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Short communication

Study of DNA damage caused by dipyrone in presence of some transition metal ions

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A B S T R A C T

The DNA damage in the presence of dipyrone (used as its sodium salt, NaDip) and some transition metal ions in an air saturated ($[O_2] \approx 0.25$ mM) non-buffered solution at T = (25.0 ± 0.5)°C was investigated by agarose gel electrophoresis. As metal ions Cu^{2+}, Fe^{3+}, Ni^{2+} and Mn^{3+} were selected and evaluated in the present study because of the important role they play in a biological system.

pUC19 plasmid DNA damage-induced by NaDip (80–600 μM) was observed in the presence of 100 μM Cu^{2+}. The damage was proportional to the NaDip concentration provided that the order of addition of reagents (pUC19 plasmid DNA + Cu^{2+} + NaDip) is obeyed. Addition in the reaction medium of ligands for Cu^{2+} and Cu^+, respectively EDTA and neocuproine, promoted total inhibition or reduction of the pUC19 plasmid DNA damage suggesting the involvement of the Cu^{2+}/Cu^+ cycle. Besides, the decrease in the pUC19 plasmid DNA damage after addition of catalase (1.0×10^{-4} mg μL^{-1}) in the same reaction medium indicates that H_2O_2 is also involved in the damage process.

In NaDip concentration range (80–600 μM), and under same the experimental conditions, it was not possible to conclude whether there was pUC19 plasmid DNA damage caused by 10 μM Fe^{3+}. No damage was observed in the presence of Mn^{3+} or Ni^{2+}.

Although the technique used in this study is sensitive to detect the pUC19 plasmid DNA damage it was not possible to identify in which DNA base this damage occurs. Further studies with other techniques should be made to unambiguously identify the oxidative intermediates that are responsible for the DNA damage.

As far as we know, this is the first study dealing with the pUC19 plasmid DNA damage-induced by NaDip in presence of copper, iron, nickel and manganese ions.

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1. Introduction

Cells must maintain an appropriate balance between the level of free radicals and antioxidants compounds to ensure its biological structural integrity. If the level of these free radicals exceeds the antioxidants compounds, acquired from diet or endogenously produced, biomolecules such as proteins and DNA can be damaged (Kawanishi et al., 2005).

Several *in vitro* studies have shown that some chemical compounds can induce DNA damage (Schweigert et al., 2000; Asad et al., 2002; Zheng et al., 2006). Among these compounds there are some drugs as L-Dopa (Husain and Hadi, 1995), N-acetylcysteine (Oikawa et al., 1999), procarbazine (Ogawa et al., 2003) gentamicin (Lesniak et al., 2003), AZT (Iwamoto et al., 2003), and chloramphenicol (Ohnishi et al., 2015) that, under specific conditions and in the presence of a transition metal ion (M^{n+}), can also lead to DNA damage. As a common sense it is believed that these drugs can reduce M^{n+} to $M^{(n-1)+}$, which in the presence of H_2O_2 generates free radicals inducing DNA damage as a consequence.

NaDip, also known as dipyrone, novalgin or metamizole (Fig. 1), is a nonsteroidal anti-inflammatory drug indicated for the treatment of painful manifestations and fever with a complex mechanism responsible for the analgesic effect (Shimada et al., 1994). Despite the suspected occurrence of agranulocytosis, a potentially fatal side

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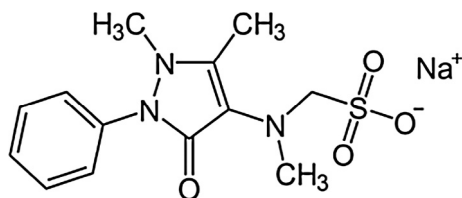Production and hosting by Elsevier

Fig. 1. Structure of sodium dipyrone (NaDip).

effect (Hedenmalm and Spigset, 2002; Wessel et al., 2006), this drug is still prescribed and used in many countries. Besides, as far as we know, no study has been done to evaluate the possible DNA damage-induced by NaDip in presence of trace elements.

