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DNA interaction with zinc(II) ions

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ABSTRACT

We focused on interactions of Zn(II) with DNA in this study. These interactions were monitored using UV/vis spectrophotometry and gel electrophoresis. Firstly, we isolated and amplified 498 bp fragment of DNA. Samples were obtained by incubation of DNA fragment with Zn(II) for 60 min at 25 °C. After incubation, the samples were dialyzed and analyzed immediately. In this way, DNA was converted into a metal bound DNA (Zn–DNA). Interaction of Zn(II) with DNA caused change in the absorption spectrum (190–350 nm) and decrease in the melting temperature (Tm) of Zn–DNA. Spectrophotometric (UV/vis) analysis showed that increasing concentrations of zinc(II) ions led to the increase in the absorbance at 200 nm and decrease in absorbance at 251 nm. Application of zinc(II) ions at 5.5 μM concentration caused decrease in Tm for app. 7.5 °C in average in comparison with control (75.5 ± 3 °C). The lowest melting temperature (60.5 ± 2.5 °C) was observed after application of zinc(II) ions at 33 μM concentration. Gel electrophoresis proved significance of Zn(II) in the renaturation of DNA. Samples of Zn–DNA (15 μM DNA + 5.5–55 μM Zn(II)) caused significant changes in the renaturation of DNA in comparison with the control, untreated DNA (15 μM DNA).

1. Introduction

Zinc is a biogenic element that plays an important role in an organism. In addition to the fact that zinc is involved in the synthesis of proteins and DNA [1], it has been shown to be essential to stabilize the structure and thus the function of a large number of biomolecules including enzymes. It has been established that zinc is involved in more than 300 enzymatic reactions [2,3]. It occurs in all tissues and fluids in relatively high concentrations. Almost 85% of total zinc occurs in muscle and bone tissues, 11% in the skin and the liver and the rest of zinc is distributed in other tissues. The average amount of zinc in a body of an adult is about 1.4–2.3 g [4,5]. Zinc is, after iron, the second most widely occurred transition metal in organisms [6]. Zinc(II) ions are transported into cells via zinc transporters, especially ZiP1 [7]. Zinc is found intracellularly in the eukaryotic organisms; 40% of total zinc is located in the nucleus, 50% in the cytoplasm, organelles, and specialized vesicles called zincosomes and the rest of zinc is bound in the cell membrane [8]. The concentration of zinc is relatively high in nucleus. It has been shown that zinc is involved in the processes of gene expression and the maintenance of gene stability by different ways. It stabilizes the structure of chromatin and thus affects the replication of DNA. In addition, zinc regulates transcription of RNA through the regulation of an activity of transcription factors and some enzymes, such as RNA and DNA polymerases [9].

The structural role of zinc(II) ions is accentuated in the stabilization of zinc finger motif [10]. It is an amino acid sequence, linked to zinc ion(II) to form a secondary structure in the shape of a finger [10]. Proteins with this motif have high affinity to DNA, thus, zinc finger directly mediates an interaction with DNA in the major groove [11]. Zinc is the main component of the zinc finger proteins, which represent the largest and the most diverse superfamily of nucleic acid-binding proteins and which play important roles in the regulation of transcription in the cellular metabolic network [12,13].

Besides structural protein, zinc(II) ion plays important role also in synthesis of metal pool maintainers called metallothioneins [14–16]. The increased amount of zinc(II) ions causes an increased expression of apo-metallothionein (apo-MT) via metal-regulatory transcription factor 1 (MTF-1). Apo-MT is able to bind up to seven atoms of Zn(II), which can likely be transported into the nucleus through the nuclear pores (NPC) (Fig. 1). MTF-1 is reversibly bound to target DNA, thus, the binding depends on changes in an availability of free Zn(II) in the cytoplasm [17–19]. The binding of MTF-1 to

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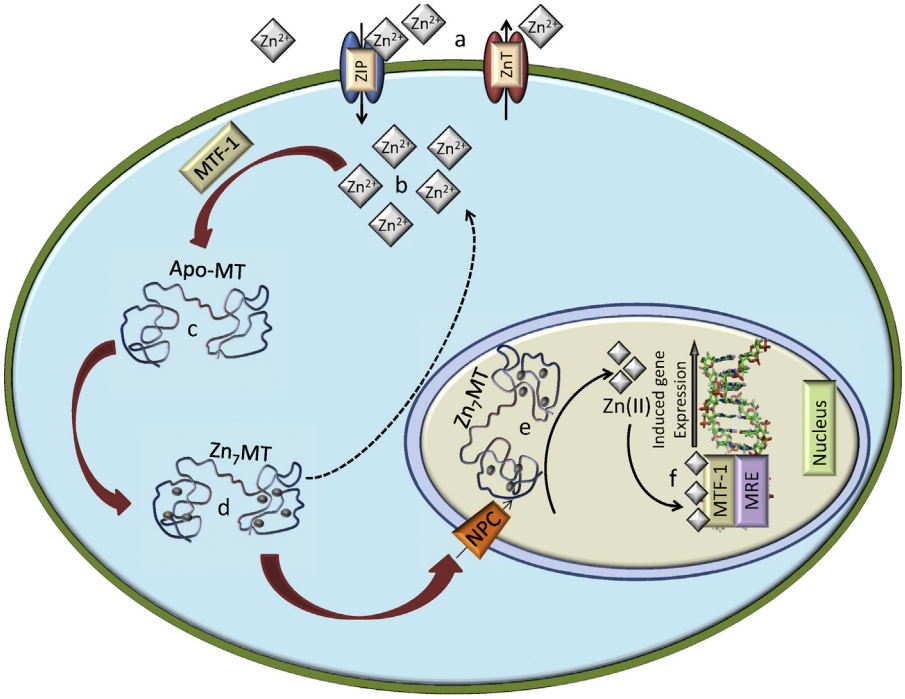

