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Quenching Effect of Metal Ions Cu(II), Hg(II) and Cr(III) on the Fluorescence of C- Phycocyanin

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Abstract:
Fluorescence spectra of C-phycocyanin, (C-PC), a protein, isolated from algae biomass, Spirulina platensis, and influence of heavy metals Cu (II), Hg (II) and Cr(III) on them have been investigated. When metals are interacting with protein, fluorescence intensity quenching takes place. The decrease of fluorescence intensity is proportional to concentration of quencher metal ions. The parameters of fluorescence quenching due to these metals are estimated quantitatively from Stern-Volmer equation. A high quenching constant of $1.0x10^6$ M^{-1} for Hg (II) ions and low values of $3.1x10^4$ M^{-1} and $4.5x10^4$ M^{-1} for Cu(II) and Cr(III) ions respectively were estimated from these data.
A blue shift of up to 12 nm was measured for Hg-ions, when this shift was only about 4 nm for Cu-ions. Chromium ion addition to C-PC aqueous solution causes spectral band shift towards short wavelengths by about 10 nm.

Keywords: *fluorescence spectra, fluorescence quenching, Stern-Volme equation, heavy metals.*

1. Introduction

Today's ecological and health international problems bring effective, cheap and ecologically pure remediation technologies to the forefront of the scientific investigation by accelerated paces. One of such methods is the remediation of heavy metals through creation their complex compounds with either living natural or nonliving algae biomasses. The mentioned applied technology implies large-volume wastewater treatment, treatment of industrial effluents, surface and groundwater decontamination, recovery of trace metals from aqueous streams in nuclear processing facilities and reclamation of decontaminated soils [1-6].

In addition to the environmental utility of these algae biomasses in the abovementioned technologies, they are used also in analytical and fundamental research fields [7].

As is known action of phycobilipigments (phycoeritrin and phycocyanin) as photosynthesis sensitizers in red and blue-green algae is related to peculiarities of spectral properties of these proteins. Effect of denaturation, fluorescence quenching and photochemical activity for these compounds' wavelength characteristics were studied early. It turned out, that denaturation causes a significant increase in photosensitization of phycocyanin and phycoeritrin [8]. This result is due to increase of penetration degree of low-molecular partners of reaction into chromophore groups of proteins.

In the present work fluorescence spectra of C-phycocyanin, the protein, isolated from algae biomass, Spirulina platensis, and influence of heavy metals, Cu(II), Hg (II) and Cr(III), on them have been investigated. The parameters of fluorescence quenching by these metals are estimated quantitatively with the aid of the Stern-Volmer equation.

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2. Experimental Conditions

Laser fluorescence spectroscopy was used to study interactions of Cu(II), Hg (II) and Cr(III) metal ions with C-phycocyanin. C-PC was isolated and purified from blue and green algae Spirulina platensis. Two different excitation wavelengths were used in the experiments - 488.0 nm argon laser and 632.8 nm He-Ne laser. A He-Ne laser is especially effective as an excitation source, due to the strong absorption band of C-PC in the red spectral region. The fluorescence spectrum of C-PC is a single intensive line around λ= 638 nm, with non-distinct long-wavelength edge.

A detailed description of the experimental setup and measurement techniques could be found in [9].

Experimental Results and Discussion

The fluorescence spectrum of C- phycocyanin water solution (the concentration of C-PC is 0.4 μM) excited by argon laser emission 488 nm shows an intensive spectral band at 638 nm with sharp shortwave edge. The long wave edge of this band is not sharp and its intensity decreases slowly. This is the fluorescence spectrum of C-PC and it is analogous to the spectra from the literature. To investigate the fluorescence quenching dynamics the spectral peak fixed at 638 nm was used.

When metal ions were added we observed a reduction of the intensity of the fluorescence and a blue shift of the spectrum. The values of both the fluorescence quenching and the blue shift of the spectra depend on the type and concentration of the metal ions involved in the interactions. Fig. 1 shows the effect of addition of Hg ion to the C-PC water solution. In this case Hg ion with concentrations 0, 1, 2, 3, 4, 5, 6, 7 μM is added to the 0.4 μM C-phycocyanin solution. Fig. 2 shows the effect of Cu ion addition when metal ions concentrations are 0, 1, 8, 26, 50, 74, 98 μM. Fig. 3 shows Cr ions effect on phycocyanin when the following concentrations of this metal is added to the solution: 0, 3, 9, 21, 49, 69 85 μM.

