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Coordination of iron ions in the form of histidinyl dinitrosyl complexes does not prevent their genotoxicity

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ABSTRACT

Formation of dinitrosyl iron complexes (DNICs) was observed in a wide spectrum of pathophysiological conditions associated with overproduction of NO. To gain insight into the possible genotoxic effects of DNIC, we examined the interaction of histidinyl dinitrosyl iron complexes (HIS-DNIC) with DNA by means of circular dichroism. Formation of DNIC was monitored by EPR and FT/IR spectroscopy. Vibrational bands for aquated HIS-DNIC are reported. Dichroism results indicate that HIS-DNIC changes the conformation of the DNA in a dose-dependent manner in 10 mM phosphate buffer (pH 6). Increase of the buffer pH or ionic strength decreased the effect. Comparison of HIS-DNIC DNA interaction with the effect of hydrated Fe^{2+} ion revealed many similarities. The importance of iron ions in HIS-DNIC induced genotoxicity is confirmed by plasmid nicking assay. Treatment of pUC19 plasmid with 1 μM HIS-DNIC did not affect the plasmid supercoiling. Higher concentrations of HIS-DNIC induced single strand breaks. The effect was completely abrogated by addition of deferoxamine, a specific strong iron chelator. Our data reveal that formation of HIS-DNIC does not prevent DNA from iron-induced damage and imply that there is no direct interrelationship between iron–NO coordination and their mutual toxicity modulation.

1. Introduction

Formation of dinitrosyl iron complexes (DNICs) has been observed in many kinds of organisms and in a wide spectrum of physiological conditions associated with inflammation, Parkinson's disease and cancer. Accumulation of DNICs coincides with intensified production of nitric oxide in macrophages, spinal cord, endothelial cells, pancreatic islet cells and hepatocytes.[1–3] DNICs are also important in NO-dependent regulation of cellular metabolism and signal transduction.[4–7] A complex relationship between iron and NO and the putative biological role of DNIC has been recently reviewed by Richardson and Lok[8] and Lewandowska et al.,[9] respectively.

In spite of many attempts, neither chemical structure nor biological importance of in vivo formed DNICs has been unequivocally explained. Two main binding sites for Fe–NO complexes with low molecular weight (LMW) ligands have been proposed: (i) sulfur (II)-containing ligands, such as glutathione, and (ii) imidazole rings present in residues, such as histidine or purines. In high molecular weight ligands, thiols are responsible for DNIC formation in non-heme iron proteins, whereas histidine can participate in the formation of iron complexes containing heme ligands.[10] The DNICs with thiol ligands, sometimes called 2.03 complexes, because of the g value of their characteristic EPR spectra have been found in many organisms, from bacteria and plants to animals.[11] However, the similarity of the EPR spectra of thiol- and histidine-derived dinitrosyl complexes in aqueous media preclude unequivocal assignment of their biological role.[3,9,12] While dinitrosyl-dithiol iron(II) complexes are well characterised species in which iron is coordinated by two sulfur atoms and two NO molecules,[3] the biological importance of complexes of histidine, NO and iron have scarcely been investigated. In the very early publication of Woolum,[13] it was proposed that the N7 atom of histidine imidazole ring is responsible for coordination of iron and DNIC formation in non-thiol proteins.

According to the literature, the toxicity of DNIC's components seems to be mutually dependent on each other, but the reported results give an ambiguous picture. Bostanci and Bagirici[14–16] reported attenuation of iron-induced neurotoxicity by nitric oxide synthases inhibitors. On the other hand, the presence of NO

Abbreviations: DNIC, dinitrosyl iron complexes; CD, circular dichroism; DFO, deferoxamine (*N*′-[5-(acetyl-hydroxy-amino)pentyl]-*N*-[5-[3-(5-aminopentyl-hydroxy-carbamoyl)propanoylamino]pentyl]-*N*-hydroxy-butane diamide); HIS-DNIC, histidine dinitrosyl iron complex; LIP, labile iron pool; MRP1, multidrug resistance associated protein 1 (an official name: ATP-binding cassette, sub-family C (CFTR/MRP), member 1, ABCC1).

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donors was reported to protect against iron-induced nephrotoxicity.[17,18] The reported ability of iron ions to rescue tumor cells from the pro-apoptotic effects of NO is in line with these results, showing mutual interrelationship of nitric oxide and iron toxicity.[19]

Another effect is attributed to coinciding active transport of iron and glutathione outside the cells[20] that was proven to be dependent on MRP1.[21] The dependence of iron release on glutathione provides an evidence that intracellular iron might be depleted via MRP1 in the form of DNIC. It was also shown that depletion of glutathione rendered the cells vulnerable to NO donors.[22]

All the above examples of mutual dependence of iron toxicity on the presence of NO (and vice versa) made us formulate a question whether the cause of these effects may simply be the mutual chemical interaction between these species, resulting from complex formation. The aim of this study was to evaluate whether formation of DNIC may participate in observed sparing effect of NO on iron toxicity. To gain insight into the possible chemical aspects of iron and nitric oxide coexistence in the form of DNIC, we decided to examine interactions of LMW histidine DNIC with DNA. Histidine DNIC (HIS-DNIC) changes the DNA conformation in dose dependent manner as revealed by circular dichroism. However, we found out that DNIC–DNA interaction strongly depends on the pH and ionic strength of the reaction buffer and is similar to the interaction of Fe^{2+} ions alone.

