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Influence of copper(II) and magnesium(II) ions on the ciprofloxacin binding to DNA

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Abstract

The influence of magnesium(II) and copper(II) ions on the binding of ciprofloxacin to double stranded calf thymus DNA was studied by fluorescence emission spectroscopy, ultraviolet- and circular dichroism (CD) spectroscopy. The interaction of ciprofloxacin and copper(II) ions was followed by strong fluorescence quenching which was almost unaffected by the presence of DNA. On the other hand, only a slight decrease in fluorescence emission intensity, which was enhanced in the presence of DNA, was observed for ciprofloxacin interaction with magnesium(II) ions. Furthermore, magnesium(II) ions increase the thermal stability of the DNA, while, in the presence of ciprofloxacin, the degree of stabilisation is smaller. In contrast, copper(II) ions destabilise double helical DNA to heat, while ciprofloxacin slightly affects only the second transition of the biphasic melting curve of calf thymus DNA. Magnesium(II) ions at 25 °C induce conformational transitions of DNA at concentrations of 1.5 mM and 2.5 M, as monitored by CD. On the other hand copper(II) ions induce only one conformational transition, at a concentration of 12.7 μM. At higher concentrations of copper(II) ions (c>700 μM) DNA starts to precipitate. Significant changes in the CD spectra of DNA were observed after addition of ciprofloxacin to a solution containing DNA and copper(II) ions, but not to DNA and magnesium(II) ions. Based on our spectroscopic results, we propose that copper(II) ions are not directly involved into ciprofloxacin binding to DNA via phosphate groups as it has been suggested for magnesium(II) ions.

Keywords: Ciprofloxacin; Magnesium(II) ions; Copper(II) ions; DNA-binding; Spectroscopic techniques

1. Introduction

Ciprofloxacin (1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid) (Fig. 1) is a synthetic antibacterial agent belonging to the family of fluoroquinolones with a very broad spectrum of activity towards Gram-negative bacteria. It is known that fluoroquinolones suppress cell growth by inhibiting one step of the multi-step activity of the enzyme DNA gyrase, which can introduce super-coils into closed-circular DNA using the free energy of ATP hydrolysis [1,2]. The detailed mechanism of inhibition of the catalytic activity of gyrases is unknown, and many different mechanisms of the activity of fluoroquinolones have been proposed [3–8]. These mechanisms rely on the fact that fluoroquinolones discriminate between single stranded, double stranded and supercoiled DNA by exhibiting different binding affinities for these structures [8,9]. The affinity of fluoroquinolone for double stranded DNA can be increased by lowering the ionic strength [9] or by adding gyrases [10] and/or ATP [7,8]. One of the proposed mechanisms suggests that the amount of fluoroquinolone bound to DNA is modulated by the concentration of magnesium(II) ions [3,5,7,8,11]. This model involves the formation of a complex between Mg^{2+}, fluoroquinolone and DNA, where the magnesium(II) ion

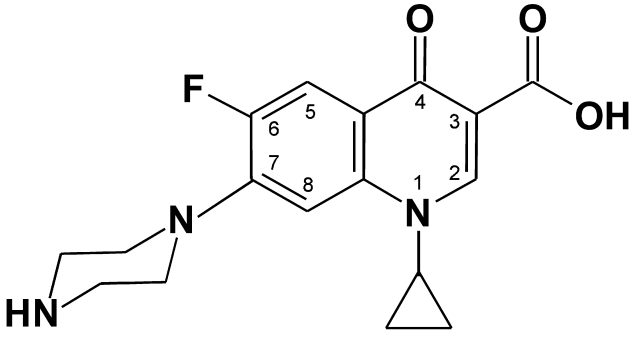

Fig. 1. Structure of ciprofloxacin.

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forms a bridge between the phosphate group of nucleic acid and the carbonyl and carboxyl groups of fluoroquinolone [5,8]. Recently, it has been observed that ciprofloxacin affects the conformational equilibrium of DNA gyrase A in the presence of Mg^{2+}. Based on this observation, it has been suggested that Mg^{2+}-mediated quinolone binding to the enzyme could be involved in the mechanism of action of this family of drugs [11].

We investigated Mg^{2+} and Cu^{2+}-mediated ciprofloxacin binding to linear genomic DNA at pH 7.0, since magnesium(II) ions play an important role in forming the gyrase–DNA complex and a crucial role in the quinolone poisoning of the catalytic activity of the bacterial gyrase. Quinolones are able to interact efficiently with gyrase or gyrase–DNA complex only in the presence of metal ions [1]. Recently, we have shown that some copper–quinolone complexes, synthesized in our laboratory [12–15], also possess antibacterial activity [14,15], even though Cu^{2+} and Mg^{2+} have different modes of DNA binding. To this end, we used a combination of spectroscopic techniques (intrinsic fluorescence emission, UV light absorption and CD spectroscopy). Our results reveal different modes of action of ciprofloxacin in the presence of Cu^{2+} and Mg^{2+}. We discuss possible correlations between Cu^{2+} and Mg^{2+} DNA binding modes and the action of ciprofloxacin.

2. Materials and methods

2.1. Chemicals

2.1.1. DNA

Genomic DNA from calf thymus was purchased from Pharmacia Biotech (USA) and thoroughly dialysed against 10 mM cacodylate buffer, pH 7.0. DNA concentration was determined spectrophotometrically at 25 °C using the molar extinction coefficient $\varepsilon_{259}=12\,800\ M^{-1}\,cm^{-1}$ (as per mole of base pairs) [16].

