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Spectral study on the unique enhanced fluorescence of guanosine triphosphate by zinc ions

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ABSTRACT

Binding effect of guanosine triphosphate (GTP) with metal ions is involved in many biologically important processes, and so its investigation has been one interesting research focus for many chemical and biochemical research groups. In this contribution, we presented the unique fluorescence recovery and enhancement of GTP induced by Zn(II) based on the spectrofluorometric method. When excited at 280 nm, GTP is hardly fluorescent at the alkaline condition. However, the presence of Zn(II) caused an obvious fluorescence emission of GTP at 346 nm, and the binding molar ratio between GTP and Zn(II) had been proved to be 1. The investigations of binding property of various nucleotides with metal ions demonstrated that this fluorescence recovery and enhancement of GTP with Zn(II) was highly specific, which could successfully discriminate GTP from other structurally similar nucleotides including GDP and GMP. Furthermore, similar fluorescence response of the bacterial alarmone ppGpp to Zn(II) had also been identified.

1. Introduction

Among various biologically important phosphate anions, guanosine triphosphate (GTP), a multifunctional nucleotide containing three negatively charged phosphate groups, has attracted much more attention in recent years due to its crucial biological functions. As one fundamental unit for all the life forms, GTP plays a key role in many major biological processes. Firstly, it can be used as the universal source of chemical energy for living organism [1]. Secondly, GTP takes part in many important processes such as citric acid cycle, transduction of cellular signal, process of eukaryotic translation termination in eukaryotic cells as well as activation control of enzymes through regulating the activity of GTP-binding proteins [1–4]. Additionally, GTP is essential for the synthesis of many paramountly important substances including RNA and the intercellular messenger (cGMP) [5,6]. Furthermore, the change of GTP concentrations in human erythrocytes can express different pathological states [7–12].

Actually, the achievement of most of these above biological processes needs the participation of metal ions such as Zn(II), Ni(II) or Mg(II) [13]. For example, metal ions are necessary for the reactions involving the most investigated activity center called G-proteins, which utilize GTP in such biological processes as cellular signaling, protein synthesis, vesicular trafficking and synaptic fusion [1,14–17]. When serving as substrate for DNA and RNA polymerases, GTP also has to be 'present as complexes of divalent metal ions. Besides, the formation of an active hydrogenase requires the hydrolysis of GTP and the binding of nickel [18]. Therefore, investigation about the binding effect between GTP and metal ions is crucial for the further understanding of relevant biological processes, which has been one interesting research focus for many chemical and biochemical research groups.

Up to now, the binding property of nucleotides with metal ions have been reported based on the theoretical studies [19–23] and experimental investigations involving in potentiometric pH titration [13,24–27], X-ray crystallographic examination [28] and other techniques [29]. However, there are still less reports in terms of spectrofluorometry despite its advantages of high selectivity, easy performance and simplicity. In this contribution, we presented the unique binding property of GTP with Zn(II) on the basis of fluorescence measurements at alkaline condition. As one strong Lewis acid with rather flexible coordination number between 4 and 6 [30], the post-transition Zn(II) ion has been one important binding center for several biological anions and plays a key role in the structure and function of nucleic acids [31,32]. Therefore, study of the binding property of GTP with zinc ions is beneficial to appreciate the role of zinc complexes of nucleotides in various biological reactions. Herein, fluorescence

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measurement shows that GTP is hardly fluorescent when excited at 280 nm. The presence of zinc ions, however, caused the fluorescence emission of GTP recovered and enhanced in a large degree. The fluorescent recovery and enhancement of GTP induced by Zn(II) was highly specific, leading to the successful discrimination of GTP from other structurally similar phosphates including GDP and GMP. Compared with the spectrofluorometry based on organic artificial sensors, this fluorescence recognition for GTP avoided the process of complicated chemical synthesis, and so could be absent of the disadvantages including considerable synthetic effect, poor solubility or bad selectivity which may limit their further biological applications.

