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INTERACTION OF Cu(II) AND Cd(II) IONS WITH DNA FROM *SPIRULINA PLATENSIS*

E. Gelagutashvili[*]

E. L. Andronikashvili Institute of Physics, Georgian Acad. Sci., 6 Tamarashvili str., Tbilisi, 0177, Georgia

Equilibrium dialysis and atomic absorption analysis were used to obtain adsorption isotherms and determine the stoichiometric binding constants of Cu(II) and Cd(II) ions to DNA from *Spirulina platensis* in solutions. The stoichiometric constants of Cu(II) and Cd(II) ions with DNA from *S. platensis* in 3 mM NaCI are $15.56 \cdot 10^4$ and $14.40 \cdot 10^4$, respectively.

Effect of ionic strength and DNA GC content on binding constants of Cu(II)- and Cd(II)-DNA complexes were studied out. It was showed that the binding constants of Cu(II)- and Cd(II)-DNA complexes decrease with increase of ionic strength. The empirical dependences of logK on the GC content has been derived for Cd(II)- and Cu(II)-DNA complexes.

Keywords: *cadmium ion, copper ion, DNA, Spirulina platensis*

Introduction

The action mechanism of antitumor and antibiotic pharmaceuticals often involves formation of complexes with DNA. Some of these DNA-binding drugs exert their effects only in the presence of a metal cation [1]. The binding of Zn-finger proteins to DNA was shown to be affected by cadmium and other metals in two major ways [2]. Cd(II), as well as Cu(II) has been shown to change the binding characteristics of the SP1 transcription factor of Zn-finger to DNA [3]. Inhibition of DNA reparative ability is an important mechanism of Cd(II) genotoxicity [4].

Although the search for innovative treatment technologies focuses its attention on the metal binding abilities of various microorganisms and their components, the mechanism of interaction of metal ions with them is not quite known. Antiviral and anticancer effects are characteristic for blue-green algae *S. platensis* that has high cadmium sorption capacity [5].

In this study the energetics of Cu(II) and Cd(II) ions binding to DNA isolated from blue-green algae *S. platensis* was determined via their binding isotherms obtained by equilibrium dialysis and atomic-absorption spectroscopy. The binding constants of Cu(II) and Cd(II) ions with DNA were estimated for various ionic strength and various G–C content in DNA.

Materials and methods

As the reagents were used chloride salts of Cu(II), Cd(II) and Na(I). All of them were of analytical grade and prepared in double-distilled water.

The technique that we used for DNA isolation from *S. platensis* was compiled according to the method described in article [6] with some modifications. The DNA preparation isolated by us were evaluated by spectral indicators, which were in accordance with literature data.

Equilibrium dialysis experiments were performed in a two-chambered Plexiglass apparatus. The chamber capacity was 5 mL. The membrane thickness was 30 μg (Visking). One chamber contained DNA (10^{-4} M) and the other one – solution of the metal ion under investigation. The initial metal concentration was varied within the range 10^{-6}–10^{-4} M. Samples were analyzed by flame atomic–absorption spectrophotometry (FAAS) ('Beckman-495') Wavelength (nm): 324.75 for Cu and 228 for Cd.

Data analysis

Binding constants were determined from the Scatchard plots. The basic binding model of Scatchard is based on the mass law, where n represents the number of binding sites per mole of DNA and K_{obs} represents the observed binding constants.

$$r = \sum_{i=1}^{N} \frac{k_i n_i m}{1 + k_i m} \quad (1)$$

* gel@iphac.ge, gelaguta@yahoo.com

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Akadémiai Kiadó, Budapest, Hungary
Springer, Dordrecht, The Netherlands

where n_i is number of binding sites for class i, k_i – the microconstant for the same class, m – number of the free molecules.

Results and discussion

The adsorption isotherms for Cu(II)-DNA complexes in the Scatchard coordinates at 3 mM NaCI and t=20°C is shown in Fig. 1. As it is seen from Fig. 1, the dependence r/m *vs.* r is nonlinear. Application of a number of mathematical models reveals that in case of Cu(II)–DNA complexes the model of two independent binding sites is the best approximation. The obtained data were treated by the Scatchard method. Microconstants k_1, k_2 and corresponding numbers of binding sites n_1, n_2 given in Table 1 were determined.