Fig. 1. Suggestion of an effect of Zn(II) in an eukaryotic cell: (a) entry of Zn(II) into the cell via ZIP transporter and exclusion of Zn(II) from the cell via ZnT transporter; (b–c) increased concentration of Zn(II) enhances expression of Apo-MT via metal regulatory transcription factor 1 (MTF-1); (d) Apo-MT binds 7 atoms of Zn(II), which can be transported in this complex into the nucleus via nuclear pores; (e) Zn(II) bound in MT (Zn7MT) may be released in the nucleus; (f) Interaction of Zn(II) with DNA, which leads to the induction of gene expression by MTF-1 and MRE; formation of Zn–DNA remains still unknown.

the metal regulatory element (MRE) requires activation by zinc(II) ions. In conclusion, zinc(II) ions can enter the nucleus to activate expression of MT genes from a dietary source. Once MTF-1 senses free Zn(II), it adopts a reversible DNA-binding conformation. This allosteric change causes an exposure of zinc fingers, and the MTF-1 can move to the nucleus and activate gene expression by an association with the gene promoters that carry MREs [20]. Study by Jiang et al. confirmed that the mediation of MT gene expression starts by a translocation of MTF-1 protein from the cytoplasm to a nucleus and bind MREs in target genes [20]. However, whether metal ions can induce the creation of MTF-1 in the cytoplasm and how do other metal ions induce MT via MTF-1 remain to be elucidated [21].

A study of DNA–metal ion complex, known as M-DNA, in which a divalent metal ion is incorporated into the center of the DNA duplex, was firstly performed in 1993 [22]. Since that time, numerous studies that describe the formation, characteristics and application of this complex, has been published [23–37]. The biological role of the interaction of Zn(II) with proteins is relatively well known and exploited. The question is what role Zn–DNA complex has in living organisms and how does it affect the regulation of transcription and replication of DNA, or what relation Zn–DNA has in a processes of cancerogenesis. The suggested structure of Zn–DNA that is based on the nuclear magnetic resonance (NMR), circular dichroism (CD), and molecular modeling studies consists of GC and AT base pairs, in which the imino-proton of G and T is replaced by Zn(II), which results in an atomically thick "wire" of zinc ions sheathed by a DNA helix [24]. Zinc(II) ions also bind to phosphate groups of DNA and are able to influence ("destroy") the basic structure of B conformation of DNA [38]. The preferred site for the coordination of the metal ion to the DNA is the N7 position of guanines [39]. Conformational change of ds or ssDNA after interacting with zinc(II) ions may be used in the regulation of transcription [40]. It has been found that the TGGGA sequence of the *Xenopus* 5S-RNA gene, which is essential for binding the transcription factor TFIIIA, has the highest affinity for zinc ions [39].

Various techniques have been applied to investigate the interactions of heavy metal ions with DNA. These techniques include Fourier transform infrared difference spectroscopy [27], Raman spectroscopy [38], and circular dichroism spectroscopy [41]. However, these techniques have high demands for special equipment which is not commonly available. The aim of this study was to study the interaction of zinc(II) ions with a fragment of DNA by UV/vis spectrophotometry (changes in absorption signals and melting temperatures in denaturation) and gel electrophoresis (changes in the mobility of DNA fragments) after purification on a semi-permeable membrane.

2. Materials and methods

2.1. Chemicals, pH measurements and MiliQ water preparation

Zinc nitrate, water and other chemicals were purchased from Sigma–Aldrich (USA) in ACS purity (chemicals meet the specifications of the American Chemical Society) unless noted otherwise. The pH value and conductivity was measured using inoLab Level 3 (Wissenschaftlich-Technische Werkstatten GmbH; Weilheim, Germany). Deionized water underwent demineralization by reverse osmosis using the instruments Aqua Osmotic 02 (Aqua Osmotic, Tisnov, Czech Republic) and then it was subsequently purified using Millipore RG (Millipore Corp., USA, 18 MΩ) – MiliQ water.

