American Journal of Nano Research and Applications
2017; 5(3-1): 5-8
http://www.sciencepublishinggroup.com/j/nano
doi: 10.11648/j.nano.s.2017050301.12

Cyanobacteria *Spirulina Platensis* Basic Protein C-Phycocyanin and Zn(II) Ions

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To cite this article:
Eteri Gelagutashvili. Cyanobacteria *Spirulina Platensis* Basic Protein C-Phycocyanin and Zn(II) Ions. *American Journal of Nano Research and Applications.* Special Issue: Nanotechnologies. Vol. 5, No. 3-1, 2017, pp. 5-8. doi: 10.11648/j.nano.s.2017050301.12

Received: July 21, 2016; **Accepted:** July 25, 2016; **Published:** September 14, 2016

Abstract: The interaction of Zn(II) ions with cyanobacteria *Spirulina platensis* basic protein C-phycocyanin (C-PC) is studied by fluorescence spectroscopy.Stern–Volmer quenching constant value for Zn(II)–C-PC is determined. The binding energy of Zn(II) ions with C-phycocyanin is determined using equilibrium dialysis and atomic absorption spectroscopy. Cooperative binding of Zn(II) ions with C-phycocyanin is observed. The binding constants diminished with increasing ionic strength, suggesting an adaptive protective response. "Nonelectrostatic" and polyelectrolyte components of binding free energy for Ag^{+}, Cu^{2+}, Cr^{3+}, Pb^{2+}, Ni^{2+}, and Zn^{2+}–C-phycocyanin (*Spirulina platensis*) complexes are determined. It is shown that "nonelectrostatic" component of binding free energy is dominating at the metal–C-PC interaction, while the polyelectrolyte contribution being less important, and the "nonelectrostatic" forces contribution for Ag^{+}–C-phycocyanin (*Spirulina platensis*) complexes exceeds that for other metal ions.

Keywords: C-phycocyanin, Zn Ions, Binding Constant

1. Introduction

The cyanobacteria cells play an important role in remediation of toxic metals through reduction of the metal ions. These biological systems have been extensively used for the biosynthesis of metal nanoparticles [1]. The application of nanoparticles as delivery vehicles for bactericidal agents represents a new paradigm in the design of antibacterial therapeutics [2]. Fourier transform infrared spectroscopic (FTIR) measurements revealed the fact that the protein is the possible bimolecular responsible for the reduction and capping of the biosynthesized nanoparticles.

More recent and detailed investigations into the use of microbes in the synthesis of different metal nanoparticles include bacteria for ZnS [3], CdS [4], yeast for PbS [5], and CdS [6] .The presence of metals can have an enhancing effect on pigment content in cyanobacteria cells. Metal exposure can be an interesting method to induce, in microalgae cells, the synthesis of target products such as pigments, lipids, nanoparticles, etc. However, stimulation of target compound production in microalgae depends on many factors such as metal type and concentration or metal combination leading to synergistic effects. Cyanobacteria *Spirulina* is a planktonic blue-green alga found in warm water alkaline volcanic lakes. It has a dark blue-green color, because it is rich in a brilliant blue polypeptide called phycocyanin. Hayashi et al. [7] reported that phycocyanin affects the stem cells found in bone marrow. Medical scientists reported that *Spirulina platensis* not only stimulates the immune system but also enhances the body's ability to generate new blood cells [8]. Cultivation of *Spirulina platensis* in the presence of Pb, showed a decrease in the biomass and pigment (chlorophyll, phycocyanin) concentration in culture volume. Nevertheless, pigment content in cyanobacteria biomass increased at some metal concentrations and cyanobacteria growth was stimulated at low Pb concentrations [9, 10]. In our previous works [11, 12], it was studied interaction of Cd(II), Hg(II), Cr(III), Ag(I), Cu(II), and Pb(II) ions with cyanobacteria *Spirulina platensis* basic protein C-phycocyanin (C-PC) and also interaction of Cd(II), Cr(III), Ag(I), and Au(III) ions with *Spirulina platensis*.

In this work, interaction of Zn(II) ions with cyanobacteria *Spirulina platensis*basic protein C-PC has been studied by fluorescence spectroscopy. The energetics of Zn(II) ions

interaction with (C-PC)from *Spirulina platensis* has been studied via determination of the stoichiometric constants at different ionic strengths under chemical equilibrium by using of equilibrium dialysis. Also, "Nonelectrostatic" and polyelectrolyte components of binding free energy for Ag^{+}, Cu^{2+}, Cr^{3+},Pb^{2+}, Ni^{2+}, and Zn^{2+}–C-phycocyanin (*Spirulina platensis*) complexeshas been determined.

