

Interaction study of arsenic (III and V) ions with metallothionein gene (MT2A) fragment



Lukas Nejdla^{a,b}, Sylvie Skalickova^a, Jiri Kudr^{a,b}, Branislav Ruttkay-Nedecky^{a,b},
Simona Dostalova^b, Monika Kremplova^a, Renata Kensova^{a,b}, Amitava Moulick^{a,b},
Marie Konecna^{a,b}, Vojtech Adam^{a,b}, Rene Kizek^{a,b,*}

^a Department of Chemistry and Biochemistry, Faculty of Agronomy, Mendel University in Brno, Zemedelska 1, CZ-613 00 Brno, Czech Republic

^b Central European Institute of Technology, Brno University of Technology, Technicka 3058/10, CZ-616 00 Brno, Czech Republic

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ABSTRACT

Arsenic compounds belong to the most controversial agents concerning human health. Arsenic (As) is considered as a top environmental element influencing human health due to its adverse effects including cancer, diabetes, cardiovascular disease, and reproductive or developmental problems. Despite the proven mutagenic, teratogenic and carcinogenic effects, the arsenic compounds are used for centuries to treat infectious diseases. In our work, we focused on studying of interactions of As(III) and/or As(V) with DNA. Interactions between arsenic ions and DNA were monitored by UV/vis spectrophotometry by measuring absorption and fluorescence spectra, atomic absorption spectrometry, electrochemical measurements (square wave voltammetry) and agarose gel electrophoresis. Using these methods, we observed a stable structure of DNA with As(III) within the concentration range 0.4–6.25 $\mu\text{g mL}^{-1}$. Higher As(III) concentration caused degradation of DNA. However, similar effects were not observed for As(V).

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1. Introduction

Arsenic is classified as a global pollutant and human carcinogen, to which humans are exposed, occurring in the environment coming from both natural and/or anthropogenic sources [1]. The toxicity depends on the oxidation state or methylation level during the biotransformation in the organism [2], whereas oxidative stress induced by arsenic exposure is suggested as a potential mode of its carcinogenic action [3]. Inorganic forms of arsenic appears in two biologically important oxidation states: As(V) (arsenate) and As(III) (arsenite), which are highly toxic. The methylated forms, monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) are much less toxic. More complex forms such as arsenobetaine (AsB) or arsenocholine (AsC) are considered as nontoxic ones [4].

For the purpose of understanding arsenic toxicity, there has been put much emphasis on the studies of arsenic binding to proteins. In humans, like in many mammalian species, inorganic arsenic is quantitatively reduced from pentavalent to

trivalent arsenic in plasma and subsequently methylated to trivalent and pentavalent metabolites in the liver [5]. The inorganic arsenic is metabolized to the monomethylarsonic acid (MMA) and monomethylarsinic acid (DMA), which are highly reactive and responsible for arsenic intoxication [6]. The toxicity of trivalent arsenicals likely occurs through the interaction of these arsenic species with sulfhydryl groups of proteins [7]. Although As(III) has high affinity to sulfhydryl compounds such as reduced glutathione (GSH) and cysteine-rich proteins, As(V) does not bind to the sulfhydryl groups [8].

Arsenicals and their applications in therapy has also attracted broadly attention [9–13]. There are currently over 100 active clinical trials involving inorganic arsenic or organoarsenic compounds registered with the Food and Drug Administration (FDA) for the treatment of cancers [14]. Arsenic trioxide is presently the most active single agent in the treatment of acute promyelocytic leukemia (APL) [15,16]. It has been shown that it is equally effective in newly diagnosed cases of APL, with much less toxicity compared to standard protocols [17]. In adult therapy, arsenic trioxide and all-trans-retinoic acid are recognized as active treatment of relapsed APL [15,18,19]. In spite of the mentioned positives, the effects of As(III) on APL, The Leukemia-associated-protein (LAP) domain, a cysteine-rich motif, is present in a wide range of proteins, including MLL, AG10, and MLLT6 proteins which has a highly cysteine-rich

* Corresponding author at: Department of Chemistry and Biochemistry, Mendel University in Brno, Zemedelska 1, CZ-613 00 Brno, Czech Republic.

Tel.: +420 5 4513 3350; fax: +420 5 4521 2044.

E-mail address: kizek@sci.muni.cz (R. Kizek).

motif (RING finger), remain poorly understood [20]. In addition, it is believed that inorganic arsenate (HAsO_4^{2-}), which is a molecular analogue of phosphate (HPO_4^{2-}), can compete for phosphate anion transporters and replace phosphate in some biochemical reactions [1]. In this field, recently published papers show the impact of the As substitution instead of P in DNA (As–DNA) [21,22]. Moreover, there was found a bacterial strain GFAJ-1 of the *Halomonadaceae* isolated from Mono Lake in California, in which arsenic was able to substitute for phosphorus to sustain its growth in hostile environment [23]. How As insinuates itself into the structure of biomolecules is unclear, and the mechanisms by which such molecules operate are unknown [23].