2.2. Amplification and isolation of DNA fragment

For amplification of bacteriophage λ Xis gene DNA fragment, Taq PCR kit and λ-DNA (New England BioLabs, USA) were used. The sequences of forward and reverse primers were 5′-CCTGCTCTGCCGCTTCACGC-3′ and 5′-TCCGGATAAAAACGTCGATGACATTTGC-3′ (Sigma–Aldrich), respectively. The 50 μL reaction mixture was composed of 1×

standard Taq reaction buffer, 0.2 mM dNTPs, 0.2 μM primers, 1.25 U Taq DNA polymerase, and 0.5 μg λ DNA. Polymerase chain reaction (PCR) was carried out in a thermocycler Mastercycler EP realplex4 S (Eppendorf AG, Hamburg, Germany), the temperature program was as follows: initial denaturation at 95 °C for 120 s; 25 cycles of denaturation at 95 °C for 15 s, annealing at 64 °C for 15 s, extension at 72 °C for 45 s and a final extension at 72 °C for 5 min. The resulting DNA fragment (498 bp) was purified using MinElute PCR Purification Kit (Qiagen, Germany). The concentration of the PCR product was determined spectrophotometrically at 260 nm (Specord 210, Analytic Jena, Germany). For Zn(II)–DNA interaction experiments (see Section 2.3), DNA was diluted in water (ACS purity). The absorbance and concentration of that DNA solution were 0.2 AU and 30 μM, respectively.

2.3. Zn(II)–DNA interaction

A stock solution of DNA (30 μM) was mixed with different concentrations of $Zn(NO_3)_2$ in 1:1 ratio. Final concentrations of compounds in aqueous solutions (ACS purity, Sigma–Aldrich, St. Louis, MO, USA) were 15 μM DNA and 0, 5.5, 11, 22, 33, 44, and 55 μM Zn(II). Then another types of aqueous solutions with final concentrations of 55 μM Zn(II) and DNA in concentrations 30, 37.5, 39, 52.5, 60, 67.5, and 75 μM were prepared. The samples were incubated for 60 min at 25 °C. After incubation, unbound zinc ions were removed using an Amicon Ultra 3 K centrifugal filter device (Millipore Corp., USA). After centrifugation at 14,000 rpm for 10 min at 20 °C (5417R Eppendorf, USA), the sample was complemented with water to the original volume (200 μl) before centrifugation.

2.4. UV/vis spectrophotometry

Spectra were recorded within the range 190–350 nm using quartz cuvettes (1 cm, Hellma, Essex, UK) on a spectrophotometer SPECORD 210 (Analytik Jena, Germany) at 20 °C Julabo (Labortechnik, Wasserburg, Germany). Spectra were recorded after 60 min of interaction (incubation). Denaturation of a complex of DNA with Zn(II) was monitored spectrophotometrically using a spectrophotometer SPECORD S600 with a diode detector (Analytik Jena, Germany). The sample was incubated for 3 min at increasing temperatures in a range from 23 to 89 °C and the absorbance was measured at 260 nm. Changes in absorbance spectra of Zn–DNA complex were recorded during denaturation. After denaturation, all Zn–DNA samples were incubated 60 min at 25 °C to renaturate.

2.5. Agarose gel electrophoresis

Renaturated samples of Zn–DNA were electrophoresed in 1.8% (*m*/*V*) agarose gel (Mercury, USA) in 1× TAE buffer (40 mM Trizma-base, 20 mM acetic acid and 1 mM ethylenediaminetetraacetic acid (EDTA), pH = 8.0). A DNA ladder (New England BioLabs, USA), within the range from 0.1 to 1.5 kbp, was used as a standard to monitor the size of the analyzed fragments of DNA. The electrophoresis (Bio-Rad, USA) was run at 70 V and 6 °C for 135 min. After electrophoresis, the gel was stained with ethidium bromide (31.3 ng/l) in 1× TAE buffer. Bands were visualized using a transilluminator at a wavelength of 312 nm (Vilber-Lourmat, France). Visualized image was recorded by a digital camera Canon G10 (Canon Inc., Japan).

3. Results and discussion

3.1. Effect of zinc(II) ions

To study the effect of different concentrations of Zn(II) on interaction with DNA, 15 μM DNA was incubated with 0, 5.5, 11, 22, 33, 44 and 55 μM Zn(II) for 60 min at 25 °C. After removal of the unbound Zn(II) (see Section 2.3), UV–vis absorption spectra were recorded. An increase of absorbance, with maximum at 200 nm according to the increasing Zn(II) concentration, can be seen from the obtained spectra (Fig. 2C a–g). A small decrease in the absorbance at 260 nm with the increasing Zn(II) concentration was observed, and the equation $y = -3 \times 10^{-4}x + 0.099$ with $R^2 = 0.88$ was determined (Fig. 2G). The decrease in absorbance at 260 nm can be explained by a binding of Zn(II) into the structure of DNA. It has been shown that Zn(II) binds to purine bases of DNA, especially to N7 of guanine [39]. Due to the presence of purine bases, DNA has the absorption maximum at 260 nm. Zn(II) ions do not absorb at 260 nm, as it is shown in Fig. 2A-c. Binding of Zn(II) into DNA caused a decrease in absorption coefficient of purine bases and this resulted in a small decrease in absorbance at 260 nm after the interaction with Zn(II) ions (Fig. 2G). All these findings are in a compliance with previously published findings [39]. On the other hand, no changes in the electrophoretic mobility of the Zn–DNA were demonstrated using gel electrophoresis, because all measured bands corresponded to the control DNA (498 bp), which is shown in Fig. 2D and F. For monitoring of Zn–DNA interactions, gel electrophoresis was carried out also by Devrim and colleagues [42].

