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A N I N T E R N A T I O N A L J O U R N A L

Effect of Hydrostatic Pressure on the Fluorescence of Tryptophan in the Presence of Metal Ions Running title: Effect of pressure on the tryptophan

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ABSTRACT

The effect of pressure on the fluorescence of tryptophan in the presence of metal ions was studied by fluorescence spectrometry. It was found that at 60 MPa, the fluorescence intensity of M/Trp mixtures (M represented metal ions) increased compared to that at atmosphere pressure. The relative fluorescence efficiency of M/Trp mixtures increased with pressure. When the M/Trp ratio was above 10:1, the relative fluorescence efficiency in decreasing order was Cu2+/Trp mixtures, Ni2+/Trp mixtures and Mg2+ (K+)/Trp mixtures. When the ratio was below 10:1, the decreasing order was Cu2+/Trp mixtures and Ni2+ (Mg2+, K+)/Trp mixtures. The relative fluorescence efficiency increased with the concentration of Cu2+ and Ni2+. The variation was relate to the quenching of tryptophan fluorescence in the presence of metal ions. A red shift was also observed, but the red shift was independent of metal ions.

Keywords: hydrostatic pressure, tryptophan, fluorescence, metal ions

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INTRODUCTION

In extreme environments such as hydrothermal vent, spectacular biological communities were discovered[1-2]. These organisms were exposed to high hydrostatic pressure (HHP) and the multiple effects of HHP on microbial physiology had been described[3-4]. Many researches showed that the organisms retrieved from the depths of 2000-3000 m or more didn't allow their survival under normal pressure[5-7]. As one of the most important biomolecular, the structure and interactions of protein under HHP had been widely studied[8-10]. In previous researches, tryptophan was the main intrinsic fluorophore as it was very sensitive to changes of physicochemical environment[11-14]. It had been widely used as a probe for investigation of protein structure and protein dynamics. A basic assumption for the spectral change of protein under HHP was that it was caused by the effect of pressure on protein, rather than the direct effect of pressure on tryptophan residues. But in fact, the effect of high pressure on intrinsic tryptophan fluorescence must be taken into consideration. Ruan et al studied the effect of pressure on pure tryptophan[15-16].

For living organisms, metal ions play important roles in the transport of message, folding and unfolding of protein[17-18]. Although many researches had been studied the effects of pressure on protein in the presence of some ions, the effect of pressure on tryptophan in the presence of metal ions were not be studied[19-20]. This should also be taken into consideration to explain the protein fluorescence variations as a function of pressure.

Therefore, in this article, we studied the fluorescence of tryptophan in the presence of different metal ions under pressures. The effect of the concentration of metal ions on tryptophan under high pressure was also studied.

MATERIAL AND METHODS

Tryptophan was purchased from Sigma Company. All solutions and Tris-HCl buffer were prepared in deionized water. The concentration of tryptophan was 2×10^{-5} M in 0.05 M Tris-HCl buffer. The solutions of metal ions were prepared from their chlorides. All the reagents were of analytical reagent grade.

The fluorescence spectra of tryptophan were measured using Hitachi F-2500 spectrometer equipped with an optical high pressure cell[21]. The excitation wavelength was 287 nm. All the spectra were recorded at room temperature. The samples were incubated for more than 15 mins. The shift of tryptophan fluorescence spectra was calculated using the center of spectral mass(CSM)[22,23]. The CSM was defined by the equation(1):

$$(vg) = \sum vi * Fi / \sum Fi \quad (1)$$

where *Fi* stands for the fluorescence intensity at wavenumber *vi*. The CSM was calculated from the wavelength of 300 nm to 450 nm.

RESULTS AND DISCUSSION

Effect of Pressure on Tryptophan Fluorescence in the Presence of Metal Ions

The fluorescence spectra of tryptophan in the presence of Mg^{2+} and Ni^{2+} for the 40:1 M/Trp ratio under atmosphere pressure and 60 MPa were presented in Figure. 1. It showed that at 60 MPa, the fluorescence intensity increased compare to that at

atmosphere pressure. But the effect of pressure on the fluorescence intensity was different for Mg^{2+}/Trp mixtures and Ni^{2+}/Trp mixtures. These results were also found for Cu^{2+}/Trp mixtures and K^{+}/Trp mixtures. Figure.2 showed the effect of pressure on the relative fluorescence efficiency of M/Trp mixtures. The relative fluorescence efficiency of M/Trp mixtures were increased with pressure. The value of the relative fluorescence efficiency in decreasing order was Cu^{2+}/Trp mixtures, Ni^{2+}/Trp mixtures and Mg^{2+} (K^{+})/Trp mixtures when M/Trp ratio was above 10:1. And the decreasing order was changed (Cu^{2+}/Trp mixtures > Ni^{2+} (Mg^{2+}, K^{+})/Trp mixtures) when M/Trp ratio was lower to 10:1.

A red shift of the spectra was also found for M/Trp mixtures. The red shift of the fluorescence spectra for M/Trp mixtures when pressure increased from atmosphere pressure to 60 MPa were shown in Table. 1. The fluorescence spectra of different mixtures shifted to red for about 73cm^{-1}. And there was almost no obvious difference for different M/Trp mixtures.

Table 1. The red shift of tryptophan in the presence of metal ions when pressure increased to 60 MPa.