For all the reasons concerning the toxicity and unknown biological effects of arsenic, the monitoring of this element in biological matrices is necessary. The techniques most widely used for detecting the total form of arsenic include inductively coupled plasma optical emission spectrometry (ICP-OES), inductively coupled plasma mass spectrometry (ICP-MS), atomic absorption spectrometry (AAS) of electrothermal or hydride generation [24–26]. Although arsenic is one of the volatile analytes, the most common form of biological tissue decomposition is acid digestion. Therefore, elevated pressure and temperature control alleviate the issues during microwave digestion. Besides, the speciation of different arsenic forms is often carried out by using hyphenated techniques. From this point of view, one of the most frequently used techniques is the combination of ICP-MS with liquid or gas chromatography. This technique, particularly nanoscale high-resolution secondary ion mass spectrometry (NanoSIMS), was applied to identify As in extracted, gel-isolated genomic DNA in the experiment with bacterial strain GFAJ-1 of the *Halomonadaceae* mentioned above [23].

The aim of this work was to compare the spectroscopic, electrochemical and electrophoretic methods for studying of As(III) and As(V) interaction with DNA fragment of metallothionein gene (MT2A). Metallothionein is a cysteine-rich metal-binding protein in which we are interested, and therefore we have chosen MT2A fragment for this experiment. The ability to influence the secondary structure of DNA with arsenic may play a major role in the induction of apoptosis in tumor cells, and therefore it is important to investigate these interactions in greater details.

2. Materials and methods

2.1. Chemicals

Standards of As(III) as Arsenic chloride (AsCl_3) and As(V) as Potassium hydrogenarsenate dihydrate ($\text{K}_2\text{HAsO}_4 \cdot 2\text{H}_2\text{O}$) as well as other chemicals used in this study were purchased from Sigma–Aldrich (St. Louis, MO, USA) in ACS purity. Standards were dissolved in ACS water (Sigma–Aldrich).

2.2. MT2A DNA fragment amplification and isolation

Human genomic DNA was isolated from blood via MagNA Pure Compact (Roche, Mannheim, Germany) using Nucleic Acid Isolation Kit I and protocol DNA_Blood_100_400 (Roche, Mannheim, Germany). Primers for MT2A PCR were obtained from Sigma–Aldrich. The sequences of forward and reverse primers were 5'-ACTCGTCCCGGCTCTTCTA-3' and 5'-ATCCCCAGCCTCTACCG-3' respectively. Taq polymerase chain reaction (PCR) kit was purchased from New England BioLabs (MA, USA) and the PCR reaction mixture (100 μL) was composed of 10 μL of isolated DNA, 1 \times standard Taq reaction buffer, 0.2 μM of each deoxynucleotide, 0.4 μM of each primer and 4U of Taq DNA polymerase. The PCR reaction was carried out using mastercycler ep realplex4

(Eppendorf AG, Germany) and the thermal profiles were as follows: initial denaturation at 95 °C for 4 min; 35 cycles of denaturation at 95 °C for 1 min, annealing at 56 °C for 30 s and extension at 72 °C for 30 s; and final denaturation at 72 °C for 7 min followed by 3 min at 10 °C. The amplicons were purified by MinElute PCR Purification Kit (250) (Qiagen, Germany) according to manufacturer's instructions.

2.3. Preparation of As–DNA

Stock solutions of 400 $\mu\text{g mL}^{-1}$ As(III and/or V) were prepared. A stock solution of DNA (10 $\mu\text{g mL}^{-1}$) was mixed with different concentrations of As(III and/or V) in 1:1 ratio. The final concentrations of compounds in aqueous solutions (ACS purity) were 5 $\mu\text{g mL}^{-1}$ of DNA and 0, 0.4, 0.8, 1.6, 3.1, 6.3, 12.5 and/or 25 $\mu\text{g mL}^{-1}$ of As(III and V). The samples were incubated for 0, 10, 20, 30, 40, 50 and/or 60 min at 25 °C and then immediately analyzed. Then, other experiment was performed with aqueous solutions with final concentrations of 5 $\mu\text{g mL}^{-1}$ DNA and 50, 100 and/or 200 $\mu\text{g mL}^{-1}$ As(III and V). The samples were incubated for 0, 24 and 48 h at 7 °C. After the incubation, unbound As 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 resuspended in 500 μL of water and centrifuged again under the same conditions. Finally, the sample was resuspended in 200 μL water.

2.4. Determination of arsenic by atomic absorption spectrometry

Arsenic was determined on 280Z Agilent Technologies atomic absorption spectrometer (Agilent, USA) with electrothermal atomization. Arsenic hollow cathode lamp (Agilent) was used as the radiation source (lamp current of 10 mA). The spectrometer was operated at 193.7 nm resonance line with spectral bandwidth of 0.5 nm. The sample volume 20 μL was injected into the graphite tube. Zeeman background correction was used with field strength 0.8 T.

2.5. UV/vis spectrophotometry

Spectra of As(III and V) and As–DNA were recorded within the range from 190 to 400 nm by a spectrophotometer SPECORD 210 (Analytik Jena, Jena, Germany) using quartz cuvettes (1 cm, Hellma, Essex, UK) at 25 °C maintained by Julabo (Labortechnik, Wasserburg, Germany). The changes in absorbance spectra of the samples were recorded and evaluated using the program WinASPECT version 2.2.7.0.

