Journal of Photochemistry and Photobiology B: Biology 120 (2013) 104–110

Contents lists available at SciVerse ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

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

In vitro DNA binding studies of Aspartame, an artificial sweetener

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ARTICLE INFO

Article history:
Received 21 September 2012
Received in revised form 2 January 2013
Accepted 4 January 2013
Available online 11 January 2013

Keywords:
CT-DNA interaction
Artificial sweeteners
Aspartame (APM)
Groove binding

ABSTRACT

A number of small molecules bind directly and selectively to DNA, by inhibiting replication, transcription or topoisomerase activity. In this work the interaction of native calf thymus DNA (CT-DNA) with Aspartame (APM), an artificial sweeteners was studied at physiological pH. DNA binding study of APM is useful to understand APM–DNA interaction mechanism and to provide guidance for the application and design of new and safer artificial sweeteners. The interaction was investigated using spectrophotometric, spectrofluorometric competition experiment and circular dichroism (CD). Hypochromism and red shift are shown in UV absorption band of APM. A strong fluorescence quenching reaction of DNA to APM was observed and the binding constants (K_f) of DNA with APM and corresponding number of binding sites (n) were calculated at different temperatures. Thermodynamic parameters, enthalpy changes (ΔH) and entropy changes (ΔS) were calculated to be +181 kJ mol^{-1} and +681 J mol^{-1} K^{-1} according to Van't Hoff equation, which indicated that reaction is predominantly entropically driven. Moreover, spectrofluorometric competition experiment and circular dichroism (CD) results are indicative of non-intercalative DNA binding nature of APM. We suggest that APM interacts with calf thymus DNA via groove binding mode with an intrinsic binding constant of $5 \times 10^{+4}$ M^{-1}.

1. Introduction

Aspartame (Fig.1) is the name for an artificial, non-saccharide sweetener. APM is the methyl ester of a dipeptide of natural amino acids L-Aspartic acid and L-Phenylalanine. This sweetener is marketed under a number of trademark names, including Equal, NutraSweet and Canderel [1].

APM is found in more than 6000 products, including soft drinks, chewing gum, candy, yoghurt, and some pharmaceuticals such as vitamins and sugar-free cough drops [2].

APM was discovered in 1965 by James M. Schlatter. He synthesized APM in the course of producing an anti-ulcer drug candidate. Schlatter discovered its sweet taste accidentally when he licked his finger, which had serendipitously become contaminated with APM. He postulated the sweetness may be due to a simple molecule of two amino acids [3]. APM is metabolized in the gastrointestinal tract by esterases and peptidases into three components: the amino acids phenylanine, aspartic acid, and methanol [4]. Since APM breaks down into these amino acids in the body, it behaves like a protein, providing an energy value of 4 kcal/g. This energy value is the same as sugar. Nevertheless, APM is used in only very small quantities, so food and beverage manufacturers can advertise their products as "calorie-free" [5]. The aim of this study was to search about the whole APM molecule, because in many studies has been reported that, it is absorbed directly. For example, APM in chewing gum could be absorbed directly through the buccal mucsa of the tongue, mouth and gums, making it a far worse poisoning than even if it was given intravenously [6]. Researchers have been reported that a small proportion of the APM (10–12% of the intake) might be absorbed without metabolization but this result requires more confirmation [7]. In this study, we investigated the interaction of mentioned molecule with DNA, using several spectroscopic methods including: fluorimetry, competition experiment, circular dichroism (CD) and UV absorption techniques.

2. Experimental

2.1. Chemicals and materials

The highly polymerized CT-DNA, Tris–HCl and APM were purchased from Sigma Co.

All solutions were prepared using double-distilled water. Tris–HCl buffer solution was prepared from (Tris-(hydroxymethyl)-amino-methane-hydrogen chloride) and pH was adjusted to 7.4. The stock solution of DNA was prepared by dissolving CT-DNA in 10 mM of Tris–HCl buffer at pH 7.4 and dialyzing exhaustively

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http://dx.doi.org/10.1016/j.jphotobiol.2013.01.001

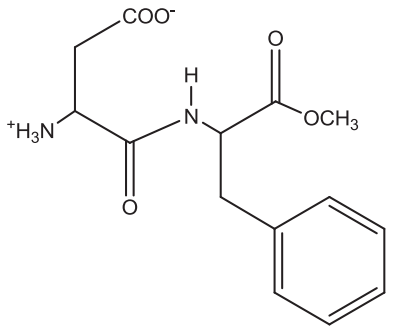

Fig. 1. Molecular structure of APM, (Aspartyl-phenylalanine methyl ester).

against the same buffer for 24 h and used within 5 days. A solution of CT-DNA gave a ratio of UV absorbance at 260/280 nm more than 1.8, indicating that DNA was sufficiently free from protein [8,9]. The concentration of the nucleotide was determined by UV absorption spectroscopy using the molar absorption coefficient (ε = 6600 M^{-1} cm^{-1}) at 260 nm [10]. The stock solution was stored at 4 °C. The APM stock solution (1×10^{-3} M) was prepared by dissolving an appropriate amount of the compound in water.