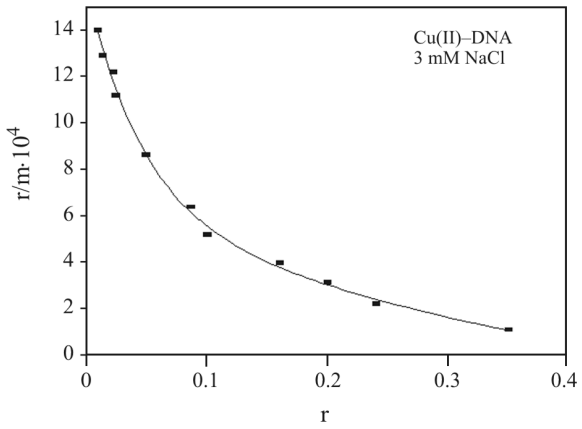

Fig. 1 Binding isotherms of Cu(II)–DNA complexes (the dependence r/m *vs.* r, where r is the concentration of bound metal ions, m – the concentration of free metal ions). The points are experimental data and the plot is received using modified Scatchard equation in the case of two types of binding

Table 1 Binding parameters for Cu(II)- and Cd(II)–DNA complexes at ionic strengths of 3 mM, t=20°C

	Cu(II)–DNA	Cd(II)–DNA
Microconstant $k_1 \cdot 10^4$ M^{-1}	205.02	–
Microconstant $k_2 \cdot 10^4$ M^{-1}	5.18	–
No. of binding sites n_1	0.06	–
No. of binding sites n_2	0.43	–
No. of binding sites n	0.49	0.4
Stoichiometric binding constant $K \cdot 10^4$ M^{-1}	15.56	14.4
log$K$	5.19	5.16
Gibbs free energy $-\Delta G_0$ kcal mol^{-1}	7.06	7.02
χ^2 distribution	0.11	–
Correlation coefficient R	0.97	0.99

As it is seen, $k_1 > k_2$ for Cu(II) ions i.e. association of these ions with DNA can be described by the model with two patterns of binding, one of them corresponding to the strong binding, the other corresponding to the weak one. The same nature of binding was obtained for Pb(II)-DNA complexes earlier [7, 8].

For Cd(II)–DNA complexes a positive slope was observed, indicative of positive cooperativity of Cd(II) binding to DNA (Fig. 2). When the ratio of cadmium(II) ions to DNA phosphorus fell in the range between 1:25 and 1:2, an approximate linear dependence with negative slope was obtained. This means that interaction of Cd(II) ions with DNA from *S. platensis*, follows the 'independent binding site' principle. The best fit slope for the descending part of the binding curve revealed stoichiometric binding constant K=1.44·10^5 M^{-1}. At low proportion of occupied sites one copper molecule is bound per 17 phosphorus and one cadmium per 25 DNA phosphorus. At saturation one binding site per two bases for both metal ions (n) is accounted. The same value of n was obtained in [7–10]. When

$$r/m = 0.5[B(r) + \sqrt{B^2(r) + 4C(r)}] \qquad (1)$$

where $B(r)=k_1n_1+k_2n_2-(k_1+k_2)r$; $C(r)=k_1k_2r(n_1+n_2-r)$.

Investigating the interaction of Mn(II) ions with the bases of DNA of different origin Zimmer [10] showed that the DNA conformation plays a major role for the number of binding sites. Several models for metal ions binding with DNA have been proposed, including: a) inter- or intra-strand cross-links of adjacent bases; b) chelate complexes, involving N7 of

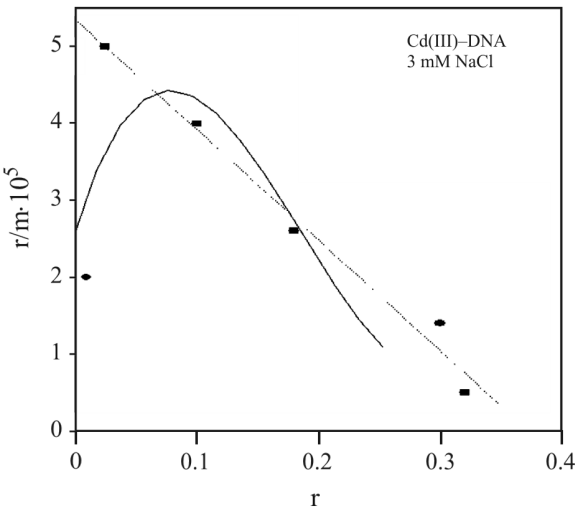

Fig. 2 Binding isotherms of Cd(II)–DNA complexes. The parameters are the same, as described in Fig. 1. The points are experimental data and the plot is received using equation: $r/m=K(n-r)$. (In both cases each point represents the average of three independent determinations and the standard deviations were ≤9% of the averages)