2.6. Fluorescence measurement

Fluorescence spectra were acquired by multifunctional microplate reader Tecan Infinite 200 PRO (TECAN, Männedorf, Switzerland). Briefly, 50 μL of sample (DNA and As–DNA) and 2 μL of ethidium bromide (625 $\mu\text{g mL}^{-1}$) were placed in a transparent 96 well microplate with flat bottom by Nunc (Thermo Scientific, Waltham, USA). 545 nm was used as an excitation wavelength and the fluorescence scan was measured within the range from 585 to 830 nm per 2-nm steps. Each intensity value was an average of three measurements. The detector gain was set to 100. All measurements were performed after 10 min of incubation at 25 °C, controlled by Tecan Infinite 200 PRO (TECAN, Männedorf, Switzerland).

2.7. Agarose gel electrophoresis

Samples of As–DNA were electrophoresed in 2% (*m/v*) agarose gel (Chemos CZ, s.r.o., Czech Republic) in 1 \times TAE buffer (40 mM

Trizma-base, 20 mM acetic acid and 1 mM ethylenediaminetetraacetic acid, pH 8.0) with ethidium bromide. 10 μL of samples was mixed with 2 μL of 30% glycerol (*v/v*) and bromphenol blue. A 100 bp 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 60 V and 6 $^{\circ}\text{C}$ for 160 min. 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).

2.8. Electrochemical measurements of As–DNA

Determination of DNA was performed with 797 VA Stand instrument connected to 889 IC Sample Center (Metrohm, Switzerland). The analyser (797 VA Computrace, Metrohm, Switzerland) employs a conventional three-electrode configuration with a hanging mercury drop electrode (HMDE) with a drop area of 0.4 mm^2 which was the working electrode. An Ag/AgCl/3 M KCl electrode was the reference and glassy carbon electrode was auxiliary. For data processing, GPES 4.9 software was employed. The analyzed samples were deoxygenated prior to measurements by purging with argon (99.999%). Acetate buffer (0.2 M CH_3COONa and CH_3COOH , pH 5) was used as a supporting electrolyte. The supporting electrolyte was exchanged after each analysis. The parameters of the square wave voltammetry were as follows: purging time 120 s, frequency 280 Hz, accumulation time 2 min, initial potential 0 V, end potential -1.8 V, step potential 0.005 V, modulation amplitude 0.025 V.

3. Results and discussion

3.1. Spectroscopic characterization of the interaction between As(III) and DNA

Interaction of As(III) with DNA (fragment MT2A) were primarily monitored spectrophotometrically. The absorption spectra of 0–50 $\mu\text{g mL}^{-1}$ As(III) were measured within the wavelength range from 190 to 400 nm (Fig. 1A). Nucleic acids have an absorption maximum at $\lambda = 260$ nm, and therefore we investigated whether As(III) at studied concentrations does not absorb in the wavelength range 260–290 nm. Whereas the determined values of absorbances came under the borderline noise, can be said that As(III) does not absorb electromagnetic oscillation at this wavelength range, Fig. 1B. This finding is important in order to be able to compare the changes in the absorption DNA signal after application of various concentrations of arsenic. The same concentration range of As(III) measured in Fig. 1A was applied to prepare the mixture with DNA (5 $\mu\text{g mL}^{-1}$). Immediately after dilution, the absorption spectra were measured within the same wavelength range. For better illustration of the interaction between As(III) and DNA, the absorption spectrum of DNA (5 $\mu\text{g mL}^{-1}$) was subtracted from the absorption spectra of the mixture according to our previously published paper [27]. The results are shown in Fig. 1C in the form of differential spectra, whereas the wavelengths of interest are shown in the inset. Differential spectra contained two absorption maxima, at $\lambda = 196$ nm and $\lambda = 284$ nm (Fig. 1C). In this case, it was demonstrated that the increasing concentrations of As(III) lead to an increase of the signal measured at $\lambda = 284$ nm. For this reason in all experiments two wavelengths $\lambda = 260$ nm (DNA signal) and $\lambda = 284$ nm (signal

