Journal of Photochemistry and Photobiology B: Biology 117 (2012) 207–213

Contents lists available at SciVerse ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

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

Molecular aspects on the specific interaction of homoisoflavonoids to DNA

Shohreh Nafisi [a,b,c,*], Roshanak Namdar [a,d]

[a] Department of Chemistry, Islamic Azad University, Central Tehran Branch (IAUCTB), Tehran, Iran
[b] Department of Chemistry, San Jose state University, San Jose , CA
[c] Research Institute for Islamic and Complementary Medicine, Tehran University of Medical Sciences, Tehran, Iran
[d] Young Researchers Club, Islamic Azad University, Central Tehran Branch, Tehran, Iran

ARTICLE INFO

Article history:
Received 26 July 2012
Received in revised form 23 September 2012
Accepted 25 September 2012
Available online 11 October 2012

Keywords:
DNA
Homoisoflavonoids
3-benzylidenechroman-4-ones
FTIR
UV–Visible spectroscopy

ABSTRACT

Homoisoflavonoids (3-benzylidenechroman-4-ones) are related to flavonoids and occur as natural products and exhibit biological activity. These compounds have been reported to possess antioxidant, antifungal, hypocholesterolemic, antimutagenic and antiviral activities. This study was designed to examine the interactions of four synthetic homoisoflavonoids (BMC, BPC, HBC and HBMC) with calf-thymus DNA in aqueous solution at physiological conditions, using constant DNA concentration (6.25 mM) and various homoisoflavonoids/polynucleotide (phosphate) ratios of 1/120, 1/80, 1/40, 1/20, 1/10 and 1/5. Fourier transform infrared (FTIR) and UV–Visible spectroscopic methods were used to determine the ligand binding modes, the binding constants and the stability of homoisoflavonoids–DNA complexes in aqueous solution. Spectroscopic evidence showed major binding of homoisoflavonoids to DNA with overall binding constants of $K_{BMC-DNA} = 9.37(\pm0.34) \times 10^3\ M^{-1}$, $K_{BPC-DNA} = 1.8(\pm0.09) \times 10^4\ M^{-1}$, $K_{HBC-DNA} = 1.3(\pm0.19) \times 10^3\ M^{-1}$ and $K_{HBMC-DNA} = 4.7(\pm0.41) \times 10^3\ M^{-1}$. The affinity of homoisoflavonoids–DNA binding is in the order of BPC > BMC > HBMC > HBC. No biopolymer secondary structural changes were observed upon homoisoflavonoids interaction and DNA remains in the B-family structure in these complexes.

1. Introduction

Homoisoflavonoids (3-benzylidenechroman-4-ones) constitute a small class of natural products isolated from *Eucomis* species such as *E. bicolour*, *E. autumnalis*, *E. punctata*, *Ophiopogan japanicus*, *O. jaburan* [1–6]. These are structurally related to flavonoids and display a wide spectrum of biological activities. These compounds are well known for their anti-inflammatory [7], antiproliferative [8], antifungal [9], antiviral [10], phosphidiesterase isoenzyme-inhibiting [11], antiallergic and antihistaminic [12]. Some natural derivatives display antimutagenic [13,14] and protein tyrosine kinase (PTK) inhibitor activities [15]. They are mainly known for their antioxidant activities [16–18]. Among this category of flavonoids, compounds, for example, sappanone and intricatinol showed high antioxidant activity [17,18].

In an effort to develop novel antioxidants, synthesis of several homoisoflavonoids (E)-3-(3,4-dihydroxybenzylidene)-7-methoxychroman-4-one (BMC), (E)-3-(3,4-dihydroxybenzylidene)-7-propoxychroman-4-one (BPC), (E)-3-(4-hydroxybenzylidene)-7-methoxychroman-4-one (HBMC) and (E) 3-(4-hydroxybenzylidene)-chroman-4-one (HBC) (Fig. 1) have been reported [19,20]. Synthesis of these compounds is based usually on the condensation of 4-chromanones with aromatic aldehydes in the presence of acidic or basic catalyst [21,22].