In the present study pUC-19 plasmid DNA was exposed to some transition metal ions (Cu^{2+}, Fe^{3+}, Ni^{2+} and Mn^{3+}), which play important roles in a biological system. Copper is an important element for growth and many enzymes require it to function properly. Iron is essential for hemoglobin formation (macronutrient in pregnant women) and most of this metal is ingested as Fe^{3+} in human diet. Nickel is a micronutrient able to prevent anemia by aiding the absorption of iron. Manganese, also a trace element, is a cofactor for a number of important enzymes. Although present as Mn^{2+} in human diet an *in vitro* study pointed out that the trivalent state (Mn^{3+}) appear to be more cytotoxic (Chen et al., 2001).

Oxidative damage to the pUC-19 plasmid DNA was verified by agarose gel electrophoresis through the conversion of supercoiled pUC-19 plasmid DNA (supercoil, SC, native conformation) to open circular (OC) form, resulting from single break, in solution containing NaDip in an unbuffered and air saturated solution ($[O_2] \approx$ 0.25 mM) (Moreno et al., 2007).

In all experiments the quantification of pUC-19 plasmid DNA damage was performed by the ratio between the total amount of OC (normalized with respect to the background produced by pUC-19 plasmid DNA alone) and the total amount of pUC-19 plasmid DNA present. In none of the experiments was observed the linear form (L), which is the result of the double-strand breaks.

Under these experimental conditions a marked damage to DNA was observed in the presence of Cu^{2+} and NaDip, being proportional to the drug concentration. Addition of EDTA and neocuproine, chelating agents for Cu^{2+} and Cu^+ respectively, inhibited or diminished the pUC-19 plasmid DNA damage suggesting the participation of the Cu^{2+}/Cu^+ redox cycle. Moreover, the decrease in the pUC-19 plasmid DNA damage after the introduction of catalase allows inferring that H_2O_2 is also involved in this damage process.

2. Materials and methods

2.1. Apparatus

Electrophoresis experiments were performed in a mini submerged horizontal gel chamber using a PowerPac™ Basic power supply.

A transilluminator UV ZT-21 (BioGlow® Crystal) coupled with a digital camera PowerShot G10 (Canon®) was used to obtain the photograph of gels.

pUC-19 plasmid DNA bands were quantified with the AlphaEase® FC Software (Pharmacia Biotech, San Francisco, CA, USA).

2.2. Reagents

Deionized water was purified with a Milli-Q Plus Water System (Millipore®) and used to prepare all analytical-grade chemicals (unless stated otherwise).

Supercoiled pUC-19 plasmid DNA (native form) 0.5 µg µL^{-1} and agarose electrophoresis were from MBI Fermentas®.

Ethidium bromide ($C_{21}H_{20}BrN_3$, 394.31 g mol^{-1}, 500 µg mL^{-1}), Ficoll type 400, bromophenol blue ($C_{19}H_{10}Br_4O_5S$, 669.96, g mol^{-1}), boric Acid (H_3BO_3, 61.82 g mol^{-1}, $\geq$99.5%), Neocuproine (NC, $C_{14}H_{12}N_2$, 208.26 g mol$^{-1} \geq$ 98%), sodium dipyrone (NaDip, $C_{13}H_{16}$-N_3NaO_4S, 333.34 g mol^{-1}, >99.9%), catalase from bovine liver (13,500 U mg^{-1}) were from Sigma®.

1,10-phenanthroline monohydrate (phen, $C_{12}H_8N_2 \cdot H_2O$, 198.26 g mol^{-1}, 99.5%) and ethanol (CH_3CH_2OH, 46.06 g mol^{-1}, 99.5%), TRIS (tris(hydroxymethyl)aminomethane, $C_4H_{11}NO_3$, 121.14 g mol^{-1}), disodium EDTA (Na_2EDTA, $C_{10}H_{14}N_2O_8Na_2 \cdot 2H_2O$, 372.24 g mol^{-1}), manganese(III) acetate dihydrate ($C_6H_9MnO_6 \cdot H_2$-O, 268.10 g mol^{-1}, >98%), copper disodium EDTA ($Na_2Cu[EDTA]$, 397.74 g mol$^{-1} \geq$ 99.5%) were from Merck®.

