Dyes and Pigments 132 (2016) 405–411

Contents lists available at ScienceDirect

Dyes and Pigments

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

Interaction of hypericin with guanine-rich DNA: Preferential binding to parallel G-Quadruplexes

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ARTICLE INFO

Article history:
Received 22 March 2016
Received in revised form
13 April 2016
Accepted 9 May 2016
Available online 10 May 2016

Keywords:
Hypericin
G-Quadruplexes
DNA
Binding model
Molecular docking

ABSTRACT

Hypericin (Hyp) is one of the principal active constituents of a traditional folk medicine, Hypericum (Saint John's wort), and possesses variety biological activities. The interaction of Hyp with DNA may play an important role in biological activity, however only a few investigations were reported on this issue. In this paper, we studied the interaction of Hyp with different DNA forms. Hyp was found to selectively bind parallel DNA G-Quadruplexes (G4) over other DNA forms, such as ss-/ds-DNA, antiparallel G4s and mix-type G4s. Binding studies suggest that Hyp bind on G-quartet surface of G4s through end-stacking interaction. These results provide new information for the investigation of action mechanism of Hyp.

1. Introduction

Hypericin (Hyp), a natural polycyclic quinone pigment, is one of the principal active constituents of Hypericum (Saint John's wort) that is extensively used in traditional folk medicine (Fig. 1) [1,2]. Hyp has been reported to have variety biological activities, such as antiviral, antiretroviral, anticancer, proteasome inhibition [3], antidepressant, anti-inflammatory and photodynamic/photodiagnostic activities [4]. Because Hyp accumulates preferentially in cancerous tissues, currently it is under research as a promising photosensitizer agent used in anticancer photodynamic therapy and as a dye in cancer fluorescence imaging [4–9]. Although the photosensitive activity of Hyp is considered to result in many bioactivities of Hyp, the real molecular mechanism of action still remains unclear [10]. The investigation of the direct interaction of Hyp with biomacromolecules in cells will provide useful information for understanding the molecular mechanism of Hyp.

The interaction of Hyp with cellular components (membranes, proteins, nucleic acids) has been widely reported [8]. In 1995, Miskovsky and coworkers observed that Hyp reaches the inside of the cell nucleus after a long term incubation [11], which suggests that the interaction of Hyp with DNA may play an important role in a biological activity. In their further investigations by resonance Raman study, Miskovsky et al. proposed that Hyp preferentially interacts with guanine in nucleotide doublets 5′-AG-3′ (stronger interaction) and 5′-GA-3′ (weaker interaction) through the external hydroxyl and carbonyl groups of Hyp [12]. They also reported that the DNA structure plays an important role in the formation of the Hyp/DNA complex. Sequence of poly(rG) showed stronger interaction with Hyp than sequence of poly(rA) [13]. However no further investigation was performed to explore the interaction of DNA and Hyp. Since guanine is the most sensitive to photooxidation under physiological conditions, further investigations are needed to clarify the interaction of Hyp with guanine-rich DNA.

Since guanine-rich nucleic acid sequences can form G-quadruplex (G4) structures, a characteristic higher-order structure that is stacked by planar arrangements of four guanines stabilized by eight Hoogsteen hydrogen bonds (known as G-quartet) [14]. G4 structures adopt a variety of topologies; according to the orientation of the DNA strands, it can be divided into parallel, antiparallel or in some cases mixed type structures. G4 forming sequences are

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http://dx.doi.org/10.1016/j.dyepig.2016.05.009
0143-7208/

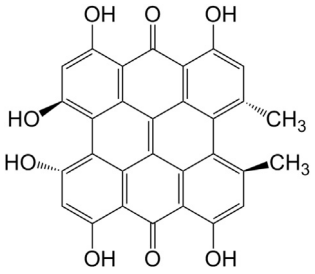

Fig. 1. Chemical structure of Hyp.

ubiquitous distributed in genomes of various organisms, such as telomeres, mitotic and meiotic double-strand break sites, and promoters [15], as well as in particular RNA domains [16]. Increasing evidence suggests the important role of G4s in vivo in the regulation of many physiological and pathological processes [17–23].

Therefore, G4s have been considered as significant targets for drug discovery [24]. Great efforts have been made to design and synthesize G-quadruplex ligands in the past decade. Most of these ligands contain an aromatic planar core that binds to G-quadruplex through π-π stacking interactions and different side chains. Hyp is a polycyclic aromatic phenanthoperylene quinone. This structure has the potential to act as a G4 ligand, because its large π-conjugated core may bind to G4s through π-π stacking interactions, and its multiple hydroxyl and keto groups may interact with adjacent nucleic acid bases and phosphate backbone of G4s.

Based on the above assumption, in this work, we studied the interaction between Hyp and G4s by absorption, fluorescence and circular dichroism spectra. The binding mode was also investigated by inhibition experiment of G4/hemin peroxidase, ^{1}H NMR experiments and molecular docking analysis.