2.1.2. Ciprofloxacin

Ciprofloxacin (1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolone carboxylic acid) (Fig. 1) was purchased from Sigma (St. Louis, MO, USA) and used without further purification. A stock solution (1 mg ml^{-1}) was prepared in dimethylsulfoxide (DMSO) and diluted to the working concentration (1 μM) in the desired buffer or triply distilled water. The final concentration of ciprofloxacin in 2% DMSO solution was determined spectrophotometrically using an extinction coefficient of $\varepsilon_{275}=35\,900\pm500\ M^{-1}\,cm^{-1}$ [9].

2.1.3. $CuCl_2{\cdot}2H_2O$ and $MgCl_2{\cdot}6H_2O$

Copper chloride dihydrate was purchased from Kemika (Zagreb, Croatia). Magnesium chloride hexahydrate was purchased from Sigma (St. Louis, MO, USA). These chemicals were of the highest grade commercially available and were used without further purification.

2.2. Fluorescence emission spectrophotometry

Fluorescence emission spectra of ciprofloxacin and the ciprofloxacin–DNA complex at molar ratio (R) of 1:5 in the presence and absence of $CuCl_2$ and $MgCl_2$ at 25 °C were measured using a 1-cm path length quartz cuvette and a Perkin-Elmer Model LS-50 luminescence spectrometer equipped with a water thermostated cell holder. The emission spectra were recorded in the range of 350–700 nm at an excitation wavelength of 330 nm. Fluorescence titration profiles were determined by incrementally adding aliquots of $CuCl_2$ and $MgCl_2$ into a cuvette containing a known amount of ciprofloxacin (1 μM) or ciprofloxacin–DNA (1:5) complex. The emission spectra of ciprofloxacin, corrected for the solvent blank were multiplied by the dilution factor and corrected for PM-tube response using the fluorescence spectrum of quinine sulphate ($c=2.5\cdot10^{-7}$ M) in 0.1 M perchloric acid as a standard. The buffer used for fluorescence measurements was 10 mM cacodylic acid/sodium cacodylate adjusted to pH 7.0 with HCl or NaOH.

2.3. Determination of the equilibrium constants by Stern–Volmer method

Fluorescence quenching refers to any process in which the fluorescence intensity of a given fluorophore decreases upon adding quencher [17]. Assuming that the fluorescence intensity of a fluorophore–quencher complex (Φ_o) is negligible compared to an unquenched fluorophore, the intensity in the presence (FI) and absence (FI^o) of the quencher is expressed by the Stern–Volmer equation:

$$\frac{FI^o}{FI} = 1 + K_{SV}[Q] \quad (1)$$

where K_{SV} is the Stern–Volmer constant, which is the equilibrium constant of the complex formation in the static quenching process. If Φ_o is not negligible, Eq. (1) must be divided by the factor $(1+\Phi_o K_{SV}[\Phi_o])$ [17]. Since the fluorescence intensity of ciprofloxacin decreases upon adding Cu^{2+} and Mg^{2+}, the Stern–Volmer approach is valid and can be used to estimate the equilibrium constant.

2.4. UV spectrophotometry

UV-absorbance measurements were conducted using a Cary 1 UV–VIS spectrophotometer (Varian, Australia) and a matched set of 1-cm path length quartz cuvettes. The spectrophotometer was equipped with a thermoelectrically controlled cell holder. Absorbance versus temperature profiles (UV-melting curves) were measured at 260 nm. The heating rate was 1.0 °C min^{-1}. For each optically monitored transition, the melting temperature (T_m) of DNA was determined as the transition midpoint. The UV-melting

experiments on calf thymus DNA (25 μM per base pairs) and ciprofloxacin–DNA complex at a molar ratio 1:5 were performed at pH 7.0 (10 mM cacodylate buffer) at concentrations of $CuCl_2$ and $MgCl_2$ from 0 to100 μM. The 1:5 molar ratio was chosen since this is the stoichiometry of the binding of ciprofloxacin to DNA (one drug molecule to five base pairs) [9]. To correct for the contribution of ciprofloxacin to the absorbance spectrum of DNA, the reference cuvette was filled with the solution of ciprofloxacin at the same concentration as in the sample cuvette.

2.5. Circular dichroism (CD) spectropolarimetry

CD spectra of DNA and the ciprofloxacin–DNA complex (with a drug to DNA molar ratio of 1:5) in the presence of different concentrations of Cu^{2+} and Mg^{2+} were measured in an AVIV Model 62A DS spectropolarimeter (Aviv Associates, Lakewood, NJ, USA) equipped with a thermoelectrically controlled cell holder and a cuvette of 1 cm path length. All spectra were recorded between 205 and 300 nm with an averaging time of 5 s. CD measurements were performed at the same buffer conditions as fluorescence emission spectroscopy and UV-melting experiments as described above. The DNA concentration was 50 μM per base pairs.