To study the effect of concentration of DNA on the interaction with zinc(II) ions, seven concentrations of DNA (30, 37.5, 45, 52.5, 60, 67.5, and 75 μM) were used in the experiment. Sample of DNA at corresponding concentration was incubated with 55 μM Zn(II) and the changes in absorption spectra were monitored (Fig. 2E). The absorbance maximum at 260 nm decreased with decreasing DNA concentration with equation $y = 0.0037x + 0.0101$ with a confidence coefficient $R^2 = 0.98$. The decreasing intensity of the bands was detected on agarose gel and the size of all bands was 489 bp (Fig. 2F).

To investigate the interaction between Zn(II) and DNA in greater details, the absorption spectrum of control (15 μM DNA) was subtracted from the absorption spectra of Zn(II)–DNA samples. The resulting differential absorption spectra (ΔAU) within the range of 190–350 nm are shown in Fig. 3A and B. We found that there was detectable change at 251 nm. Therefore, we assume that the interaction between Zn(II) and DNA is manifested as a decrease in the differential absorption spectra at 251 nm and it is shown in inset in Fig. 3A. Dependency of decrease of ΔAU on the concentration of Zn(II) had a linear trend with the equation $y = -0.2 \times 10^{-4}x + 1.6 \times 10^{-4}$ with $R^2 = 0.98$.

3.2. Denaturation experiments

In order to study an influence of Zn(II) on changes of melting point (Tm) of DNA, samples were denatured after recording the Zn(II)–DNA spectra and the absorbance at 260 nm was measured. Obtained temperature dependence was derived and Tm of Zn–DNA was calculated. As it is well evident from Fig. 4A, Tm of Zn(II)–DNA depends on the concentration of zinc(II) ions. Application of 5.5 μM Zn(II) led to a decrease in Tm by an average of 7.5 °C compared to the control (75.5 ± 3 °C). The lowest Tm (60.5 ± 2.5 °C) was observed at 33 μM Zn(II). Application of 44 and 55 μM Zn(II) induced no changes in Tm within the standard deviation. These experiments proved the binding of Zn(II) into DNA. Binding of Zn(II) ions into DNA destabilizes the hydrogen bonds between the DNA strands, which leads to the decrease in Tm. In the second set of samples, no changes in Tm were observed. For all used concentrations of DNA, the Tm of Zn–DNA was determined to be 61 ± 2.5 °C (Fig. 4B), which is in accordance with the first set of samples.

After denaturation, all samples were left to cool at 25 °C for 60 min and then were incubated for 24 h at 8 °C. The renaturated samples were analyzed by gel electrophoresis, which showed that