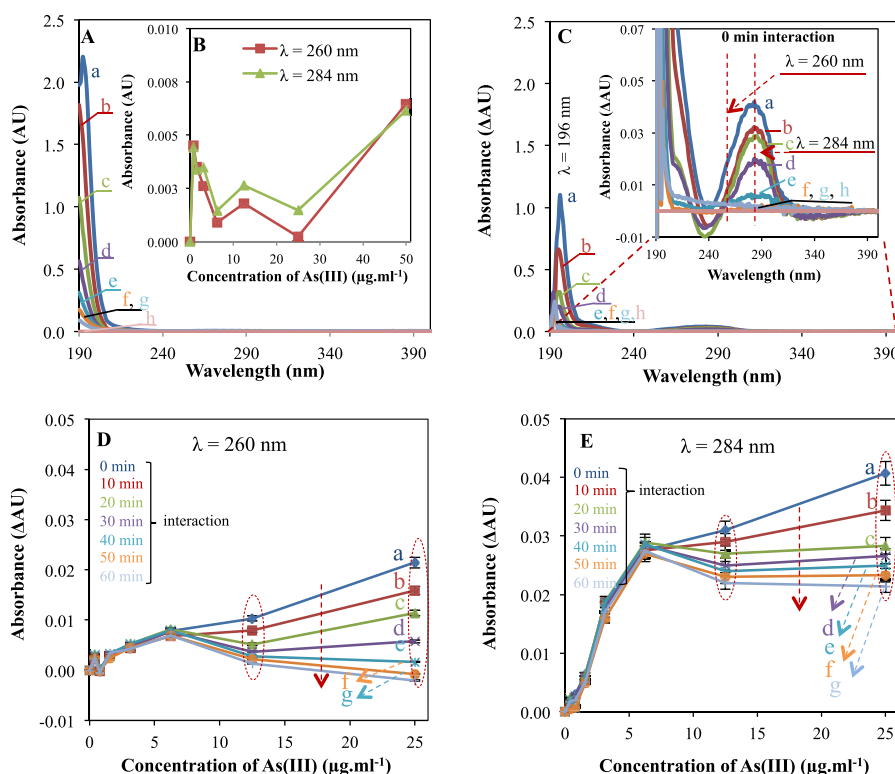


Fig. 1. (A) UV/vis spectra of As(III), concentration range 0–50 $\mu\text{g mL}^{-1}$ (a = 50, b = 25, c = 12.5, d = 6.25, e = 3.13, f = 1.56, g = 0.78 and h = 0 $\mu\text{g mL}^{-1}$) and wavelength range 190–400 nm. (B) Dependence of absorbance measured at $\lambda = 260$ nm and 284 nm on As(III) concentration (0–50 $\mu\text{g mL}^{-1}$). (C) Differential spectra (Δ) of 5 $\mu\text{g mL}^{-1}$ DNA with 0–25 $\mu\text{g mL}^{-1}$ As(III) (a = 25, b = 12.5, c = 6.25, d = 3.13, e = 1.56, f = 0.78, g = 0.39 and h = 0 $\mu\text{g mL}^{-1}$) measured within the wavelength range from 190 to 400 nm. (D) Dependence of the absorbance of As–DNA complex measured at $\lambda = 260$ nm in the various times of interaction as (a) 0, (b) 10, (c) 20, (d) 30, (e) 40, (f) 50 and (g) 60 min on the As(III) concentration 0–25 $\mu\text{g mL}^{-1}$. (E) Dependence of the absorbance of As–DNA complex measured at $\lambda = 284$ nm in the various times of interaction as (a) 0, (b) 10, (c) 20, (d) 30, (e) 40, (f) 50 and (g) 60 min on the As(III) concentration 0–25 $\mu\text{g mL}^{-1}$. For all analyses mean from three measurements are given and in D and E, RSD = 5%.