3. Results and discussion

3.1. The influence of copper(II) and magnesium(II) ions on fluorescence properties of ciprofloxacin at 25 °C

Fig. 2A and B show the fluorescence emission spectra of ciprofloxacin at different concentrations of Cu^{2+} and Mg^{2+} at pH 7.0 (10 mM cacodylic buffer). No changes in λ_{max} were observed. The fluorescence intensity of ciprofloxacin is decreased in the presence of Cu^{2+}—but not Mg^{2+} (Fig. 2). The changes can be analysed by plotting the relative fluorescence emission intensity of ciprofloxacin (FI/FI^o) at $\lambda_{max}=415$ nm versus the concentration of Cu^{2+} and Mg^{2+} (Fig. 3). Copper(II) ions, at concentrations above 3 μM, cause an almost complete loss of fluorescence of ciprofloxacin and the stoichiometry of complex formation between ciprofloxacin and Cu^{2+} can therefore not be determined precisely. A similar decrease in FI/FI^o, although less pronounced, was observed for Mg^{2+} (Fig. 3B) up to concentration of 1 μM. From this curve, a stoichiometry of 1:1 can be determined for the binding of Mg^{2+} to ciprofloxacin. At higher concentrations of Mg^{2+} the fluorescence intensity of ciprofloxacin increases (see Fig. 3B). This increase, which is absent in the presence of Cu^{2+} or Na^+ may be related to the formation of a new type of magnesium–ciprofloxacin complex with a stoichiometry of 2:2. Such a complex has been observed previously [18,19].

The Stern–Volmer constant—equilibrium constants (Eq. (1)) for the ciprofloxanin–Cu^{2+} and ciprofloxacin–Mg^{2+} complexes can be obtained from a reciprocal plot of data presented in Fig. 3 (as FI^o/FI versus concentration). The observed values of K_{SV} suggest that ciprofloxacin has a higher binding affinity for Cu^{2+} ($K_{SV}=(4.3\pm0.1)\times10^5$ M^{-1}) than for Mg^{2+} ($K_{SV}=(1.4\pm0.4)\times10^4$ M^{-1}), although the value for ciprofloxacin–Mg^{2+} complex formation is probably underestimated because the fluorescence intensity of quenched ciprofloxacin in this complex is not negligible. Crystal structure of copper–quinolone complex-

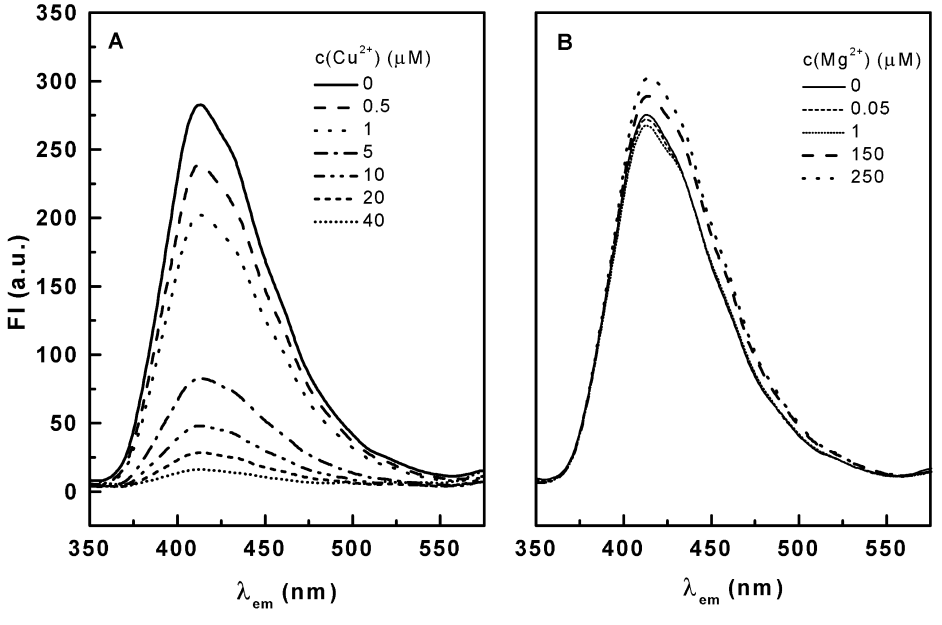

Fig. 2. Fluorescence emission spectra of ciprofloxacin at different concentrations of Cu^{2+} (A) and Mg^{2+} (B) at pH 7.0, $\lambda_{ex}=330$ nm, $c_{CF}=1$ μM, $T=25$ °C.