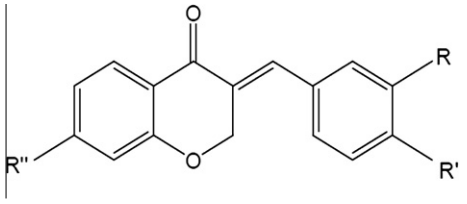
* Corresponding author at: Department of Chemistry, Islamic Azad University, Central Tehran Branch (IAUCTB), Tehran 14676-86831, Iran.
E-mail address: drshnafisi@gmail.com (S. Nafisi).

BMC, BPC and HBMC have been measured for their antioxidant activities. BMC and BPC exhibited excellent antioxidant activity more than HBMC [20]. The high antioxidant strength of these compounds can be assumed due to the presence of ortho-dihydroxy groups (catechol structure) and O-alkyl group bound to C-7 chroman ring [20].

HBC has been tested for its antifungal and antiviral activities in vitro. This compound displayed good activity against the pathogenic fungi *Cryptococcus neoformans* [23]. The experiments on HBC showed low cytotoxicity, and a good antiviral activity against all the Coxsackie viruses studied, and Echo30, which might prove useful as additional antiviral drugs against these virus infections. Furthermore, this compound showed a broad spectrum of activity against both genera of HRVs (rhinoviruses) and EVs (enteroviruses) [10,19,24], and this compound was also found to be potent and selective human monoamine oxidase isoforms B (hMAO-B) inhibitor [25].

Even though much is reported about biological activities of homoisoflavonoids, there is no study on the interaction of homoisoflavonoids with DNA. The aim of this study was to characterize the DNA structural changes in the presence of four synthetic homoisoflavonoids: BMC, BPC, HBC and HBMC. Here, we compared the interactions of these compounds with DNA in aqueous solution at pH 7 with homoisoflavonoids/DNA (P) molar ratios of 1/120–1/5 using FTIR and 0.004–0.1 mM by UV measurements.

1011-1344/$ - see front matter © 2012 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.jphotobiol.2012.09.016



	R	R'	R''
BMC(A)	OH	OH	OMe
BPC(B)	OH	OH	OPr
HBC(C)	H	OH	H
HBMC(D)	H	OH	OMe

Fig. 1. Chemical structure of homoisoflavonoids.

Structural analyses regarding the homoisoflavonoids binding site, binding constant and DNA secondary structure are provided.

2. Materials and methods

2.1. Materials

Highly polymerized type I calf-thymus DNA sodium salt (7% Na content) was purchased from Sigma Chemical (St. Louis, MO) and deproteinated by the addition of $CHCl_3$ and isoamyl alcohol in NaCl solution. To check the protein content of DNA solutions, the absorbance bands at 258 and 280 nm were used. The A_{258}/A_{280} ratio was 2.10 for DNA, showing that DNA samples were sufficiently free from protein [26]. Other chemicals were of reagent grade and used without further purification.

The synthesis of BMC, BPC and HBMC was achieved. The reaction of resorcinol with 3-chloropropionic acid using trifluoromethane sulfonic acid furnished 2′,4′-dihydroxy-3-chloro propiophenone which was cyclized to give 7-hydroxy-4-chromanone. Alkylation of 7-hydroxy-4-chromanone gave 7-alkoxy chroman-4-one. Condensation of 7-alkoxy chroman-4-one with different aryl aldehydes afforded BMC, BPC and HBMC [20]. HBC was synthesized by acid-catalyzed condensation of the appropriate chroman-4-one with substituted benzaldehydes (Purity 99%) [19].

2.2. Preparation of stock solutions

DNA was dissolved to 0.5% w/w (0.0125 M) polynucleotide (phosphate) (pH 7) in NaCl solution for 24 h with occasional stirring to ensure the formation of a homogeneous solution. The final concentration of the DNA solution was determined spectrophotometrically at 258 nm using a molar extinction coefficient ε_{258} = 9250 $cm^{-1}\,M^{-1}$ (DNA) (expressed as molarity of phosphate groups) [27].

Due to low stability of BMC, BPC, HBC and HBMC in water, appropriate amount of BMC and BPC ethanol/water (40/60), HBC and HBMC ethanol/water (10/90) (0.05–12.5 mM) were prepared and added dropwise to DNA solution (homoisoflavonoids–DNA solutions). The alcohol concentration were reduced to half to attain the desired homoisoflavonoids/DNA(P) molar ratios (r) of 1/120, 1/80, 1/40, 1/20, 1/10 and 1/5 with a final DNA(P) concentration of 6.25 mM. At higher concentrations (for HBMC and HBC more than 1/5 and for BMC and BPC more than 1/10), the experiments could not be continued due to DNA gel formation. The pH values of solutions were adjusted at 7.0 ± 0.2 using $NaHCO_3$ solution. The infrared spectra were recorded 2 h after mixing of homoisoflavonoids with DNA solution. For UV measurements, the homoisoflavonoids concentrations of 0.004–0.1 mM were used with a constant DNA concentration of 0.51 mM.

