Journal of Pharmaceutical and Biomedical Analysis 149 (2018) 290–295

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Identification of the binding between three fluoronucleoside analogues and fat mass and obesity-associated protein by isothermal titration calorimetry and spectroscopic techniques

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ARTICLE INFO

Article history:
Received 6 July 2017
Received in revised form 27 October 2017
Accepted 1 November 2017
Available online 8 November 2017

Keywords:
Fat mass and obesity-associated protein
Analogues
Isothermal titration calorimetry
Fluorescence

ABSTRACT

In this work, the interactions between fat mass and obesity-associated (FTO) protein and three fluoronucleoside analogues (three-member-ring compound (**1a**), five-member-ring compound (**1b**) and six-member-ring compound (**1c**)) have been investigated by fluorescence, ultraviolet-visible absorption spectroscopy, isothermal titration calorimetric (ITC) and molecular modeling. Analysis of fluorescence data showed that the binding between three analogues and FTO occurred via a static quenching mechanism. Both ITC and fluorescence results indicated that **1b** is the strongest quencher. In contrast to spectroscopy techniques, ITC results suggested that there is no binding for **1c**. ITC results showed that the binding between FTO and **1a** (or **1b**) were exothermic. Fluorescence results showed that the binding between three analogues and FTO were endothermic. Results of thermodynamic analysis and molecular modeling suggested that it was entropy driven event between FTO and **1a** (or **1b**).

1. Introduction

Recent studies [1,2] have unequivocally associated the fat mass and obesity-associated (FTO) gene with the risk of obesity. In vitro FTO protein is an AlkB-like DNA/RNA demethylase with a strong preference for 3-methylthymidine in single-stranded DNA or 3-methyluracil (3-meU) in single-stranded RNA [3]. FTO comprises an amino-terminal AlkB-like domain and a carboxy-terminal domain. Biochemical assays show that these two domains interact with each other, which is required for FTO catalytic activity. Structural information of FTO provides a starting point for the successful development of FTO inhibitors that holds promise for developing therapeutic agents to treat obesity or even diabetes [4]. How the modulation of nucleic acid methylation status by FTO relates to increased body mass has remained elusive. A FTO inhibitor has been synthesised and found to have anticonvulsant activity [5]. The interactions between FTO proteins and small molecules were investigated by fluorescence spectroscopy [6,7]. Currently, nucleoside antiviral drug discovery was one of the most promising areas for developing potential therapeutics for the treatment of chronic hepatitis B [8]. A series of fluoronucleosides had been synthesized in our group [9].

In this work, the interactions between fluoronucleoside analogues and FTO protein were investigated for the first time. Three fluoronucleoside analogues (**1a**, **1b** and **1c**) have different functional groups as shown in Fig. 1. The comparison between fluoronucleoside analogues was performed. These results will be helpful in understanding the effect of analogues in the field of developing new drugs for the treatment of obesity or even diabetes.

2. Materials and methods

2.1. Reagents

FTO protein was obtained from Prof. Jijie Chai (Tsinghua University, Beijing, China). The FTO protein (residues 31-505) was expressed as previously described [10]. Briefly, cells (BL21 (DE3)) were induced for 12 h at room temperature. The cells were then harvested, pelleted, and resuspended. The cells were lysed by sonication, and the lysate was then centrifuged at 14,000 rpm for 1 h. The soluble proteins were first purified using Ni^{2+}-resin (Novagen) and then further by ion exchange (Source-15Q, Pharmacia) and gel-filtration chromatography (Superdex-200, Pharmacia).

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https://doi.org/10.1016/j.jpba.2017.11.007
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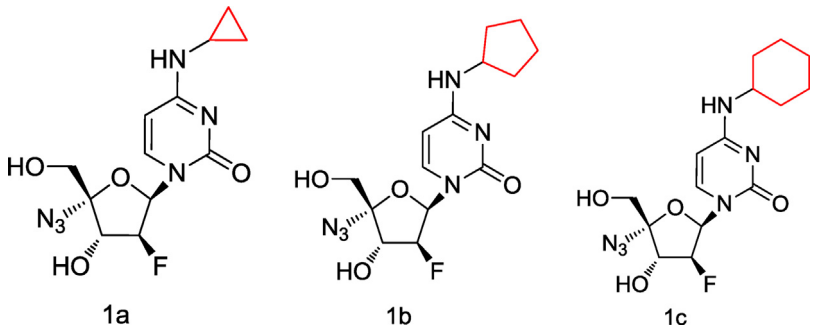

Fig. 1. The chemical structure of three fluoronucleoside analogues.

Protein purification was performed at 4 °C. The stock solution of FTO (3.0×10^{-6} M) was preserved at −80 °C. Fluoronucleoside analogues (**1a**, **1b** and **1c**) were dissolved in dimethyl sulfoxide (DMSO). The concentration of fluoronucleoside analogues was 2.0×10^{-3} M. All chemical reagents were of analytical grade and were used without further purification. Doubly distilled water was used throughout the experiments.