2. Materials and Methods

Organisms, culture techniques, metal analysis methods, data analysis are the same as have been described previously [12].

3. Results and Discussions

The fluorescence spectra of C-PC in the presence of Zn^{2+} ions are shown in Fig. 1. The intensity of C-PC fluorescence, observed in the range 580 – 690 nm, was measured as a function of the added metal concentration. Metal free form of C-PC has a fluorescence maximum at 635 nm. The increase of the metal concentration causes the decrease in the peak amplitude and was accompanied by a blue shift of fluorescence peak. It is clear from Fig. 1, that quenching has not been saturated even at high concentrations of Zn ions. The absence of saturation in the quenching plot even when the binding is saturated indicates that only a fraction of the binding sites quenches the fluorescence.

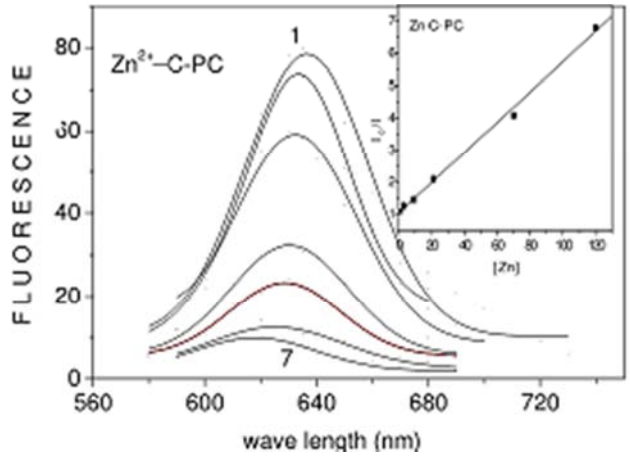

Figure 1. *Fluorescence spectra of C-PC (0.4 μM) in the presence of different concentrations of Zn(II) ions. 1 → 7 [Zn] = 0 → 120 (mM). Insert: Stern–Volmer plot.*

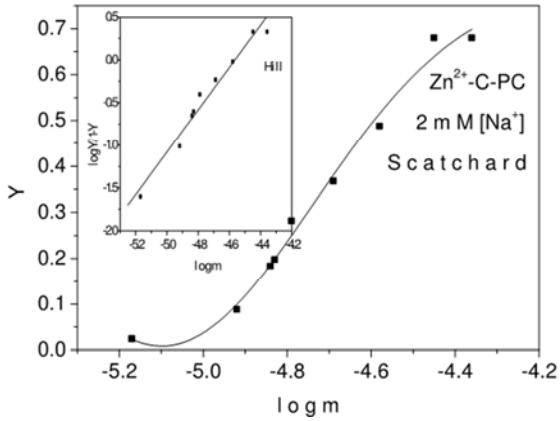

Figure 2. *Binding isotherm of Zn(II) ions with C-PC in the Scatchard coordinates [14] at 2 mM ionic strength (dependence of Y against $\log m$, where $Y = r/n$ and $r = c_{\text{Bound}}/[C-PC]$. Parameter r is the concentration of bound metal ions per mole of C-PC, m is concentration of free metal ions, n is the number of binding sites for the metal ions per mol of C-PC at saturation). Points stand for experimental data, while lines are from the hypothetic fit with $\chi^2 = 0.007$ and $R^2 = 0.98$. Each point represents the mean from three independent determinations. Standard deviations were < 13% of the means. Insert: Hill plot [15] and linear least-square fit of the Hill equation to determine K and n_H, the index of cooperativity (other parameters are the same, as in the case Scatchard analysis).*

The quantitative analysis of quenching efficiency ("accessibility of the fluorophore") is based on the classic Stern–Volmer equation ($I_0/I = 1 + K_{SV}[C]$), where I_0 and I are the fluorescence intensities without and with metal ions, $[C]$ is the metal cation concentration and K_{SV} is the collision quenching (Stern–Volmer) constant [13]. From The Insert of Fig. 1, it was determined: $K_{SV} = 4.6 \cdot 10^4$ M^{-1} for C-PC fluorescence quenching by Zn ions.

The flame atomic absorption spectral analysis was applied to register the equilibrium binding of Zn(II) ion to C-PC at different ionic strengths (0.002, 0.02, and 0.05 M) and to determine the number of free (m) and bound (C_b) Zn(II) ions.