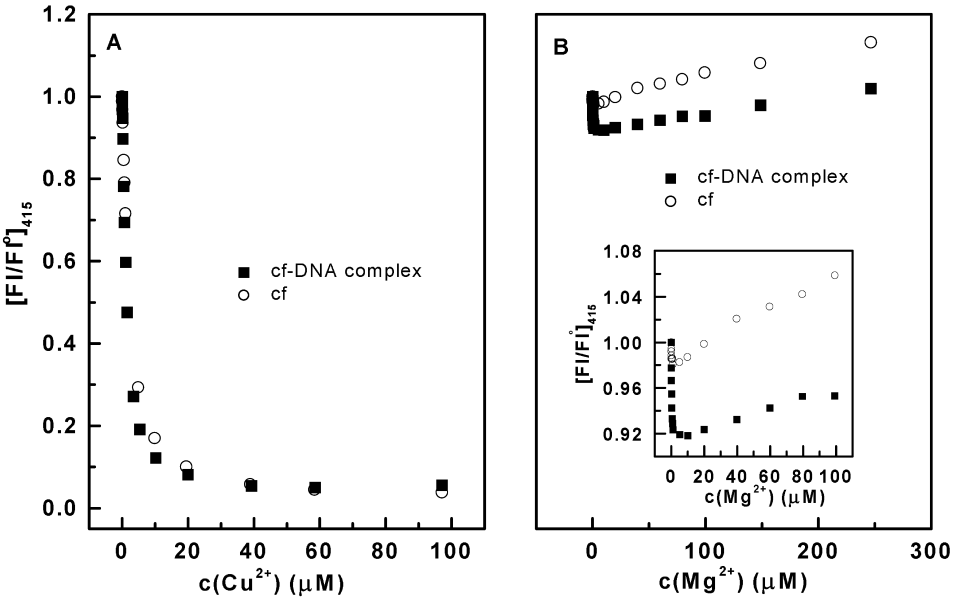

Fig. 3. Relative fluorescence emission intensity $(FI/FI^{o})_{415}$ of ciprofloxacin (○) and ciprofloxacin (CF)–DNA complex (■) (CF/DNA = 1:5) at 415 nm versus concentration of Cu^{2+} (A) and Mg^{2+} (B). The inset panel B shows the $(FI/FI^{o})_{415}$ of ciprofloxacin in the Mg^{2+} concentration range from 0 to 100 μM. FI^{o} and FI stand for fluorescence emission intensity of ciprofloxacin in the absence or presence of metal ions, respectively. pH 7.0, λ_{ex} was 330 nm, $c_{CF} = 1$ μM, $T = 25$ °C.

es show that metal atoms are usually bonded to the oxygen atoms of carbonyl and carboxylic groups of the quinolone and exhibit octahedral [12], or square pyramidal geometry [13,20,21], with additional water molecules or counter ions in the remaining coordination sites. The same oxygen atoms are involved in binding magnesium in the solid state at elevated temperatures [22]. It is proposed, that quinolones in solution form the same chelate bonds with metal ions [23].

3.2. The influence of copper(II) and magnesium(II) ions on fluorescence properties of ciprofloxacin in the presence of DNA at 25 °C

Fig. 3A and B illustrate the influence of Cu^{2+} and Mg^{2+}, respectively, on the changes in the relative fluorescence emission intensity of ciprofloxacin in the presence of calf thymus DNA. The fluorescence of ciprofloxacin in the presence of DNA decreases for approximately 1% of the original value (Fig. 3A and B). The Cu^{2+} titration profiles of ciprofloxacin and ciprofloxacin–DNA complex are practically super-imposable and yield closely similar values of K_{SV} of $(4.3\pm0.1)\times10^{5}$ M^{-1} and $(4.7\pm0.1)\times10^{5}$ M^{-1}, respectively. The relatively small changes in the fluorescence of ciprofloxacin–DNA complex compare to ciprofloxacin on titration by Cu^{2+} are, probably, due to the fact that paramagnetic Cu^{2+} represents a strong quencher of the emission fluorescence of ciprofloxacin. It is also possible that different steps in the binding may make opposite contributions to the fluorescence emission intensity of ciprofloxacin (e.g. a release of some Cu^{2+} bound to ciprofloxacin would increase the fluorescence intensity, but the binding ciprofloxacin to DNA would decrease it again). In contrast, the Mg^{2+} titration profiles of ciprofloxacin and ciprofloxacin–DNA complex are significantly different (see Fig. 3B). The quenching of ciprofloxacin fluorescence intensity by Mg^{2+} is less pronounced for the free ciprofloxacin than for the ciprofloxacin–DNA complex, corresponding to a 5-fold increase in the value of the Stern–Volmer constant (from $K_{SV} = (1.4\pm0.4)\times10^{4}$ M^{-1} for ciprofloxacin to $K_{SV} = (7.0\pm0.4)\times10^{4}$ M^{-1} for ciprofloxacin–DNA complex). These results suggest that magnesium(II) ions play a role in the interaction between DNA and ciprofloxacin. How magnesium(II) ion is involved into the interaction between DNA and ciprofloxacin still remains unknown. There are many different possibilities, e.g.: (i) Mg^{2+} could directly interact with the phosphate oxygen atoms and neutralise the negative charge on DNA allowing the ciprofloxacin to bind to the minor/major groove of DNA or to intercalate between DNA bases; (ii) Mg^{2+} could be involved in formation of the ternary complex between ciprofloxacin, magnesium, and DNA and act as a bridge between the phosphate groups of the DNA and the carbonyl and carboxyl moiety of ciprofloxacin [3,5]. On the other hand, copper(II) ions are known to bind preferentially to purine bases, mostly guanine, by forming covalent coordination bonds to the nucleophilic N7 atoms and no binding of Cu^{2+} to phosphate oxygen has been observed [24,25].