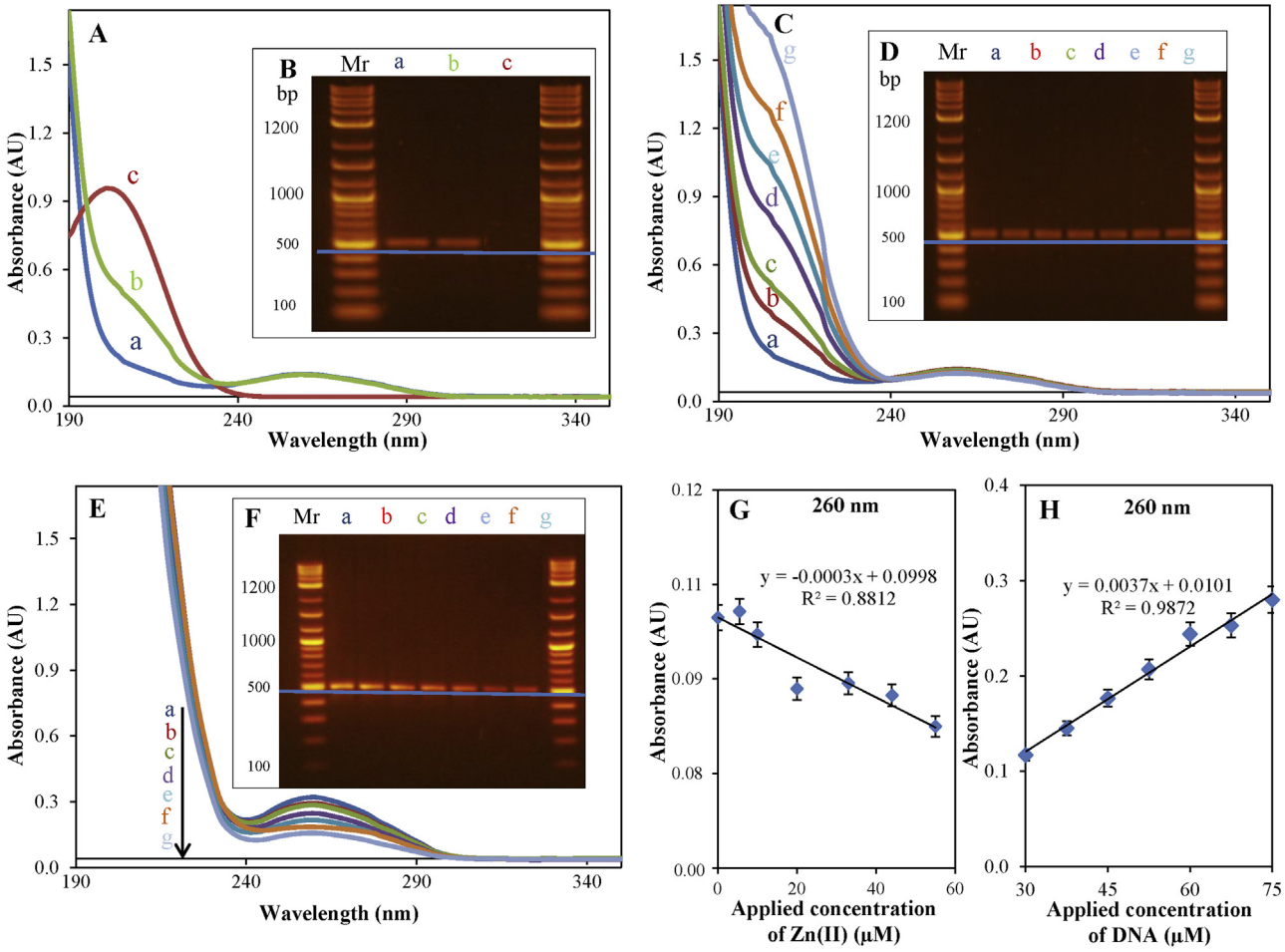

Fig. 2. (A) Spectrophotometric records of absorption spectrum measured within the range 190–350 nm, (a) 15 μM DNA, (b) 15 μM DNA with 20 μM Zn(II), and (c) 20 μM Zn(II). (B) 1.8% agarose gel electropherogram, labeling of the samples is the same as in A. (C) Spectrophotometric records of absorption spectrum measured within the range 190–350 nm, (a) 15 μMDNA, (b) 15 μM with 5.5 μM Zn(II), (c) 15 μM DNA with 11 μM Zn(II), (d) 15 μM DNA with 22 μM, (e) 15 μM DNA with 33 μM Zn(II), (f) 15 μM DNA with 44 μM Zn(II), and (g) 15 μM DNA with 55 μM Zn(II). (D) 1.8% agarose gel electropherogram, labeling of the samples is the same as in C. (E) Spectrophotometric records of absorption spectrum measured within the range 190–350 nm, (a) 75 μM DNA with 55 μM Zn(II), (b) 67.5 μM DNA with 55 μM Zn(II), (c) 60 μM DNA with 55 μM Zn(II), (d) 52.5 μM DNA with 55 μM Zn(II), (e) 39 μM DNA with 55 μM Zn(II), (f) 37.5 μM DNA with 55 μM Zn(II), and (g) 30 μM DNA with 55 μM Zn(II). (F) 1.8% agarose gel electropherogram, labeling of the samples is the same as in E. (G) Absorption maxima obtained from graph C (λ = 260 nm). (H) Absorption maxima obtained from graph E (λ = 260 nm).