2.3. Solutions

pUC-19 plasmid DNA 1.875×10^{-2} µg µL^{-1} solution was obtained transferring 6 µL pUC-19 plasmid DNA 0.5 µg µL^{-1} (freshly thawed) to a 1.5 mL microtube and adding 154 µL water.

NaDip 7.5 mM stock solution was prepared by transferring 0.2500 g into a 100 mL volumetric flask and completed with water. Working solutions (0.5–800 µM) were obtained by appropriate dilution.

$Cu(NO_3)_2$, $Fe(ClO_4)_3$ and $Ni(NO_3)_2$ 0.20 M stock solutions (respectively at pH 6.3, 3.5 and pH 4.5) were prepared from reaction of pure electrolytic metal (99.99%, Sigma®) with doubly distilled nitric acid (99.99% Sigma®) as described elsewhere (Moreno et al., 2007; Alipázaga et al., 2009, 2010). A 0.75 mM diluted solution of each ion was daily prepared by diluting 375 µL of 0.20 M stock solution in a 100.0 mL volumetric flask.

$Cu[EDTA]^{2-}$ 0.75 mM complex solution was prepared by dissolving 0.0140 g $Na_2Cu[EDTA]$ in a 50.0 mL volumetric flask (pH 7.5).

$Cu(NC)_2^{2+}$ 0.75 mM complex solution was prepared by mixing 0.0156 g NC and 188 µL $Cu(NO_3)_2$ 0.2 M stock solution in a 50.0 mL volumetric flask (pH 7.5).

$Fe[EDTA]^-$ 0.75 mM complex solution was prepared by dissolving 0.0140 g Na_2EDTA and mixing with 188 µL $Fe(ClO_4)_3$ 0.20 M in a 50.0 mL volumetric flask (pH 4.5).

Solution mixture containing Fe^{3+} and phen, both 0.75 mM, was prepared by dissolving 0.0223 g phen and mixing with 188 µL Fe($ClO_4)_3$ 0.2 M in a 50.0 mL volumetric flask (pH 5.0).

$Mn(H_3C-COO)_3$ 0.75 mM was prepared just before use transferring 0.020 g manganese(III)-acetate-dihydrate to a 100.0 mL volumetric flask containing 50 mL of water (pH 4.5).

Cu^{2+} (1.0–100 µM), Fe^{3+} (5.0–100 µM), Ni^{2+} and Mn^{3+} (10–100 µM) working solutions were all obtained by appropriate dilution.

Catalase freshly solution was prepared by dissolving 1.0 mg in 2.0 mL water.

The preparation of TBE buffer (pH 8.0) and agarose gel 0.8% are described in Supplementary Material.

3. Procedures

3.1. Strategy for preparation of mixtures

In all experiments, pUC-19 plasmid DNA were exposed to a transition metal ion (complexed or not) in air saturated solution ($[O_2] \approx$ 0.25 mM) followed by the addition of NaDip solution. The percentages of pUC-19 plasmid DNA strand breaks were observed when the freshly prepared solutions of the reagents were mixed.

Three series of experiments were carried out in the following order:

(a) In the presence of metal ion and absence of NaDip: 20 μL of pUC-19 plasmid DNA 1.875×10^{-2} μg μL^{-1} were transferred to eight 1.5 mL microtubes. From the second microtube 10 μL a transition metal ion solution of different concentrations were added.

(b) In the presence of a fixed concentration of metal ion containing NaDip: 20 μL of pUC-19 plasmid DNA 1.875×10^{-2} μg μL^{-1} were transferred to eight 1.5 mL microtubes. From the second microtube 10 μL a transition metal ion solution (complexed or not) were added and from the third microtube 10 μL of NaDip solution of different concentrations were added.

(c) In the presence of a fixed concentration of NaDip containing metal ion: 20 μL pUC-19 plasmid DNA 1.875×10^{-2} μg μL^{-1} were transferred to eight 1.5 mL microtubes. From the second microtube 10 μL of NaDip solution were added and from the third microtube 10 μL a transition metal ion solution (complexed or not) of different concentrations were added.