2. Materials and methods

L-(+)-Histidine hydrochloride monohydrate (98%) was obtained from Lancaster Synthesis UK. All other chemicals were supplied by Sigma–Aldrich. CD spectra in the range of 240–320 nm were recorded on Jasco 600 spectrophotometer. FT/IR spectra of histidine hydrochloride and HIS-DNIC (240 μM) in D_2O were recorded in ZnSe flat cell in the range of 2000–1260 cm^{-1} on Jasco FT/IR 470 PLUS spectrophotometer. The baseline was corrected for D_2O. EPR spectra were recorded on X-band Bruker ESP 300 in 77 K, at microwave power 1 mW, microwave frequency 9.3 GHz and modulation ampitude 0.3 mT. Formation of HIS-DNIC was also monitored at room temperature (293 K). In this case EPR spectra were recorded at 20 mW of microwave power and 0.06 mT of modulation amplitude. Evaluation of g factors was performed using SimFonia 1.25 software (Bruker, DE). To assess HIS-DNIC stability in the presence of DNA, 120 μM salmon testes DNA (10-fold molar excess) was added for 2 min prior to recording.

2.1. Preparation of histidinyl dinitrosyl iron complex

HIS-DNIC was prepared according to.[13] Briefly, iron salt was added to a deoxygenized solution of histidine hydrochloride (20 mM, pH 6) under an atmosphere of N_2 to the final concentration: 1 mM, 100 μM, 10 μM, 1 μM of Fe^{2+}. NO was prepared via reduction of $NaNO_2$ by $FeSO_4$ in acidic solution, anaerobically. Forming gas was then bubbled twice through concentrated NaOH solution to eliminate traces of NO_2. NO was then passed through the solution of Fe/His (20 min), followed by N_2 (5 min) to evacuate excess of NO. The reaction yield was estimated to be more than 99%, based on the concentration of the bathophenantroline chelatable iron ions at the end of the reaction. Bathophenanthroline does not disrupt the HIS–DNIC complex, thus the amount of iron ions chelated by bathophenanthroline corresponds to the amount of iron ions not bound in the form of HIS-DNIC. This was further confirmed by UV–VIS spectroscopy that revealed the reaction yield to be 95% (data not shown).

2.2. Measurement of HIS-DNIC–DNA interaction

A calf thymus DNA stock solution (1 mg/mL in phosphate buffer) was mixed with solutions containing either histidine hydrochloride (20 mM), iron(II) salt, iron(II) salt in the presence of 20 mM histidine or its dinitrosyl complex in phosphate buffer. The HIS-DNIC–DNA interaction was evaluated by CD spectroscopy. All CD spectra were recorded at 293 K, 2 min after sample preparation. Influence of pH or ionic strength of the reaction buffer on HIS-DNIC–DNA interaction was evaluated by changing pH from 6 to 7 or use of 10 mM, 20 mM and 100 mM buffer, respectively.

2.3. Plasmid nicking assay

In order to estimate the extent of DNA damage caused by different concentrations of the histidine dinitrosyl iron complex a plasmid cleavage test was carried out. In this test, the proportion of abundance of DNA bands, corresponding to supercoiled (CCC), open circular (OC) and linear (L) forms of the plasmid, visualised after electrophoresis is directly related to DNA damage.

Plasmid pUC19 was incubated at 37 °C for 40 min with different concentrations of iron(II) ions in the presence or absence of 20 mM histidine, or HIS-DNIC in 10 mM phosphate buffer (pH 6 or 7). Aquated iron ions were incubated in water of adjusted pH to avoid iron(II) phosphate precipitation. The incubation mixtures in a total volume of 20 μL contained 400 ng pUC19 plasmid DNA. For each HIS-DNIC concentration a control reaction was set with ten times molar excess of deferoxamine (DFO), a selective and strong iron chelating agent, that was proven by EPR to decompose DNIC, to test if the observed DNA damage was induced by iron ions. All experiments were done in a glove bag (Sigma–Aldrich) under N_2 atmosphere.

Reaction products were resolved electrophoretically on 1.5% agarose gel containing 0.25 μg/mL ethidium bromide. To obtain a linear form, the plasmid was cleaved with SmaI endonuclease (Fermentas). All the procedures were performed in a glovebag (Sigma–Aldrich) filled with nitrogen. The DNA bands were visualized under UV light, photographed and the intensity of bands was estimated by ImageJ, a public domain Java image processing program (http://rsbweb.nih.gov/ij/index.html).

3. Results

3.1. Spectra of histidinyl dinitrosyl iron complex

Formation of the HIS-DINC was confirmed by EPR spectroscopy (Fig. 1). The EPR spectra recorded at 77 K revealed anisotropic singlet, which was characterised by three g values (calculated by simulation): $g_x = 2.051$, $g_y = 2.033$, $g_z = 2.014$ (Fig. 1A). The EPR spectra of water solutions recorded at 293 K were all isotropic singlet with $g = 2.032$, peak-to-peak width of 1.1 mT and well-resolved isotropic hyperfine splitting, characterized by 13 lines with $A_{iso} = 0.21$ mT (Fig. 1B). The structure of the EPR signal obtained for histidinyl DNIC at 293 K implies that coordination sphere of iron in DNIC with histidine contains two nitrogen atoms of nitrosyl ligands and two imidazole nitrogen atoms of two histidine molecules, as proposed by Woolum and Commoner.[23] and as it takes place in dinitrosyl complex with methylimidazolate.[24] The 13-line signal appears to be a result of interaction of unpaired electron with four nitrogen nuclei—two of NO ligands with hyperfine splitting of 0.42 mT and two of histidine with HFS of 0.21 mT. Thus, upon the analysis of the hyperfine structure of EPR signal and comparison to the data of Reginato et al.[24] concerning complexes of the similar structure, one can conclude that histidinyl DNIC coordination sphere is likely composed of two imidazolate nitrogen atoms