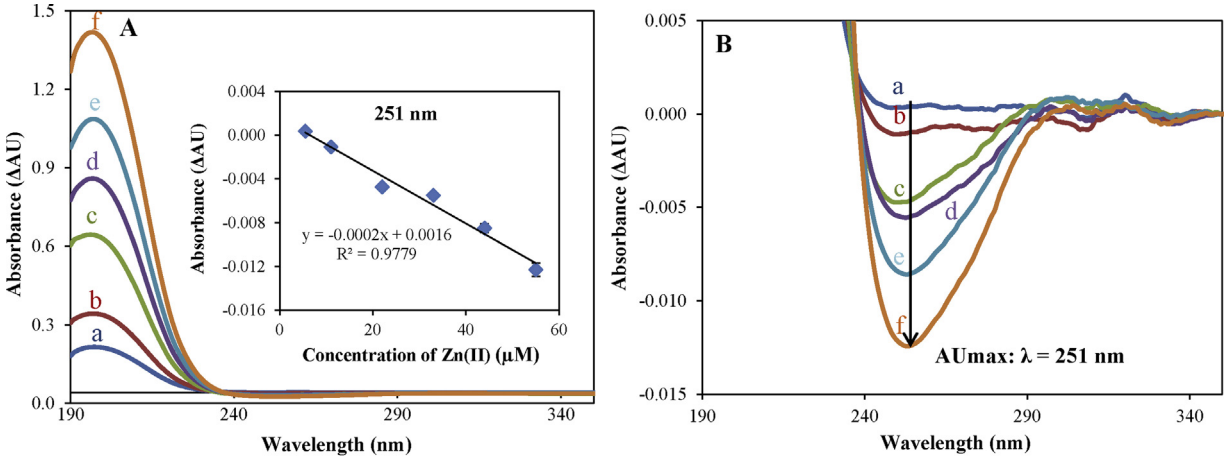

Fig. 3. (A) Spectrophotometric record of differential absorption spectra measured within the range 190–350 nm, (a) 15 μM with 5.5 μM Zn(II), (b) 15 μM DNA with 11 μM Zn(II), (c) 15 μM DNA with 22 μM, (d) 15 μM DNA with 33 μM Zn(II), (e) 15 μM DNA with 44 μM Zn(II), and (f) 15 μM DNA with 55 μM Zn(II); in inset: differential absorption maxima measured at 251 nm. (B) Spectrophotometric records of differential absorption spectra measured within the range 190–350 nm with special focus on the area at 251 nm, labeling of the samples is the same as in (A).