2.3. FTIR spectroscopy measurements

Infrared spectra were recorded on a Jasco FTIR spectrometer (Japan, Tokyo) equipped with a liquid–nitrogen-cooled HgCdTe (MCT) detector and a KBr beam splitter. The spectra of homoisoflavonoids/DNA solutions were taken using a cell assembled with AgBr windows. Spectra were collected and treated using the Spectra Manager software supplied by the manufacturer of the spectrophotometer. The spectra of the solutions were recorded after 2 h incubation of the homoisoflavonoids with DNA solution, using AgBr windows. The bands were measured in triplicates (three individual samples of the same DNA, and homoisoflavonoids concentrations, and the demonstrated spectra were the average of these triplicates). For each spectrum, 100 scans were collected at a resolution of 4 cm^{-1}. The difference spectra [(polynucleotide solution + homoisoflavonoids solution) – (polynucleotide solution)] were obtained using a sharp DNA band at 968 cm^{-1} as internal reference [28,29]. This band, which is due to sugar C–C and C–O stretching vibrations, exhibits no spectral change (shifting or intensity variation) upon homoisoflavonoids–DNA complexation, and cancelled out upon spectral subtraction.

The intensity ratios of the bands due to several DNA in-plane vibrations related to A–T, G–C base pairs and the PO_2 stretching vibrations were measured with respect to the reference band at 968 cm^{-1} (DNA) as a function of homoisoflavonoids concentrations with an error of ±3%. Similar intensity variations have been used to determine the ligand binding to DNA bases and backbone phosphate groups [30].

The plots of the relative intensity (R) of several peaks of DNA in-plane vibrations related to A–T and G–C base pairs and the PO_2 stretching vibrations such as 1714 (guanine), 1661 (thymine), 1608 (adenine), 1490 (cytosine), and 1227 cm^{-1} (PO_2 groups), versus ligand concentrations were obtained after peak normalization using $R_i = I_i/I_{968}$, where I_i is the intensity of the absorption peak for pure DNA in the complex with i as the ligand concentration, and I_{968} is the intensity of the 968 cm^{-1} peak (DNA internal reference).

2.4. Absorption spectroscopy

The absorption spectra were recorded on a LKB model 4054 UV–Visible spectrometer; quartz cuvettes of 1 cm were used. The absorption spectra recorded with ligand concentrations of 0.004–0.1 mM and constant polynucleotide concentration of 0.51 mM. The binding constants of the ligand–DNA complexes were calculated as reported [31]. It is assumed that the interaction between the ligand [L] and the substrate [S] is 1:1; for this reason a single complex SL (1:1) is formed.

The relationship between the observed absorbance change per centimeter and the system variables and parameters is as follows;

$$\frac{\Delta A}{b} = \frac{S_t\, K_{11} \Delta\, \varepsilon_{11}[L]}{1 + K_{11}[L]} \tag{1}$$

where $\Delta A = A - A_0$, from the mass balance expression $S_t = [S] + [SL]$, we get $[S] = S_t/(1 + K_{11}[L])$, Eq. (1) is the binding isotherm, which shows the hyperbolic dependence on free ligand concentration. The double-reciprocal form of plotting the rectangular hyperbola $\frac{1}{y} = \frac{f}{d} \cdot \frac{1}{x} + \frac{e}{d}$, is based on the linearization of Eq. (1) according to the following equation,

$$\frac{b}{\Delta A} = \frac{1}{S_t\, K_{11}\, \Delta\varepsilon_{11}[L]} + \frac{1}{S_t \Delta\varepsilon_{11}} \tag{2}$$

Thus the double reciprocal plot of $1/\Delta A$ versus $1/[L]$ is linear and the binding constant can be estimated from the following equation:

$$K_{11} = \frac{\text{intercept}}{\text{slope}} \tag{3}$$