2.2. Absorbance and fluorescence measurements

The UV–vis spectra were recorded on Agilent 8453 UV–vis spectrophotometer equipped with 1.0 cm quartz cells (Agilent, Santa Clara, CA, USA). Fluorescence quenching, synchronous fluorescence and three-dimensional fluorescence spectra were performed on F-4600 fluorescence spectrophotometer (Hitachi High-Technologies Co., Tokyo, Japan) equipped with a 3×10^{-3} dm^{-3} quartz cell. The excitation and emission slit widths were fixed at 5 nm.

A FTO protein solution was titrated by successive addition of nucleoside analogues. To investigate the effect of temperature, experiments were carried out at different temperatures (298, 307 and 315 K). The fluorescence spectra were measured in the range of 290–450 nm. The excitation wavelength was set at 280 nm. Synchronous fluorescence spectra were recorded with $\Delta l = 15$ nm and 60 nm in the absence and presence of analogues. The three-dimensional fluorescence spectra were obtained by scanning excitation wavelength in the range of 210–290 nm, and emission wavelength in the range of 310–390 nm, respectively. The scan speed was 1200 nm min^{-1}. PMT voltage was 700 V.

2.3. Isothermal titration calorimetry

The Isothermal titration calorimetry (ITC) experiments were performed using a MicroCal ITC200 (Microcal Inc., Northampton, MA, USA) at 25 °C. All these solutions were thoroughly degassed prior to the titrations to avoid the formation of bubbles in the calorimeter cell. Purified FTO protein stock solution was prepared in a buffer containing 25 mM HEPES (pH 7.5), 100 mM NaCl, and 10 mM β-mercaptoethanol. Water was in the reference cell. The FTO protein and analogue **1a** (or **1b**, **1c**) solutions were prepared from the stock solution to contain 10% DMSO. In a typical experiment, FTO solution was placed in the sample cell of the calorimeter and analogue solution was loaded into the injection syringe. Fluoronucleoside analogue solution was titrated into the sample cell by means of syringes via 20 individual injections. The amount of each injection was 2.0 μL. The first injection of 0.4 μL was ignored in the final data analysis. After the background dilution heats were subtracted from the experimental data, the titration data were analyzed with the Microcal ORIGIN V7.0 software (Microcal Software, Northampton, MA, USA).

3. Results and discussion

3.1. Absorption spectra

UV–vis absorption technique was used to investigate the interaction between protein and ligand [11,12]. The absorption spectral change of FTO in the absence and presence of analogue was shown in Fig. S1. The sole theoretical absorption spectrum of FTO ([FTO-**1a**]-**1a**) in the presence of analogue **1a** was obtained by subtracting the spectrum of **1a** from that of FTO-**1a** complex. If no interactions occurred between FTO and compound **1a**, the spectrum of [FTO-**1a**]-**1a** and that of FTO should be identical theoretically. However, it was found that the spectrum of FTO was obviously different from the spectrum of [FTO-**1a**]-**1a**. Results indicated that the interactions occurred between FTO and fluoronucleoside analogue (**1a**, **1b** and **1c**).

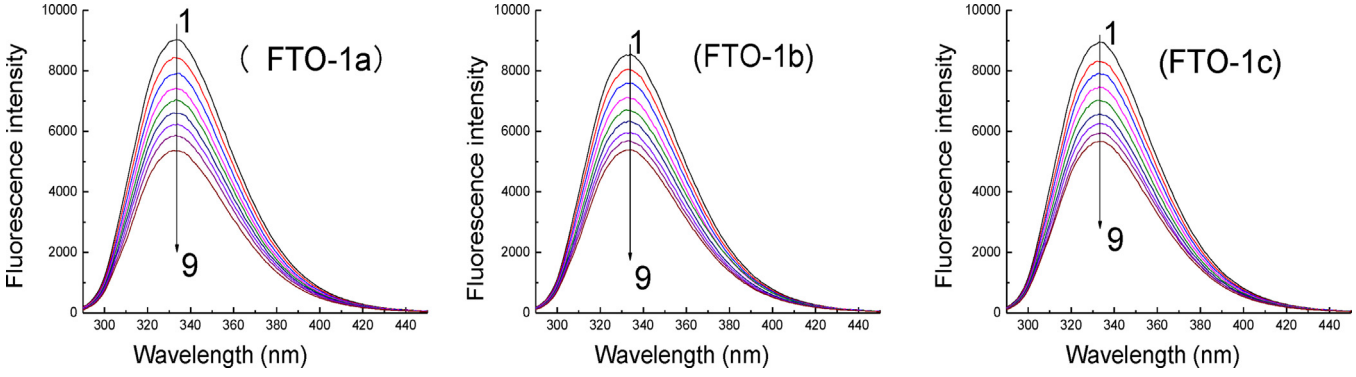

Fig. 2. Effect of **1a** (**1b**, **1c**) on fluorescence spectra of FTO (T = 298 K, pH = 7.4, λ_{ex} = 280 nm). $C_{FTO} = 1.53 \times 10^{-6}$ M. The concentration of **1a** (**1b**, **1c**) is 0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 14.0 and 16.00×10^{-6} M (from top to bottom).