2. Experimental

2.1. Materials and apparatus

Guanosine 5′-triphosphate sodium salt (GTP), guanosine diphosphate (GDP), guanosine monophosphate (GMP), adenosine 5′-triphosphate sodium salt (ATP), cytosine 5′-triphosphate sodium salt (CTP) and uracil 5′-triphosphate sodium salt (UTP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Guanosine 3′-diphosphate-5′-diphosphate (ppGpp) was commercially purchased from Trinlink (USA). Stock solutions of GTP, GDP, GMP, ATP, CTP, UTP, ppGpp, PPi and Pi were prepared by dissolving them respectively in water and diluted to the final concentration of 3.0×10^{-4} M. All the nucleotide solutions were stored in refrigerator at 4 °C and used within one month.

Zinc nitrate solution of 3.0×10^{-4} M was obtained by dissolving definite commercial product (Xiangzhong Geology Institute, Hunan, China) in 100 mL of distilled water. 50 mM borate buffer solution was used to adjust the acidity of the solutions. All reagents were of analytical grade and used without further purification. Milli-Q purified water (18.2 MΩ) was used throughout.

Absorption and fluorescence measurements were made with a U-3010 spectrophotometer and an F-2500 spectrofluorometer (Hitachi Ltd., Tokyo, Japan), respectively, by keeping the excitation wavelength at 280 nm. A QL-901 vortex mixer (Qilinbeier Instrument Manufacture Ltd., Haimen, China) was employed to blend the solutions. The pH values of the test solution were measured with a glass electrode connected to a pH 510 pH meter (made in Singapore). All experiments were carried out at room temperature.

2.2. Experimental procedures

To a 1.5 mL tube, solutions were added in the sequence of 100 μL 50 mM borate buffer solution, an appropriate aliquot of 0.3 mM $Zn(NO_3)_2$ solution, then various 0.3 mM anion solutions. Finally, relevant volume of water was added to make the final volume 1000 μL. The mixture was vortexed for about 5 s and transferred for fluorescence detection immediately. All Fluorescent spectra were excited at 280.0 nm and keeping the excitation and emission slit widths 5.0 nm, PMT Voltage 400 V and scan speed 3000 nm/min at room temperature.

3. Results and discussion

3.1. Spectral characteristic of GTP with zinc ions

GTP displays one absorption peak at 252 nm (Fig. 1(A)), which decreased with the addition of Zn(II). This spectral change stopped until the metal/ligand ratio reached 1. Curve-fitting analysis of the absorbance intensity at 252 nm gave a 1:1 binding model between