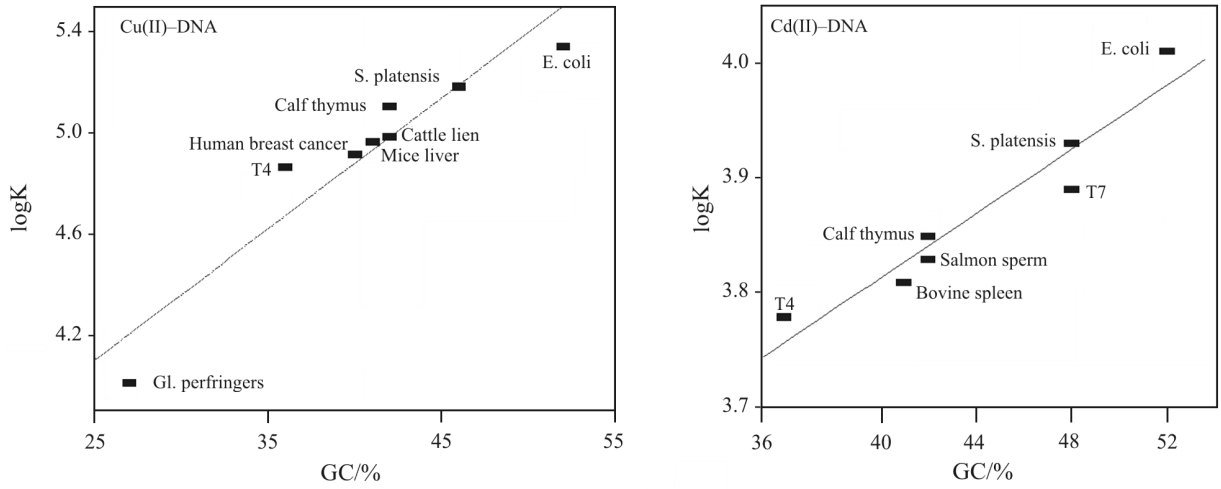

Fig. 3 Dependence of log*K* on the GC content DNA for Cu(II)- and Cd(II)–DNA complexes

guanine and phosphate groups; c) sandwich-type intercalation of a metal. In general, binding between cations and poly-ions (DNA) may be inner-sphere (direct interaction with active groups of DNA), outer-sphere (territorial-interaction of metal ions via the bridging action of water molecules) and 'atmospheric' (through long-range electrostatic interaction only) bindings [9].

Table 1 summarizes the obtained data as standard Gibbs energy. These results show that the value of ΔG^0 is of the same order as the energy of hydrogen bonding i.e. Cu(II) and Cd(II) ions mainly form outer sphere complexes with DNA.

Evidently, there is certain balance between all types of metal-DNA complexes (inner sphere, outer sphere and atmospheric) and their constants depend on the DNA nucleotide composition, the nature of the metal ion and ionic strength of the solution. The quantitative characteristics of interaction of Cu(II) and Cd(II) ions with DNA (binding constants) were estimated by equilibrium dialysis for various ionic strength and various G-C content in DNA.

Effect of GC content in DNA on the binding constants of Cu(II) and Cd(II) ions with DNA is presented in Fig. 3 (we have limited ourselves to the comparison of stoichiometric constants (*K*)). As one can see, log*K* increases with the increase of the GC content due to preferred association of Cu(II) and Cd(II) ions with GC pairs. For the native DNA the empirical dependence of log*K* on GC content has been derived for metal (II)-DNA complexes:

