International Journal of Biological Macromolecules 62 (2013) 405–410

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Studies on the interactions of bioactive quinone avarone and its methylamino derivatives with calf thymus DNA

Miroslava T. Vujčić[a,*], Srđan Tufegdžić[a], Irena Novaković[a], Daniela Djikanović[b], Miroslav J. Gašić[c], Dušan Sladić[d]

[a] Institute of Chemistry, Technology and Metallurgy, Department of Chemistry, University of Belgrade, Njegoševa 12, 11000 Belgrade, Serbia
[b] Institute for Multidisciplinary Research, Kneza Višeslava 1, 11000 Belgrade, Serbia
[c] Serbian Academy of Sciences and Arts, Knez Mihailova 35, Belgrade, Serbia
[d] Faculty of Chemistry, University of Belgrade, Studentskitrg 16, Belgrade, Serbia

ARTICLE INFO

Article history:
Received 15 March 2013
Received in revised form
13 September 2013
Accepted 17 September 2013
Available online 28 September 2013

Keywords:
Avarol
Avarone
3′-(Methylamino)avarone
4′-(Methylamino)avarone
Calf thymus DNA
Fluorescence measurements

ABSTRACT

The interactions of avarone, a quinone from the marine sponge Dysideaavara, and the methylamino derivatives of avarone (**2**), 3′-(methylamino)avarone (**3**) and 4′-(methylamino)avarone (**4**) with calf thymus DNA (CT-DNA) were studied. Agarose gel electrophoreticanalysis showed that binding of the quinones quenched fluorescence of ethidium bromide (EB). The extent of fluorescence quenching of intercalator EB by competitive displacement from EB–CT-DNA system and of groove binder Hoechst 33258 (H) from H–CT-DNA system with the quinones was analyzed by fluorescence spectroscopy. The obtained results demonstrated that the quinones reduced binding of both the intercalator EB and the minor groove binder H, indicating possible degradation of DNA. The substituent on the quinone moiety determined the extent of DNA damaging effect of the quinone, which was the most extensive with 3′-(methylamino)avarone and the least extensive with its regioisomer 4′-(methylamino)avarone. The results were confirmed by the observed hyperchromic effects in UV–visible spectra measured after interactions of the derivatives with CT-DNA.

1. Introduction

Deoxyribonucleic acid (DNA), which contains the genetic instructions for the development and functioning of living organisms is an obvious focus of attraction for many researchers. DNA is the target molecule of many drugs, especially antitumor agents. A large number of low molecular weight molecules, synthetic and natural products or their derivatives recognize DNA and interact with DNA double helix structures in different non-covalent modes: intercalation, groove binding, external electrostatic effects and three-way junction binding [1,2]. Intercalation occurs by inserting a molecule between stacked base pairs thereby distorting the DNA backbone conformation. Minor groove binders cause little distortion of the DNA backbone. When DNA-reactive molecules form complexes with double-stranded helices it results in marked changes in absorbance and fluorescence properties compared to their spectral characteristics when free in solution [3,4]. On the other side, DNA damage may be a result of covalent modification of nucleobases and/or strand cleavage by a variety of oxidizing or alkylating/arylating species such as quinones [5]. Many sesquiterpenoid quinones from marine origin have attracted much interest in recent years due to their interesting pharmacological properties including antitumor, anti-inflammatory, antipsoriasis, and antiviral activity [6]. Avarol is a sesquiterpenoid hydroquinone from the marine sponge *Dysideaavara* and avarone is the corresponding quinone. The antitumor activity of the quinone/hydroquinone couple avarone/avarol (compounds **1** and **2**, Fig. 1) [7] is well known. Biological activity of derivatives of the quinone avarone such as antibacterial action, brine shrimp toxicity and cytotoxicity to tumor cells have been demonstrated recently [8]. Among these compounds methylamino derivatives proved to be of special interest due to their strong activity and difference in effects of regioisomers. Therefore, the methylamino derivatives of avarone (**2**) 3′-(methylamino)avarone **(3)** and 4′-(methylamino)avarone **(4)** were synthesized following the scheme represented in Fig. 1. The study of the binding of these compounds to calf thymus DNA (CT-DNA) would provide an opportunity to understand the selectivity and efficiency of DNA damage by quinone compounds. The damage to CT-DNA by **2**, **3** and **4** was analyzed by agarose gel electrophoresis. The different structural features on the quinone ring may result in different DNA binding modes. The possibilities of the compounds