The binding isotherm of Zn(II) ions with C-PC under equilibrium conditions (as an example) at 2 mM ionic strength is presented in Fig. 2 as dependence Y *vs* $\log m$. In Fig. 2 points stand for experimental data while line is hypothetic fit with $\chi^2 \rightarrow 0.007$, from which the apparent affinity is estimated. The binding parameters of Zn(II) ions to C-PC derived from the fit with the parameters at 50 and 20 mM ionic strengthare presented in Table 1. It is seen from Table 1 that the number of Zn(II) binding sites per C-phycocyanin at saturation is within the range $n = 4 – 5$, i.e. the studies have shown that C-PC is able to bind 4 or 5 mol of Zn(II) per mol of protein.

Table 1. *Parameters of Zn(II) ions binding with C-PC at 20 °C.*

	Ionic strength, M	**0.050**	**0.020**	**0.002**
Analysis by the Scatchard method	Binding constant K, 10^4 M^{-1}	1.15	1.92	4.10
	Gibbs free energy $-\Delta G^0$, kJ/mol	23.14	24.39	26.22
	Number of binding sites $-\Delta G^0$, kJ/mol	4.10	4.00	5.20
Analysis by the Hill method	Binding constant K, 10^4 M^{-1}	1.66	2.32	3.72
	Gibbs free energy $-\Delta G^0$, kJ/mol	24.05	24.88	26.05
	Hill coefficient n_H	3.11	2.30	2.48

By reducingionic strength from 50 to 2 mM, the stoichiometry of Zn(II) protein binding is increased from 4 to 5. The type of Y *vs* $\log m$ dependence observed implies that there exists positive cooperativity between bound Zn(II) ions in C-PC [14, 16], i.e. binding of the first metal ion increases affinity of the site for the second one. So that, the binding at one site, along the protein chromophore, may alterthe probability of among at the neighboring sites.

To elucidate such possibilities, the presentdata were replotted according to Hill equation [15]. The Hill plot forbinding of Zn(II) to C-PC is shown in Fig. 2 (Insert). From this figure the binding parameters K and n_H were determined. It is seen that index of cooperativity $n_H > 1$, denoting, that the binding of Zn(II) ions with C-PC iscooperative process. So, Zn(II) binding to C-PC is cooperative at various ionic strengths; 2, 20, and 50 mM with the Hill coefficients equal to 2.48, 2.30, and 3.11, respectively. In all the cases, the Hill coefficient is less than the number of active binding sites, i.e.the interaction does not occur at "all-or-none" principle, suggesting that Zn(II) has low affinity for binding to C-PC at various ionic strengths.

Table 1 shows also that when increasing the sodium concentration the binding constants for Zn(II) complexed toC-PC decrease. It is clear that binding constant at ionic strength of 2 mM for Zn^{2+}–C-phycocyanin (*Spirulina platensis*) complexesis in good agreement with Stern–Volmer quenching constant.

It is known that phycocyanin is a blue phycobiliprotein composed of two relatively homologous subunits: the -chain with one phycocyanobilin attached at cysteine 84 and the -chain with two phycocyanobilins attached at cysteines 84 and 155. The two subunits form monomers, which aggregate into 33 trimers and further into disc-shaped 66 hexamers, the functional unit of phycocyanin [17]. Cys-84 and Cys-155 residues as cysteine thiolates may be potent binding sites for zinc ions. The decrease of Zn(II) ion binding constant may be caused by screening of the thiol groups by sodium ions.

On the other hand, it is known that the principal phycobiliproteins, which consist of α- and β-subunit polypeptides form well defined, helical globin-like domains with six helices. For C-PC the dominant electrostatic field is negative, particularly in the center of the hexamers [18]. This charge distribution may be important for interaction of metal ions with C-PC. The increase of ionic strength most likely makes the penetration of Zn(II) into the C-PC structure more difficult. Thus, it is possibly assumed, that the effect of Zn(II) upon C-PC may be carried out by interaction at the apoprotein and prosthetic group levels, as other metal ions [12].