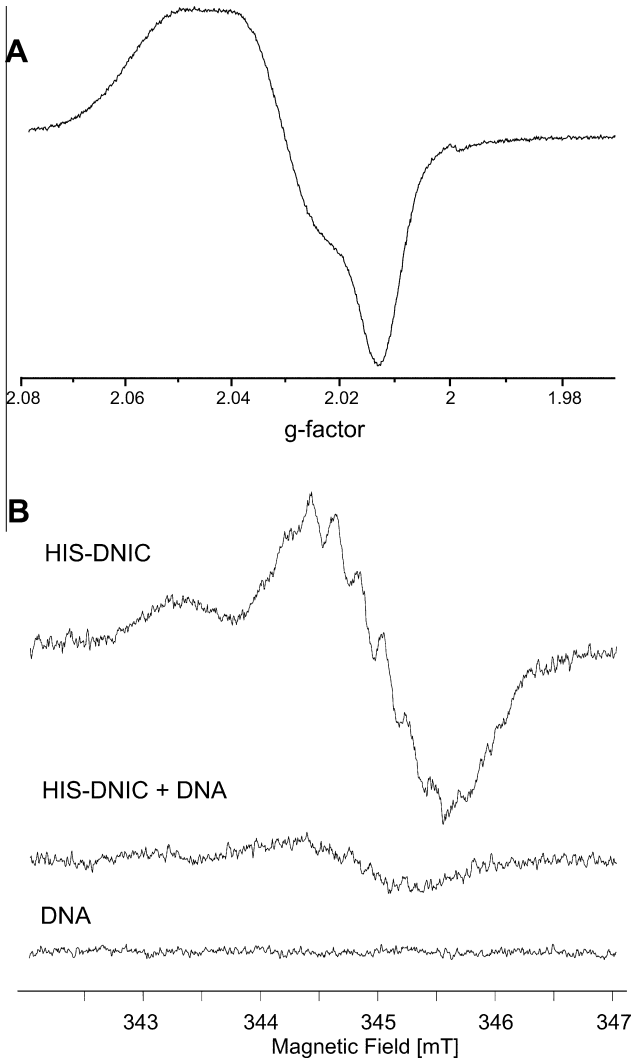

Figure 1. EPR spectra of histidinyl dinitrosyl iron complex: at 77 K (A) and at 293 K (B).

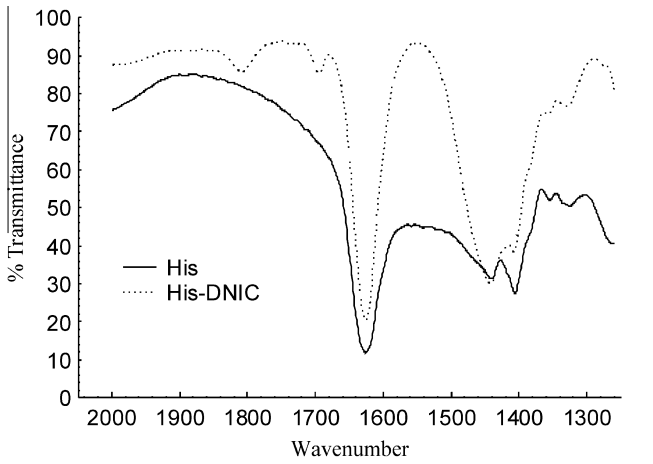

Figure 2. FT/IR spectra of histidine (240 μM) (solid line) and histidinyl dinitrosyl iron complex (240 μM) (dotted line) in D_2O, pH 5. Solvent spectrum subtracted. For band assignment see Table 1.

Table 1
Vibrational band assignment for histidinyl chloride vs histidinyl dinitrosyl iron complex in the range of 1260–2000 cm^{-1}.

HisCl (herein)	HisCl[23]	$Fe(NO)_2$(His) (herein)	Band assignment[a]
–	–	1807	ν_{sym} N–O
–	–	1696	ν_{as} N–O
1439	1439	1439	β CH_2
1407	1410	1407	ν_{sym} COO^-
1354	1340	1354	R_4[a]
1322	–	1322	δ (NH_3^+)

[a] ν—stretching, δ—deformation, β—bending, R_4[a]—imidazole ring vibrations.

and two nitrogens of the two NO molecules. On the other hand the spectroscopic data do not allow to exclude the possibility that the histidinyl ligand is bidentately bound to the central ion via the nitrogen atom of the imidazole moiety and the nitrogen of the amino-group. Such coordination was proposed in the already mentioned early work of Woolum and Commoner.[23] The EPR spectrum of DNIC in an excess of DNA was of much lower intensity and its hyperfine structure was hardly visible. DNA solution in the range of *g*-factor about 2 was completely EPR silent (Fig. 1B).

Another approach to the structure evaluation was made using IR spectroscopy. Infrared spectra of histidine and HIS-DNIC are presented in Figure 2. Assignment of ligand bands was done according to[25] and is presented in Table 1. Two peaks at 1807 and 1690 cm^{-1} observed in the HIS-DNIC spectrum were assigned to symmetric and asymmetric stretching vibration of NO groups, respectively.[24–27]

3.2. Plasmid nicking assay

Dose-dependent induction of plasmid DNA strand breaks by HIS-DNIC was observed at pH 6, as indicated by higher fluorescence of bands assigned to the OC form and appearance of the linear form of the plasmid (Fig. 3A). For the samples incubated with the highest (1 mM) concentration of either iron ions or HIS-DNIC about 80% of the plasmid was found in its OC or L form. However, no induction of strand breaks was observed in the 1 μM HIS-DNIC concentration that corresponds to cellular LIP concentrations.[28] No statistically important differences were found between the amount of DNA strand breaks induced by HIS-DNIC and iron ions alone or iron ions in the presence of 20 mM histidine.

Induction of DNA breaks by HIS-DNIC treatment was completely inhibited by the presence of the iron ion chelator, DFO (Fig. 3B). Neither histidine alone, nor the HIS–NO complex induced DNA strand breaks. As expected from the CD experiment, at pH 7 DNA breaks formed by the treatment with HIS-DNIC were less pronounced. At this pH a marked induction of DNA damage (ca. 34% of OC) was observed only after treatment with 1 mM HIS-DNIC (Fig. 3C).