* Corresponding author at: Institute of Chemistry, Technology and Metallurgy, Department of Chemistry, Njegoševa 12, POB 815, 11001 Belgrade, Serbia. Tel.: +381 11 2636 061; fax: +381 11 2636 061.
E-mail addresses: mvujcic@chem.bg.ac.rs, miravujcic61@gmail.com (M.T. Vujčić).

0141-8130/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijbiomac.2013.09.013

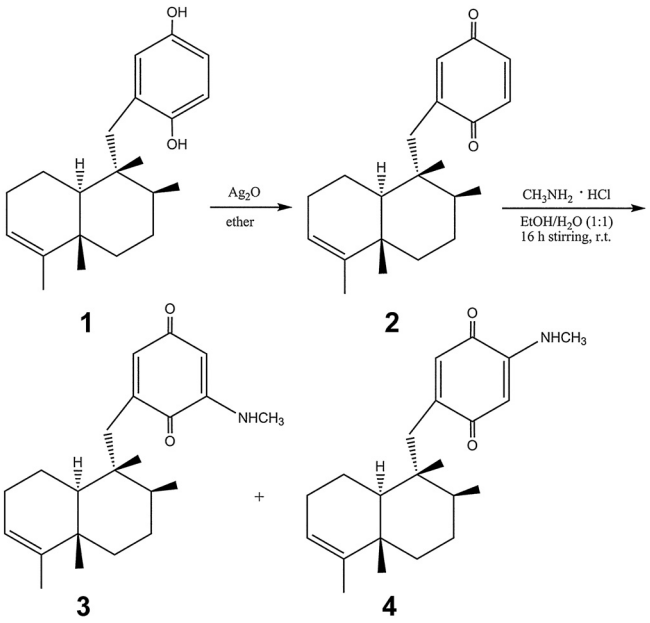

Fig. 1. Scheme for synthesis of avarone and methyl(amino) derivatives; **1** – avarol, **2** – avarone, **3** – 3′-(methylamino)avarone, **4** – 4′-(methylamino)avarone.

to interfere with intercalator of DNA ethidium bromide and with groove binder Hoechst 33258 were investigated by fluorescence spectrometry. In addition, the interaction with CT-DNA was investigated by UV–vis spectroscopy.

2. Experimental

2.1. Chemicals

Avarol **(1)** was isolated from the sponge *Dysideaavara*, collected in the Bay of Kotor (Montenegro) as previously described [9]. Avarone was obtained from its corresponding hydroquinone avarol by oxidation with silver oxide [10]. 3′-(Methylamino)avarone and 4′-(methylamino)avarone were prepared from avarone and methylaminehydrochloride [11]. The supplementary information to this paper provides ^{1}H NMR, ^{13}C NMR and mass spectroscopic data of the compounds (Figs. S1–S6). ^{1}H and ^{13}C NMR spectra were recorded at 200 MHz (Oxford NMR YH) in deuterated methanol and mass spectra were recorded on Mass Spectrometer 6210 Time-of-Flight LC–MS system (Agilent Technologies). Calf thymus DNA (lyophilized, highly polymerized) was obtained from Serva, Heidelberg. Agarose was purchased from Amersham Pharmacia-Biotech, Inc.

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijbiomac.2013.09.013.