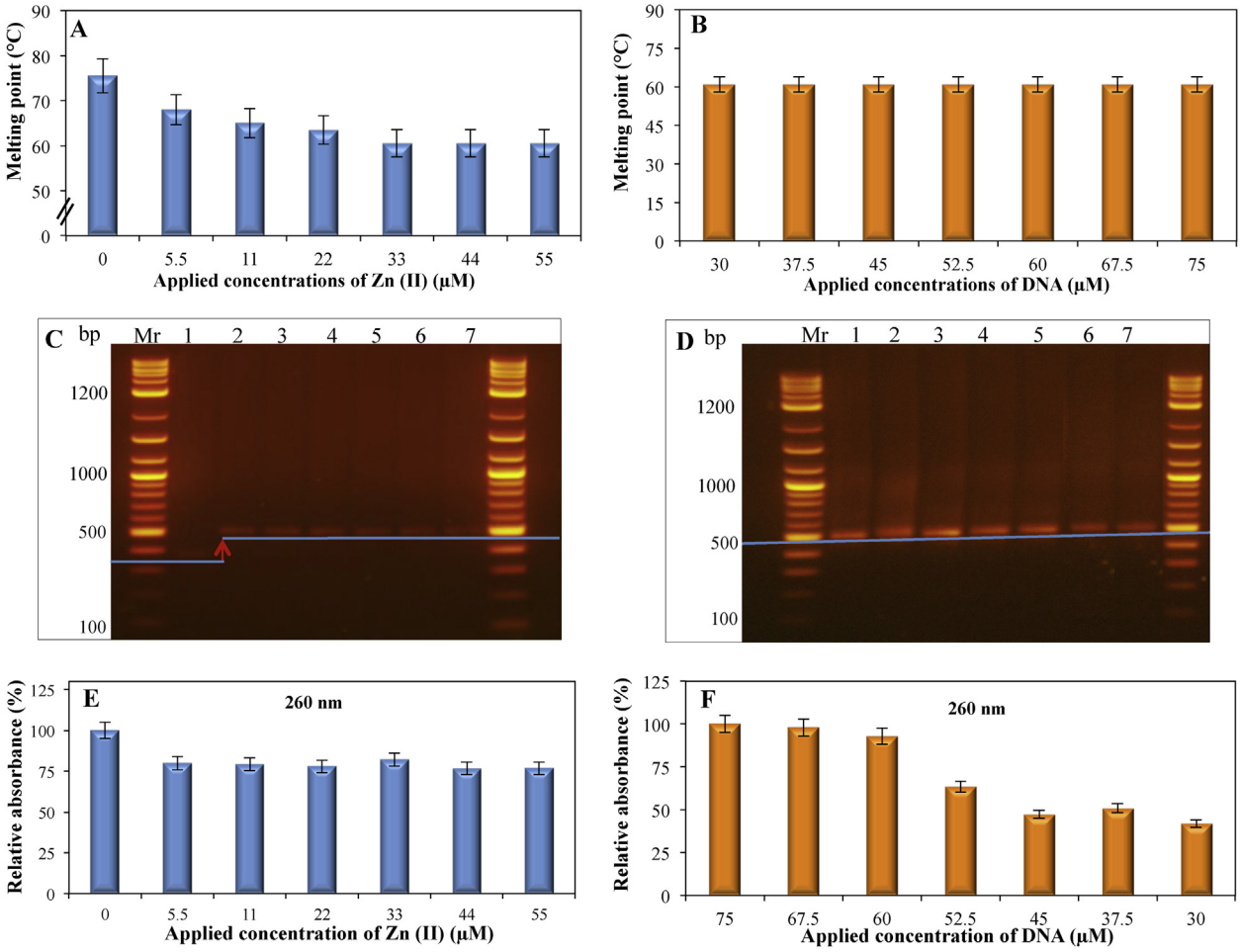

Fig. 4. (A) Column graph that represents decrease in Tm, 15 μM DNA (75.5 °C) and 15 μM Zn–DNA (68–60.5 °C) with Zn(II) ions (5.5–55 μM). (B) Column graph that represents a decrease in Tm, 30–75 μM Zn–DNA (61 °C) after application of 55 μM Zn(II). (C) 1.8% agarose gel electropherogram after 24 h of renaturation of samples, (1) 15 μM DNA, (2) 15 μM DNA with 5.5 μM Zn(II), (3) 15 μM DNA with 11 μM Zn(II), (4) 15 μM DNA with 22 μM, (5) 15 μM DNA with 33 μM Zn(II), (6) 15 μM DNA with 44 μM Zn(II), and (7) 15 μM DNA with 55 μM Zn(II). (D) 1.8% agarose gel electropherogram after 24 h of renaturation of samples, (1) 75 μM DNA with 55 μM Zn(II), (2) 67.5 μM DNA with 55 μM Zn(II), (3) 60 μM DNA with 55 μM Zn(II), (4) 52.5 μM DNA with 55 μM Zn(II), (5) 39 μM DNA with 55 μM Zn(II), (6) 37.5 μM DNA with 55 μM Zn(II), and (7) 30 μM DNA with 55 μM Zn(II). (E) Column graph that represents height of signals at absorption spectrum (λ = 260 nm) expressed in % after 24 h renaturation, marking of the samples is the same as in C (100% corresponds to the control). (F) Column graph that represents height of signals of absorption spectra (λ = 260 nm) expressed in % after 24 h renaturation, marking of the samples is the same as in (D) (100% corresponds to the 75 μM).

the DNA with bound Zn(II) ions renaturated in a different way than control (15 μM DNA) for the first set of the samples. Newly created dsDNA, which was renaturated without Zn(II), migrated at approximately 400 bp in comparison with the original fragment (489 bp) (shown in Fig. 4C). This may be caused by an alternative rehybridization of repetitive sequences in the fragment of DNA as a result of low ionic strength solution [43]. The second set of samples (constant Zn(II), different DNA concentrations) demonstrated no changes in the shift of band at renaturation, but smears, that correspond to double length (498 bp) of the fragment of DNA, appeared. Smears began to be observable from the concentration of 30 μM, and the increasing concentration of DNA accentuated their visibility (Fig. 4D). This is probably caused by an alternative rehybridization of ssDNA fragments at high concentration of DNA. Absorption spectra were recorded for renaturated samples of both the sets. We determined that in the first set of the samples, the control (15 μM DNA without Zn(II)) had about 20% higher absorption signal (λ = 260 nm) than that of Zn–DNA (15 μM DNA + 5.5–55 μM Zn(II)), see Fig. 4E. Only concentration-dependent decrease in absorbance at 260 nm was observed for the second set of the samples. This fact indicates that the observed phenomenon is caused by the interaction of Zn(II) ions with DNA and is not affected by its increasing concentration (Fig. 4F).

3.3. Suggestion of Zn–DNA complexes formation

Zinc(II) ions influence many cellular processes including cell proliferation, differentiation, and apoptosis. Therefore, we can expect that zinc(II) ions can significantly contribute to develop a prostate cancer (process of cancerogenesis), and to promote the ability of primary tumor to metastasize [44]. Prostate cells specifically maintain homeostasis of zinc(II) ions [45,46], thus, zinc(II) ions play extraordinary role in these cells. However, further studies of the role of zinc(II) ions in cancerogenesis, and dysregulation of the homeostasis of zinc(II) ions must be carried out. Fluorescence techniques are the most commons methods to monitor the interactions between Zn(II) ions and DNA [22,29,47], however, they demand special equipment and are not commonly available. Presented method of studying the interaction of Zn(II) with DNA is more simple in comparison with above-mentioned methods. Our method does not use special and expensive fluorescent probes, but it focuses only the absorption spectra and melting temperature during denaturation [48,49]. The samples (Zn(II) with DNA) were purified using a semi-permeable membrane Amicon Ultra – 0.5 ml 3 K [50]. Purified samples were subjected to spectrophotometric evaluation that has shown that application of zinc(II) ions causes an increase in the absorbance at 200 nm and a decrease