3.3. DNA–Fe interaction

Influence of increasing amounts of Fe^{2+} ions on the DNA conformation was investigated by CD spectroscopy in different pH and ionic strength conditions. The CD spectra of DNA in the presence of increasing amounts of $FeSO_4$ are shown in Figure 4. The right band of the DNA spectrum monotonically decreased with the increase of the $FeSO_4$ concentration. An identical effect was seen in the presence of $Fe(NO)_2$ (data not shown). This effect is completely suppressed at pH 7 or by increasing the ionic strength of the reaction buffer (Fig. 5, Supplementary Fig. 1).

3.4. HIS-DNIC–DNA interaction

CD spectra of DNA treated with increasing amounts of HIS-DNIC are shown in Figure 6. Increase of the HIS-DNIC concentration resulted in increased changes of the DNA helix structure that

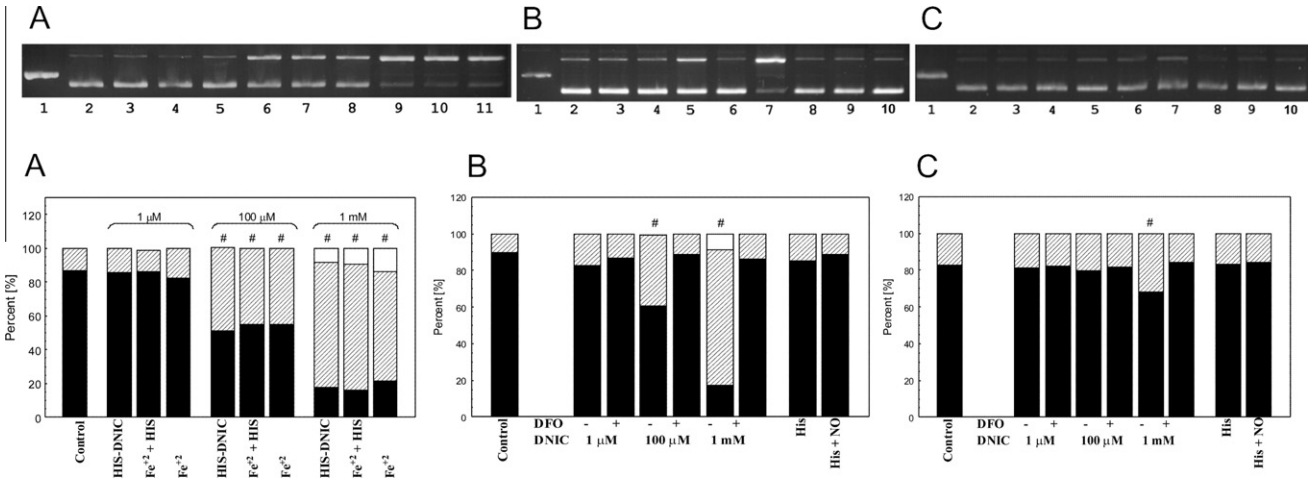

Figure 3. (A) Nicking of the plasmid DNA by different concentrations of HIS-DNIC, iron in the presence of 20 mM histidine or iron ions alone at pH 6. (B) Nicking of the plasmid DNA by HIS-DNIC at pH 6 in the presence or absence of DFO. (C) Nicking of the plasmid DNA by HIS-DNIC at pH 7 in the presence or absence of DFO. pUC19 plasmid (400 ng) was treated with solutions indicated on the figure. Upper panel: gel images; Lower panel: plasmid DNA bands intensity. Filled box – supercoiled form (CCC); hatched box – open circular form (OC); empty box – linear form (L). For numerical values and statistical evaluation see Supplementary Table 1. Hash denotes statistically significant difference vs control. Upper panel - gel images: in lane one position of linear form of Puc19 cleaved by SmaI endonuclease is shown. Lanes 2-11 correspond to the bands intensity graph in the lower panel.

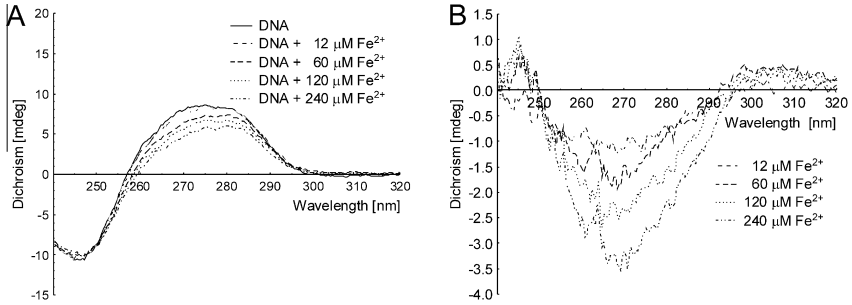

Figure 4. CD spectra of DNA (200 μM) in the presence of increasing amounts of Fe^{2+} in 10 mM phosphate buffer, pH 6 (A); DNA spectrum subtracted (B).

points to the interaction of the complex with DNA. In order to establish whether the effect comes distinctively from DNIC or from the complex components, the observed effect was compared to the interaction of DNA with increasing amounts of iron(II) alone (Fig. 4) or in the presence of 20 mM histidine (Supplementary Fig. 2). The influence of the HIS–DNIC complexes on the DNA helix structure was identical to that of the hydrated iron ions or iron(II) histidine mixture. As in case of hydrated Fe^{2+}, this effect of HIS-DNIC on DNA was suppressed by increasing pH or buffer ionic strength (Fig. 7, Supplementary Fig. 3).

