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INVESTIGATION OF DNA BINDING AND CLEAVAGE ACTIVITIES OF BORON COMPLEXES IN THE PRESENCE OF HYDROGEN PEROXIDE

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ABSTRACT. Magnesium [di(hydroxy)mono(citratoborate)]dihydrate ($Mg[B(Cit)(OH)_2]_2{\cdot}2H_2O$), Lithium [di(hydroxy)mono(citratoborate)]hydrate $Li[B(Cit)(OH)_2]{\cdot}H_2O$, Sodium [di(hydroxy)mono(salicylato) borate)hydrate $Na[B(Sal)(OH)_2]{\cdot}H_2O$ and Magnesium[bis(salicylatoborate)]decahydrate $Mg[B(Sal)_2]_2{\cdot}10H_2O$) complexes have been synthesized and characterized. The cattle genomic DNA (CG-DNA) interaction of the complexes was studied by spectroscopic methods, viscosity, and electrophoresis measurements. The complexes partially intercalated to CG-DNA. Furthermore, DNA cleavage activity of these complexes was also investigated using agarose gel electrophoresis and the complexes show moderate ability of cleavage to the DNA.

Keywords: *Boron complexes, DNA-binding cleavage, DNA cleavage*

INTRODUCTION

Cancer is still most common cause of death and may grow into the most common disease in the future in spite of remarkable therapeutic achievements [1]. Thereby, the discovery and development of new therapeutic agents have a crucial importance. Drug researches propose that a lot of anticancer, antiviral and antiseptic compounds act through binding to DNA [2–4], because the interaction between small molecules and DNA can usually

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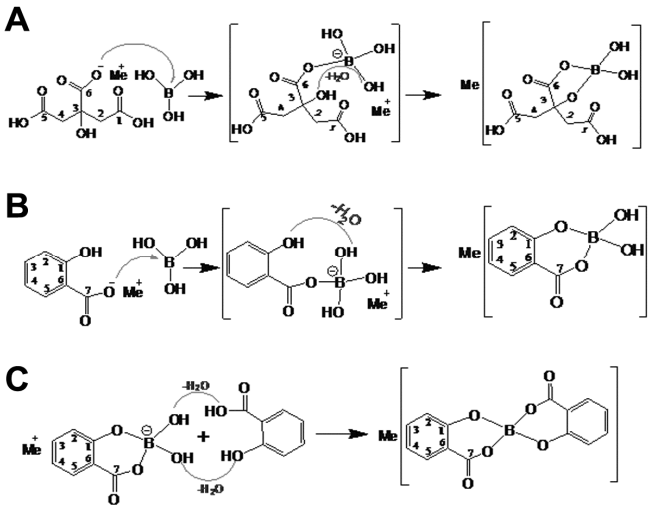
cause DNA damage in cancer cells, blocking cell division and resulting in cell death [5–7]. Compounds can interact non-covalently with DNA by intercalation, groove binding or external electrostatic binding [8]. Many significant applications of the compounds demand that they could attach to DNA in an intercalative mode [9].

Boron complexes of inorganic and organic molecules have important pharmacological properties, such as hypolipidemic, anti-inflammatory, anti-osteoporosis, and antineoplastic activities [10, 11]. Nowadays, there is interest in the synthesis of boron complexes with potential use for the treatment of some types of malignant cancers, such as melanoma and glioblastoma multiform brain tumors [12]. The synthesis of boron complexes of biomolecules like amino acids, peptides, nucleosides is one of the main fields of cancer research [13]. Actions of boron molecule include arthritis alleviation, bone growth and maintenance of central nervous system function, hormone facilitation, immune response, inflammation, and oxidative stress modulation were previously reported [14]. There is a significant literature supporting the application of artificial DNA binding and cleaving agents in biotechnology. Compounds showing the properties of effective binding as well as cleaving double stranded DNA under physiological conditions are of great importance since these could be used as diagnostic agents in medicinal and genomic research. Therefore, it is obvious that the nature of the ligand plays significant roles in their interaction with DNA [15–19].



Scheme 1. *(A) Proposed complexation mechanism of boric acid with citric acid, Me=Mg or Li, (B) Proposed mechanism for the complexation of boric acid with salicylic acid, Me=Na, (C) Me=Mg.*

In this study, DNA binding and interaction activities of synthesized [20] complex 1 ($Mg[B(Cit)(OH)_2]_2 \cdot 2H_2O$) (Scheme 1A.), complex 2 ($Li[B(Cit)(OH)_2] \cdot H_2O$) (Scheme 1A.), complex 3 ($Na[B(Sal)(OH)_2] \cdot H_2O$) (Scheme 1B.) and complex 4 ($Mg[B(Sal)_2]_2 \cdot 10H_2O$) (Scheme 1C.) were investigated with the cattle genomic DNA (CG-DNA) to demonstrate their ability to bind and cleave the DNA [21] by UV absorption, agarose gel electrophoresis, and viscosity measurement methods.