All of microtubes were made up to 50 μL with water. The temperature was kept at (25.0 ± 0.5) °C. The concentrations of the final solutions are indicated in the legends of the figures.

The gel electrophoresis experiments are described in Supplementary Material.

4. Results and discussion

4.1. Electrophoresis experiments in the presence of Cu^{2+}

Several control experiments were carried out in the present work. No pUC-19 plasmid DNA damage was observed when Cu^{2+} ranges from 10 to 120 μM in the absence of NaDip (Fig. 1, Supplementary Material) under these experimental conditions. Since the Cu^{2+} concentration used is not strictly established (Husain and Hadi, 1995; Ogawa et al., 2003; Iwamoto et al., 2003; Lesniak et al., 2003), in the studies involving DNA damage in the presence of drugs, a Cu^{2+} 100 μM solution (Frelon et al., 2003) was chosen to be used in the present study (Fig. 2C).

Under the experimental conditions used in the present study no pUC-19 plasmid DNA damage was markedly observed when NaDip ranges from 0.5 to 100 μM (Fig. 2A), however the damage can be clearly noted from 200 μM (Fig. 2B, lane 4). Fig. 2C shows the results of an experiment by varying Cu^{2+} concentration and main-taining fixed NaDip at 500 μM in which it is possible to notice that the pUC-19 plasmid DNA damage is complete when Cu^{2+} is 100 μM (Fig. 2C, lane 8). It can be noted from these results that there is a minimum [NaDip:Cu^{2+}] concentration ratio for pUC-19 plasmid DNA damage be initiated (2:1) and then completed (5:1).

Experiments have shown that when Cu^{2+} (100 μM) is added as $Cu[EDTA]^{2-}$ (log β = 18.8) (Smith and Martell, 2004) no pUC-19 plasmid DNA damage was observed (Fig. 2, Supplementary Material). In addition, when Cu^{2+} (100 μM) is added along with NC (a ligand for Cu^+) the pUC-19 plasmid DNA damage indicated by OC formation is significantly repressed (Fig. 2D). The decrease of NaDip/Cu^{2+}-mediated pUC-19 plasmid DNA strand breaks by NC addition occurs as a result of the $Cu(NC)_2^+$ complex formation (log β$_2$ = 19.1) (Smith and Martell, 2004) removing Cu^+ from the solution. This corroborates that the Cu^{2+}/Cu^+ redox cycle occurs (in the presence of O_2) and is essential for the pUC-19 plasmid DNA damage in presence of NaDip.

In another experiment the enzyme catalase was added in the following order: pUC-19 plasmid DNA, catalase (final concentration 1×10^{-4} mg μL^{-1}), Cu^{2+} and NaDip (experimental conditions of Fig. 2). Since the addition of the enzyme catalase inhibited entirely the pUC-19 plasmid DNA damage (Fig. 3, Supplementary Material) is possible to infer that the presence of H_2O_2 in the reaction medium is necessary to cause this damage.

In the experiments where pUC-19 plasmid DNA damage occurred it was noted that the order of addition of reagents (i.e. pUC-19 plasmid DNA + Cu^{2+} + NaDip) is critical. In fact, when it was added pUC-19 plasmid DNA + NaDip + Cu^{2+} no damage was observed. This suggests that the copper ions must be linked to the pUC-19 plasmid DNA before the NaDip-mediated damage occurs. In fact, it was reported that Cu^{2+} ions are able to interact with DNA in-between the bases (Lloyd and Phillips, 1999) yielding DNA-copper-hydroperoxo complexes which generates highly reactive species as O_2 and ·OH (Schweigert et al., 2000). The experiments described above seem to show that there is no formation of complexes between NaDip and Cu^{2+} under these experimental conditions.
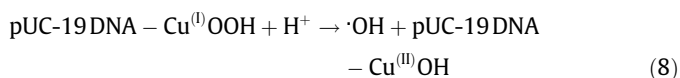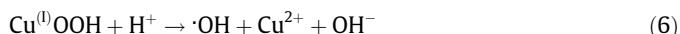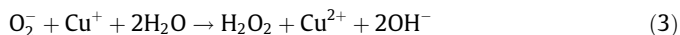
In addition, it has been previously shown that resveratrol (Win et al., 2002), hydroquinone (Li and Trush, 1993) and catechol (Schweigert et al., 2000), all non-drugs, are also capable of inducing DNA strand breaks (OC and L configurations) in the presence of Cu^{2+} with effective contribution of H_2O_2 and the Cu^{2+}/Cu^+ redox cycle.