Using these data we have calculated the quenching constants of the metal ions. The well-known Stern-Volmer equation was used for this purpose:

$$I_0/I=1+k_q\tau_0C_M=1+K_{din}C_M \qquad (1)$$

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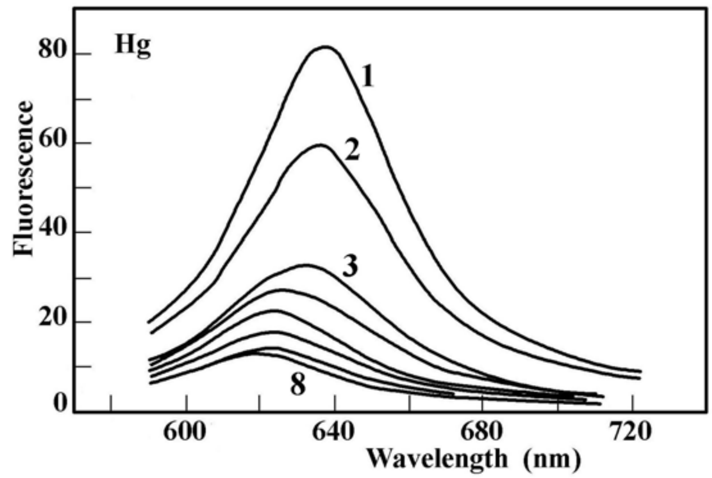

Figure 1 Fluorescence spectra of C-PC (0.4 μM) in the presence of different concentration of Hg(II) ions: 1→8[Hg] = 0, 1, 2, 3, 4, 5, 6, 7 μM

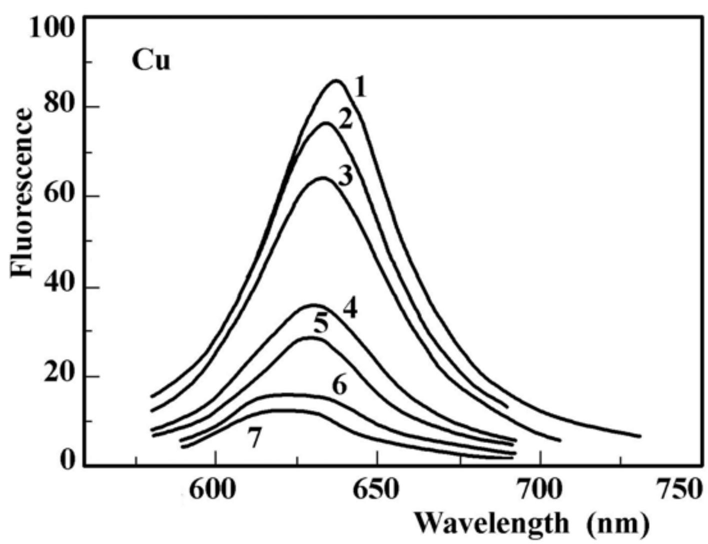

Figure 2 Fluorescence spectra of C-PC (0.4 μM) in the presence of different concentration of Cu(II) ions: 1→7[Cu] = 0, 1, 8, 26, 50, 74, 98 μM

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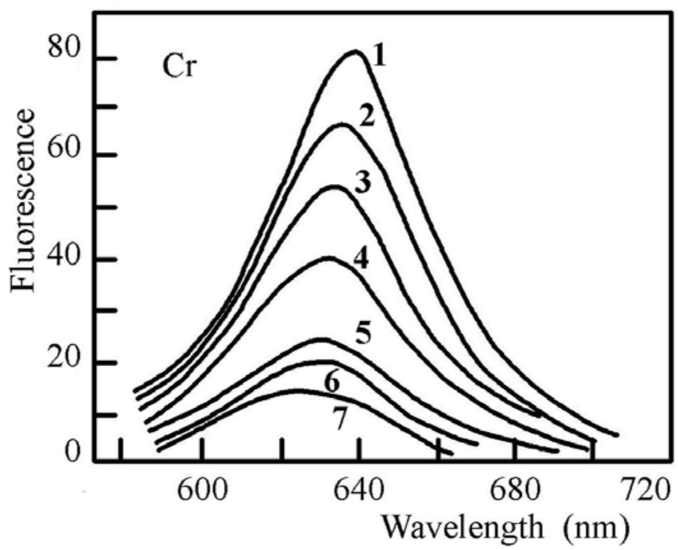

