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Interaction of a copper (II) complex containing an artificial sweetener (aspartame) with calf thymus DNA

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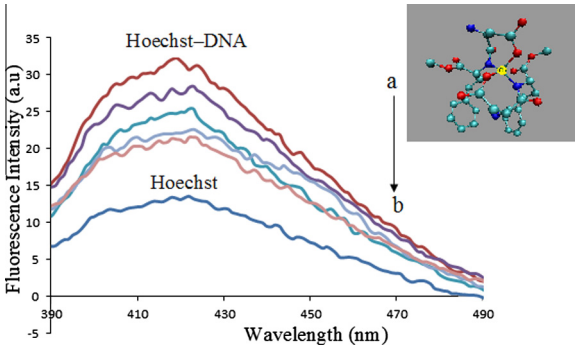
HIGHLIGHTS

- Copper is an essential trace element in plants and animals which binds to aspartame and makes stable complex.
- Aspartam rapidly picks up toxic metals and carries into the body.
- All the results showed that $Cu(APM)_2Cl_2{\cdot}2H_2O$ complex was a DNA groove binder.

GRAPHICAL ABSTRACT

A copper (II) complex containing aspartame (APM) as ligand, $Cu(APM)_2Cl_2{\cdot}2H_2O$, was synthesize and characterized. In vitro binding interaction of this complex with native calf thymus DNA (CT-DNA) was studied at physiological pH.



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ABSTRACT

A copper (II) complex containing aspartame (APM) as ligand, $Cu(APM)_2Cl_2{\cdot}2H_2O$, was synthesized and characterized. In vitro binding interaction of this complex with native calf thymus DNA (CT-DNA) was studied at physiological pH. The interaction was studied using different methods: spectrophotometric, spectrofluorometric, competition experiment, circular dichroism (CD) and viscosimetric techniques. Hyperchromicity was observed in UV absorption band of $Cu(APM)_2Cl_2{\cdot}2H_2O$. A strong fluorescence quenching reaction of DNA to $Cu(APM)_2Cl_2{\cdot}2H_2O$ was observed and the binding constants (K_f) and corresponding numbers of binding sites (n) were calculated at different temperatures. Thermodynamic parameters, enthalpy change (ΔH) and entropy change (ΔS) were calculated to be +89.3 kJ mol^{-1} and +379.3 J mol^{-1} K^{-1} according to Van't Hoff equation which indicated that reaction is predominantly entropically driven. Experimental results from spectroscopic methods were comparable and further supported by viscosity measurements. We suggest that $Cu(APM)_2Cl_2{\cdot}2H_2O$ interacts with calf thymus DNA via a groove interaction mode with an intrinsic binding constant of $8 \times 10^{+4}$ M^{-1}. Binding of this copper complex to DNA was found to be stronger compared to aspartame which was studied recently.

Introduction

Aspartame ($C_{14}H_{18}N_2O_5$) (Fig. 1a) is the methyl ester of the dipeptide of the natural amino acids L-aspartic acid and L-phenyl-

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alanine [1]. The mentioned molecule is an artificial sweetener and widely used in many fields such as medicine and food [2]. There are many reports concerning aspartame (APM) and its harmful effects ranging from brain damage to pre-term delivery [3–5]. APM has carboxylate and amino groups suitable for binding metal ions. Aspartame also chelates the toxic heavy metals from the ingested food and drink, as well as from the very containers from which it is served in, and carries them directly into the body as their highly absorbed chelated heavy metal forms.

Copper is an essential trace element. It is essential to all living organisms and is a universally important cofactor for many hundreds of metalloenzynes. Copper deficiency is widespread and appears in many forms. Copper is required in many physiological functions (RNA, DNA, lysil oxidase cofactor, melanin production (hair and skin pigment), electron transfer of oxygen subcellular respiration, tensile strength of elastic fibers in blood vessels, skin, vertebral discs, etc.) [6,7]. Deficiencies of copper can result in hernias, aneurysms, and blood vessel breakage manifesting as bruising or nosebleeds. The diet must be as low as possible in sweets, fruits and sugars as possible. These foods, along with all stimulants, stress the adrenal glands and tend to make copper imbalance worse. Stimulants include sugars, caffeine and food additives such as aspartame and other excitotoxins in the diet.

Literature review proves the interaction between copper complexes and DNA [8]. There have also been recent developments in the genetic and non-genetic abnormalities of copper [9]. The interaction of aspartame with copper as a biologically significant metal has been studied in [10] where the emphasize is on the pathological mechanism of aspartame interactions.