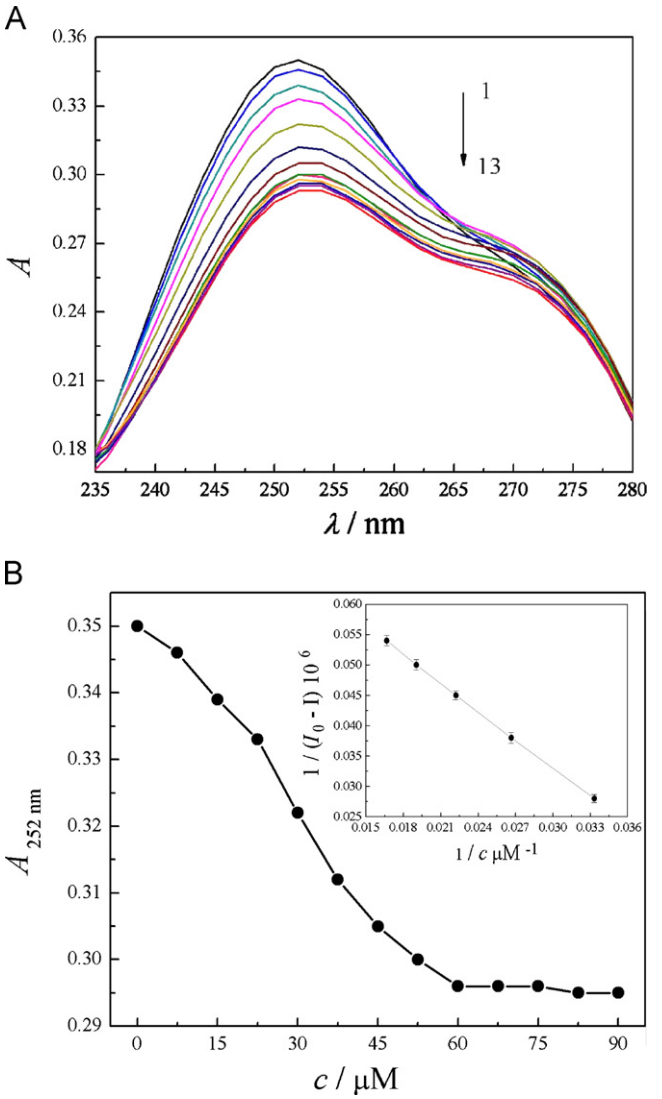

Fig. 1. Absorption decrease of GTP induced by Zn(II). (A) Absorption spectra of GTP upon the addition of Zn(II); (B) A dynamic response of the absorption intensity at 252 nm against the molar ratios of Zn(II) and GTP. Inset shows Benesi–Hildebrand fit for absorption decrease of GTP by Zn(II). Concentrations: GTP, 60 μM; Zn(II): 0, 7.5, 15, 22.5, 30, 37.5, 45, 52.5, 60, 67.5, 75, 82.5, 90 μM (1–13); 50 mM boric acid buffer, pH 10.0. λ_{ex}, 280 nm.

GTP and Zn(II) for the newly formed species (Fig. 1(B)). Additionally, the Benesi–Hildebrand analysis of the absorption data also gave a 1:1 stoichiometry for the zinc complex of GTP (inset of Fig. 1(B)), with an association constant (K_{ass}) of $5.56\times10^4\ M^{-1}$.

The formation of zinc complex of GTP was further investigated by fluorescence examination. Fluorescence titration of GTP with Zn(II) shows that GTP was hardly fluorescent at the alkaline condition when excited at 280 nm, while the presence of Zn(II) induced an obvious recovery and enhancement of fluorescence emission of GTP at 346 nm (Fig. 2(A)). Meanwhile, the relative fluorescence increase at 346 nm shown by F/F_0 had a linear relationship with Zn(II), which could be expressed as $F/F_0=-2.38+2.67c$ (μM, $n=5$) with a correlation coefficient of 0.9946 in a range of 7.5–37.5 μM (Fig. 2(B)). Just as the results from absorption examination, fluorescence emission of GTP remained unchanged when the metal/ligand ratio reached 1, demonstrating a 1:1 binding molar ratio between GTP and Zn(II). To confirm the molar ratio of zinc to GTP, we kept the total concentration unchanged while changing the concentration of both Zn(II) and GTP simultaneously. It can be seen

