International Journal of Biological Macromolecules 51 (2012) 406–411

Contents lists available at SciVerse ScienceDirect

International Journal of Biological Macromolecules

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

Spectroscopic and molecular docking studies on chlorambucil interaction with DNA

Sonika Charak[a], Manish Shandilya[b], Gunjan Tyagi[a], Ranjana Mehrotra[a,*]

[a] Quantum Optics and Photon Physics, National Physical Laboratory (Council of Scientific and Industrial Research), Dr. K.S. Krishnan Road, New Delhi 110012, India
[b] Department of Biochemistry, University of Delhi South Campus, Benito Juarez Road, New Delhi 110021, India

ARTICLE INFO

Article history:
Received 21 April 2012
Received in revised form 7 June 2012
Accepted 10 June 2012
Available online 16 June 2012

Keywords:
Chlorambucil
FTIR spectroscopy
UV–visible spectroscopy
Circular dichroism spectroscopy
Molecular docking

ABSTRACT

Chlorambucil (CMB) is an anticancer drug used for the treatment of variety of cancers. Structural and conformational changes associated with DNA after binding with CMB were explored using spectroscopic techniques to get insight into the mechanism of action of CMB at molecular level. Different molar ratios of CMB–DNA complex were prepared with constant DNA concentration under physiological conditions. FTIR spectroscopy, UV–visible spectroscopy, CD spectroscopy and molecular docking studies were employed to determine the binding site and binding constant of CMB with DNA. The results show CMB binds DNA through nitrogenous bases (thymine, guanine and cytosine). The binding constant was calculated to be $1.3 \times 10^3\ M^{-1}$, which suggests weak binding of CMB with DNA double helix. FTIR and CD results show that CMB do not disturb native B-conformation of DNA and it continues to remain in its B conformation even at higher concentrations of CMB. The molecular docking results are in corroboration with our experimental results and provides structural insight into the interaction site.

1. Introduction

Chlorambucil 4-(4-[bis(2-chlorethyl)amino]phenyl)butyric acid is an alkylating antineoplastic drug belonging to nitrogen mustard family (Fig. 1). It is used for chemotherapy of lymphocytic leukemia, ovarian and breast carcinomas, and Hodgkin's disease [1–3]. It is also used as an immunosuppressive drug for the treatment of autoimmune and inflammatory diseases [4]. It is used in conjunction with other anticancer drugs for the treatment of advanced breast cancers [5,6].

Various mechanisms are known for the cytotoxicity induced by alkylating drugs but their exact mechanism of action inside the cell is still not delineated. It is believed that CMB exerts its cytotoxic effects by inhibiting the normal function of biological molecules. DNA, the genetic material which replicates very fast in the cancer cells is the major target of alkylating drugs. It is proposed that CMB alkylates the nitrogenous bases of DNA and forms inter and intra strand crosslinks. Formation of crosslinks, between DNA strands and drug results in uncoiling and twisting of the DNA helix [7,8]. These structural changes in DNA duplex result in the inhibition of DNA synthesis and DNA replication and finally lead to cell death. It is evident from the in vitro and in vivo experiments that CMB inhibit cell proliferation and causes cell death in carcinoma cell lines [9].

Although CMB is very effective for the treatment of various types of cancer still it is associated with several adverse effects [10,11]. As cytotoxicity of CMB is associated with changes in structure and function of DNA, therefore the structural analysis of CMB–DNA complex under physiological condition require attention.

In the recent years, infrared spectroscopy has gained growing interests in elucidating the structure and conformation of biomolecules and in understanding the interaction mechanism of biomolecules and drugs [12–17]. This technique is very efficient in determining the binding site and sequence specificity of the drug binding with the biomolecule. Along with FTIR, circular dichroism (CD) spectroscopy is another powerful technique in determining the change in structural conformational state of the biomolecules after formation of complex with drug. UV–visible spectroscopy provides information on binding mode and stability of the DNA–drug complex [18–20].

In the present study, the interaction of CMB with double stranded DNA has been examined using various spectroscopic techniques including Fourier transform infrared spectroscopy, UV–visible spectroscopy and circular dichroism spectroscopy. Molecular docking studies were also performed to get insight to the mechanism of action of CMB.

2. Materials

CMB and calf thymus DNA was procured from the Sigma Aldrich and was used without further purification. Purity of DNA was estimated by UV–visible spectroscopic measurement. The ratio of the

* Corresponding author. Tel.: +91 11 45608366; fax: +91 11 45609310.
E-mail addresses: ranjana@mail.nplindia.ernet.in, ranjana@nplindia.org (R. Mehrotra).

0141-8130/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijbiomac.2012.06.012

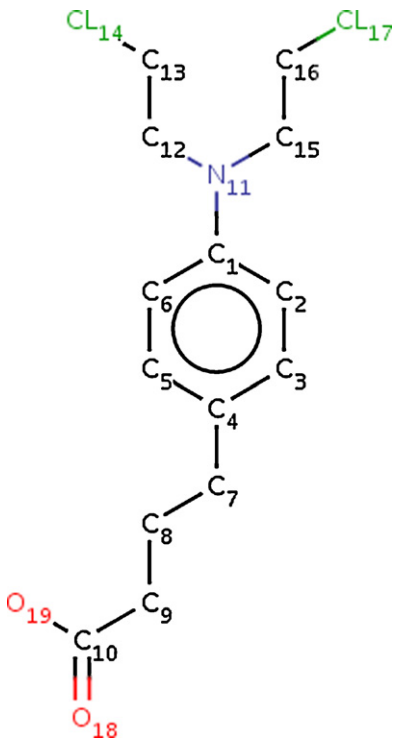

Fig. 1. Chemical structure of chlorambucil.

absorption at 260/280 nm was calculated to be 1.85, which indicates that sample was free from protein contaminations [21]. DNA, CMB and buffer solutions were freshly prepared before each experiment. Other chemicals used were of analytical grade and were used as supplied.

