Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 188 (2018) 244–251

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

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

Deciphering the mechanism of interaction of edifenphos with calf thymus DNA

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ARTICLE INFO

Article history:
Received 15 April 2017
Received in revised form 10 July 2017
Accepted 12 July 2017
Available online 13 July 2017

Keywords:
Pesticide
Edifenphos
DNA interaction
Spectrophotometry
Isothermal calorimetry
Molecular docking

ABSTRACT

Edifenphos is an important organophosphate pesticide with many antifungal and anti-insecticidal properties but it may cause potential hazards to human health. In this work, we have tried to explore the binding mode of action and mechanism of edifenphos to calf thymus DNA (CT-DNA). Several experiments such as ultraviolet-visible absorption spectra and emission spectroscopy showed complex formation between edifenphos and CT-DNA and low binding constant values supporting groove binding mode. These results were further confirmed by circular dichroism (CD), CT-DNA melting studies, viscosity measurements, density functional theory and molecular docking. CD study suggests that edifenphos does not alter native structure of CT-DNA. Isothermal calorimetry reveals that binding of edifenphos with CT-DNA is enthalpy driven process. Competitive binding assay and effect of ionic strength showed that edifenphos binds to CT-DNA via groove binding manner. Hence, edifenphos is a minor groove binder preferably interacting with A-T regions with docking score − 6.84 kJ/mol.

1. Introduction

Edifenphos (O-ethyl S, S-diphenyl phosphorodithioate) (Fig. 1) is one of the most important classes of organophosphorus pesticides due to its wide spectrum antifungal activity. However, it accumulates in various agricultural products, such as vegetables, fruits, and especially rice [1,2]. Moreover, edifenphos (EDF) shows clastogenic and karyo-toxic effects in bone marrow cells in mouse and embryonic shoot meristems in barley [3,4]. Detailed study on the genotoxicity of EDF reveals that it induces statistically significant chromosomal aberrations [5]. In spite of exhaustive research on its mutagenic effects, the actual mechanism of genotoxicity is still not clear.

Interaction of pesticides with DNA has attracted a lot of interest by various groups of investigators because various pesticides have been recognised as potent genotoxicants [6–10]. Several reports have revealed that some pesticides such as fenitrothion, pyriproxyfen, chloridazon and diazinon interact with DNA and cause structural change in DNA [11–15]. However, Ahmadi and Bakshandeh investigated the interaction of DNA with 2, 4-Dichlorophenoxy acetic acid and found significant changes in the structure of [16].

Generally, small molecules bind to DNA under the following three modes of interactions: (i) intercalative binding; (ii) major or minor groove binding involving Vander Waals interactions; and (iii) electrostatic interactions [17,18].

Present work was aimed at deciphering the mechanism of interaction of EDF with CT-DNA employing various techniques such as absorption spectroscopy, emission fluorescence spectroscopy, circular dichroism (CD), isothermal calorimetry, viscosity measurement, DNA melting and molecular docking. This combinatorial approach is expected to provide a broader understanding into the mode of binding of EDF with DNA.

2. Materials and methods

2.1. Chemicals

Edifenphos (EDF, 99.7% pure) and highly polymerized calf thymus DNA (CT-DNA) were purchased from Sigma chemicals, USA. Hoechst 33342 was purchased from Life Technologies, USA. Acridine orange (AO) and ethidium bromide (EtBr) were purchased from SRL.

2.2. Sample preparation

CT-DNA was dissolved in 20 mM sodium phosphate buffer at pH 7.4 and stored overnight at 4 °C with gentle mixing to obtain homogeneity. The purity of CT-DNA was determined by the absorbance ratio A260/A280 which was found in the range of 1.8–1.9. The CT-DNA concentration was calculated by spectrophotometer using molar extinction coefficient of 6600 mol^{-1} cm^{-1} [19]. Stock solution of EDF (10 mM) was prepared in DMSO.

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http://dx.doi.org/10.1016/j.saa.2017.07.014
1386-1425/

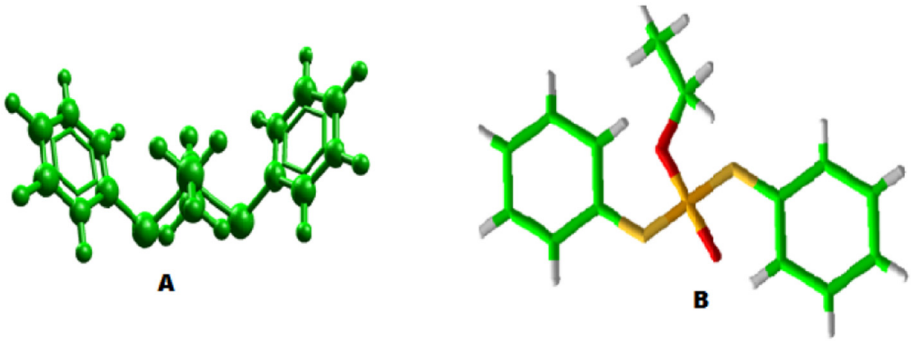

Fig. 1. Ball and stick model for chemical structure of EDF.