Figure 3 Fluorescence spectra of C-PC (0.4 μM) in the presence of different concentration of Cr(III) ions: 1→7[Cr] = 0, 3, 9, 21, 49, 69 85 μM

where I_0 and I are the intensities of the fluorescence in the absence and presence of the quencher, respectively; τ_0 -is fluorescence quenching time in the absence of the quencher; k_q – bimolecular constant of quenching velocity; C_M – concentration of quencher and $K_{din}= k_q\tau_0$ - is quenching constant of Stern-Volmer. Eq. (1) shows dynamic, collisional mechanism of fluorescence quenching. At this time excited-state fluorophore comes into collision with the ground-state quencher. As a result, a transient complex is formed. The excited-state complex undergoes dissociation due to radiation or radiationless spectral transitions and as a result both the fluorophore and the quencher pass into the ground-state energetic levels. Since the discussed complex is to be formed in excited state in lifetime period of fluorophore, dynamic or collisional quenching mechanism has a diffusive character [10]. In case of the second mechanism of fluorescence quenching, static quenching, the ground-state fluorophore and ground-state quencher form a ground-state stable complex. Spectral properties of this complex will necessarily be different from those before the complex formation. Again in case of static quenching mechanism the Stern-Volmer equation gives linear dependence between the fluorescence intensities ratio and quencher metal concentrations. In this case the equation has the form:

$$I_0/I=1+k_{St}\tau_0C_M=1+K_{St}C_M \qquad (2)$$

where K_{St} – is the equilibrium formation constant between the fluorophore and quencher. Other variables have the same meaning as in case of Eq. (1).

Generally, a linear relationship between the Stern-Volmer co-ordinates indicates that there is only one type fluorophore in the solution, equally accessible for the quencher. If there are two fluorophores and one of them is inaccessible for the quencher, then the relationship between the fluorescence intensities ratio and the concentration of the metal ions, instead of straight line becomes curvilinear. The curvature is directed towards x-axis. In case of simultaneous acting of the both mechanisms of quenching, dynamic and static, the Stern-Volmer plot is curved towards y-axis. Fig. 4, 5, 6 show the

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dependences plotted in the Stern-Volmer co-ordinates giving a quantitative picture of C-PC fluorescence quenching at various concentrations of metals Hg, Cu and Cr, respectively.

As one can see the plots are linear, there is no curvature towards any direction. According the above discussion fluorescence quencher metals interact with only one fluorophore in C-PC. Fig. 1, 2 and 3 show, that quenching was not saturated even at high concentrations of Cu(II), Hg(II) and Cr(III). Lack of total saturation in the quenching plot indicates that only a fraction of the binding sites quench the fluorescence. The quenching plots prove that fluorescence C-PC quenching by metals Hg, Cu and Cr, we have investigated, takes place due to one mechanism only. To state exactly whether this mechanism is dynamic or static some more experiments are needed.

A blue shift of up to 12 nm was measured for Hg-ions, when this shift was only about 4 nm for Cu-ions. Addition of chromium ion to C-PC water solution causes shift of spectral band towards the short wavelengths by about 10 nm. Blue shifts in the fluorescence spectra were observed when Hg(II), Cu(II) or Cr(III) binds to C-PC.

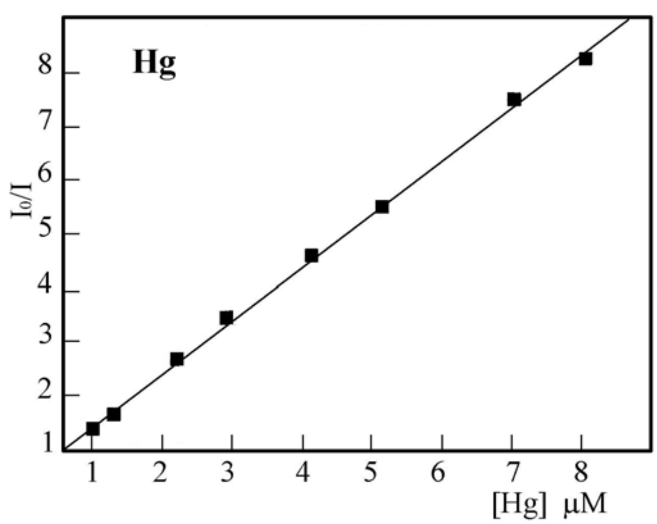

Figure 4 Stern-Volmer dependence for Hg metal ions

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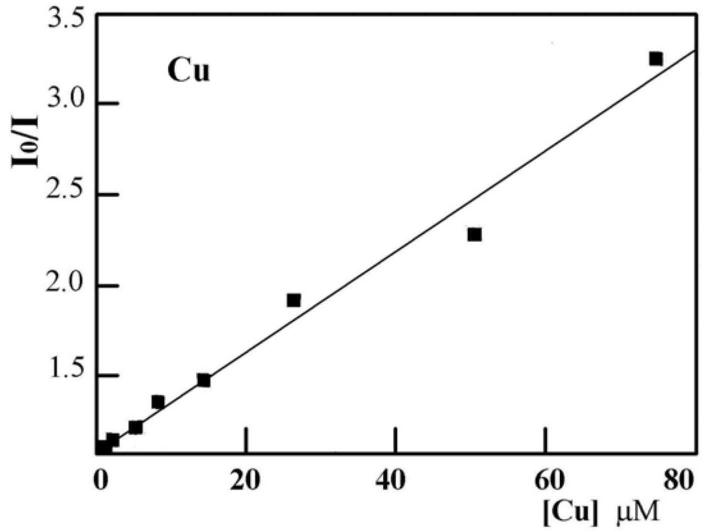