Pressure (MPa)	K^{+}/Trp mixtures (cm^{-1})	Mg^{2+}/Trp mixtures (cm^{-1})	Ni^{2+}/Trp mixtures (cm^{-1})	Cu^{2+}/Trp mixtures (cm^{-1})
0	277.725	277.737	277.720	277.866
60	277.005	276.984	277.047	277.166
Red shift	72	75	77	70

Effect of the Concentration of Metal Ions on Tryptophan Fluorescence Under High Pressure

When pressure increased to 60 MPa, the relative fluorescence efficiency of M/Trp mixtures for different ratios was presented in Figure. 3. The results showed that the relative fluorescence efficiency increased with the concentration of copper ions for Cu^{2+}/Trp mixtures. And when Cu^{2+}/Trp ratio exceed 5:1, the increase was declined. For Ni^{2+}/Trp mixtures, there was almost no changes for the 2:1, 5:1 and 10:1 Ni^{2+}/Trp ratios, but obviously increased for 20:1 and 40:1 Ni^{2+}/Trp ratios. For Mg^{2+}/Trp mixtures and K^{+}/Trp mixtures, the relative efficiency was not influenced by the concentration of Mg^{2+} and K^{+}. It was found that the variation was in line with the Stern-Volmer plots (Fig. 4). Figure. 4 showed that Cu^{2+} and Ni^{2+} were the quencher of tryptophan, and the quenching was appeared for the 2:1 and 10:1 M/Trp ratios respectively. The fluorescence spectra of M/Trp mixtures shifted to red for about 73 cm^{-1}. But the red shift was not influenced by the concentration of metal ions.

As the main intrinsic fluorophore of protein, tryptophan was widely used as a probe in high pressure researches. Although the fluorescence of pure tryptophan under high pressure had been reported, the effect of pressure on tryptophan in the presence of metal ions was not mentioned. The results described above showed that the fluorescence efficiency of M/Trp mixtures increased with pressure. According to the theory forwarded by Ruan[15], the increase of fluorescence efficiency was contributed to the ionization states of $–NH_2$ and –COOH groups in solutions. Pressure promoted the transition of the groups from low quantum yield form to high quantum yield form. At the same time, the polarity of water increased with pressure. If the increase in quantum yield caused by the transition of the ionization state was higher than the

decrease caused by the polarity change in water, the relative fluorescence efficiency increased. It was also suitable to explain the fluorescence efficiency of M/Trp mixtures under high pressure.

An interesting result was that the relative fluorescence efficiency influenced different metal ions and also influenced by the concentration of metal ions. The variation of relative fluorescence efficiency was associated with the quenching of tryptophan fluorescence in the presence of metal ions. This could explained by the binding of metal ions with tryptophan. It was generally known that the quenching of tryptophan fluorescence due to the binding of the Cu^{2+} and Ni^{2+} [13,24]. They formed complex and decreased the fluorescence intensity of tryptophan. But the complex might be not stable under high pressure. When pressure increased, the complex dissociated and the relative fluorescence efficiency increased. It could be proved by calculating the binding constant of metal ions through fluorescence intensity. Equilibrium between free and bound molecules was given by Equation(2) [25,26].

$$\log(F_0\text{-}F)/F = \log Ka + n \log[Q] \qquad (2)$$

where F_0 and F are the fluorescence intensity before and after the addition of quencher. Ka is the binding constant and n is the number of binding site. Values of Ka can thereby be determined from the intercept by plotting $\log(F_0\text{-}F)/F$ versus $\log[Q]$.

As can be seen from Table 2, the value of Ka decreased for Cu^{2+} (Ni^{2+})/ mixtures when pressure increased to 60 MPa. This indicated that the stability of M/Trp complex declined when pressure increased. With the adding of Cu^{2+} and Ni^{2+}, more complex formed, and more complex dissociated when pressure increased. This explained the result that the relatively fluorescence efficiency increased with the concentration of Cu^{2+} and Ni^{2+}, but didn't increased with the concentration of Mg^{2+}/ and K^{+}. This also explained the effect of pressure on the fluorescence intensity was different for Mg^{2+}/Trp mixtures and Ni^{2+}/Trp mixtures. The results also showed that the relative fluorescence efficiency of Cu^{2+}/Trp mixtures higher than Ni^{2+}/mixtures when pressure increased. This was contribute to the value of Ka of Cu^{2+} /Trp mixtures was much higher than Ni^{2+}/Trp mixtures(Table 2). When pressure increased, the decrease of Ka for Cu^{2+} /Trp mixtures was higher than the decrease of Ka for Ni^{2+}/Trp mixtures.

Table 2. The binding constant of M/Trp mixtures.(Ka represented binding constant, R^2 represented correlation coefficent)

Metal ions	Pressure (MPa)	Ka ($\times 10^3\ M^{-1}$)	R^2
Ni^{2+}	0.1	3.991	0.996
	60	2.765	0.966
Cu^{2+}	0.1	3489	0.987
	60	2781	0.986

For different M/Trp mixtures, a red shift was observed when pressure increased. But the red shift was not influenced by metal ions. According to the theory forwarded by Lippert, an increase in dielectric constant should result in an increase in Stokes

shift[15,16]. The dielectric constant of water at atmosphere pressure was about 89.When pressure increased to 60 MPa, the dielectric constant of water was about 92. At the same time, the increase of pressure enhanced the non-specific interations between water and tryptophan, and the specific interactions between the fluorophore and solvent molecules[27]. All these would cause the red shift of tryptophan under high pressure.

CONCLUSIONS

The effect of pressure on the fluorescence of tryptophan in the presence of four metal ions was studied. The results showed the relative fluorescence efficiency of M/Trp mixtures increased with pressure. It was also found that the relative fluorescence efficiency was influenced by metal ions and the ratio of M/Trp. The interesting result was that the variation of M/Trp mixtures fluorescence under pressures was relate to the quenching of tryptophan fluorescence in the presence of metal ions. A red shift was also found for the fluorescence spectra of M/Trp mixtures and it was irrelevant with metal ions.

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