INTERACTION OF ZINC(II) IONS WITH DNA - BIOPHYSICAL STUDY

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INTRODUCTION

Zinc is a biogenic element that plays an important role in an organism. 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 [4].

The structural role of zinc(II) ions is accentuated in the stabilization of zinc finger motif [6]. It is an amino acid sequence linked to zinc ion(II) to form a secondary structure in the shape of a finger [6]. Proteins with this motif have high affinity to DNA, thus, zinc finger directly mediates an interaction with DNA in the major groove [2]. 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 [3, 5]. A study of DNA–metal ion complex, known as M-DNA, in which a divalent metal ion is incorporated into the centre of the DNA duplex, was firstly performed in 1993 [8]. The suggested structure of M-DNA that is based on the nuclear magnetic resonance (NMR), circular dichroism (CD), and molecular modelling 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 [1]. Zinc(II) ions also bind to phosphate groups of DNA and are able to influence (”destroy”) the basic structure of B conformation of DNA [7]. The preferred site for the coordination of the metal ion to the DNA is the N7 position of guanines [10].

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.

METHODS

a) amplification and isolation of DNA fragments: create copies of DNA fragments using polymerase chain reaction
b) spectrophotometry: changes in absorption spectrum and melting temperatures of nucleic acids
c) gel electrophoresis: changes in electrophoretic mobility of the fragment

RESULTS

To study the influence of Zn(II) concentration on Zn(II)-DNA interaction 5 μg/ml DNA was incubated with 5.5, 11, 22, 33, 44 and 55 μM Zn(II) for 60 minutes at 25 °C. After removal of the unbound Zn(II) the UV-VIS absorption spectra were recorded (Fig. 1A, C)