2.2. Physical measurements and instrumentation

Absorbance spectra were recorded using an HP spectrophotometer (Agilent 8453) equipped with a thermostated bath (Huber polysat cc1). Absorption titration experiments were conducted by keeping the concentration of APM constant (5×10^{-5} M) while varying the DNA concentration from 0 to 2×10^{-4} M (ri = [DNA]/[APM] = 0.0, 0.2, 0.4 ,0.6, 1.0, 1.4, 1.8, 2.0, 2.5, 3.0, 4.0). Equal small aliquots of DNA stock solution were added to both Aspartame and reference solutions to eliminate the effect of DNA absorbance. Fluorescence measurements were carried out with a JASCO spectrofluorimeter (FP 6200) by keeping the concentration of APM constant (5×10^{-5} M) while varying the DNA concentration from 0 to 3.5×10^{-4} M, (ri = [DNA]/[APM] = 0, 1, 2, 3, 4, 5, 6 and 7) at four different temperatures (291, 301, 310 and 318 K). All solutions were allowed to equilibrate for 5 min before measurements were made. The emission spectra were recorded from 260 to 330 nm. Iodide quenching experiments were conducted by adding stoichiometric small aliquots of potassium iodide stock solution to APM and APM–DNA complex solutions, respectively. The fluorescence intensity was recorded, and then the quenching constants were calculated [11]. Monitoring the changes of ionic strength is an efficient method to recognize the binding modes between molecules and DNA [12]. For this purpose, NaCl quenching experiments were conducted by adding stoichiometric small aliquots of sodium chloride stock solution to APM and APM–DNA complex solutions, respectively. The fluorescence intensity was recorded.

CD measurements were recorded on a JASCO (J-810) spectropolarimeter by keeping the concentration of DNA constant (8×10^{-5} M) while varying the APM concentration from 0 to 1.0×10^{-4} M (ri = [APM]/[DNA] = 0.0, 0.2, 0.5, 0.7, 1.0, 1.3, 1.5, and 2.0). In the competitive binding studies, concentrations of DNA and Hoechst 33258 or DNA and methylene blue were kept constant while varying the APM concentration.

3. Results and discussion

DNA is a molecule of great biological significance and controls the structure and function of cells [13]. These important biological activities will be started via receiving a signal to DNA, which is often in the form of a regulatory protein binding to a particular region of the DNA molecule. The binding specificity and strength of this regulatory protein may be imitated by a small molecule; consequently DNA function can be artificially modulated, inhibited or activated by binding this molecule instead of the protein [14]. Thus, this synthetic/natural small molecule can act as an uncontrolled factor. Food additives such as antioxidants, food colorants, and artificial sweeteners. have been extensively applied in recent decades. Accordingly, in our center we have studied the interactions of food additives with DNA. Some studies show that binding can occur between the DNA base pairs (intercalation) [15,16], while some results are indicative of their groove binding nature [17,18]. In this work, in order to compare APM's behavior with other food additives, we studied the interaction of this artificial sweetener with DNA.

3.1. UV/Vis spectroscopic studies

The UV spectra of APM in the absence of DNA showed the maximum absorption at 212 nm (Fig. 2). The change of the UV spectra of APM in the presence of different concentrations of DNA was studied. Different concentrations of DNA solutions, equal to the concentrations of DNA in sample solutions, were used as blank solutions. Hypochromism and red shift in the UV absorption spectra were observed upon addition of DNA increasing concentrations to the APM (5.0×10^{-5} M) solution at 212 nm. These effects are particularly pronounced for intercalators. In the case of groove binders wavelength shift is usually correlated with a conformational change on binding or complex formation [19]. In general, the extent of the hypochromism indicates the interaction binding strength. To further study of Aspartame interaction with DNA, the intrinsic binding constant between mentioned molecule and DNA was calculated; the intrinsic binding constant, K_b for APM with CT-DNA was determined according to the following equation [20]:

$$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/K_b(\varepsilon_b - \varepsilon_f) \quad (1)$$

where [DNA] is the concentration of DNA in base pairs. ε_a is the extinction coefficient for APM absorption band at a given DNA concentration, ε_f is extinction coefficient of free APM , ε_b is the extinction coefficient of APM when fully bound to DNA (it is assumed when further addition of DNA does not change the absorbance). In particular, ε_f was determined by a calibration curve of the isolated APM in aqueous solution, following Beer's law. ε_a was determined as the ratio between the measured absorbance and the APM concentration. Plot of $[DNA]/(\varepsilon_a - \varepsilon_f)$ versus [DNA] gives a slope of $1/(\varepsilon_b - \varepsilon_f)$ and a y-intercept equal to $1/K_b$ $(\varepsilon_b - \varepsilon_f)$; K_b is the ratio of the slope to the y-intercept (Fig.2 inset). The K_b value was calculated to be $5 \times 10^{*4}$ M^{-1}. The K_b value obtained here is less than that of reported for classical intercalator (for ethidium bromide whose binding constants has been found to be in the order of 10^6–10^7 M^{-1}) [21]. In comparing the intrinsic binding constant (K_b) of APM with some DNA groove binders, as observed in the literature, we can deduce that this complex binds to CT-DNA via groove binding [22,23].

3.2. Fluorescence quenching studies

Since luminescence was observed for the APM solution, it is possible to monitor the interaction of APM with DNA by employing direct fluorescence emission methods, so in order to investigate the interaction mode between APM and CT-DNA; the fluorescence titration experiments were performed. APM can emit luminescence with maximum wavelength of about 280 nm in Tris–HCl buffer. The emission intensity of APM decreases in the presence of increasing amounts of DNA (Fig.3).