Based on those reports (Win et al., 2002; Li and Trush, 1993; Schweigert et al., 2000), and from the observations of the present study, a mechanism for the pUC-19 plasmid DNA damage in the presence of Cu^{2+} and NaDip (Eqs. (1) to (8)) is proposed (despite
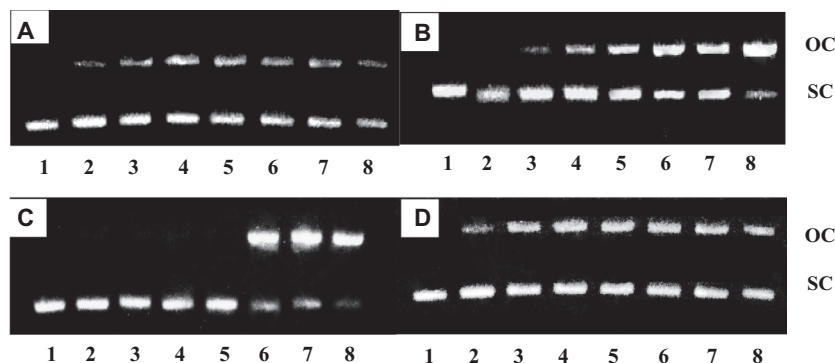
Fig. 2. Effect of Cu^{2+}. The pUC19 plasmid DNA is present in two conformations SC (native) and OC (resulting from single strand breaks). Saturated air solution ([O_2] ≈ 0.25 mM). pH 7.5. T = (25.0 ± 0.5) °C. All lanes (1) contains only pUC19 plasmid DNA 5 ng μL^{-1}. (A) Lane (2): (1) + Cu^{2+} 100 μM; Lanes (3): (2) + NaDip 0.5; 1.0, 5.0; 10; 50 and 100 μM respectively. (B) Lane (2): (1) + Cu^{2+} 100 μM; Lanes (3–8): (2) + NaDip 80; 200; 300; 400; 500 and 600 μM, respectively. (C) Lane (2): (1) + NaDip 500 μM; Lane (3): (2) + Cu^{2+} 5.0; 10; 25; 50; 75 and 100 μM; respectively. (D) Lane (2): (1) + $Cu(NC)_2^+$ 100 μM; Lanes (3–8): (2) + NaDip 80; 200; 300; 400; 500 and 600 μM, respectively.

the fact that the oxidized form of Dip⁻ has not yet been properly elucidated and that the O_2^- and ·OH free radicals were not determined in the present study):

$$Dip^- + Cu^{2+} \rightarrow Dip_{oxidized} + Cu^+ \qquad (1)$$

$$O_2 + Cu^+ \rightarrow O_2^- + Cu^{2+} \qquad (2)$$

$$O_2^- + Cu^+ + 2H_2O \rightarrow H_2O_2 + Cu^{2+} + 2OH^- \qquad (3)$$

$$O_2^- + H_2O_2 \rightarrow OH^- + \cdot OH + O_2 \qquad (4)$$

$$H_2O_2 + Cu^+ \rightarrow Cu^{(I)}OOH + H^+ \qquad (5)$$

$$Cu^{(I)}OOH + H^+ \rightarrow \cdot OH + Cu^{2+} + OH^- \qquad (6)$$

$$H_2O_2 + pUC\text{-}19\,DNA + Cu^+ \rightarrow pUC\text{-}19\,DNA - Cu^{(I)}OOH + H^+ \qquad (7)$$

$$pUC\text{-}19\,DNA - Cu^{(I)}OOH + H^+ \rightarrow \cdot OH + pUC\text{-}19\,DNA - Cu^{(II)}OH \qquad (8)$$