3.3. The influence of copper(II) and magnesium(II) ions on the thermal stability of calf thymus DNA

The absorbance versus temperature profiles of calf

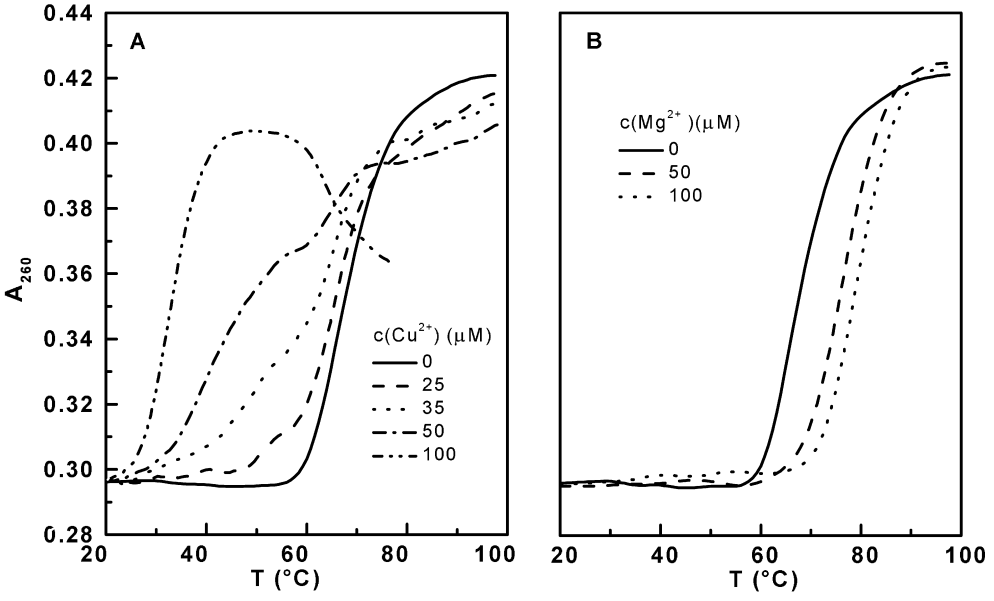

Fig. 4. UV-melting profiles of calf thymus DNA measured at 260 nm at different concentrations of Cu^{2+} (A) and Mg^{2+} (B) at pH 7.0, c_{DNA} = 25 μM.

thymus DNA in the presence and absence of Cu^{2+} and Mg^{2+} at pH 7.0 are shown in Fig. 4. The UV-melting curves of calf thymus DNA in the presence of Cu^{2+} differ significantly from that in the presence of Mg^{2+}. Furthermore, when the concentration of Cu^{2+} is increased the transition midpoint, T_m, decreases. In contrast, the T_m increases when the concentration of Mg^{2+} is increased. Copper(II) ions destabilise DNA, while magnesium(II) ions stabilise it against thermal denaturation [26–30]. The opposite effects of Cu^{2+} and Mg^{2+} on DNA stability originate from their different modes of binding to DNA [28,30]. Magnesium(II) ions shield the negative charges by contacting the phosphate oxygen atoms, copper(II) ions bind to guanine, forming covalent coordination bonds to the nucleophilic N7 atoms. The consequence of this bond formation is the rotation of the phosphate group linking the two neighbouring bases of DNA and placing the oxygen atoms of the phosphate closer to the copper bound to guanine on the other chain of the duplex. The overall conformation of the individual base pairs stays intact [25]. Another report [24] states that copper(II) ions could also be bis-coordinated to N7 of guanine, suggesting a possible N7–Cu–N7 crosslinking mechanism. Copper(II) ions pull two neighbouring guanines closer to form a bis-coordination linkage and therefore link the two DNA duplexes.

The apparent monophasic UV-melting curves of calf thymus DNA in the absence and presence of Mg^{2+} suggests that Mg^{2+} do not have any DNA sequences preferences, but binds to phosphate groups uniformly. In contrast, Cu^{2+} binds preferentially to N7 position of guanine in GC rich parts of DNA. The GC sequence preferences of Cu^{2+} can be observed as a change of the shape of UV-melting curve, which becomes apparently biphasic. The coupling between the copper binding region and the non-binding region is not infinitely strong, so individual regions melt independently from one to another. Once the DNA is saturated by Cu^{2+} and exists in a Cu^{2+}-induced conformational state (see below) the transition becomes apparently monophasic [31]. At higher temperatures ($T > 50$ °C) the absorbance starts to decrease and it is likely that DNA starts to precipitate.

3.4. The influence of copper(II) and magnesium(II) ions on the thermal stability of calf thymus DNA in the presence of ciprofloxacin

The melting temperatures, T_m, of calf thymus DNA and ciprofloxacin–DNA complex were measured at different concentrations of metal ions (Fig. 5). In the presence of Cu^{2+}, ciprofloxacin does not have any effect on the T_m of the first transition of DNA (inset of Fig. 5A), while affecting the T_m of the second transition (Fig. 5A). At lower molar ratios of Cu^{2+} to DNA base pairs ($R < 1.0$) ciprofloxacin increases the thermal stability of calf thymus DNA.