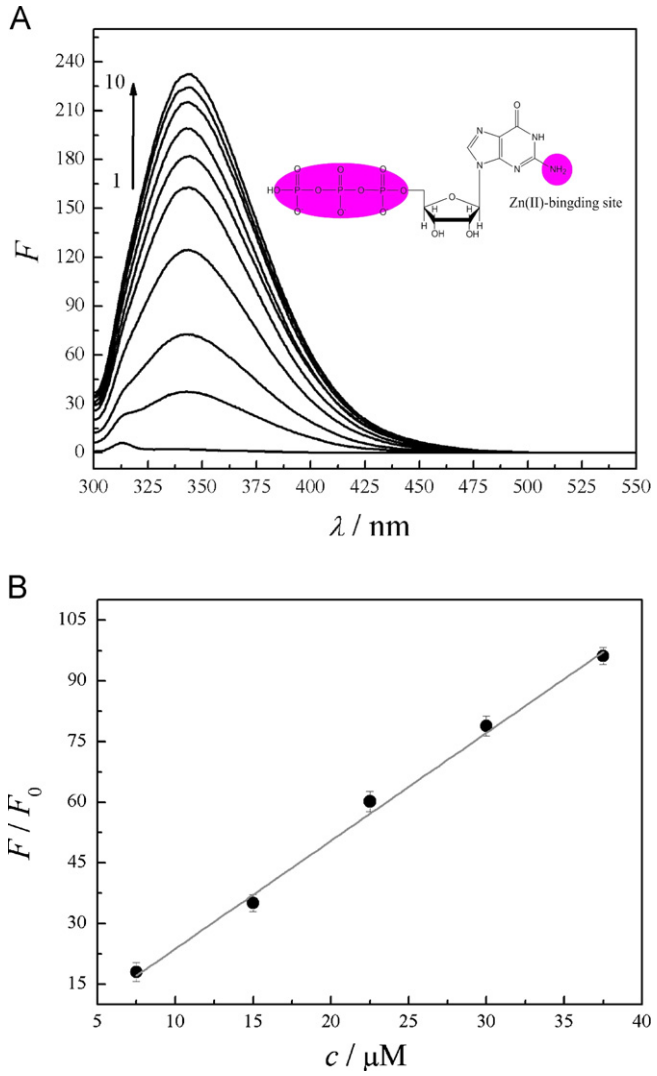

Fig. 2. Zn(II) induced enhancement of the fluorescence of GTP. (A) Fluorescence spectra of GTP with the increasing concentrations of Zn(II). Inset is the chemical structure of GTP and proposed binding site of GTP with Zn(II). (B) The linear relationship of F/F_0 at 346 nm as a function of $c_{Zn(II)}$. Concentrations: GTP, 60 μM; Zn(II): 0, 7.5, 15, 22.5, 30, 37.5, 45, 52.5, 60, 67.5 μM (1–10); 50 mM boric acid buffer, pH 10.0. λ_{ex}, 280 nm.

from Supplementary Fig. 1 that the molar ratio of zinc to GTP is 1:1, which is identical with the result given in Fig. 2(B).

Due to the accessible nitrogen and oxygen lone pairs, guanine base acting as a metal ligand is possible [33]. It is well established that the metal ions often bind to the nitrogenous sites of nucleobases, and the possible metal binding sites for guanine base are N^2 and N^7 [19–21,23,33,34]. With a view to make certain binding mechanism for such fluorescence enhancement of GTP induced by zinc ions, we further examined the fluorescence spectrum of inosine triphosphate (ITP) with Zn(II), the N^2 of which has been deleted. Just the same as GTP, ITP was hardly fluorescent at the same condition (Supplementary Fig. 2). When added with Zn(II), however, there was no fluorescence newly appeared, which was different from the situation of GTP. That is to say, the N^2 site of guanine base appeared to be crucial for the binding effect between GTP and zinc ions, which resulting in the distinct fluorescence response of GTP and ITP to Zn(II). Here, the binding of zinc ions to the N^2 site of guanine base could be ascribed to the attractive 3d electron–lone pair interaction [20,21,35]. Meanwhile, zinc ions could also interact electrostatically with the negatively charged phosphate groups [36,37]. Accordingly, the proposed binding sites of GTP with Zn(II) were the N^2 site of guanine base and the hydroxyl oxygen atoms of phosphate (inset of Fig. 2(A)). Based on the above result, it is potential for other derivative of GTP, such as guanosine 3′-diphosphate- 5′-di(tri)-phosphate [(p)ppGpp], to display a similar fluorescence response with zinc ions to GTP.