From the data obtained in the *in vitro* study presented here (Fig. 5) it can be seen that NaDip concentration that caused markedly pUC-19 plasmid DNA damage (in presence of Cu^{2+}) is about 7500 times smaller than the NaDip concentration usually available
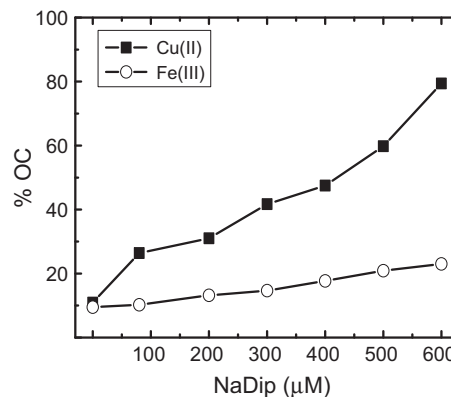
Fig. 5. pUC-19 plasmid DNA damage in the presence of NaDip and Cu^{2+} or Fe^{3+} (both 100 µM). Saturated air solution ($[O_2] \approx 0.25$ mM). pH = 7.5. T = (25.0 ± 0.5) °C. The data points represent the average of three OC% values (error between 1 and 10%). Experimental conditions shown in Figs. 2 and 3.

in most pharmaceutical formulations (500 mg mL⁻¹ ≈ 1.60 M). Nevertheless, it does not allow inferring that the same damage degree occurs *in vivo* conditions. In fact, in the human body the presence of ceruloplasmin (protein responsible for binding to the free copper) may prevent Cu^{2+} from being a catalytic inducing factor in redox reactions.
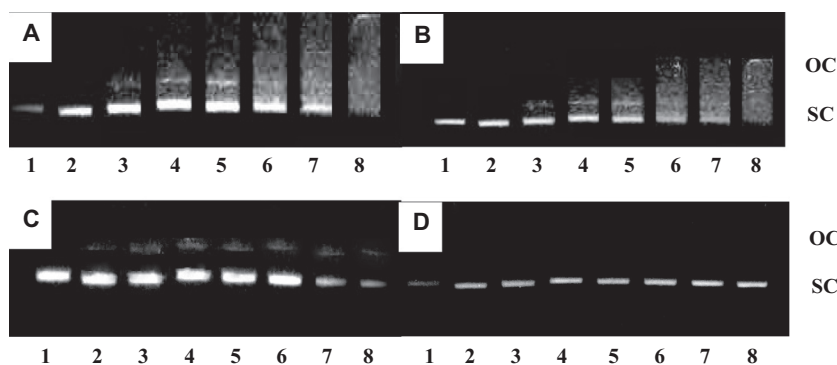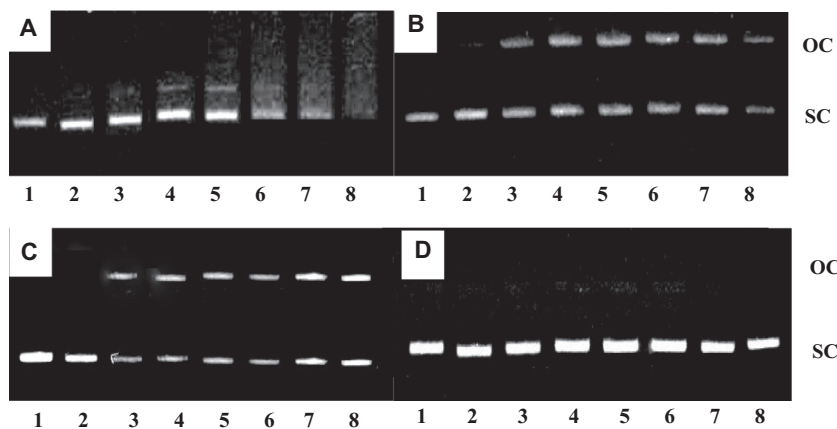