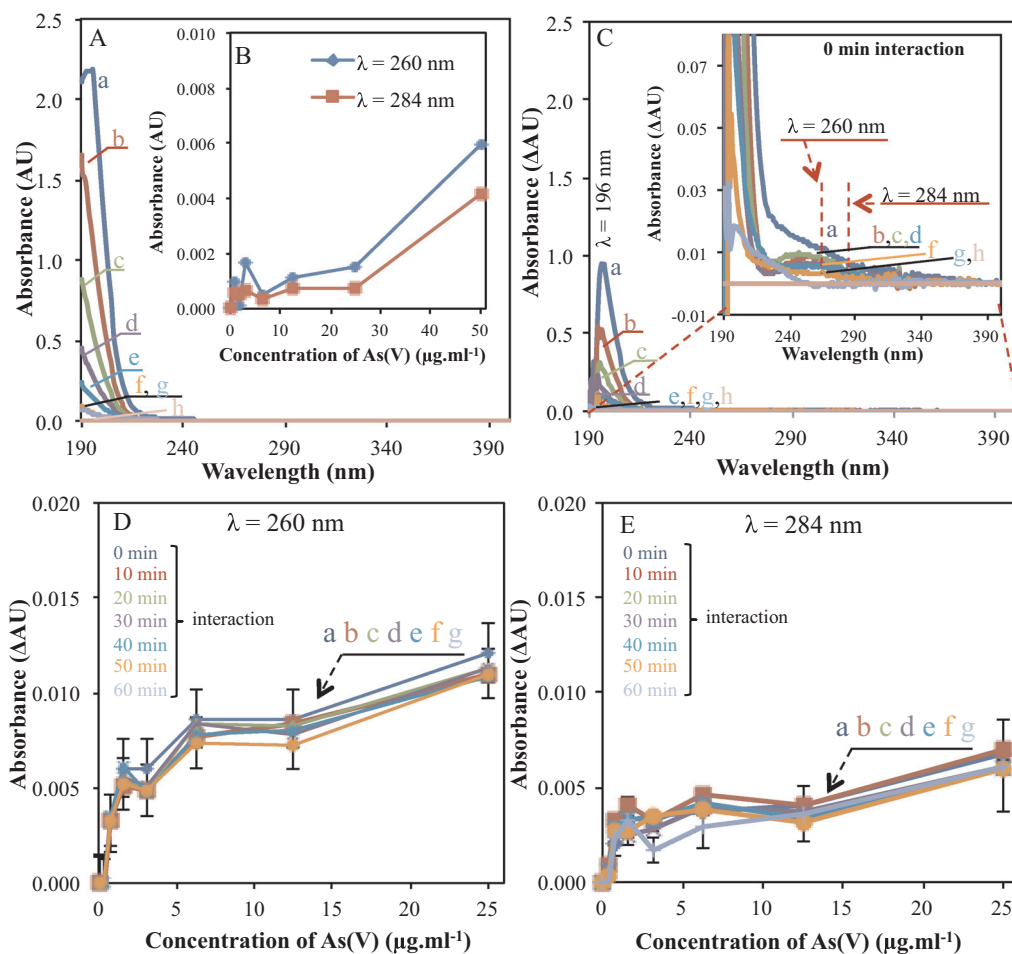


Fig. 2. (A) UV-vis spectra of As(V), concentration range 0–50 $\mu\text{g mL}^{-1}$ (a = 50, b = 25, c = 12.5, d = 6.25, e = 3.13, f = 1.56, g = 0.78 and h = 0 $\mu\text{g mL}^{-1}$) and wavelength range 190–400 nm. (B) Dependence of absorbance measured at $\lambda = 260$ nm and 284 nm on As(V) concentration (0–50 $\mu\text{g mL}^{-1}$) of 5 $\mu\text{g mL}^{-1}$ DNA with 0–25 $\mu\text{g mL}^{-1}$ As(III) (a = 25, b = 12.5, c = 6.25, d = 3.13, e = 1.56, f = 0.78, g = 0.39 and h = 0 $\mu\text{g mL}^{-1}$) measured within the wavelength range from 190 to 400 nm. (C) Differential spectra (Δ) of 5 $\mu\text{g mL}^{-1}$ DNA with 0–25 $\mu\text{g mL}^{-1}$ As(III) (a = 25, b = 12.5, c = 6.25, d = 3.13, e = 1.56, f = 0.78, g = 0.39 and h = 0 $\mu\text{g mL}^{-1}$) measured within the wavelength range from 190 to 400 nm. (D) Dependence of the absorbance of As–DNA complex measured at $\lambda = 260$ nm in the various times of interaction as (a) 0, (b) 10, (c) 20, (d) 30, (e) 40, (f) 50 and (g) 60 min on the As(V) concentration 0–25 $\mu\text{g mL}^{-1}$. (E) Dependence of the absorbance of As–DNA complex measured at $\lambda = 284$ nm in the various times of interaction as (a) 0, (b) 10, (c) 20, (d) 30, (e) 40, (f) 50 and (g) 60 min on the As(V) concentration 0–25 $\mu\text{g mL}^{-1}$. For all analyses mean from three measurements are given and in D and E, RSD = 5%.

that originated upon interaction with arsenic) were investigated (Fig. 1C). Moreover, the influence of interaction time on As–DNA interactions was studied via making differential spectra, i.e. subtracting signal of DNA only. Modifications of the differential spectra of As–DNA were observed at $\lambda = 260$ nm and $\lambda = 284$ nm after every 10 min as it is shown in Fig. 1D and 1E. The spectra showed structural changes of DNA, which appeared at concentrations of 12.5 and 25 $\mu\text{g mL}^{-1}$ of As(III). This phenomenon probably reflects the fact that the lower concentration of As(III) from 0.4 to 6.25 $\mu\text{g mL}^{-1}$ are able to bind covalently to DNA in the form of a stable structure. Earlier, it was shown spectroscopically that As(III) could bind to DNA and RNA at G – C, A – T, and A – U [28]. This finding supports the claim that arsenates might serve as a potential substitution for phosphates in the DNA backbone [21–23,29,30]. On the other hand, higher concentrations of As(III) as 12.5 and 25 $\mu\text{g mL}^{-1}$ destabilized the structure of DNA, which resulted in the decrease of the differential signal measured at these concentrations. This relates with the ability of As(III) to damage DNA playing a significant role in the induction of apoptosis in tumor cells. Nakamura et al. showed that treatment of osteosarcoma with As(III) increased occurrence of damaged DNA [31]. The damage of DNA with As was also observed in human lymphoblastoid cell [27], fibroblast [28] and lung cells [28].

3.2. Spectroscopic characterization of the interaction between As(V) and DNA

In the case of As(V)–DNA interactions, the procedure was the same as mentioned above. Absorption spectra of 0–50 $\mu\text{g mL}^{-1}$ As(V) were determined within the wavelength range from 190 to 400 nm (Fig. 2A). For two wavelengths ($\lambda = 260$ nm and $\lambda = 284$ nm) the dependence of absorbance on As(V) concentration (0–50 $\mu\text{g mL}^{-1}$) was measured and shown (Fig. 1B). The same concentration range of As(V) was used to prepare complexes with DNA (5 $\mu\text{g mL}^{-1}$). Immediately after dilution, the absorption spectra were determined within the wavelength range from 190 to 400 nm. Differential spectra showed in Fig. 2C contained two absorption maxima (at $\lambda = 196$ and $\lambda = 284$ nm) as in the case of As(III). In the case of As(V), the differential spectra did not show so marked interaction in comparison with As(III). However, slight increasing of both measured signals was determined (Fig. 2C). Fig. 2D and E shows the influence of time of interactions of As and DNA (0–60 min). During 60 min long interactions, negligible signal changes were observed only with increasing trend. These results confirmed that the interaction of As(V) with DNA is weaker compared to As(III). It is generally known that the toxicity of As(V) is lower in comparison with As(III). This effect may be caused by the fact that As(V)