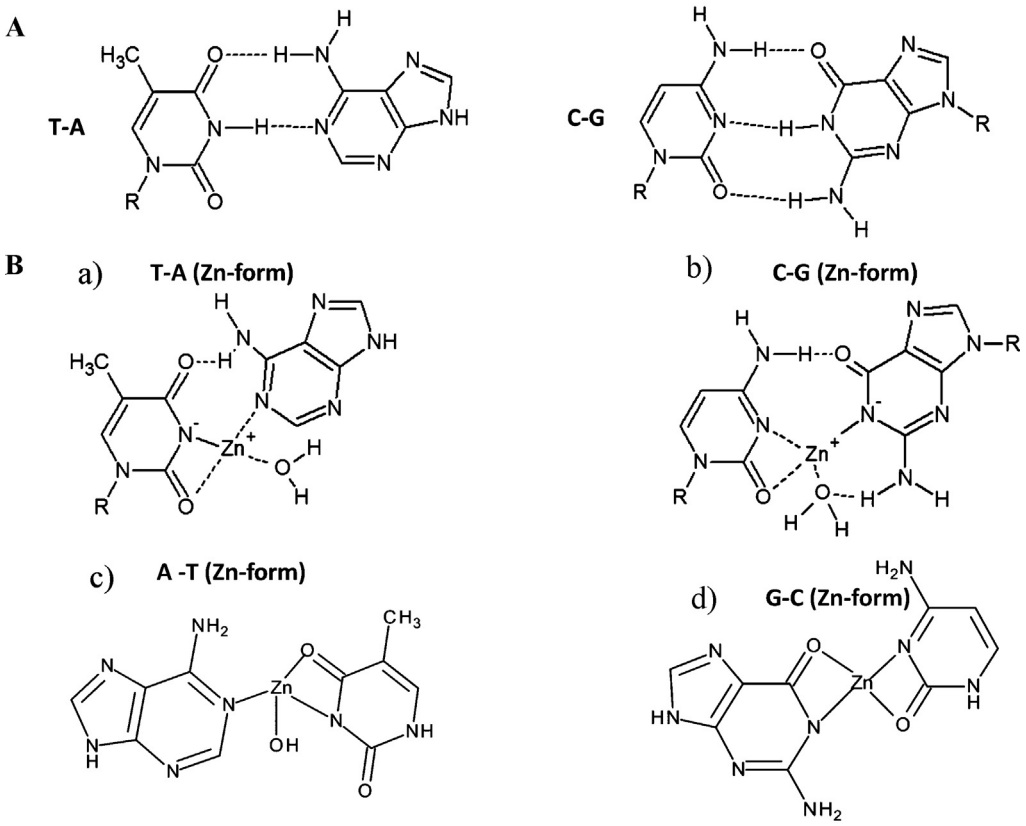

Fig. 5. Suggestion of structure of Zn–DNA adopted according to [36,37].

at 260 nm. Interaction between Zn(II) and DNA had an impact on differential absorption spectra, where a decrease at 251 nm was determined. The most probable reason for the hypochromicity is deprotonation of N3 of thymine upon creation of intramolecular chelates N1(A)-Zn(II)-N3(T). Formation of such chelates enables the transition of DNA blocks enriched with AT pairs into M-form [51]. Samples were subsequently denatured in our experiments. When studying the denaturation of DNA, hyperchromicity effect was used. Due to the interactions of the electrons in the complementary bases, solution of double-stranded DNA (dsDNA) absorb UV radiation at 260 nm in less extend than the solution, containing the same concentration of the bases in the mononucleotides or single-stranded DNA molecules. The temperature, corresponding to Tm, causes the absorbance rises suddenly during warming up the solution of dsDNA [52,53]. These changes may be observed in derivative spectra. We observed a decrease in Tm in DNA, treated with zinc(II) ions in our experiments. This is similar to experiments where possible interactions of intercalating agents, such as ethidium bromide with DNA, are monitored with fluorescent intensity. Our study shows that zinc(II) ions may interact directly with DNA (binding to DNA), or may regulate gene expression, which is consistent with previously published data [54]. Stabilizing the interactions between MTF-I and MT promoter in response to metals may involve the recruitment of chromatin remodeling factors as well as increased affinity and specificity of DNA binding in response to zinc occupancy and/or post-translational modifications [20].

DNA double helix consists of two mutually antiparallely arranged strands of DNA. Hydrogen bonds stabilize opposite bases in both DNA strands, namely between guanine and cytosine and between adenine and thymine (Fig. 5A). There are other ways also to organize the strands of DNA that differ from the traditional concept of the double helix. A-DNA, B-DNA and Z-DNA are well known, and they have been observed in vivo. These alternative conformations of DNA are involved in the regulation of transcription and DNA replication. Furthermore, there is also a parallel arrangement of DNA strands, three- (triplex) and four-stranded (quadruplex) DNA, which is expected to occur limitedly in living organisms. It is well known that the interaction of the DNA bases with intercalating and chelating compounds affects the structure of DNA. DNA structure can be changed via the interaction of various compounds with sugar-phosphate skeleton. Zinc(II) ions probably bind into both DNA bases and sugar-phosphate skeleton in our experiment (Fig. 5B).

4. Conclusions

We studied binding of zinc(II) ions into DNA by the use of the simple and well available methods (spectrophotometry and gel electrophoresis). Interactions between DNA and zinc(II) ions were shown as changes in the absorption spectra (190–350 nm) and as the decrease in Tm of denaturation of Zn–DNA. Structural changes at renaturation of DNA and Zn–DNA were monitored using the gel electrophoresis. This study showed that Zn–DNA might be created also under physiological conditions. This fact has a possible impact on a plenty of biochemical processes and pathways in eukaryotic cells. Considering the fact that zinc(II) ions play essential role in prostate cells, we can assume that possible formation of Zn–DNA may be related closely with development and progression of a prostate cancer. However, further studies are necessary.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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