Figure 5 Stern-Volmer dependence for Cu metal ions

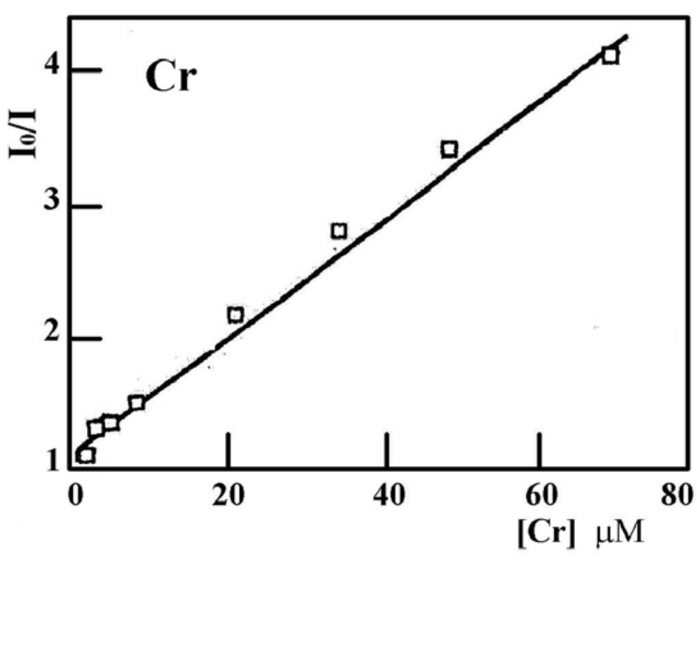

Figure 6 Stern-Volmer dependence for Cr metal ions

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Stern-Volmer quenching constants obtained from the linear quenching plots (1), in Fig. 4. 5 and 6, strongly depended on the metal ions. A high quenching constant of
$1.0x10^6$ M^{-1} for Hg(II) ions and low values of $3.1x10^4$ M^{-1} and $4.5x10^4$ M^{-1} for Cu(II) and Cr(III) ions respectively were estimated from this data.

4. Conclusion

The C-PC fluorescence spectrum includes intensive spectral peak at 638 nm. When metal solutions of various concentrations are added to the phycocyanin, maxima of these band shift towards the short wavelengths. Magnitudes of these shifts depend on metal types and concentrations.

The fluorescence of C-phycocyanin is quenching gradually when interacting with metals Cu(II), Hg(II) and Cr(III). Quenching intensity depends upon the metal concentration. This dependence in Stern-Volmer co-ordinates is linear. Stern-Volmer quenching constants for metals Cu(II), Hg(II) and Cr(III) has been estimated with the aid of these plots. It has been stated that the quenching occurs by only one mechanism and the metals interact with only one fluorophore of the C-phycocyanin.

References

1. V. Dushenkov, P.B.A.N. Kumar, H. Moro, and I. Raskin,
   Environ. Sci. Technol, (1995) **29,** 1239.
3. S.L. Brown, R.L. Chaney, J.S. Angle, and A.J.M. Baker,
   Environ. Sci. Technol. (1995), **29,** 1581.
4. B. Volesky and I. Prasetyo, Biotechnol. Bioeng. (1994), **43,** 1010.
   P.J. Jackson, W.L. Anderson, J.G. DeWitt, H.Y. Ke, C.R. Kuske,
   R.M. Moncrief, and G.D. Rayson, (1993), In Vitro Cell, Dev, Biol. **29P,** 220.
5. C.D. Scott, Biotechnol. Bioeng. (1992), **39,** 1064.
6. A. Nakajima and T. Sakaguchi, Biomass, (1990), **21,** 55,
7. M. Shengjun and J.A. Holcombe, (1990), Anal. Chem. **62,** 1994.
8. A.A. Krasnovsky and Erokhina, Doklady AN SSSR, , (1970) , **193,** 6.
9. S. Gotoshia Bulletin of the Georgian Academy of Sciences, 1999, **159**, 241.
10. Joseph R. Lakowicz, Principles of Fluorescence Spectroscopy
    Moscow, "Mir", 1986, pp. 262-279.

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