4. Discussion

4.1. Spectral properties of nitrosyl histidinyl iron complex and implications for electronic charge distribution in the complex

The EPR signal in the range around $g = 2.03$ is characteristic for the nitrosylated iron ion. Ligands and the free iron(II) itself cannot be observed in X-band ESR, thus this signal is commonly used as the indication of nitrosyl iron. Also the observed shape of the signal, suggesting rhombic to axial symmetry of the ligand field, is commonly explained by the distorted tetrahedral structure of the coordination sphere.[9]

Nitrosyl iron complex of histidine was obtained also by Reginato et al.[24] and EPR spectra indicating formation of the paramagnetic complex were recorded, as noted in the mentioned work. Although, no crystallographic data were provided for the histidinyl complex, Reginato et al.[24] published therein a detailed report on the formation of crystalline nitrosyl methylimidazole complex, showing that before the formation of diamagnetic d^{10} species an intermediate $Fe(NO)_2(1\text{-MeIm})_2^+$ was formed, paramagnetic properties of which were lost upon crystallization. The EPR spectra of herein examined HIS-DNIC, as well as the mode of its interaction with DNA suggest similar electronic nature of the central ion as described by Reginato et al.[24] for the paramagnetic form of imidazolate complex. The phenomenon of dinitrosyl iron complexes changing their electronic proprieties during solubilization was observed before for thiol-containing ligands.[12] The change of diamagnetic complexes to paramagnetic ones is likely due to the disproportionation reaction of iron atoms. The precise mechanism for the disproportionation step is not known, but possible mechanism was proposed in[29] (see also Ref. 9 and references therein).

According to calculations of,[26] in DNICs covalent bonding is formed between the iron atom and the two NO groups (see also Enemark and Feltham's theory of the electronic structure of Me–N–O groups[30]). The iron becomes formally negatively charged, and the two nitrosyl groups take on the nature of nitrosyl cations (NO^+). Depending on the electron-donating properties of the two non-nitrosyl ligands, a partial back-donating effect occurs with the transfer of electron density back to antibonding π^* orbitals of NO groups. Thus, higher wavenumbers of NO vibrations indicate weaker electron donating proprieties of non-nitrosyl ligand in case of DNICs. The difference in the frequencies of

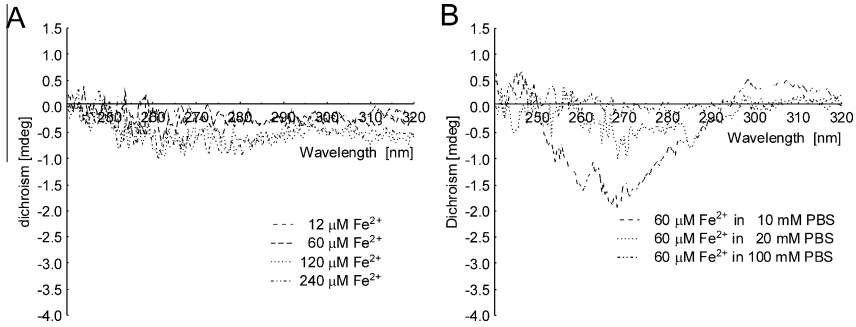

Figure 5. CD spectra of DNA (200 μM): (A) in the presence of increasing amounts of Fe^{2+} in phosphate buffer, pH 7; (B) in the presence of 60 μM Fe^{2+} in phosphate buffer (pH 6) of the indicated ionic strength; DNA spectrum subtracted. Raw spectra are shown on Supplementary Fig. 1.

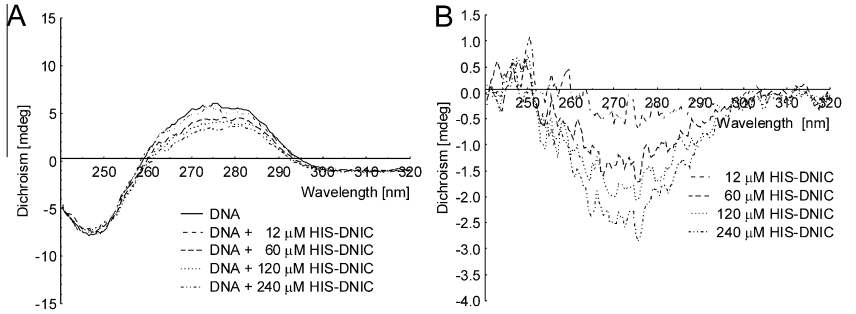

Figure 6. CD spectra of DNA (200 μM) in the presence of increasing amounts of HIS-DNIC in 10 mM phosphate buffer, pH 6 (A); DNA spectrum subtracted (B).

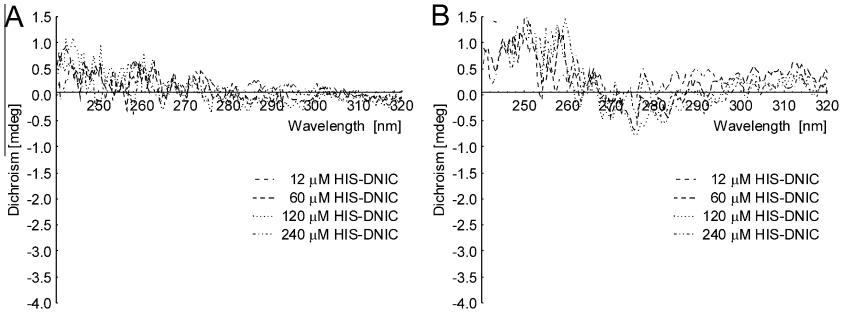

Figure 7. CD spectra of DNA (200 μM): (A) in the presence of increasing amounts of HIS-DNIC in phosphate buffer pH 7; (B) in the presence of increasing amounts of HIS-DNIC in 100 mM phosphate buffer, pH 6; DNA spectrum subtracted. Raw spectra are shown on Supplementary Fig. 3.