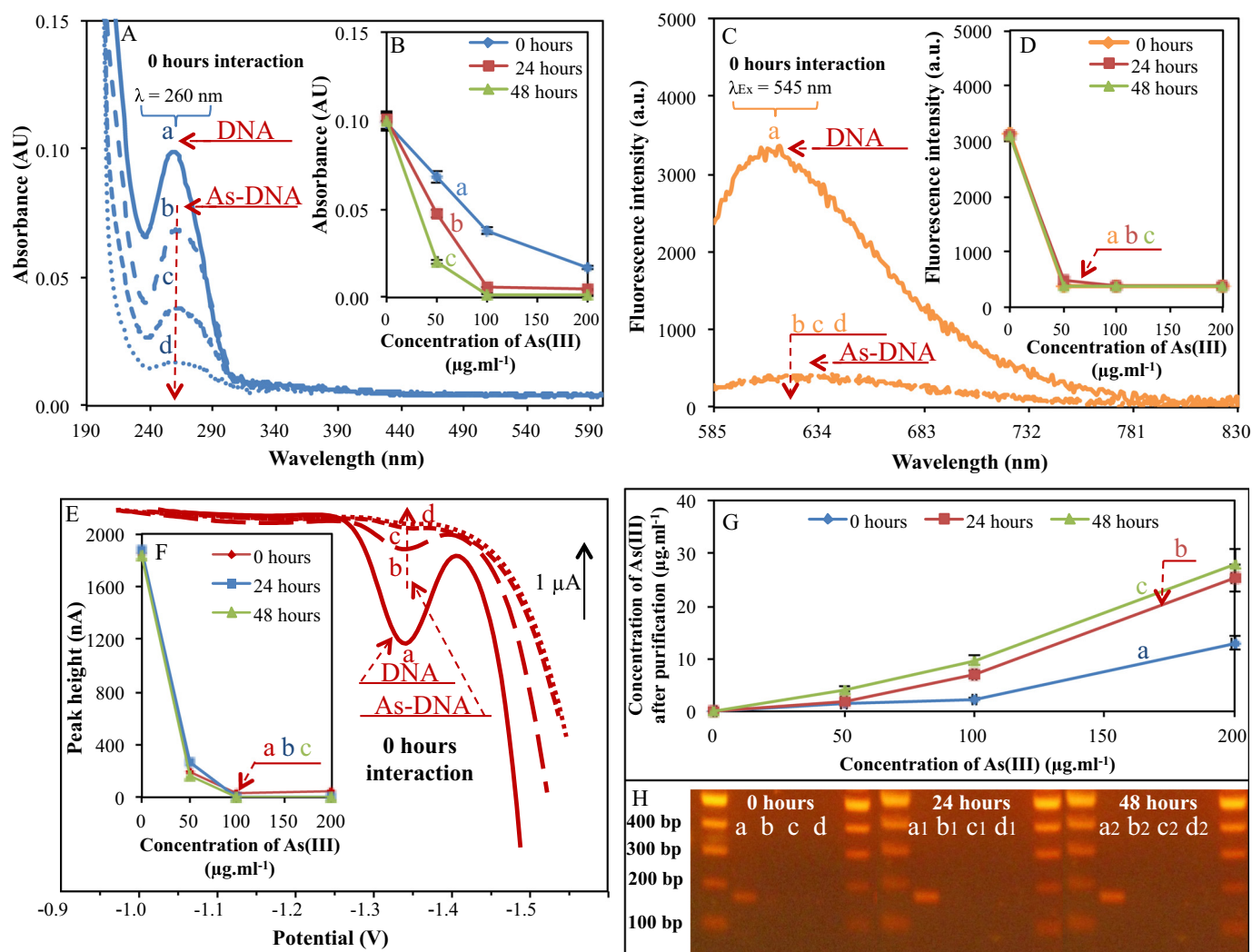


Fig. 3. (A) UV/vis spectra of (a) $5 \mu\text{g mL}^{-1}$ DNA and DNA with As(III) in concentration of (b) 50, (c) 100 and (d) $200 \mu\text{g mL}^{-1}$ measured with the wavelength range from 190 to 600 nm. (B) The influence of As(III) concentration (0–200 $\mu\text{g mL}^{-1}$) on the signal of DNA ($5 \mu\text{g mL}^{-1}$) measured at $\lambda = 260 \text{ nm}$ in various time intervals as (a) 0 h, (b) 24 h and (c) 48 h. (C) Fluorescence spectra of (a) $25 \mu\text{g mL}^{-1}$ EtBr and $5 \mu\text{g mL}^{-1}$ DNA and DNA with As(III) at concentration of (b) 50, (c) 100 and (d) $200 \mu\text{g mL}^{-1}$ ($\lambda_{\text{Ex}} = 545 \text{ nm}$, $\lambda_{\text{Em}} = 585\text{--}830 \text{ nm}$). (D) The influence of As(III) (0–200 $\mu\text{g mL}^{-1}$) on the intensity of fluorescent signal of DNA ($5 \mu\text{g mL}^{-1}$) interacted with EtBr ($25 \mu\text{g mL}^{-1}$) measured in various time intervals as (a) 0 h, (b) 24 h and (c) 48 h. (E) SWV voltammogram of (a) $5 \mu\text{g mL}^{-1}$ DNA and DNA with As(III) in concentration of (b) 50, (c) 100 and (d) $200 \mu\text{g mL}^{-1}$. (F) The influence of As(III) concentration (0–200 $\mu\text{g mL}^{-1}$) on CA peak of $5 \mu\text{g mL}^{-1}$ DNA in various time intervals as (a) 0 h, (b) 24 h and (c) 48 h. (G) The total concentration of As(III) interacted with $5 \mu\text{g mL}^{-1}$ DNA (As–DNA) determined in various time intervals as (a) 0 h, (b) 24 h and (c) 48 h. (H) Electropherogram of $5 \mu\text{g mL}^{-1}$ DNA and DNA with As(III) in concentration of (b) 50, (c) 100 and (d) $200 \mu\text{g mL}^{-1}$.

generates lower amount of the free radicals [1,29] and it has a lower affinity to DNA, which was confirmed by the results obtained here.