Deoxyribonucleic acid (DNA) plays an important role in living organisms, such as gen expression, gen transcription, mutagenesis and carcinogenesis. Studies toward the interactions between small molecules and DNA will be valuable for preventing and caring disease. DNA is known to be a major target for drugs and some harmful chemicals to be attacked. The studies of complex – DNA interactions are of current general interest and significance [11,12], particularly, for the designing of new DNA-targeted complex and the experience of these in vitro. During recent years, the interest for metal complexes containing planar aromatic ligands has increased tremendously, mainly for their usage as probes capable to utilize the nucleic acid structures and as DNA-molecular light switches [13].

The above mentioned studies motivated us to investigate the interaction of a copper (II) complex containing APM as ligand, $Cu(APM)_2Cl_2{\cdot}2H_2O$, with DNA. The main goal is to study DNA damage and understand the toxic effect of aspartame in the presence of copper. In this study, different spectroscopic methods including: fluorimetry, competition experiment, circular dichroism (CD), UV absorption and viscometric techniques were considered and the results are compared thoroughly.

Experimental

Material and methods

Aspartame and CT-DNA were purchased from Sigma, Tris—HCl buffer was purchased from Merck. $Cu(APM)_2Cl_2{\cdot}2H_2O$ was prepared according to the method published in [14]. Experiments were performed in Tris—HCl buffer at pH = 7.4. Stock solution of CT-DNA was prepared by dissolving approximately 1–2 mg of CT-DNA fibers in 2 mL Tris—HCl buffer (10 mM), shaken gently and stored at 4 °C for 24 h. Concentration of DNA solution was expressed in monomer units, determined by spectrophotometry to be 260 nm using an extinction coefficient (ε_p) of 6600 $M^{-1}cm^{-1}$. DNA solutions were used after 4 days at most. A solution of CT-DNA in the buffer gave a ratio of UV absorbance at 260 and 280 nm of ~1.8–1.9:1, indicating that the DNA was sufficiently protein-free. Stock solution of $Cu(APM)_2Cl_2{\cdot}2H_2O$ was prepared by dissolving sufficient amount of the complex in 2.0 mL of Tris—HCl buffer (10 mM) (final concentration = 10^{-3} M). Aliquots of the DNA solution were treated with the complex at several input molar ratios (r_i) (r_i = [DNA]/[complex]) with a constant complex concentration. The final volume of samples was made with Tris—HCl (10 mM).

Synthesis of the $Cu(APM)_2Cl_2{\cdot}2H_2O$

The complex (Fig. 1b) was prepared as a mononuclear complex. A solution of $CuCl_2{\cdot}2H_2O$ (85.24 mg, 0.5 mmol) in 20 mL methanol was added to a solution containing APM (290.4 mg, 1 mmol) in 30 mL methanol, and the mixture was stirred at room temperature (RT) for 4 h and then left at RT for 6 h. Resulting solution was stirred at RT for 24 h. The solution was evaporated to dryness. A crude green solid, $[Cu(APM)_2Cl_2{\cdot}2H_2O]$ was obtained.

Elemental Analysis for $C_{28}H_{40}CuN_4O_{13}Cl_2$ Calc C, 43.28; H, 5.19; N, 7.21. Found: C, 42.45; H, 5.19; N, 6.8%.

IR ν_{max}: 3600–2400 Cm^{-1} (hydrogen bond H—N/O); 1738–1420 Cm^{-1} (symmetric and anti symmetric C=O stretching);

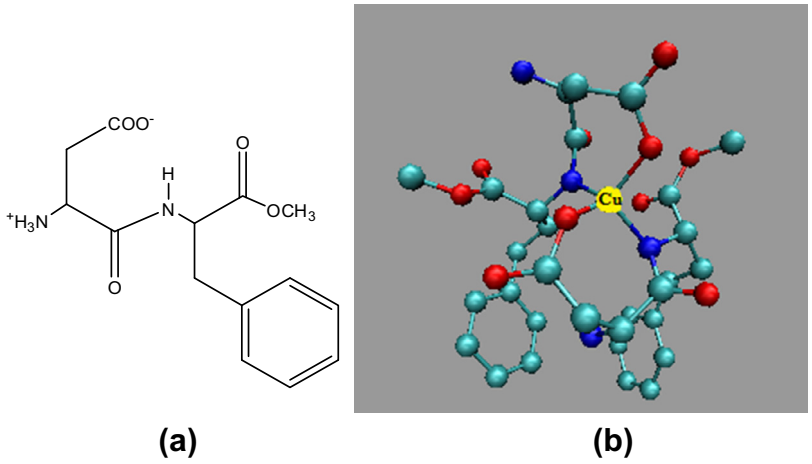

Fig. 1. (a) Molecular structure of APM and (b) molecular structure of $Cu(APM)_2Cl_2{\cdot}2H_2O$.