symmetric and asymmetric stretching vibrations of NO groups in hydrated $[Fe(His)_2(NO)_2]$ reported here is greater than in the case of other iron nitrosyl complexes described in the literature and shows that the two NO groups are non-equivalent to a great extent (Supplementary Table 2). The wavenumber of the symmetric vibration (1807 cm^{-1}) falls in the range between N–O vibration in $[Fe(H_2O)_5(NO)]^{2+}$ (1810 cm^{-1})[31] and that in $[Fe(EDTA)(NO)]^{2-}$ (1777 cm^{-1}).[32] Slight differences in the structures of the Fe–N–O fragments can be explained by differences in the electron density transfer from the iron atom to the NO due to the dative interaction $d\pi(Fe) \rightarrow p\pi^*(NO)$. At the same time the wavenumber of the symmetric stretching vibration is much higher in the studied complex than in the iron nitrosyl complex with 1-methylimidazole (1673 cm^{-1}).[24] The difference of NO stretching vibrations as compared to methylimidazole and observed non-equivalence of the two NO-groups, suggest a different mode of coordination. The wavenumber of the asymmetric stretching vibration band (1690 cm^{-1}) for the studied histidinyl complex is low as compared to other iron nitrosyl complexes,[24–27,33] however it is much higher than this band in the methylimidazole complex. The wavenumbers of N–O stretching vibrations for the histidinyl complex are closest to those of the thiolate-bridged dinuclear iron complex $(NO)_4Fe_2(\mu\text{-cysteamine})_2$,[26] however the IR spectra similarity does not imply the similar mode of coordination of HIS–DNIC complex.

4.2. Interaction of Fe^{2+} and its nitrosyl complexes with DNA

The small decrease in the intensity of the right band of the CD spectrum of DNA upon addition of either iron ions or the His–DNIC, indicates that the interaction between the iron complex and DNA induces only slight modifications to the native conformation of the DNA (Figs. 4 and 6). DNA intercalation as a major binding interaction, usually produces much more pronounced changes in the intensity and position of the left and/or right band of the CD spectrum of native DNA.[34,35] Indeed, it has been reported that iron ions bind in a sequence specific manner DNA bases and sugar–phosphate backbone.[36] Small changes of the CD spectrum of DNA ob-

served in this work upon addition of HIS-DNIC suggest that the HIS-DNIC interactions with DNA are of similar nature like that of the iron ions alone, rather than DNA intercalation. Influence of the buffer ionic strength supports this explanation. Similar spectra were found by Silvestri et al.[37] for Fe(Salen) complexes and also attributed to ionic interaction. The change of the DNA CD spectrum upon addition of iron(II) ions, observed by us, resembles changes reported by Baase and Johnson[38] for DNA in presence of various concentrations of metal salts. Correlation with crystallographic data allowed these authors to attribute these changes to transfer of DNA coiling from 'B' form to 'C' form with the change in the number of base pairs per turn of DNA helix. Therefore, changes in the CD spectrum of DNA reported in this paper are likely to be due to changes in the DNA conformation resulting from ionic interaction.

The effect disappears at pH 7 and along with increasing buffer ionic strength, what further confirms the labile character of the observed interaction. The weaker effect of HIS-DNIC and iron ions on DNA at the lower pH was also observed in plasmid nicking assay. At pH 6, 100 μM and 1 mM HIS-DNIC or iron ions produced a significant increase in plasmid damage as compared to the control. This effect was diminished at pH 7, where the significant effect was observed only for treatment with 1 mM HIS-DNIC (Fig. 3 and Supplementary Table 1). Similar change in the capability of iron ions to induce plasmid DNA cleavage in relation to the changes of phosphate buffer acidity was recently reported by Kim and Kim,[39] and was attributed to the rapid autooxidation of Fe^{+2} ions. Interestingly, the effect was also profound in N_2 atmosphere, suggesting that remaining dissolved oxygen is an efficient reactant for Fe^{2+} ion-mediated DNA cleavage reaction.[39]

The interactions between cellular levels of iron and nitric oxide have been widely studied and mutual relationship between iron and NO toxicity is presently discussed.[40–42] Recent reports have shown, that among the most effective biological scavengers of nitrosyl iron are glutathione transferases.[43–45] Therefore, the formation of a dinitrosyl iron complex could prevent DNA from strand breakage induced by radicals produced in Fenton reaction, via forming high-molecular complexes outside the nucleus. This would have a particular meaning in circumstances such as oxidative stress, a situation accompanied by formation of NO.

On the other hand, it is well proven, that increased NO levels cause an increase of the genotoxic labile iron pool (LIP), that is cellular iron which remains in the form of complexes with low molecular mass ligands, labile enough to enter Fenton reaction.[28] The iron ions are likely released from iron containing proteins, such as iron–sulfur cluster proteins that are prone to nitric oxide action.[46,47] According to our studies up to 50% of NO scavenged by Fe to form dinitrosyl complexes is bound by LIP,[48,49] likely in the form of low molecular weight (LMW) complexes, such as complexes with glutathione or histidine, both ligands abundantly present in the cellular milieu. Thus, formation of LMW DNIC involving LIP might protect cellular components against toxic action of NO and iron. Although the actual composition and the net charge of the histidinyl DNIC molecule is not clear,[3] our results show that this LMW complex of nitrosylated iron does not form any stable conjugates with DNA but is electrostaticaly attracted to polyanionic DNA strand. To test the hypothesis that HIS-DNIC formation might protect DNA from iron-induced damage, we performed DNA nicking assays. No significant reduction of formation of DNA breaks was observed in HIS-DNIC treated plasmid as compared to hydrated iron ions (Fig. 3), which agrees well with the discussed above CD results. Moreover, we observed that presence of DNA facilitates decomposition of HIS-DNIC (Fig. 1B). This implies that DNIC does not protect DNA from the toxic action of iron. Thus, there is no effect coming distinctively from physicochemical nature of DNIC that could account for the mutual protective role of iron and nitric oxide in toxicity for living cells. On the contrary, our results emphasize the labile character of the complexes of the studied type. This is in accordance with the well known biological relevance of DNICs, which act as nitric oxide transducers, and it supports the conclusions that the aforementioned mutual interrelationship of iron and NO toxicity depends rather on the modulation of signaling pathways or the other not yet identified effects than direct genotoxicity.