RESULTS AND DISCUSSION

DNA Binding and Electronic Absorption Spectra

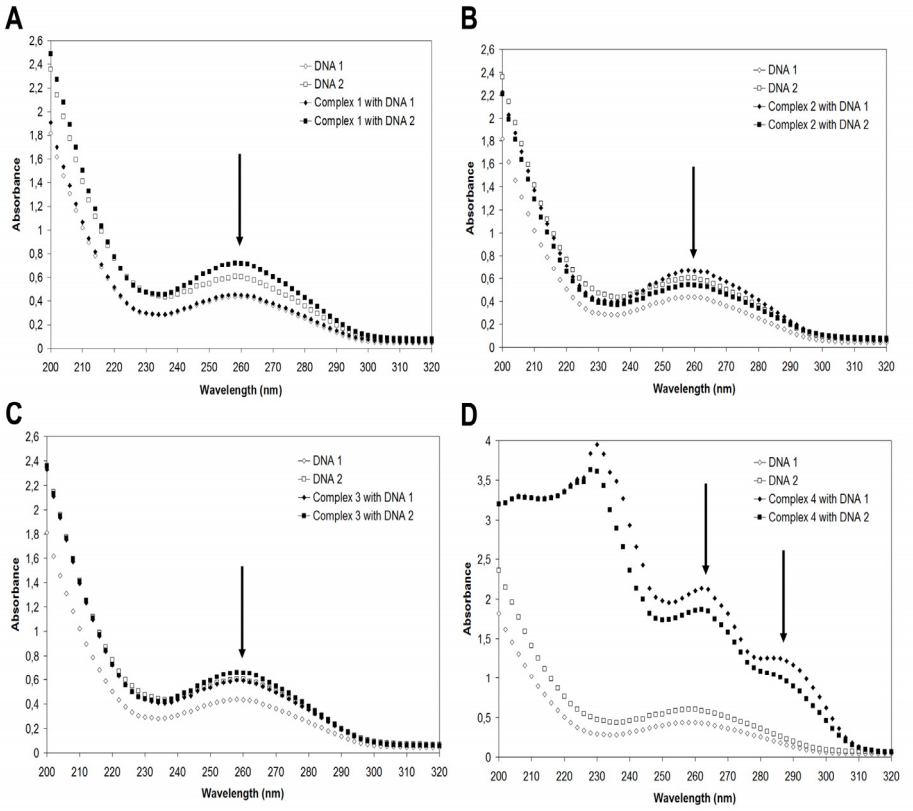


Figure 1. (A) Electronic absorption spectra of complex **1** (100 µM), (B) complex **2** (100 µM), (C) complex **3** (100 µM) and (D) complex **4** (100 µM) in the absence and presence of increasing amounts of the cattle genomic DNA (CG-DNA) (DNA 1 (11.25 µg) and DNA 2 (22.50 µg). Arrows show the changes in absorbance with respect to an increase in the DNA concentration

The absorption spectra of CG-DNA in the absence and presence of the complexes are shown in Fig. 1A-D. On the addition of CG-DNA, hypo and hyperchromism effect were observed. The absorption bands of $Mg[B(Cit)(OH)_2]_2{\cdot}2H_2O$, $Li[B(Cit)(OH)_2]{\cdot}H_2O$, $Na[B(Sal)(OH)_2]{\cdot}H_2O$ and $Mg[B(Sal)_2]_2{\cdot}10H_2O$ complexes at around 260 nm show hypochromism. In fact, the hypochromic impact, characteristic of intercalation, is generally connected to the interaction between the electronic states of the complexes and those of DNA bases [22, 23]. The observed spectroscopic shifts suggest that the complex molecules have interaction with DNA.

DNA Cleavage Activities

The interaction of the cattle genomic DNA (CG-DNA) with $Mg[B(Cit)(OH)_2]_2{\cdot}2H_2O$, $Li[B(Cit)(OH)_2]{\cdot}H_2O$, $Na[B(Sal)(OH)_2]{\cdot}H_2O$ and $Mg[B(Sal)_2]_2{\cdot}10H_2O$ complexes was investigated in order to specify the DNA cleavage efficiencies of these complexes. The aim was succeeded by observing through agarose gel electrophoresis of CG-DNA. After 3 h incubation at 37 °C, in the concentration range of 5, 0.5, 0.05 mM complexes, the results of the experiments implemented and DNA bands are shown in Fig. 2.