2.1. Stock solution preparation

DNA stock solution was prepared by dissolving 10 mg of calf thymus DNA in 1 ml of Tris–HCl buffer, pH = 7 at 4 °C. DNA solution was stirred at repeated intervals to form homogenous solution for 24 h. The concentration of DNA was determined spectrophotometrically using molar extinction coefficient of 6600 cm^{-1} M^{-1} at 260 nm [22]. Due to low solubility of CMB in water, it was dissolved in ethanol/water solution (1:1). Final concentration of ethanol (25%) in the mixture of CMB–DNA solution did not affect the conformation of DNA [23]. It is known that ethanol concentration (more than 70%) induces DNA conformational changes (B to A-form) [24]. Varying concentrations of CMB were prepared and added dropwise to DNA solutions. For FTIR studies different molar ratios of CMB/DNA = 1/20, 1/40, 1/80, 1/100 and 1/150 were prepared with final DNA concentration of 13 mM. Circular dichroism studies were carried out with CMB/DNA molar ratios of 1/20, 1/40, 1/80, 1/100 and 1/150 with constant DNA concentration of 5 mM. UV–visible spectroscopic measurements were performed using various CMB concentrations in the range of 0.01–0.06 mM with constant DNA concentration of 0.1 mM.

2.2. FTIR spectroscopic measurements

Infrared spectroscopic measurements of DNA and CMB–DNA complexes were performed using FTIR spectrophotometer (Varian-660 model) equipped with deuterated triglycine sulfate (DTGS) detector and KBr beamsplitter using horizontal attenuated total internal reflection (HATR) crystal. Spectra of DNA and CMB–DNA complexes were collected after incubation of complex for 2 h at room temperature. Interferograms were accumulated in the spectral range of 1800–600 cm^{-1} with resolution of 4 cm^{-1}. All the spectroscopic measurements were performed in triplicate and average were taken. Two hundred fifty six scans were collected to accomplish better signal to noise ratio. Spectrum of Tris-buffer at pH = 7.4 was recorded and then subtracted from the spectrum of free DNA and CMB–DNA complexes to carry out water subtraction [25]. A satisfactory water subtraction is considered to achieve when a flat baseline around 2200 cm^{-1} is produced, where water combination bands are located. This is a rough estimation method for water subtraction, but removes water spectral features in a satisfactory way [25]. Spectrum of calf thymus DNA (13 mM) in buffer and in ethanol (25%) was recorded to ensure that ethanol does not alter B-DNA structure [26].

2.3. UV–visible spectroscopic measurements

The absorption spectra of free DNA and CMB–DNA complex were recorded using Cary 100 Bio UV-visible spectrophotometer. Quartz cuvette of 1 cm path length was used. The binding constant of CMB–DNA complex was calculated by the method described by Kanakis et al. [27]. It was assumed that CMB–DNA complex forms in a ratio of 1:1 [28,29]. On the basis of this assumption, Eqs. (1) and (2) can be established.

$$\mathrm{DNA} + \mathrm{CMB} \rightleftharpoons \mathrm{DNA} : \mathrm{CMB} \tag{1}$$

The equilibrium constant is given by

$$K = \frac{\mathrm{DNA} : \mathrm{CMB}}{(\mathrm{DNA})(\mathrm{CMB})} \tag{2}$$

Eq. (2) can be written as

$$K = \frac{C_{DC}}{[C_D][C_C]} \tag{3}$$

C_{DC}, C_D, and C_C are the analytical concentration of CMB–DNA complex, DNA and CMB in solution respectively.

Beer Lambert law for the absorption of light is assumed to be followed by the ligand substrate binding.

$$C_D = C_{Do} - C_{DC} \tag{4}$$

$$C_{DC} = \frac{A - A_0}{\varepsilon_{DC} \cdot L} \tag{5}$$

and

$$C_{Do} = \frac{A_0}{\varepsilon_D \cdot l} \tag{6}$$

where C_{Do} is the concentration of pure DNA, A_0 and A are the absorbance of pure DNA and in the presence of CMB respectively at 260 nm. ε_D and ε_{DC} are the molar extinction coefficient of DNA and CMB–DNA complex respectively. l is the path length of the cuvette (1 cm).

By putting the values of C_D and C_{DC} from above equations into Eq. (3), following equation can be deduced:

$$\frac{A_0}{A - A_0} = \frac{\varepsilon_D}{\varepsilon_{DC}} + \frac{\varepsilon_D}{\varepsilon_{DC} \cdot K} \times \frac{1}{C_C}$$

The double reciprocal plot of $1/(A - A_0)$ versus $1/C_C$ is linear and the binding constant (K) can be estimated by calculating the ratio of the intercept to the slope [30].

2.4. Circular dichroism spectroscopic measurements

CD spectroscopic measurements were performed with Jasco J812 CD spectrophotometer. Quartz cuvette with a path length of 1 mm was used for spectra collection in the UV region