It has been well established in the literature that photoinduced electron transfer (PET) process can be occurred from the N-donors to the π-system of fluorophores upon excitation, which is the reason for the quenching of fluorescence [38–40]. GTP was potential to undergo a PET process due to the existence of lone pairs in exocyclic N^2 site and π-system of guanine, which resulted in the no fluorescence of GTP. During involvement of the same lone pairs of N^2 site in forming bonds to metal ions, the PET quenching effect could be lessened, leading to the chelation enhanced fluorescence (CHEF) effect [38]. Therefore, the binding of N^2 site of guanine with Zn(II) caused the suppression of PET process of guanine base, and then finally induced the fluorescence recovery and enhancement of GTP due to the CHEF effect. On the other hand, it has been reported that Zn(II) is photophysically inactive because of its completely filled d^{10} electronic configuration, and so it does not display any one-electron redox activity and cannot be involved in any electron transfer or electronic energy transfer process [39,41]. Consequently, Zn(II) can exert an indirect effect on the emitting activity of a proximate fluorophore, which can stop the electron transfer process of ligands and lead to the regeneration or enhancement of fluorescence [41]. Therefore, the fluorescence recovery and enhancement of GTP could be ascribed to the CHEF effect induced by Zn(II), which depending on the PET process of guanine.

To gain an insight into the mechanism of this unique binding property of GTP with zinc ions, we treated this zinc complex with metal chelator of EDTA. As shown in Supplementary Fig. 3, the obvious fluorescence emission of zinc complex of GTP gradually decreased with the addition of EDTA and finally disappeared, testifying the coordination effect between GTP and Zn(II).

3.2. Effect of pH on the fluorescence property of GTP with Zn(II)

Firstly, we investigated the effect of pH on the fluorescence emission of GTP and found that, except for metal coordination, the fluorescence of GTP was also strongly affected by pH condition. When pH was less than 4.15, the fluorescence spectrum of GTP displayed one obvious emission (Fig. 3(A)). Once pH was larger than 5.1, however, GTP was hardly fluorescent. The comparison of fluorescence emission in different pH conditions illuminated that the protonation process of GTP at acidic conditions gave rise to the recovery of fluorescence emission of fluorophore. It is well known that the proton, just as Zn(II), can prevent the fluorescence quenching based on electron transfer involving the lone pair of an amine group [39]. Hence, the large increase of fluorescence of GTP at acidic conditions could be explained in terms of the suppression of PET process of guanine base by proton, which leading to the CHEF effect as metal binding.

Next, the effect of pH on the binding of GTP with Zn(II) has also been examined during the pH range of 7.9–11.3. Fig. 3(B) shows the changes of emission intensity of GTP at 346 nm before and after the addition of Zn(II) with different pH values. Results demonstrated that the fluorescence of GTP kept unchanged in the pH range of 7.9–11.3, while the addition of Zn(II) into GTP caused a gradual increase in 7.9–9.8 and kept a relative stabilization in 9.8–10.5. Accordingly, the binding interaction between GTP and Zn(II) should be easier at the alkaline condition because the binding site of guanine base was easier to be accessed upon the deprotonation process [33].