3.3. The influence of As(III) on the structural changes in DNA

In this part of the work, As(III) (50 , 100 and $200 \mu\text{g mL}^{-1}$) was interacted with DNA ($5 \mu\text{g mL}^{-1}$) to observe DNA structural changes using spectroscopy and electrochemistry. The procedure of As–DNA purification using the filtration columns Amicon Ultra 3K is described in Material and Methods section and it was processed after 0, 24 and 48 h of incubation. After the purification, the sample was divided into five aliquots and analyzed immediately. Differences in the absorption spectra of As–DNA were observed within the wavelength range from 190 to 600 nm. It follows from the results obtained that the increasing concentration of As(III) caused a decrease in As–DNA absorption signals ($\lambda = 260 \text{ nm}$), which is shown in Fig. 3A, as well as the increasing time of interaction showed in Fig. 3B. Further, the samples were mixed with $24 \mu\text{g mL}^{-1}$ of ethidium bromide (EtBr) and the

fluorescence analysis was performed (Fig. 3C). EtBr is a typical mutagen with the ability to intercalate into DNA [32], however, it was demonstrated that EtBr was unable to intercalate into DNA structure due to the presence of As(III) in the structure. This was found by Zhu et al. when studying interactions of DNA with 5-Hydroxymethyl-2-furfural [33]. Based on these, we expected the same and found that the decrease in fluorescence signal with the increasing As(III) concentration and time of interaction is shown Fig. 3D. The results obtained are in good agreement with those published by Zhu et al. [33]. Moreover, As–DNA interaction was also studied using square wave voltammetry (SWV) to confirm the formation of As–DNA complex. First reports about electrochemical reduction and oxidation signal of nucleic acids were published by the end of 1950s and in the beginning of 1960s [34]. It was pointed out that these signals are due to the residues of bases in DNA. Adenine and cytosine in DNA yielded common reduction signals at the same potential called CA peak [35]. In the case of As–DNA, the great decrease of CA peak with the increasing concentration of As(III) in DNA in comparison with DNA itself were determined (Fig. 3E). This