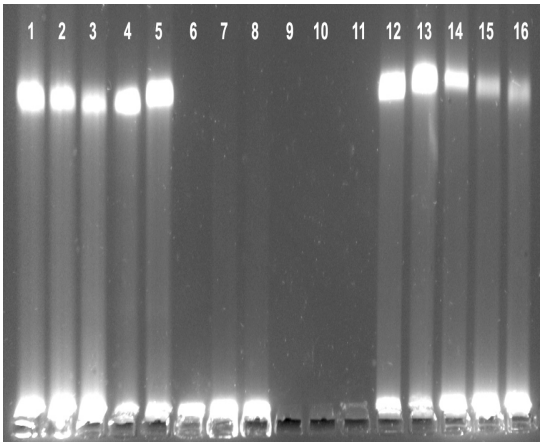


Figure 2. DNA cleavage by the complexes 1, 2, 3, and 4 obtained using agarose gel electrophoresis. The chemical activity of the cattle genomic DNA (CG-DNA) incubated at 37 °C for 3 h with varied concentrations of the complexes in the presence of H_2O_2 as an oxidizing agent: lane 1, 2, and 3, CG-DNA + H_2O_2 (60 mM) + complex 1 (5, 0.5 and 0.05 mM); lane 4, control CG-DNA; lane 5, CG-DNA + H_2O_2 (60 mM); lane 6, 7 and 8, CG-DNA + H_2O_2 (60 mM) + complex 2 (5, 0.5 and 0.05 mM); lane 9, 10, and 11, CG-DNA + H_2O_2 (60 mM) + complex 3 (5, 0.5 and 0.05 mM); lane 12, control CG-DNA; lane 13, CG-DNA + H_2O_2 (60 mM); lane 14, 15 and 16, CG-DNA + H_2O_2 (60 mM) + complex 4 (5, 0.5 and 0.05 mM)

Nevertheless, a raise in concentration of the complexes resulted in increased DNA cleavage. It was found that complex 1 and 4 have a moderate DNA cleavage ability than either complex 2 and 3. The results show that complex 2 almost can slightly promote DNA cleavage at given complex concentrations and complex 3 has the most cleavage activity than other three complexes because DNA bands disappeared completely in the presence of complex 3 (Fig. 2 lanes 9–11). The complexes 1, 2, 3 and 4 show cleavage potential because of the increased reaction of complexes with H_2O_2, thereby creating hydroxyl radicals or molecular oxygen, both of which have ability of damaging DNA [24, 25].

Viscosity Experiments

Optical photophysical studies give important, but not provide enough evidence to promote a binding model. The complexes bind particularly in DNA grooves with partial non-classical intercalation, under the same conditions, generally lead to negative or no shift in DNA solution viscosity [26, 27]. In order to verify the binding mode of the complexes 1, 2, 3 and 4 with CG-DNA, the viscosity measurements of the complexes with CG-DNA were carried out by varying the concentration of complexes. The effect of $Mg[B(Cit)(OH)_2]_2{\cdot}2H_2O$, $Li[B(Cit)(OH)_2]{\cdot}H_2O$, $Na[B(Sal)(OH)_2]{\cdot}H_2O$ and $Mg[B(Sal)_2]_2{\cdot}10H_2O$ complexes on the viscosity of DNA is shown in Fig. 3.

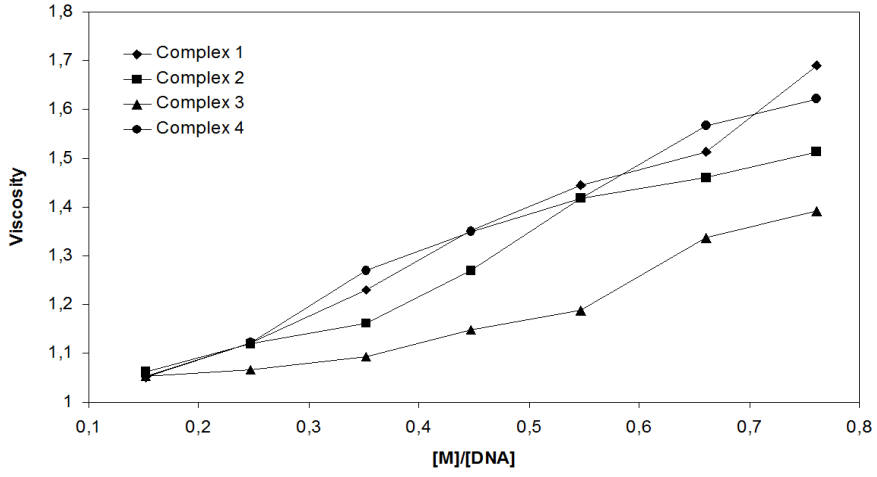


Figure 3. Effect of increasing concentrations of the complexes on the relative viscosity of CG-DNA at 25 °C