2. Experimental section

2.1. Materials and reagents

Hyp was obtained from Nanjing Spring & Autumn Biological Engineering Co.,Ltd. (Nanjing, China). Hemin was obtained from Beijing XinJingKe Biotechnology Ltd. (Beijing, China). The stock solutions of Hyp (10 mM) and hemin (20 mM) were prepared in DMSO and stored in the dark at −20 °C. ABTS was purchased from Amresco. H_2O_2(30%) and other reagents used were from commercial source without further purification. All solutions were prepared with deionized water purified by a UPHW-90T UP water purification system (Chengdu, China). DNA sequences (Table 1.) were purchased from Sangon Biotech Co., Ltd. (Beijing, China). Stock solutions of DNA sequences (100 μM) were prepared in Tris-HCl buffer (10 mM Tris-HCl, 100 mM NaCl, 20 mM KCl, and 0.1 mM EDTA, pH 7.4) except for 22AG. 22AG was dissolved in either Na^+ buffer (10 mM Tris-HCl, 100 mM NaCl, 0.1 mM EDTA, pH 7.4) or K^+ buffer (10 mM Tris-HCl, 20 mM KCl, 0.1 mM EDTA, pH 7.4). The purity and concentration of the DNA mentioned below were determined by absorption spectra collected on SpectraMax M5 instrument (Molecular Devices). Before testing, all DNA were annealed at 95 °C for 10 min followed by cooling to 4 °C and kept at this temperature overnight. ds-DNA was prepared by heat denaturing and annealing the mixture of ss-DNA1 and ss-DNA2 (1:1).

2.2. UV–visible spectroscopy

The absorption spectra were measured on a UV2550 UV/VIS spectrophotometer (Shimadzu). Absorption spectra of Hyp in mixture of DMSO and Tris-HCl buffer: the final concentration of Hyp was fixed at 4 μM, and the ratio of DMSO/buffer was 5:0, 4:1, 3:2, 2:3, 1:4, and 0:5. Tris-HCl buffer, DMSO and Hyp stock solutions were mixed to a final volume of 400 μL and spectra recorded immediately at room temperature.

Table 1
DNA Sequences used in this study.

Name	Sequence (from 5′ to 3′)	DNA structure
Pu22	TGAGGGTGGGTAGGGTGGGTAA	parallel G4
Pu27	TGGGGAGGGTGGGGAGGGTGGGGAAGG	parallel G4
ckit2	CGGGCGGGCGCGAGGGAGGGT	parallel G4
22AG in Na^+	AGGGTTAGGGTTAGGGTTAGGG	antiparallel G4
22AG in K^+	AGGGTTAGGGTTAGGGTTAGGG	mixed type/hybrid G4
TBA	GGTTGGTGTGGTTGG	antiparallel G4
SPB1	GGCGAGGAGGGGCGTGGCCGGC	antiparallel G4
ss-DNA1	CCAGTTCGTAGTAACCC	single stranded
ss-DNA2	GGGTTACTACGAACTGG	single stranded
ds-DNA	ss-DNA1 + ss-DNA2	double stranded

Absorption spectra of Hyp in the presence of different DNA: 4 μM Hyp mixed with 24 μM different DNA to a final volume of 200 μL. The absorption spectra were measured after mixed with DNA for 3 h at room temperature in dark. Titration experiment: Hyp was fixed at 4 μM, the final concentrations of Pu27 and ckit2 were 4.0, 6.0, 8.0, 10, 12, 14, 16, 18, 20, 22, and 24 μM. The absorption spectra were measured after mixed with DNA for 3 h at room temperature in dark. The apparent binding constants from the spectral titrations were analyzed according to the following equation

$$A/A_0 = 1 + \frac{P-1}{2}\left[M + 1 + x - \sqrt{(M+1+x)^2 - 4x}\right] \quad (1)$$

Where A_0 and A correspond to the absorption intensity of free and DNA-binding Hyp at 605 nm, respectively. $P = A_{max}/(A_0)$, $M = (K_a C_{Hyp})^{-1}$ and $x = nC_{DNA}(C_{Hyp}^{-1})$, and n is the putative number of binding sites on a given DNA matrix. The parameters P and M were found by Levenberg–Marquardt fitting routine in the Origin 8.5 software, whereas n was varied to obtain a better fit.

2.3. Fluorescence spectroscopy

The fluorescence spectra were carried out on a Hitachi F-4600 fluorescence spectrofluorometer (Kyoto, Japan.). Fluorescence spectra of Hyp in mixture of DMSO and Tris-HCl buffer: Hyp were fixed at 4 μM and the ratio of DMSO and Tris-HCl buffer were 5:0, 4:1, 3:2, 2:3, 1:4, and 0:5. Spectra were collected with excitation at 555 nm.

Fluorescence spectra of Hyp in the presence of DNA: 4 μM Hyp mixed with 24 μM different DNA to a final volume of 200 μL. The fluorescence spectra were measured after mixed with DNA for 3 h at room temperature in dark. Titration experiment: Hyp was fixed at 4.0 μM, and the concentrations of ckit2 were 2.0, 4.0, 6.0, 8.0, 10, 12, 14, 16, 18, 20, 22, and 24 μM. The spectra were measured after mixed with DNA for 3 h at room temperature in dark. The titration curves fit well to a 1:1 binding model (Hyp/DNA) and the K_a was calculated according to the following equation

$$F/F_0 = 1 + \frac{Q-1}{2}\left[A + 1 + x - \sqrt{(A+1+x)^2 - 4x}\right] \quad (2)$$

Where F_0 and F_{max} correspond to the fluorescence intensity of free and DNA-binding Hyp, $A = (K_a C_{Hyp})^{-1}$ and $x = nC_{DNA}(C_{Hyp}^{-1})$, and n is the putative number of binding sites on a given DNA matrix. The