Studies of Interaction between Heavy Metal Ions and C-phycocyanin from S.platensis.

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One of the basic proteins of *S. platensis* - C-phycocyanin (C-PC) - is the component mainly responsible for the antioxidant, hepatoprotective, anti-inflammatory and anti-arthritic activity and also anticancer effects.

In this work the interaction of metal ions with C-PC was investigated by optical and thermodynamic methods**.**

Cu(II)–C-PC (using equilibrium dialysis) binding isotherms are given in the Scatchard coordinates at different ionic strengths in fig.1. The points represent experimental data whereas solid curves are hypothetic functions fitted by χ^2 criterion. Binding constants were estimated by these isotherms . Both *n* values and the binding constants are given in the table 1. Comparison of *K* values at different ionic strengths shows that within the error limits they do not differ virtually at 0.02M and 0.05M, whereas at 0.002 M NaCl $K_{0.002\ M\ NaCl}$ is greater than $K_{0.02\ M\ NaCl}$ and $K_{0.05\ M\ NaCl}$ i.e. *K* decreases with ionic strength increase.

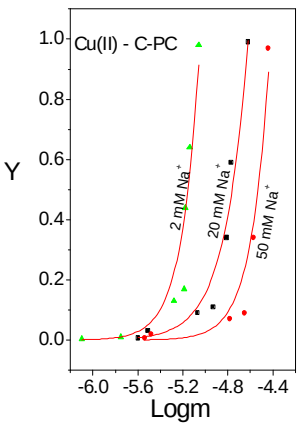
The type of *y* versus *log m* dependence means that there exists positive cooperation of interaction of metal ions bound with C-PC, i.e. binding of the first metal ion increases affinity of the site for the second one. To illustrate the possibility, the obtained data were additionally plotted in the Hill coordinates. The following binding parameters were determined: the binding constant *K*, the Hill coefficient n_H, which is the cooperativity index. All the results are given in the table 1. As it is seen, the binding constants *K* determined by both the Scatchard and Hill methods are in good agreement, which is also valid for ΔG°. It is seen from the table 1, that at saturation the number of Cu(II) binding sites per C-PC is within the range n= 3÷4 . ΔG^0 is approximately 7 kcal/mol that is characteristic for hydrogen bonds.

Whereas tetrapyrrol group (phycocianobilin) in C-PC is chromophore, the possibility of complex formation between Metal and chromophore of C-PC can be supposed. It is known from the literature data that Cu(II) preferentially bind with thiol groups of proteins. Naturally, the metal effect depends also on the structural position of the chromophore in the protein. In phycocyanobiline the main prosthetic group of C-PC is fixed in α-84 and β-84 positions by cystein thioester groups[1]. It was shown by applying the Forster theory [2] that β-84 chromophore is fluorescent [3]. Proceeding from our data on C-PC fluorescence quenching by copper ions, it is quite possible that cystein in β-84 position is one of the specific binding sites for copper ions in C-PC. At the same time, hexamers of α and β-subunits form globule mostly, so in C-PC the negative electrostatic field of hexamer is dominant. It is quite possible that such a distribution of the charge also plays an important role in the interaction with Cu(II) ions.

The dependence of *pK* (for Me-C-PC complexes using absorption titration) upon the covalent index $X_m^2 r$ is presented in Fig.2. Correlation is observed between *pK* and the covalent index $X_m^2 r$.

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

	Ionic strength	0.05 M	0.02 M	0.002 M
Analysis by the Scatchard method	Binding constant, $K \times 10^5$ M^{-1}	7.41	8.1	10.98
	Gibbs free energy, $-\Delta G^0$ kcal/mol	6.62	6.68	6.86
	Number of binding sites n	4.1	3.94	3.54
	χ^2	0.03	0.02	0.005
Analysis by the Hill method	Binding constant, $K \times 10^5$ M^{-1}	7.2	8.2	11.2
	Gibbs free energy, $-\Delta G^0$ kcal/mol	6.6	6.68	6.87
	Hill coefficient n_H	3.1	2.3	2.57
	Correlation coefficient R	0.87	0.84	0.95



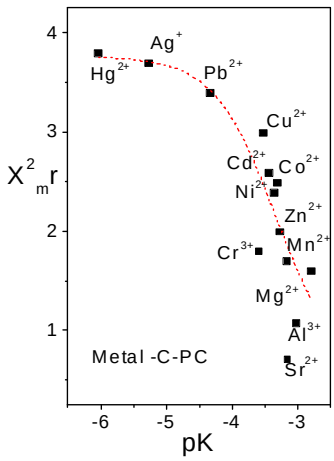


Fig. 1. The binding isotherm of Cu(II) ions with C-PC at various ionic strengths. (The dependence *Y vs Log m*, where $Y = r/n$ and $r = C_{bound}$/ [C-PC] is the concentration of bound metal ions per mol C-PC, m is the concentration of free metal ions, n is the number of binding sites for the metal ions per mol C-PC at saturation). Points stand for experimental data while lines are from the hypothetic fit with χ^2 0.005 ÷ 0.03. Each point represents the mean from three independent determinations and standard deviations are < 12 % of the means.

Fig.2. Correlation between the covalent index $X_m^2 r$ and binding constant logarithm for metal-C-PC complexes.

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