The results of viscosity measurements obviously show that all complexes have an intercalative intercation between next to DNA base pairs and it led to an extension in the DNA helix and also increased the viscosity of DNA with a raising concentration of the complexes. The viscosity of DNA is slightly increased with the increase of the concentration of the complexes 1, 2, 3 and 4, in contrast to that of proven DNA intercalator ethidium bromide (EtBr). Based upon all the spectroscopic studies together with the viscosity measurements, it was found that the boron complexes can bind to CG-DNA through an intercalative interaction.

CONCLUSIONS

In conclusion, the citric acid and salicylic acid-based ligands and their boron (boric acid) complexes have been prepared and fully characterized. In agreement with UV-Vis absorption and viscosity measurements, there is an intercalative interaction between the complexes and CG-DNA. DNA cleavage studies reveal that entire four complexes have the capability to cleave the DNA.

EXPERIMENTAL SECTION

Synthesis of $Mg[B(Cit)(OH)_2]_2{\cdot}2H_2O$, $Li[B(Cit)(OH)_2]{\cdot}H_2O$ complexes

The molecular structure of citric acid provides various coordination sites for complex formation with metals. Controlling the molar ratios, mono-chelate complexes were prepared in this work. As for the *bis*-chelate complexes, binding of citrate ligands was suggested to occur through the carboxylate group and the nearest OH group forming five-membered rings. Mono- and bis-chelates of citric acid with boron were prepared in salt form with Mg and Li ions. The compounds were obtained as crystallites and found to be stable at room conditions. The compositions and melting points of the products are summarized in Table 1 [20].

Table 1. Chemical compositions[a] and melting points of citrate-borate complexes

Compound	M.p.(ºC)	C(%)	H(%)	H_2O(%)
Citric acid	153	37.46(37.48)	4.14(4.17)	-
$Li[B(Cit)(OH)_2].H_2O$	95	32.14(31.89)	3.54(4.13)	7.50(8.00)
$Mg[B(Cit)(OH)_2]_2{\cdot}2H_2O$	115	27.49(27.63)	4.42(4.60)	7.80(7.00)

[a] Calculated in parentheses (Cit=$C_6H_6O_7$)

FTIR Spectra of Boric acid Complexes with Citric Acid

FTIR spectral data of boric acid-citrate complexes are given in Table 2. In the spectra of the complexes, in addition to the -OH stretching vibrations of the acidic, alcoholic groups and water molecules at 3600-3200 cm^{-1}, ν_a(C=O) of non ionized carboxylic acid groups (≈1740 cm^{-1}); ν_a(COO) (≈1590 cm^{-1}) andν_s(COO) (1350 cm^{-1}) of carboxylate groups were observed. Asymmetric B-O vibrations were observed around 1100 $cm^{-1}$and the specific tetrahedral B-O band appeared in the range of 825 cm^{-1}– 784 cm^{-1} [20].

Table 2. summary of the FT-IR spectral data of citric acid-borate complexes

Compound	ν(O-H)	ν_a(C=O)	ν_aCOO	ν_sCOO	ν_a(B-O)/BO_4 & ν(C-O)	ν_s(B-O)/BO_4
Citric acid	3496, 3293	1739, 1686	_	_	1252	_
Borax	~3300	_	_	_	1220	834,815 doublet
$Li[B(Cit)(OH)_2]\cdot H_2O$	3503	1715	1597	1347	1111, 1060	835, 787
$Mg[B(Cit)(OH)_2]_2\cdot 2H_2O$	3429	1728	1571	1356	1110, 1073	820, 776

Synthesis of $Na[B(Sal)(OH)_2]\cdot H_2O$ and $Mg[B(Sal)_2]_2\cdot 10H_2O$) complexes

Mono- and bis-chelates of salicylic acid with boron were prepared in salt form with Na and Mg ions. The compounds were obtained as white crystallites and found to be stable at room conditions. X-ray quality crystals were obtained only for the 1:1 complex with Na and for the 1:2 complex with Mg. The compositions and melting points of the products are summarized in Table 3 [20].