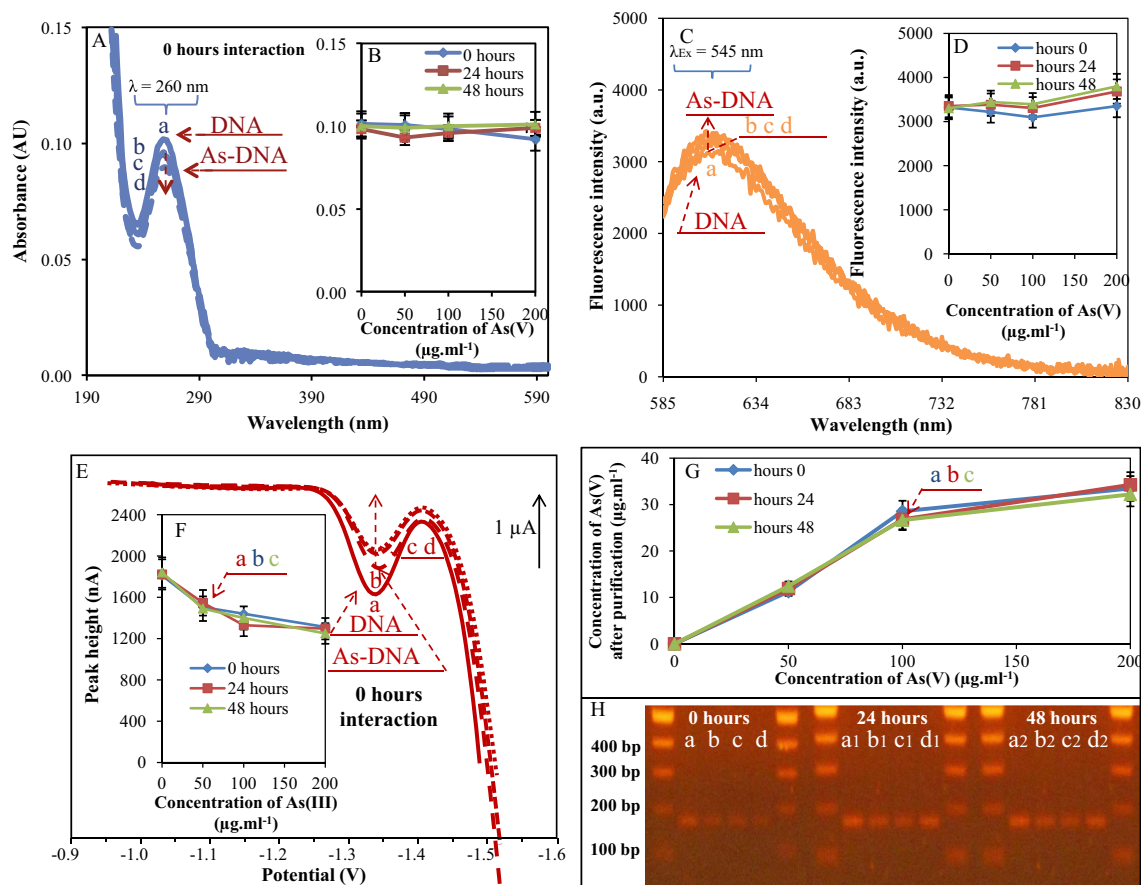


Fig. 4. (A) UV/vis spectra of (a) $5 \mu\text{g mL}^{-1}$ DNA and DNA with As(V) in concentration of (b) 50, (c) 100 and (d) $200 \mu\text{g mL}^{-1}$ measured with the wavelength range from 190 to 600 nm. (B) The influence of As(V) concentration ($0\text{--}200 \mu\text{g mL}^{-1}$) on the signal of DNA ($5 \mu\text{g mL}^{-1}$) measured at $\lambda = 260 \text{ nm}$ in various time intervals as (a) 0 h, (b) 24 h and (c) 48 h. (C) Fluorescence spectra of (a) $25 \mu\text{g mL}^{-1}$ EtBr and $5 \mu\text{g mL}^{-1}$ DNA and DNA with As(V) at concentration of (b) 50, (c) 100 and (d) $200 \mu\text{g mL}^{-1}$ ($\lambda_{\text{EX}} = 545 \text{ nm}$, $\lambda_{\text{EM}} = 585\text{--}830 \text{ nm}$). (D) The influence of As(V) ($0\text{--}200 \mu\text{g mL}^{-1}$) on the intensity of fluorescent signal of DNA ($5 \mu\text{g mL}^{-1}$) interacted with EtBr ($25 \mu\text{g mL}^{-1}$) measured in various time intervals as (a) 0 h, (b) 24 h and (c) 48 h. (E) SW voltammogram of (a) $5 \mu\text{g mL}^{-1}$ DNA and DNA with As(V) in concentration of (b) 50, (c) 100 and (d) $200 \mu\text{g mL}^{-1}$. (F) The influence of As(V) concentration ($0\text{--}200 \mu\text{g mL}^{-1}$) on CA peak of $5 \mu\text{g mL}^{-1}$ DNA in various time intervals as (a) 0 h, (b) 24 h and (c) 48 h. (G) The total concentration of As(V) interacted with $5 \mu\text{g mL}^{-1}$ DNA (As-DNA) determined in various time intervals as (a) 0 h, (b) 24 h and (c) 48 h. (H) Electroferogram of $5 \mu\text{g mL}^{-1}$ DNA and DNA with As(V) in concentration of (b) 50, (c) 100 and (d) $200 \mu\text{g mL}^{-1}$.

electrochemical method confirmed that the investigated concentrations of As(III) can damage DNA significantly by strong binding of As into DNA. The changes in CA peak of As–DNA after 0, 24 and/or 48 h interaction are shown in Fig. 3F. In all cases of As–DNA samples after purification, the total arsenic concentration was determined. As concentration was increasing in time, this proves the ability of As(III) to bind into the DNA structure (Fig. 3G). The gel electrophoresis showed only the presence of the control DNA and the As–DNA bands were not observed here (Fig. 3H), which can be also considered as the confirmation of interactions between As and DNA

3.4. The influence of As(V) to the structural changes of DNA

The same procedure as described in the previous section was used for As(V). The absorption spectra of As–DNA were determined within the range from 190 to 600 nm. The signals of As–DNA ($\lambda = 260 \text{ nm}$) decreased (Fig. 4A) with the increasing concentration, but the decrease was not significant (Fig. 4B). Moreover, the fluorescence spectra did not exhibit a marked change of As–DNA fluorescence signals (Fig. 4C). Significant change in the fluorescence signals did not occur even after 24 and 48 h of the incubation (Fig. 4D). Electrochemical observation of CA peaks showed differences in the peak height of As–DNA (Fig. 4E). This change was high, but in average it was $450\times$ lower in comparison with As(III). The

differences in CA peaks after 24 or 48 h of incubation were not detected (Fig. 4F). Quantification of total As in purified samples after 0, 24 or 48 h of incubation did not show the increase in As(V) concentration (Fig. 4G) and the gel electrophoresis showed the presence of As–DNA, but the shifts for different sizes of As–DNA were not detected (Fig. 4H). All these results confirm that As(V) interacts slightly with DNA.

4. Conclusions

In this study, the interactions of As(III and V) with DNA were investigated by spectroscopic and electrochemical methods. It was found that As(III) in lower concentrations ($0.4\text{--}6.25 \mu\text{g mL}^{-1}$) forms stable structure with DNA. In the case of higher concentrations ($\text{As(III)} > 6.25 \mu\text{g mL}^{-1}$) the structure of DNA is significantly damaged. The interactions of As(V) with DNA were not spectroscopically observed, but the electrochemical investigation showed interactions. Therefore, it can be concluded that As(III) has great potential to somewhat intercalate into DNA.

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