2.2. Preparation of reagents

All buffer solutions for reactions of the quinones with DNA were prepared in deionized water and filtered through 0.2 μm filters (Nalgene, USA). Stock solutions (10 mg/mL) of **2**, **3** and **4** were prepared by dissolving the substance in ethanol. Lyophilized calf thymus DNA (CT-DNA) was dissolved in 20 mM Tris-HCl pH 7.5/20 mM NaCl overnight at 4 °C. DNA concentration was adjusted with buffer to 3 mg/mL of CT-DNA (9.24 mM, calculated per phosphate). This stock solution was stored at 4 °C and was stable for several days. A solution of CT-DNA in water gave a ratio of UV absorbance at 260 and 280 nm, A_{260}/A_{280} of 1.89–2.01, indicating that DNA was sufficiently free of protein. The concentration of DNA was determined from the UV absorbance at 260 nm. One optical unit corresponds to 50 μg mL^{-1} of double stranded DNA (based on the known molar absorption coefficient value of 6600 M^{-1}cm^{-1}) [12].

2.3. Electrophoretic analysis

An incubation mixture (1 mL) consisted of 5 μL of stock solution of CT-DNA and 5 μL of the ethanolic quinone solution in 40 mM bicarbonate solution (pH 8.4). Reaction mixtures were incubated at 37 °C for 90 min with vortexing from time to time. To 10 μL of the reaction mixtures were added 5 μL of the loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol FF and 30% glycerol in water). The samples were subjected to electrophoresis on 0.8% agarose gel prepared in TAE buffer pH 8.24 (40 mM Tris-acetate, 1 mM EDTA, pH 8.0). The electrophoresis was performed at a constant voltage (80 V) for about 1.5 h (until bromophenol blue had passed through 75% of the gel). After electrophoresis, the gel was stained for 30 min by soaking it in an aqueous ethidium bromide solution (0.5 μg mL^{-1}). The stained gel was illuminated under a UV transilluminator Vilber-Lourmat (France) at 312 nm and photographed with a Panasonic DMC-LZ5 Lumix Digital Camera.

2.4. Fluorescence measurements

The competitive interactions of quinones and fluorescence probe ethidium bromide with CT-DNA have been studied by measuring the change of fluorescence intensity of each DNA–quinone solution after addition of ethidium bromide. Reaction mixtures containing 92.4 μM of CT-DNA (calculated per phosphate) and different concentrations of the quinone in 1 mL of 40 mM bicarbonate solution (pH 8.4) were incubated for 90 min with occasional vortexing. 1 μL of 1% ethidium solution (25 μM final concentration) was added to each solution, and the incubation was prolonged for the next 30 min and the mixture was analyzed by fluorescence measurement. The control was DNA–ethidium bromide solution. The solutions of quinones did not have fluorescence under applied conditions. Fluorescence spectra were collected using a Fluorolog-3 spectrofluorometer (Jobin Yvon Horiba, Paris, France) equipped with a 450 W xenon lamp and a photomultiplier tube. The slits on the excitation and emission beams were fixed at 4 and 2 nm respectively. The emission spectrum of the solvent was subtracted. All measurements were performed at controlled temperature of 25 °C by means of a Peltier element, by excitation at 500 nm (optimum wavelength) in the range of 520 nm to 700 nm.

The competitive interactions of quinones and fluorescence probe Hoechst 33258 (28 μM at final concentration) with CT-DNA were performed as describedabove for ethidium bromide. The control was CT-DNA–Hoechst 33258 solution. Fluorescence spectra were collected using a Thermo Scientific Lumina Fluorescence spectrometer (Finland) equipped with a 150 W Xenon lamp. The slits on the excitation and emission beams were fixed at 5 nm. All measurements were performed by excitation at 350 nm in the range of 390–600 nm. The details are given in figure legends.

2.5. UV–visible measurement

For an UV–vis measurement, to DNA solution (10 μL of CT-DNA stock solution) was added a small volume of a concentrated solution of quinone (final concentration 300 μM) and the volume was adjusted up 1 mL with 40 mM bicarbonate buffer pH 8.4. Reaction mixtures were incubated at 37 °C during 2 h with occasional vortexing. Spectra of CT-DNA of the same concentrations were also recorded. UV–vis spectra were recorded in a UV Cintra 40 UV/Visible spectrometer operating from 200 to 800 nm in 1.0 cm quartz cells. UV–vis spectroscopic study was not performed with