In the presence of Mg^{2+}, at $R > 0.5$, ciprofloxacin shifts the melting temperature of calf thymus DNA to lower values. At lower molar ratios of Mg^{2+} to DNA base pairs ($R < 0.5$), ciprofloxacin does not exert any significant influence on T_m (Fig. 5B). Within the range of concentrations employed in this work, ciprofloxacin does not affect the thermal stability of calf thymus DNA in the absence of Mg^{2+} (Fig. 5). The observed Mg^{2+}-induced decrease in the thermal stability of DNA in the presence of ciprofloxacin suggests a shift in the equilibrium towards the single stranded DNA conformation. At low concentrations, Mg^{2+} bind to phosphate oxygens. By extension, we propose that coordination of Mg^{2+} with the carbonyl

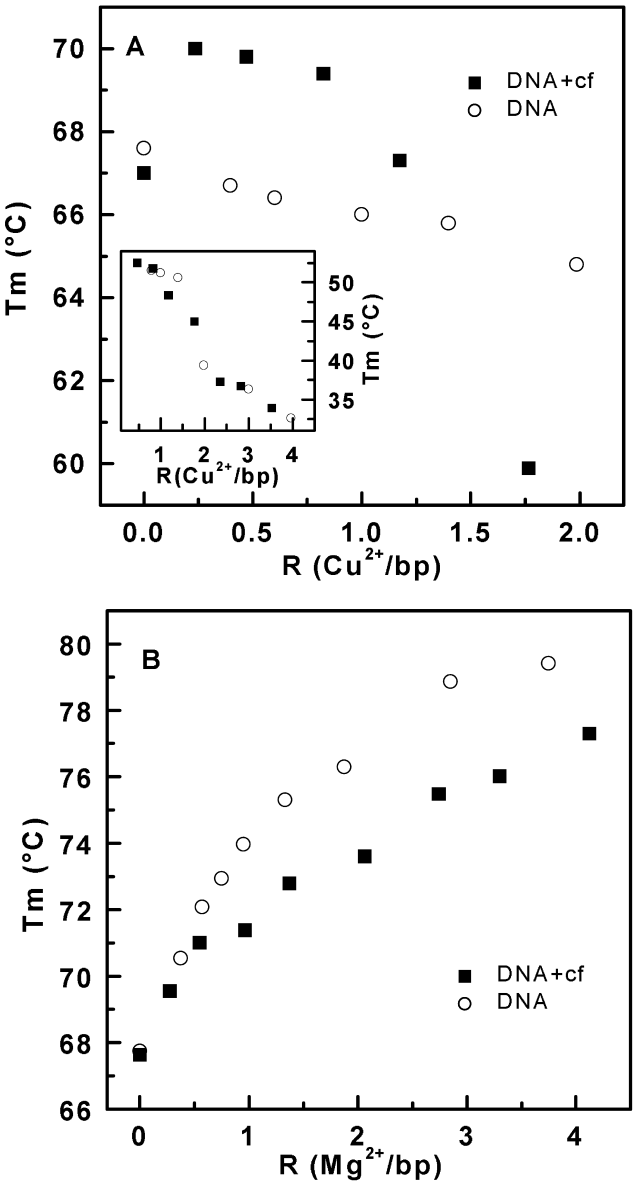

Fig. 5. Melting temperatures, T_m, of calf thymus DNA (○) and ciprofloxacin–calf thymus DNA complex (molar ratio 1:5) (■) plotted as a function of molar ratio of Cu^{2+} (A) and Mg^{2+} (B) per DNA base pairs, *R*. The inset of panel A shows the effect of Cu^{2+} on the T_m of the first transition of DNA and ciprofloxacin–DNA complex observed in the biphasic UV-melting curve plotted in Fig. 4A. Solution conditions were 10 mM cacodylic acid/sodium cacodylate (pH 7.0), $c_{DNA} = 25$ μM.

and carboxyl moieties of ciprofloxacin would pull the two DNA strands apart [32]. It has been suggested that the stacking interactions between norfloxacin (another member of the group of fluoroquinolones) aromatic rings and nucleic bases of single stranded domains of DNA stabilise the ternary norfloxacin–Mg^{2+}–DNA complex [5,33]. Another possibility is that ciprofloxacin or the ciprofloxacin–Mg^{2+} complex may bind into the minor groove of DNA or intercalate between its bases at low concentrations of Mg^{2+} [34].

3.5. The influence of copper(II) and magnesium(II) ions on CD spectrum of calf thymus DNA at 25 °C

The CD spectra of calf thymus DNA were measured at different concentrations of metal ions (Fig. 6). The molar ellipticity of DNA, $[\Theta]_{280}$, depend on the concentration of metal ions in a manner depending on the presence or absence of ciprofloxacin (Fig. 7). These titrations were performed under the same experimental conditions as fluorimetric and UV-melting experiments. Copper(II) ions change the molar ellipticity of DNA at very low concentrations (from 5 to 50 μM) (Figs. 6A and 7A). At higher concentrations of Cu^{2+} (700 μM), DNA begins to precipitate. Magnesium(II) ions cause similar changes in the molar ellipticity of DNA, but at much higher concentrations (15 mM). At very high concentrations of Mg^{2+} ions, above 2.5 M, a second transition of calf thymus DNA occurs that is not followed by precipitation (Fig. 6B and inset of Fig. 7B).