Whether HIS-DNIC is formed in vivo is a matter of further investigations. The majority of in vivo works deals with thiolate DNIC. However, it is known for a long time[13] that in vitro also other DNICs may form, thus it cannot be excluded that other DNICs are also formed in vivo. This could take place in case of low availability of thiolate ligands in the biological compartments. The non-thiol species are supposed to be in the balance with the prevailing thiol-DNICs and in some conditions the non-thiol DNICs might have an important part to play in the FeNO equilibrium. Moreover, paramagnetic DNICs found in biological systems are characterized by EPR spectra with isotropic g values of around 2.03. In the case of high-molecular DNICs an axial symmetry of g-factor tensor is reported, for example: $g_{\perp} = 2.04$, $g_{\parallel} = 2.014$ for protein-bound DNIC,[50,51] decreasing to rhombic in case of DNICs with low-molecular ligands.[4] The anisotropy of the g values is displayed in the EPR spectra of low-molecular-weight DNICs as well, if acquired from frozen solutions. As g-values vary only slightly according to the nature of the ligand, the spectra for each one is not distinctive in a manner that would allow the treatment of each signal as the fingerprint of a given complex. Thus, the same signal can originate from a DNIC incorporating only one thiol-containing ligand along with a non-thiol ligand, as well as from a dithiolate complex, ergo it is not possible to distinguish whether published in vivo data regarding biological formation and effects of DNIC result from thiolate or non-thiolate DNIC.

5. Conclusion

The difference in frequencies of symmetric and asymmetric stretching vibrations of NO groups in $[Fe(His)_2(NO)_2]$ is greater than in case of other iron nitrosyl complexes described in the literature, and shows that the two NO groups are non-equivalent to a great extent. The wavenumbers of N–O stretching vibrations for the histidinyl complex are closest to those of the dinucleated iron complex $[(NO)_4Fe(\mu\text{-cysteamine})]_2$. Our data suggest that the nature of the HIS-DNIC influence on the DNA conformation appears to be identical to that of iron(II) ion and is of electrostatic nature. These complexes affect DNA similarly to other salts.[38] CD spectral changes are identical for aquated, histidine or histidine and NO complexed iron and these changes are suppressed by pH 7 and high ionic strength. Therefore, we suggest that no covalent binding of DNA by DNIC is observed and DNIC formation does not affect the interaction of iron ions with polyanionic DNA. DNA nicking assay suggests also no effect of DNIC formation in iron-induced DNA damage. It is plausible to assume that this is caused by low stability of HIS-DNIC, especially in the presence of nucleic acids, as was shown by addition of DNA to the HIS-DNIC preparation. Thus, it is unlikely that the observed sparing effect of NO on iron toxicity is mediated by HIS-DNIC.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.09.032.