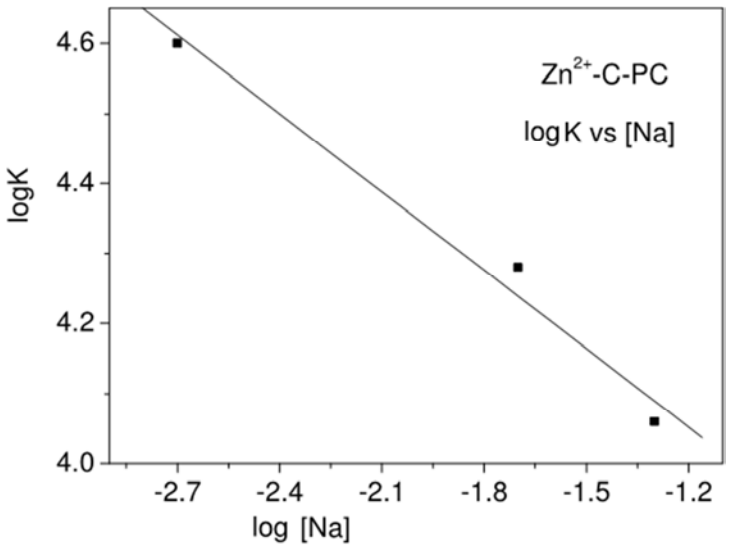

Figure 3. *Plot of* $\log K$ *vs* $\log[Na^+]$*, with the linear least-squares fit of the equation* $\log K = -\Delta G_t / 5.7 - b \log[Na^+]$ *(derived from equations by Record and co-workers [19, 20]).*

The dependence of Zn(II) binding constant for C-PC upon the concentration of Na(I) ion is shown in Fig. 3. Record and co-workers showed [19, 20] that the ionic strength dependence of the binding constantmay be used tocalculate the polyelectrolyte contribution (ΔG_{pe}) to ΔG^0 at a given NaCl concentration by the relation $\Delta G_{pe} = bRT \ln[Na^+]$, where b is an empirical parameter. The difference between the Gibbs frees energy and ΔG_{pe} is the "nonelectrostatic" free energy contribution ΔG_t: $\Delta G_t = \Delta G^0 - \Delta G_{pe}$. The value of ΔG_t refers to that portion of the binding free energy that is independent of salt concentration and contains minimal contributions from polyelectrolyte effects. The binding constant K was used to calculate the Gibbs free energy from the standard relation $\Delta G^0 = -RT \ln K$, where R is the gas constant, T is the absolute temperature and ΔG^0 – the Gibbs free energy. Calculated ΔG^0 is characteristic for hydrogen bonds (see Table 1).

These equations were used [19, 20] to distinguish "nonelectrostatic" and polyelectrolyte component of binding free energy. The results are presented in Table 2. In 50 mM Na(I), ΔG_{pe}, and ΔG_t derived from the experimental data for Zn^{2+}–C-PC complexes are –2.6 and –20.52 kJ/mol, respectively.

Table 2. *"Nonelectrostatic" and polyelectrolyte component of binding free energy for metal–C-PC.*

Metal ion	Ag^{+}	Pb^{2+}	Cu^{2+}	Cr^{3+}	Ni^{2+}	Zn^{2+}
$-\Delta G^{0}$, kJ/mol	32.6	31.06	27.76	20.64	23.88	23.14
$-\Delta G_{pe}$, kJ/mol	0.053	1.08	0.96	1.65	1.37	2.6
$-\Delta G_{t}$, kJ/mol	32.55	29.98	26.79	22.29	22.51	20.52

Also "nonelectrostatic" forces contribution for Ag^{+}, Cu^{2+}, Cr^{3+}, Pb^{2+}, Zn^{2+}, and Ni^{2+}–C-phycocyanin (*Spirulina platensis*) complexes were calculated. The comparison of these results indicate that the "nonelectrostatic" forces contribution for Ag^{+}–C-phycocyanin (*Spirulina platensis*) complexes exceeds that for other metal ionsand according to the "nonelectrostati" component of binding free energy for Ag^{+}, Cu^{2+}, Cr^{3+}, Pb^{2+}, Zn^{2+}, and Ni^{2+}–C-phycocyanin (*Spirulina platensis*) complexes are arranged in the following sequence: $Ag^{+}>Pb^{2+}>Cu^{2+}>Ni^{2+}>Cr^{3+}>Zn^{2+}$.

Thus, the calculations revealed that "nonelectrostatic" free energy is dominating at the metal–C-PC interaction, while the polyelectrolyte contribution being less important.

4. Conclusion

Zn(II) binding to C-PC has low affinity and C-PC is able to respond to the rise of Na(I) level decreasing the affinity. Stern–Volmer quenching constant value for Zn(II)–C-PC was determined by fluorescence spectroscopy.

"Nonelectrostatic" and polyelectrolyte components of binding free energy for Ag^{+}, Cu^{2+}, Cr^{3+}, Pb^{2+}, Ni^{2+}, and Zn^{2+}–C-phycocyanin (*Spirulina platensis*) complexes were determined.

It was shown, that "nonelectrostatic" component of binding free energy is dominating at the metal–C-PC interaction, while the polyelectrolyte contribution being less important, and the "nonelectrostatic" forces contribution for Ag^{+}–C-phycocyanin (*Spirulina platensis*) complexes is more than for other metal ions.

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