$$\text{Cd(II)–DNA: } \log K = 3.25 + 0.01(\text{GC\%})$$

$$\text{Cu(II)–DNA: } \log K = 2.52 + 0.06(\text{GC\%})$$

It can be expected that such tendency of stronger complex formation with DNA electron-donor groups may be caused by increase of GC content in the DNA that, in its turn, increases formation probability of N(7)–Cu(II) and N(7)–Cd(II) complexes. This is in good agreement with literature data [11–13]. In particular, it was shown in [11] that transition metal ions have low affinity to phosphates and are able to condense GC-rich DNA. Divalent alkali-earth metal ions have high affinity to phosphates but do not induce DNA condensation, while transition metal ions easily precipitate GC-rich DNA [12]. Using X-ray diffraction analysis of oligonucleotides it was shown that investigation of Z-DNA structure of Cu(II)-soaked CGCGTG crystal revealed that the Cu(II) ion is bis-coordinated to N7 position of G10 and G12 bases with a trigonal bipyramid geometry, suggesting a possible N7-Cu-N7 crosslinking mechanism [13]. Copper ions bind almost exclusively by coordinating to the N7 position of purine, especially of guanine [14]. The interaction of calf thymus DNA was studied in aqueous solution at pH 6.5 using FTIR spectroscopy [15]. Spectroscopic evidence has shown that at low metal/DNA (P) ratios copper ions bind mainly to the PO_2 of the backbone resulting in increased base-stacking interaction and duplex stability. The major copper ion-base binding via GC base pairs starts at $r>1/40$.

Effect of ionic strength on the binding constants of Cu(II)- and Cd(II)–DNA complexes is presented in Fig. 4. The dependences show that with increase of salt concentration the binding constants for Cu(II)- and Cd(II)–DNA complexes decrease. Such decrease of metal ion binding constant is caused by screening of nucleotide bases and phosphates by sodium ions. In [16] gel electrophoretic study of monovalent counterion effect on the degree of divalent counterion binding to DNA was carried out. Ionic strength effects for Zn(II) and Cu(II) ions were observed.

The counterions in the immediate vicinity of a highly charged cylindrical ion (such as DNA) are be-

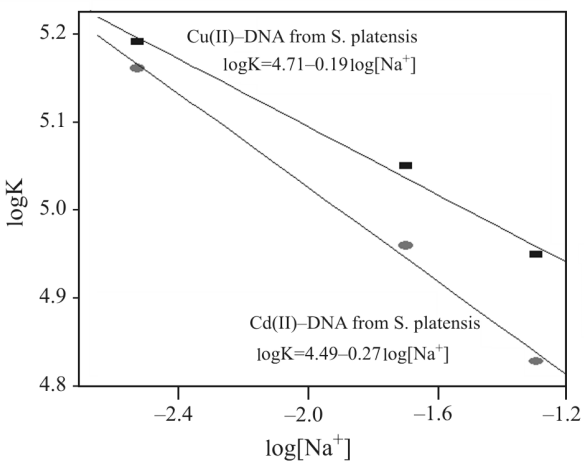

Fig. 4 Plots of log*K vs.* log[Na^+] and linear least-squares fit

lieved to be electrostatically associated rather than fixed at specific sites. Most studies are based on two known models: the Poisson-Boltzman (PB) cell model [17] and the counterion condensation (CC) [18] model. Detailed theoretical comparisons of CC and PB theories applied to ion distributions and thermodynamic properties in different concentration regimes have been reviewed by Anderson and Record [19]. They have also carried out a detailed thermodynamic analysis of effects of salt in ligand-nucleic acid interactions on the basis of preferential interaction coefficients [20]. We have used Record and Anderson models for investigations of ion effects on Cu(II)- and Cd(II)–DNA interactions.

Record and co-workers have shown [21, 22], that the salt dependence of the binding constant (*SK*) may be used to calculate the polyelectrolyte contribution (ΔG_{pe}) to ΔG^0 at a given NaCl concentration. (ΔG_{pe} is the portion of the free energy of the binding.) The difference between the Gibbs free energy and ΔG_{pe} defines the 'non-electrostatic' free energy contribution (ΔG_t). In our case ΔG_t(Cu)= –6.03 kcal mol^{-1} and ΔG_t(Cd)= –5.55 kcal mol^{-1}. Comparison of ΔG_t^0 of Cu(II) and Cd(II) ions indicates that ΔG_t^0 for Cd(II) is less than that for Cu(II) ions. This result is in good agreement with literature data [23], where high specificity of DNA for copper comparing to other metal ions is observed.

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