in 10-mM Tris buffer. ctDNA-LMF and (ctDNA-H_1) LMF complexes were titrated with KI and NaCl with increasing concentration of KI and NaCl. LMF was added to a 0.5 ml reaction mixture containing 10-mM Tris buffer (pH = 6.8) and the emission spectra were recorded after increasing the concentration of KI and NaCl.

ctDNA melting experiments were performed by monitoring the absorption of ctDNA at 260 nm in the presence and absence of LMF at different temperatures using a spectrophotometer coupled to a thermocouple. The T_m values for ctDNA, ctDNA-LMF and (ctDNA-H_1) LMF complexes were obtained from the transition midpoint of melting curves based on absorbance against temperature. CD spectra of ctDNA, H1, and the ctDNA-H1 complex and the enhancement of the LMF concentrations were recorded using a CD spectrophotometer. All the CD spectra were recorded in a range from 220 nm to 330 nm. The background spectrum of the buffer solution (10 mM Tris, pH = 6.8) deduced from the spectra of ctDNA-LMF and (ctDNA-H_1) LMF and H_1 with LMF. The inner filter effect was carried out for all experiments [29,30].

2.2.2. Viscosity measurements

Viscosity measurements were performed using an Ostwald viscometer which was thermostated at 298 K in a constant temperature bath. The concentrations of ct DNA and ct DNA-H_1 complexin buffer solution (pH = 6.8) in the absence and presence of LMF were fixed and the flow time were measured using a digital stopwatch. The mean values of three replicated measurements were used to evaluate the relative specific viscosity $(\eta/\eta_0)^{1/3}$, where η_0 and η are the specific viscosity contributions of ct DNA and ct DNA-H_1 in the absence and presence of LMF, respectively.

2.2.3. Molecular modeling

The molecular docking studies were performed with the MOE software. The structure of LMF was drawn in ChemBioOffice-ChemDraw followed by MM energy minimization in ChemBioOffice-Chem3D while the ct DNA and histone H_1 files were taken from a protein data bank (PDB), codes: 1bna and 5NLO, respectively. The base pair sequence of ct DNA was: DC-DG-DC-DG-DA-DA-DT-DT-DC-DG-DC-DG/DC-DG-DC-DG-DA-DA-DT-DT-DC-DG-DC DG. To create the complex of ct DNA-H_1 the HEX8 software was taken into account while the correlation type was “shape only” and the receptor and ligand range was set to 180° whereas the twist range was set to 360°. The result complex was saved as a PDB file and became the receptor source for the final docking with LMF in the MOE software. LMF was docked in MOE with ct DNA, histone H_1, ct DNA-H_1 complex after protonation of all atoms and requesting 30 final docking results in MOE. Other parameters were set according to the following: the docking placement methodology was a triangle matcher; the initial scoring methodology was London