Fig. 3. Effect of Fe^{3+}. The pUC19 plasmid DNA is present in two conformations SC (native) and OC (resulting from single strand breaks). Saturated air solution ($[O_2] \approx$ 0.25 mM). pH 7.5; T = (25.0 ± 0.5) °C. All lanes (1) contains only pUC19 plasmid DNA 5 ng µL⁻¹. (A) Lanes (2) a (8): (1) + Fe^{3+} 5.0; 10; 20; 25; 50; 75 and 100 µM, respectively (No NaDip). (B) Lane (2): (1) + NaDip 500 µM; Lanes (3–8): (2) + Fe^{3+} 10; 20; 25; 50; 75 and 100 µM, respectively. (C) Lane (2): (1) + Fe^{3+} 10 µM; Lanes (3) a (8): (2) + NaDip 300; 400; 500; 600; 700 and 800 µM, respectively. (D) Lane (2): (1) + Fe^{3+} and phen (both 10 µM); Lane (3–8): (2) + NaDip 300; 400, 500, 600, 700 and 800 µM, respectively.

Fig. 4. Effect of Ni^{2+} or Mn^{3+}. The pUC19 plasmid DNA is present in two conformations SC (native) and OC (resulting from single strand breaks). Saturated air solution ($[O_2] \approx$ 0.25 mM). pH 7.5. T = (25.0 ± 0.5) °C. Solutions containing Ni^{2+} (Figures A and C) or Mn^{3+} (Figures B and D). All lanes (1) contains only pUC19 plasmid DNA 5 ng µL⁻¹. (A) Lanes (2) a (8): (1) + Ni^{2+} 10; 20; 30; 40; 50; 75 and 100 µM; respectively (No NaDip). (B) Lane (2): (1) + Mn^{3+} 10; 20; 30; 40; 50; 75 and 100 µM; respectively (No NaDip). (C) Lane (2): (1) + Ni^{2+} 10 µM; Lanes (3–8): (2) + NaDip 300; 400; 500; 600; 700 and 800 µM, respectively. (D) Lane (2): (1) + Mn^{3+} 10 µM; Lanes (3–8): (2) + NaDip 300; 400; 500; 600; 700 and 800 µM, respectively.

4.2. Electrophoresis experiments in the presence of Fe^{3+}

In the absence of NaDip and in air saturated solution ($[O_2] \approx$ 0.25 mM) the pUC-19 plasmid DNA damage occurs from addition of Fe^{3+} 10 μM (Fig. 3A, lane 3). An observable smear, a damage noted when DNA is divided into small fragments, begins at Fe^{3+} 50 μM and it is complete at 100 μM (Fig. 3A, lane 8). The repetition of the same experiment in a solution NaDip 500 μM does not produce any appreciable effect which seems surprisingly to show that NaDip does not reduce Fe^{3+} promoting the Fe^{3+}/Fe^{2+} redox cycle (Fig. 3B) under these experimental conditions (similar reaction represented by Eq. (1)).

Moreover, keeping Fe^{3+} 10 μM, minimum concentration at which the damage was observed (Fig. 3A, lane 3), and NaDip in the range 300–800 μM, the % OC formed remains constant, $(15 \pm 5)\%$ up to 600 μM (Fig. 3C, lane 6). The damage in the presence of Fe^{3+} cannot be surely concluded in these experimental conditions ($[O_2] \approx 0.25$ mM and T = $(25.0 \pm 0.5)\,°C$) due to small increases of OC (21 and 23%) in presence of NaDip (700 and 800 μM), respectively.

On the other hand, if 10 μM Fe^{3+} is added complexed with EDTA (in presence of NaDip 300–800 μM) the Fe[EDTA]$^-$ complex formed ($\log \beta = 25$) (Smith and Martell, 2004) totally suppress this small pUC-19 plasmid DNA damage (Fig. 4, Supplementary Material) observed in Fig. 3A and B. Similarly, if a mixture containing 10 μM Fe^{3+} and 10 μM phen (which binds stronger with Fe^{2+}, $\log \beta_3 = 21.5$, but also can form complex with Fe^{3+}, $\log \beta_3 = 14.1$) (Smith and Martell, 2004), was added in presence of NaDip (300–800 μM) no pUC-19 plasmid DNA damages is observed (Fig. 3D).