Circular dichroism of DNA in the UV region above 200 nm is induced by the electronic transitions of four nucleobases (adenine, thymine, guanine and cytosine) and is due to two effects: the intrinsic CD of the nucleotide monomers caused by the interaction of symmetrical bases with asymmetrical sugar-phosphate groups, and the extrinsic CD resulting from the interaction between neighbouring bases [35]. It is difficult to interpret the DNA CD spectrum as it arises from two different sources. However, due to the different conformation in the helical backbone, various forms of DNA have unique spectral characteristics that are easy to recognise. B-DNA has a CD spectrum with a positive band at 275 nm and a negative band at 240 nm [36]. Our CD spectra of calf thymus DNA in the absence of Mg^{2+} and Cu^{2+} show the characteristics of B-DNA (Fig. 6A and B). At low concentrations of Mg^{2+}, the ellipticity of the positive band at 275 nm decreases, while above 2.5 M, the positive band splits into two bands at 265 and 285 nm (Fig. 6B). The CD spectrum of DNA at high concentrations of Mg^{2+} is characteristic of Z-DNA [37]. These results are in agreement with some other studies of conformational changes of nucleic acids that showed two conformational transitions of DNA induced by Mg^{2+} [37–40]. The first transition is due to a stabilisation of B-DNA by magnesium(II) ions binding to the negatively charged phosphate groups [25] and can be described as a B to C transition of DNA [40,41]. At a critical concentration of Mg^{2+} (2.5 M), all the phosphate sites are occupied, and binding of the ions to the bases starts to occur which, in turn, induces the second transition described as a C to Z transition.

Cu^{2+} induces a conformational transition of calf thymus DNA that is similar to the first Mg^{2+}-induced transition. The Cu^{2+}-induced transition takes place at $c_{1/2} = 12.4$ μM. The CD spectra in Fig. 6A show that the positive band at 275 nm loses most of its intensity, as was observed previously [41]. The first monophasic transition of B-DNA

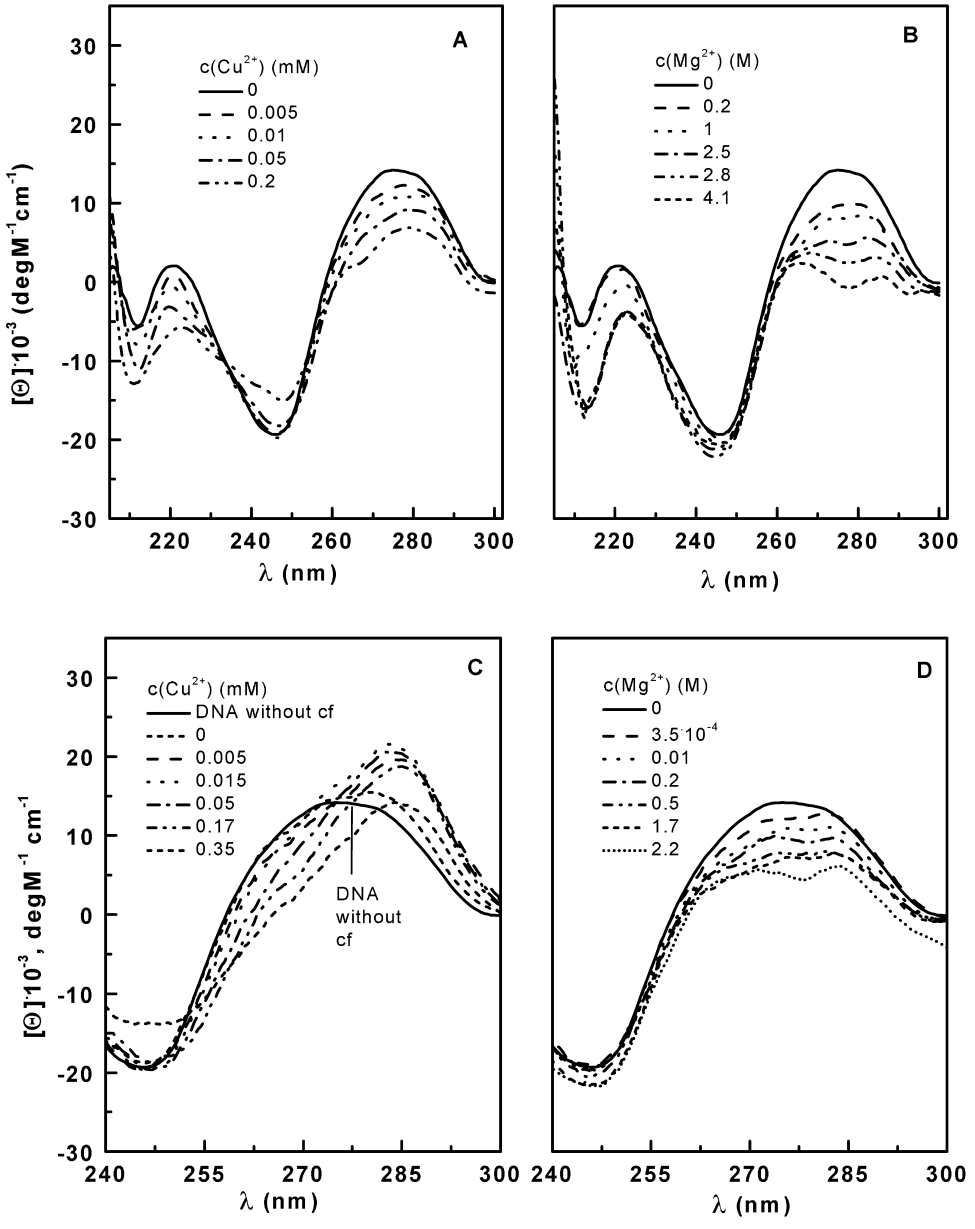

Fig. 6. CD spectra of calf thymus DNA in the presence of Cu^{2+} (A) and Mg^{2+} (B). CD spectra of ciprofloxacin–DNA complex (molar ratio 1:5) in the presence of Cu^{2+} (C) and Mg^{2+} (D) at pH 7.0, $c_{DNA}=50$ μM and $T=25$ °C.

induced by Cu^{2+} [40] is not followed by a second transition, instead, DNA precipitates, which could be the consequence of intramolecular N7–Cu–N7 crosslinking [29].