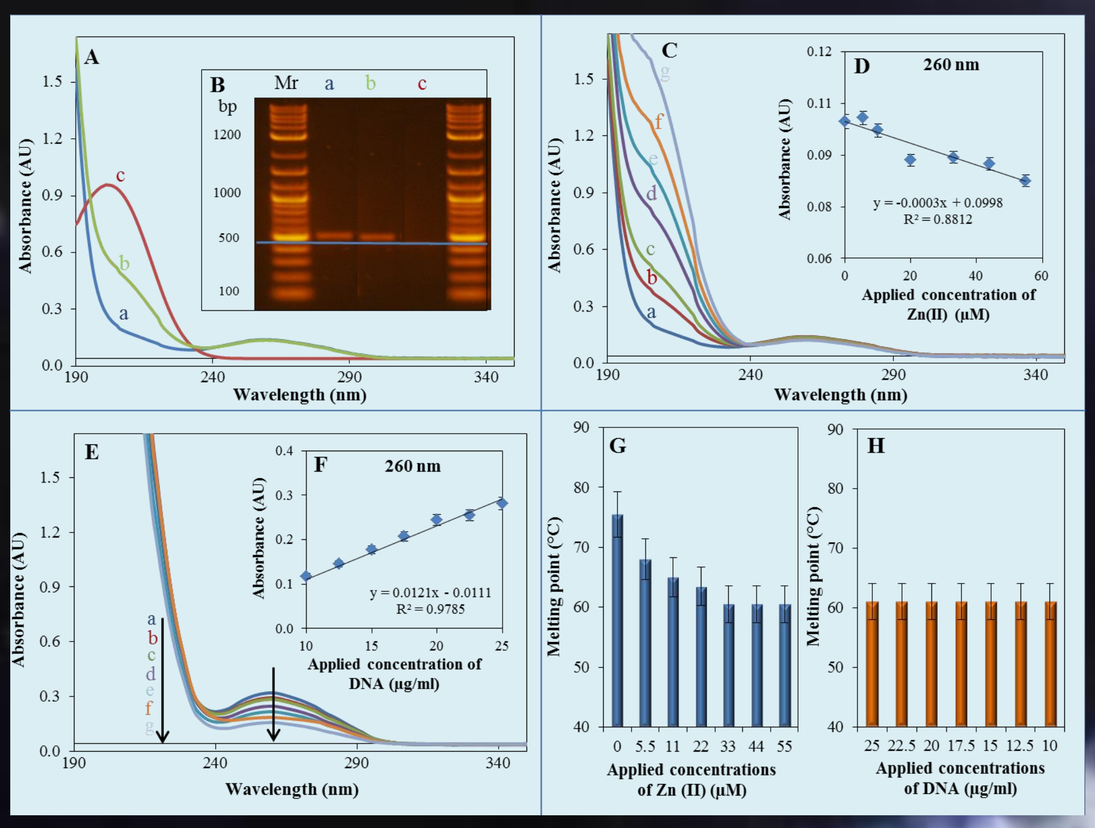

Figure 1.(A) Spectrophotometric records of absorption spectrum measured within the range 190 – 350 nm, (a) = 5 μg/ml DNA, (b) = 5 μg/ml DNA with 20 μM Zn(II), and (c) = 20 μM Zn(II). (B) 1.8 % agarose gel electropherogram, labelling of the samples is the same as in A. (C) Spectrophotometric records of absorption spectrum measured within the range 190 – 350 nm, (a) = 5 μg/ml DNA, (b) = 5 μg/ml with 5.5 μM Zn(II), (c) = 5 μg/ml DNA with 11 μM Zn(II), (d) = 5 μg/ml DNA with 22 μM, (e) = 5 μg/ml DNA with 33 μM Zn(II), (f) = 5 μg/ml DNA with 44 μM Zn(II), and (g) = 5 μg/ml DNA with 55 μM Zn(II). (D) Absorption maxima obtained from graph C (λ = 260 nm). (E) Spectrophotometric records of absorption spectrum measured within the range 190 – 350 nm, (a) = 25 μg/ml DNA with 55 μM Zn(II), (b) = 22.5 μg/ml DNA with 55 μM Zn(II), (c) = 20 μg/ml DNA with 55 μM Zn(II), (d) = 17.5 μg/ml DNA with 55 μM Zn(II), (e) = 15 μg/ml DNA with 55 μM Zn(II), (f) = 12.5 μg/ml DNA with 55 μM Zn(II), and (g) = 10 μg/ml DNA with 55 μM Zn(II). (F) Absorption maxima obtained from graph E (λ = 260 nm). (G) Dependence of Tm of DNA (5 μg/ml) on applied concentration of Zn(II) ions (5.5-55 μM). (H) Dependence of Tm of DNA after application of 55 μM Zn(II) on DNA concentration (10-25 μg/ml) M-DNA.

A decrease in the absorbance at 260 nm of the 5 μg/ml DNA with the increasing concentration of Zn(II) ions (5.5-55 μM) was observed, and the equation y = -3x10-4x + 0.099 with R^2 = 0.88 was determined (Fig. 1D). The decrease in absorbance at 260 nm can be explained by a binding of Zn(II) ions into the DNA structure. It has been shown that Zn(II) binds to purine bases of DNA, especially to N7 of guanine [9]. On the other hand, no changes in the structure of M-DNA were demonstrated using gel electrophoresis, because there was no change in band mobility after addition of Zn to DNA. Both measured bands corresponded to the control DNA fragment (498 bp), which is shown in Fig. 1B. To study the effect of concentration of DNA on the interaction with Zn(II) ions, seven concentrations of DNA (10, 12.5, 15, 17.5, 20, 22.5, and 25 μg/ml) were used in the experiment. A linear decrease in the absorbance at 260 nm with the decreasing DNA concentration incubated with Zn(II) ions of the same concentration (55 μM) was observed (Figs. 1E a-g) and the equation y = 0.0121x – 0.0111 with a confidence coefficient R^2 = 0.98 was determined (Fig. 1F). Binding of Zn(II) ions into DNA did not affect a linear decrease in absorbance of DNA at 260 nm with decreasing DNA concentration in comparison with DNA control (not shown). In order to study an influence of Zn(II) ions on changes of DNA melting point (Tm), 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 M-DNA was calculated. As it is well evident from Fig. G, Tm of Zn(II)-DNA depends on the concentration of Zn(II) ions. Decrease in Tm was observable in the Zn (II) concentration range of 5.5. – 33 μM. Application of 44 and 55 μM Zn(II) induced no further changes in Tm. In the second set of samples, where the highest concentration of Zn(II) ions (55 μM) were used no further changes in Tm with increasing DNA concentration were observed (Fig.H).

CONCLUSION

We proved binding of zinc(II) ions in DNA using the simple methods (spectrophotometry and gel electrophoresis) in our study. This study verified the significance of zinc ions in all eukaryotic organisms. Interactions between DNA and Zn(II) presented by changes in absorption spectra (190 – 350 nm) and by decrease in Tm at denaturation of M-DNA. Structural changes of DNA and M-DNA were monitored using gel electrophoresis. This study proved that M-DNA may develop at physiological conditions, which may affect many biochemical pathways in eukaryotic cells.

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