Table 3. Chemical compositions[a] and melting points of salicylic acid-borate complexes

Compound	M.p.(ºC)	C(%)	H(%)	H_2O(%)
$Na[B(Sal)(OH)_2]\cdot H_2O$	140	38.44(37.83)	3.75(3.60)	8.3(8.1)
$Mg[B(Sal)_2]_2\cdot 10H_2O$	110	43.84(44.66)	3.99(4.52)	22.3(21.6)

[a] Calculated in parentheses (Sal=$C_7H_4O_3$)

FTIR Spectra of Boric Acid-Salicylic Acid Complexes

One of the most significant changes is the narrowing in the O-H stretching band on going from salicylic acid itself (a) to the complexes. The intermolecular hydrogen bonds formed by the carboxylic acid and OH groups of salicylic acid were cleaved in the complexes since these groups were involved in esterification reaction. The disruption of the hydrogen bonded network resulted in a narrowing in the O-H stretching band. Some important shifts and intensity changes, due to complexation with boron, in ν(CO), $\nu(CC)_{arom}$ vibrations of salicylic acid and ν_a(COO)andν_s(COO) of sodium salicylate, are summarized in Table 4. There are also changes in the δ_{OH} vibrations around 1600 cm^{-1} due to the participation of the phenol group in esterification with boron, however these changes are difficult to clarify due to the overlapping ν(CC) and ν_a(COO) vibrations in the same region. The characteristic vibrations of B-O bond in the tetra hedral boron complexes in the range 900-1000 cm^{-1}were also overlapped with the ν(C-O-) band [20].

Genomic DNA Isolation

Peripheral blood samples (9 ml) of healthy, male, Holstein calves at 6 months of age were collected from *vena jugularis* in EDTA containing blood tubes and CG-DNA were extracted using Wizard® Genomic DNA Purification kit (Promega, Medison WI, USA). The amounts and purity (260/280 nm ratio) of extracted DNA samples were detected on a microplate spectrophotometer (Epoch, BioTek, Vermont, USA) and 250 ng/µl concentration was used in DNA binding experiments. The concentration of DNA was determined by absorption spectroscopy using the molar absorptivity of 6600 M cm^{-1} at 260 nm [28].

Table 4. A summary of the FT-IR spectral data of salicylic acid-borate complexes

Compound	ν(O-H)	ν(CO) &ν(CC)	ν_a(COO) &ν_s(COO)	ν(C-O-) & ν_a(B-O)/BO_4	ν_s(B-O)/BO_4
Salicylic Acid	3350-2400	1685s, 1613		_	_
Na-salicylate		1597	1583, 1376	1250w	_
Borax	~3300	_	_	1220s	834+815 doublet
$Na[B(Sal)(OH)_2]\cdot H_2O$	3700-3000 (3436,3068)	1687s	1613s, 1351s	260-900 sharp (max. at 1150)	753, 695
$Mg[B(Sal)_2]_2\cdot 10H_2O$	3700-2800 (3424,3070)	1662vs	1611s, 1362m	1200-900,b (max. at 1144)	753, 698

DNA Binding Experiment

In DNA binding experiments, in order to adjust the desired concentrations, the complexes were dissolved in sterile deionized ultrapure water. The extracted CG-DNA (11.25 and 22.50 µg) was mixed with a solution of the complex (100 µM) at a fixed concentration and UV-Vis spectra were obtained using the microplate spectrophotometer in 96-well UV microtiter plates (Thermo Scientific) [21, 29, 30].

DNA Cleavage Experiments

The level of DNA cleavage was observed by agarose gel electrophoresis. A solution containing 25 µl of CG-DNA (1.67 ng/µl), the complexes (5, 0.5 and 0.05 mM), and H_2O_2 (60 mM) was incubated at 37 °C for 3 h. The gel electrophoresis experiment was conducted at 75 V for 3 h in 0.7% (w/v) agarose gel containing 0.5× TBE buffer. After electrophoresis, the gel was stained with 3× GelRed (Biotium) fluorescent nucleic acid dye in 0.5× TBE buffer for 30 min by shaking and visualized using Doc EZ gel imaging system (Bio-Rad) [31].

Viscosity measurements

Viscosity measurements were carried out using an Ubbelodhe viscometer, which was immersed in a thermostatic water-bath that kept at a constant temperature at 25 °C. The compounds were added into CG-DNA solution presented in the viscometer. The flow time of each compound was measured by a digital stopwatch. Data were shown as $(\eta/\eta_o)^{1/3}$ vs. binding ratio [32], where η and η_o were the viscosity of DNA in the presence and absence of the complexes. Viscosity values were calculated from the observed flow time of CG-DNA containing solutions corrected from the flow time (t_o), $\eta = t\text{-}t_o$ [32–34].

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