3.6. The influence of copper(II) and magnesium(II) ions on CD spectrum of calf thymus DNA in the presence of ciprofloxacin at 25 °C

Ciprofloxacin induces an increase in the molar ellipticity of DNA (Fig. 6C). Comparison of the spectra in the absence (Fig. 6B) and presence of ciprofloxacin (Fig. 6D) at different concentrations of Mg^{2+} show no significant differences. As can be seen from Fig. 7B, an increase in the concentration of Mg^{2+} up to 1000 mM causes a decrease in the molar ellipticity of DNA, indicating that the first conformational transition induced by Mg^{2+} is affected in the presence of ciprofloxacin, while the second transition remains unchanged (data not shown).

On the other hand, low concentrations of Cu^{2+} cause significant changes in the CD spectrum of calf thymus DNA in the presence of ciprofloxacin (Fig. 6C). The molar ratios of Cu^{2+} to ciprofloxacin to DNA at the point of maximum change in the CD signal of calf thymus DNA are 1:1:5 (Fig. 7A). At the same molar ratios, the maximal increase in thermal stability, T_m, of calf thymus DNA was

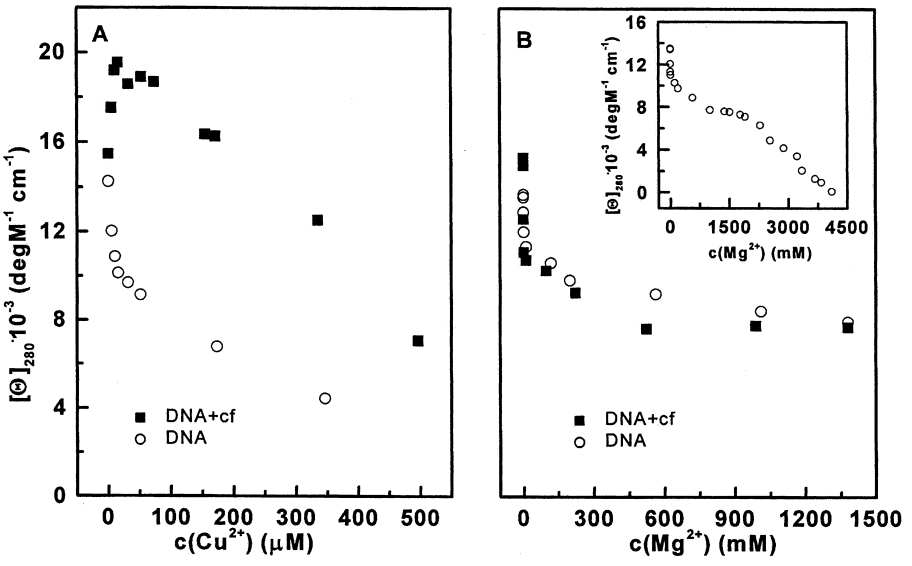

Fig. 7. Molar ellipticity of DNA (○) and ciprofloxacin–DNA complex (molar ratio 1:5) (■) versus concentration of Cu^{2+}(A) and Mg^{2+} (B). The inset of panel B shows the whole titration range performed by Mg^{2+}. Solution conditions were 10 mM cacodylic acid/sodium cacodylate (pH 7.0), $c_{DNA}=50$ μM and $T=25$ °C.

observed (T_m was determined from the second temperature-induced transition) (Fig. 5A). Cu^{2+} binding to double stranded DNA results in thermal destabilisation as was confirmed in the UV-melting experiments (Figs. 4A and 5A). Our CD data suggest that ciprofloxacin probably does not bind to the same binding sites as Cu^{2+} (to N7 position of purine bases [24]), but instead to the copper-unbound part of DNA. Based on our UV-melting curves, we propose that this binding at low concentration of Cu^{2+} results in thermal stabilisation of the non copper-bound regions of DNA.

4. Conclusions

We have studied the effect of Mg^{2+} and Cu^{2+} on the binding of ciprofloxacin to the calf thymus DNA by applying fluorescence emission, UV- and CD-spectroscopy. Our results show that magnesium(II) ion forms 1:1 complex with ciprofloxacin, which is likely to be involved in the process of ciprofloxacin binding to DNA via phosphate groups of the DNA. The UV-melting profile reveals that the thermal stability of DNA induced by Mg^{2+} decreases by addition of ciprofloxacin. On the other hand, the association constant of Cu^{2+} with ciprofloxacin is an order of magnitude greater than that for the Mg^{2+} association. Copper(II) ions bind preferentially to N7 position of purine bases on DNA and no binding of copper(II) ions to phosphate oxygen were observed [24,25]. Based on our CD and UV results, we propose that copper(II) ions are not directly involved into ciprofloxacin binding to DNA via phosphate groups. Instead, it is likely that ciprofloxacin binds to non copper-bound regions of DNA.

Results of the present study also suggest the necessity of further experiments that would examine the antagonistic effects of metals on the interaction between quinolones and DNA as well as DNA gyrase.

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