The reagent addition order strategy used in this study, (item 3.1), allows the metal ion to associate with the pUC-19 plasmid DNA before the addition of NaDip. The small pUC-19 plasmid DNA damage in the presence of Fe^{3+} might be attributed to the final pH of the mixtures containing pUC-19 plasmid DNA, Fe^{3+} and NaDip because in this experimental condition (pH 7.5) ferrichydroxo complexes should be present (Baes and Mesmer, 1986).

4.3. Electrophoresis experiments in the presence of Ni^{2+} and Mn^{3+}

Ni^{2+} and Mn^{3+} cause pUC-19 plasmid DNA damage (in the absence of NaDip) after 20 μM addition of each metal ion (Fig. 4A and B) and smear was observed from Ni^{2+} 50 μM (Fig. 4A, lane 5). For Mn^{3+} the% OC remained constant $(50 \pm 6)\%$ up 100 μM and no occurrence of smear was observed (Fig. 4B).

Ni^{2+} experiments carried out at 10 μM (fixed concentration) and NaDip from 300 to 800 μM did not form significant amounts of OC form $(10 \pm 2)\%$ (Fig. 4C). It was pointed out that Ni^{2+} complex preferentially with phosphate groups on the DNA backbone (Lloyd and Phillips, 1999). Since Ni^{2+} cannot be readily reduced in aqueous solutions, even in presence of suitable complexing agents, it can be inferred that under these experimental conditions the addition of NaDip does not promote the formation of the Ni^{2+}/Ni^+ redox cycle. In fact, Ni^{2+} was able of causing pUC-19 plasmid DNA damage, induced by autoxidation of S(IV), when complexed with glycylglycylhistidine (GGH), forming the Ni^{3+}/GGH complexes with formation of none Ni^+ complexes (Alipázaga et al., 2005).

Despite of the high value of the standard reduction potential of the Mn(III)/Mn(II) couple in aqueous solution ($E^0 = 1.51$ V vs. NHE) (Moya et al., 1997) the pUC-19 plasmid DNA damage in the presence of the reducing agent such NaDip was not observed.

In fact, for Mn(III) the increase of OC form was also insignificant in presence of NaDip, $(10 \pm 4)\%$. Here again the presence of hydrolysed species of Mn^{3+} can prevent the formation of Mn^{3+}-pUC-19 plasmid DNA interaction since there is considerable evidence that Mn^{3+} hydrolyses even in concentrated acid solution (Baes and Mesmer, 1986). Besides, previous work observed that DNA damage does not occur in a solution containing Mn^{3+} and AZT even when exposed to UVB (Iwamoto et al., 2003).

5. Conclusions

Supercoiled of pUC 19 plasmid DNA configuration becomes converted into OC after exposure to 100 μM Cu^{2+} and (80–600) μM NaDip. There is a critical order in the addition of reagents (pUC-19 plasmid DNA + Cu^{2+} + NaDip) for the pUC-19 plasmid DNA damage. No damage was observed when NaDip was added before Cu^{2+}.

No marked pUC-19 plasmid DNA damage was observed in the presence of NaDip and Fe^{3+}, Ni^{2+} or Mn^{3+} under the experimental conditions of this study (air saturated solution with $[O_2] \approx$ 0.25 mM and 25.0 °C). This may be because in these experimental conditions, and with order of addition of reagents, Fe^{2+}, Ni^+ and Mn^{2+} are not originated after addition of NaDip and do not return to their oxidized states, which prevents the formation of the $M^{n+}/M^{(n-1)+}$ redox cycle. In oxygen saturated solutions and in other temperatures these remarks could be different.

In spite of the gel electrophoresis study performed here has been efficacious to detect the pUC-19 plasmid DNA damage it was not possible to find out which DNA base was damaged.

Further studies with other techniques (e.g. HPLC) should be made to identify in which pUC-19 plasmid DNA base this damage occurs since, at this point, it is still difficult to unambiguously identify the oxidative intermediates that are responsible for the damage.

Author contributions

B.C. Roriz carried out the experiments and wrote a first draft of the paper. H.D. Moya conceived and designed the experiments and wrote the final draft of the paper.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jsps.2017.02.010.

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