2.3. Instrumentation

Ultraviolet-visible absorption spectra were carried out by using a dual beam UV-1800 spectrophotometer (Shimadzu, Japan) using 1.0 cm quartz cell, fitted with a thermostat bath. Fluorescence studies were performed with the use of a 1.0 cm quartz cell on a Shimadzu RF-5301PC spectrofluorophotometer (Japan) equipped with a xenon flash lamp.CD spectra were carried out by using CD spectrophotometer (JASCO-J-815 spectropolarimeter Japan). Further, to eliminate the inner filter effects, the fluorescence data of EDF were corrected according to the equation.

$F_C = F_m{}^{e\,(A1+A2)}$.

Where Fc and Fm are the corrected and measured fluorescence, respectively, and A1 and A2 are the absorbance of the CT-DNA solutions at 225 and 371 nm, respectively.

2.3.1. Ultraviolet-visible spectroscopic method

Ultraviolet-visible absorption spectra were performed on a UV-1800 spectrophotometer (Shimadzu, Japan) using a 1 cm × 1.0 cm cell. The UV-visible absorption measurements of EDF and EDF-CT-DNA complex were measured in the wavelength range of 190–260 nm. Experiments were performed by fixed EDF concentration (20 μM) and titrated with increasing CT-DNA concentrations (0–12 μM). All recorded ultraviolet-visible absorption spectra were corrected for the buffer absorbance.

2.3.2. Fluorescence studies

Fluorescence emission measurements of EDF were carried out by using RF-5301PC spectroflurophotometer (Shimadzu, Japan) fitted with xenon flash lamp using 1.0 cm cell. The fluorescence experiments were measured by fixed EDF concentration (50 μM) and increasing CT-DNA concentration (0–45 μM). Emission spectra of fluorescence were observed in the wavelength range of 230–550 nm while exciting wavelength at 225 nm.

2.3.3. Competitive displacement assay

EtBr displacement assay was carried out with a solution containing concentration of EtBr (3 μM), CT-DNA (50 μM) and varying concentration of EDF. EtBr–CT-DNA complex was excited at 471 nm and emission spectra were observed from 500 to 700 nm. Similarly, Acridine orange and Hoechst 33342 dye displacement assays were carried out under same concentrations of dye and CT-DNA like EtBr. The emission spectra of AO-CT-DNA complex were recorded from 490 to 600 nm with the excitation at 480 nm. However, the emission spectrum and excitation wavelength of CT-DNA-Hoechst 3342 complex were commanded at 400–650 nm and 343 nm respectively.

2.3.4. Effect of ionic strength

Effect of ionic strength was performed by recorded the spectra of EDF-CT-DNA complex and titrated with increasing the concentration of sodium chloride (0–30 mM). The emission and excitation range of wavelengths were recorded from 250 to 500 nm and 225 nm respectively.

2.3.4. Circular dichroism spectroscopy

All CD spectra were performed by using JASCO J-815 CD spectrophotometer. The spectra of CT-DNA and EDF–CT-DNA complex were measured in the range of 220 nm to 320 nm in Tris-HCl at pH 7.4.

2.3.5. Isothermal titration calorimetry

Isothermal titration calorimetry was used to measure binding affinity of EDF with CT-DNA on a model VP-ITC titration microcalorimetry system (MicroCal, Northampton, MA). For ITC measurements, samples (EDF, CT-DNA) and buffer solution were degassed prepared and experiment was carried out at 25 °C with constant stirring speed of 307 rpm. Syring was loaded with 1 mM EDF, sample cell filled with 0.5 mM CT-DNA and buffer contained in the reference cell and then titration was carried out between EDF and CT-DNA.

2.3.6. Viscosity measurement

Viscosity experiments were performed by fixed CT-DNA concentration (120 μM) and increasing the concentration of EDF. For viscosity measurements, an Ubbelohde viscometer (Canon, Model-9721-K56, Coleparmer, USA) with a thermostat was used. The flow time of sample was measured with a digital stopwatch.

2.3.7. DNA melting studies

Mode of interaction between EDF and CT-DNA were further monitored by DNA melting profile. For determination of melting temperature, CT-DNA (30 μM) was conducted in the absence and presence of EDF at different temperature using a spectrophotometer attached with peltier temperature controller thermocouple. Absorbance at 260 nm was then plotted as a function of temperature ranging from 25 to 100 °C and Tm of CT-DNA was determined as the transition midpoint.

2.3.8. Density functional theory (DFT) analysis and molecular docking studies

Density Functional Theory (DFT) is presently the most successful computational method. This theory determines electron density distribution and provides the ground state properties of the molecules [20]. DFT studies were performed for the lowest energy optimized gas phase geometry of EDF applying the GAUSSIAN 03 set of codes [21]. The optimized structure of EDF in ground state was computed by performing DFT (B3LYP) [22,23] with 6-311G, 6-311G (d,p) + basis sets by applying the Berny optimization algorithm under tight convergence criteria. The selected level of theory (B3LYP/6311G + (d,p) has been validated in various studies to be accurate and efficient in predicting geometries, frontier molecular orbital interaction energies of organic compounds [24,25]. In the B3LYP level of theory, Becke's three-parameter was employed for exchange interactions and for electron correlation, the Li-Yang–Parr (LYP) functional was used.

Auto Duck 4.2 software was used to measure docking calculations between EDF and CT-DNA [26]. The PDB structure of DNA duplex