References and notes

1. Henry, Y.; Ducrocq, C.; Drapier, J. C.; Servent, D.; Pellat, C.; Guissani, A. *Eur. Biophys. J.* **1991**, *20*, 1.
2. Singel, D. J.; Lancaster, J. R., Jr. Electron Paramagnetic Resonance Spectroscopy and Nitric Oxide Biology. In *Methods in Nitric Oxide Research*; Feelisch, M., Stamler, J. S., Eds.; John Wiley: Chichester, 1996; pp 341–356.
3. van Faassen, E.; Vanin, A. F. *Radicals for Life: the Various Forms of Nitric Oxide*; Elsevier Science Ltd: Amsterdam, 2007.
4. Vanin, A. F. *Biochemistry (Mosc.)* **1998**, *63*, 782.
5. Bouton, C.; Drapier, J. C. *Sci. STKE* **2003**, *2003*, e17.
6. De Maria, F.; Pedersen, J. Z.; Caccuri, A. M.; Antonini, G.; Turella, P.; Stella, L.; Lo, B. M.; Federici, G.; Ricci, G. *J. Biol. Chem.* **2003**, *278*, 42283.
7. Turella, P.; Pedersen, J. Z.; Caccuri, A. M.; De Maria, F.; Mastroberardino, P.; Lo, B. M.; Federici, G.; Ricci, G. *J. Biol. Chem.* **2003**, *278*, 42294.
8. Richardson, D. R.; Lok, H. C. *Biochim. Biophys. Acta* **2008**, *1780*, 638.
9. Lewandowska, H.; Kalinowska, M.; Brzoska, K.; Wojciuk, K.; Wojciuk, G.; Kruszewski, M. *Dalton Trans.* **2011**, *40*, 8273.
10. Cooper, C. E. *Biochim. Biophys. Acta* **1999**, *1411*, 290.
11. Vanin, A. F.; Serezhenkov, V. A.; Mikoyan, V. D.; Genkin, M. V. *Nitric Oxide* **1998**, *2*, 224.
12. Vanin, A. F.; Sanina, N. A.; Serezhenkov, V. A.; Burbaev, D. S.; Lozinsky, V. I.; Aldoshin, S. M. *Nitric Oxide* **2007**, *16*, 82.
13. Woolum, J. C.; Tiezzi, E.; Commoner, B. *Biochim. Biophys. Acta* **1968**, *160*, 311.
14. Bostanci, M. O.; Bagirici, F. *Cell. Mol. Neurobiol.* **2007**, *27*, 933.
15. Bostanci, M. O.; Bagirici, F. *Neurotoxicology* **2008**, *29*, 130.
16. Bostanci, M. O.; Bagirici, F. *Brain Res. Bull.* **2008**, *76*, 57.
17. Gupta, A.; Sharma, S.; Chopra, K. *Food Chem. Toxicol.* **2008**, *46*, 537.
18. Kadkhodaee, M.; Gol, A. *Hum. Exp. Toxicol.* **2004**, *23*, 533.
19. Feger, F.; Ferry-Dumazet, H.; Mamani, M. M.; Bordenave, J.; Dupouy, M.; Nussler, A. K.; Arock, M.; Devevey, L.; Nafziger, J.; Guillosson, J. J.; Reiffers, J.; Mossalayi, M. D. *Cancer Res.* **2001**, *61*, 5289.
20. Watts, R. N.; Richardson, D. R. *Eur. J. Biochem.* **2002**, *269*, 3383.
21. Watts, R. N.; Hawkins, C.; Ponka, P.; Richardson, D. R. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 7670.
22. Fass, U.; Panickar, K.; Williams, K.; Nevels, K.; Personett, D.; McKinney, M. *Brain Res.* **2004**, *1005*, 90.
23. Woolum, J. C.; Commoner, B. *Biochim. Biophys. Acta* **1970**, *201*, 131.
24. Reginato, N.; McCrory, C. T. C.; Pervitsky, D.; Li, L. *J. Am. Chem. Soc.* **1999**, *121*, 10217.
25. Wellner, N.; Zundel, G. *J. Mol. Struct.* **1994**, *317*, 249.
26. Costanzo, S.; Menage, S.; Purrello, R.; Bonomo, R. P.; Fontecave, M. *Inorg. Chim. Acta* **2001**, *318*, 1.
27. D'Autreaux, B.; Horner, O.; Oddou, J. L.; Jeandey, C.; Gambarelli, S.; Berthomieu, C.; Latour, J. M.; Michaud-Soret, I. *J. Am. Chem. Soc.* **2004**, *126*, 6005.
28. Kruszewski, M. *Mutat. Res.* **2003**, *531*, 81.
29. Vanin, A. F.; Papina, A. A.; Serezhenkov, V. A.; Koppenol, W. H. *Nitric Oxide* **2004**, *10*, 60.
30. Enemark, J. H.; Feltham, R. D. *Coord. Chem. Rev.* **1974**, *13*, 339.
31. Wanat, A.; Schneppensieper, T.; Stochel, G.; Van Eldik, R.; Bill, E.; Wieghardt, K. *Inorg. Chem.* **2002**, *41*, 4.
32. Wolak, M.; Eldik, R. *Coord. Chem. Rev.* **2002**, *230*, 263.
33. Sanina, N. A.; Rakova, O. A.; Aldoshin, S. M.; Shilov, G. V.; Shulga, Y. M.; Kulikov, A. V.; Ovanesyan, N. S. *Mendeleev Commun.* **2004**, *14*, 7.
34. Vaidyanathan, V. G.; Nair, B. U. *J. Inorg. Biochem.* **2003**, *95*, 334.
35. Xi, P. X.; Xu, Z. H.; Liu, X. H.; Cheng, F. J.; Zeng, Z. Z. *Spectrochim. Acta, Part A* **2008**, *71*, 523.
36. Ouameur, A. A.; Arakawa, H.; Ahmad, R.; Naoui, M.; Tajmir-Riahi, H. A. *DNA Cell Biol.* **2005**, *24*, 394.
37. Silvestri, A.; Barone, G.; Ruisi, G.; Lo Giudice, M. T.; Tumminello, S. *J. Inorg. Biochem.* **2004**, *98*, 589.
38. Baase, W. A.; Johnson, W. C., Jr. *Nucleic Acids Res.* **1979**, *6*, 797.
39. Kim, J. M.; Kim, S. K. *Bull. Korean Chem. Soc.* **2011**, *32*, 964.
40. Chenais, B.; Morjani, H.; Drapier, J. C. *J. Neurochem.* **2002**, *81*, 615.
41. Ramachandran, A.; Ceaser, E.; Darley-Usmar, V. M. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 384.
42. Cornejo, P.; Fernandez, V.; Vial, M. T.; Videla, L. A. *Nitric Oxide* **2007**, *16*, 143.
43. Cesareo, E.; Parker, L. J.; Pedersen, J. Z.; Nuccetelli, M.; Mazzetti, A. P.; Pastore, A.; Federici, G.; Caccuri, A. M.; Ricci, G.; Adams, J. J.; Parker, M. W.; Bello, M. L. *J. Biol. Chem.* **2005**, *280*, 42172.
44. Lo Bello, M.; Nuccetelli, M.; Caccuri, A. M.; Stella, L.; Parker, M. W.; Rossjohn, J.; McKinstry, W. J.; Mozzi, A. F.; Federici, G.; Polizio, F.; Pedersen, J. Z.; Ricci, G. *J. Biol. Chem.* **2001**, *276*, 42138.
45. Pedersen, J. Z.; De, M. F.; Turella, P.; Federici, G.; Mattei, M.; Fabrini, R.; Dawood, K. F.; Massimi, M.; Caccuri, A. M.; Ricci, G. *J. Biol. Chem.* **2007**, *282*, 6364.
46. Lipinski, P.; Starzynski, R. R.; Drapier, J. C.; Bouton, C.; Bartlomiejczyk, T.; Sochanowicz, B.; Smuda, E.; Gajkowska, A.; Kruszewski, M. *Biochem. Biophys. Res. Commun.* **2005**, *327*, 349.
47. Soum, E.; Drapier, J. C. *J. Biol. Inorg. Chem.* **2003**, *8*, 226.
48. Lewandowska, H.; Meczynska, S.; Sochanowicz, B.; Sadlo, J.; Kruszewski, M. *J. Biol. Inorg. Chem.* **2007**, *12*, 345.
49. Meczynska, S.; Lewandowska, H.; Sochanowicz, B.; Sadlo, J.; Kruszewski, M. *Hemoglobin* **2008**, *32*, 157.
50. Timoshin, A. A.; Vanin, A. F.; Orlova, T. R.; Sanina, N. A.; Ruuge, E. K.; Aldoshin, S. M.; Chazov, E. I. *Nitric Oxide* **2007**, *16*, 286.
51. Kleschyov, A. L.; Sedov, K. R.; Mordvintcev, P. I.; Vanin, A. F. *Biochem. Biophys. Res. Commun.* **1994**, *202*, 168.