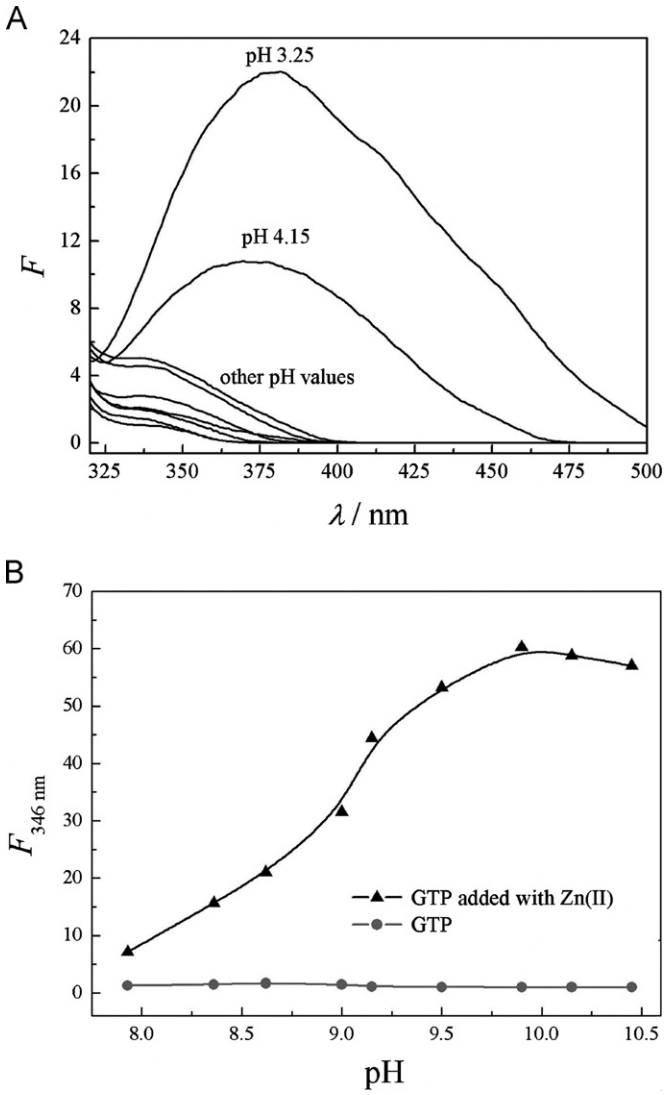

Fig. 3. Effects of pH on the fluorescence emission of GTP with Zn(II). (A) Fluorescence spectra of GTP with different pH values. (B) Intensity of fluorescence emission of GTP at 346 nm before and after the addition of Zn(II) in different pH condition. Concentrations: Zn(II) ion, 30μM; GTP, 30μM. λ_{ex}, 280 nm.

3.3. Spectral studies of GTP with various metal ions

To further understand the coordination effect of GTP with zinc ions, we studied the binding property of GTP with other metal ions by fluorescence examination. The relative fluorescence increase of GTP at 346 nm (F/F_0) shows that Cd(II) can cause a definite fluorescence enhancement of GTP, but it is much less than that induced by Zn(II) (Fig. 4). This fluorescence increase of GTP induced by Cd(II) might be related to the same fulfilled d^{10} configuration of Cd(II), just as Zn(II) ions, which restrained the PET process of guanine base and led to the CHEF effect. However, other metal cations including the alkali and alkaline-earth metal such as K(I), Li(I), Mg(II), Ca(II) etc., and the heavy metal such as Pb(II), Mn(II), Cu(II) and Hg(II) etc. could not cause any fluorescence increase of GTP.

3.4. Binding properties of various nucleotides with zinc ions

Fluorescence spectra of other nucleotides with zinc ions were measured to testify the unique fluorescence property of GTP with Zn(II). As shown in Fig. 5(A), Zn(II) can induce the fluorescence

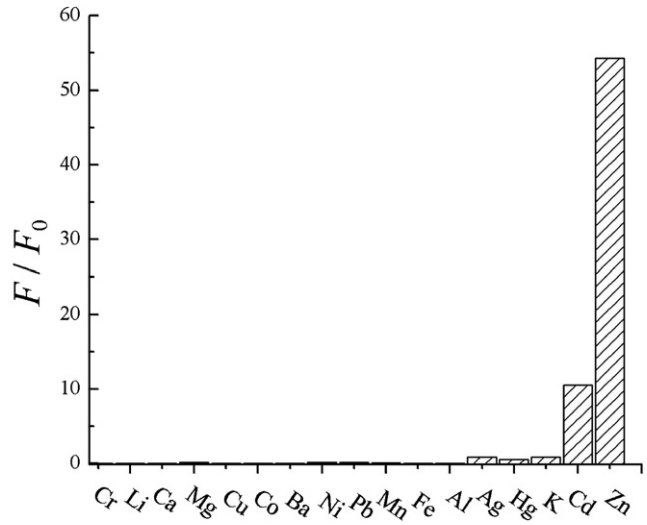

Fig. 4. A comparison of F/F_0 by adding different metal ions into the solution of GTP. Concentrations: GTP, 30 μM, metal ions, 30 μM. λ_{em}, 346 nm.

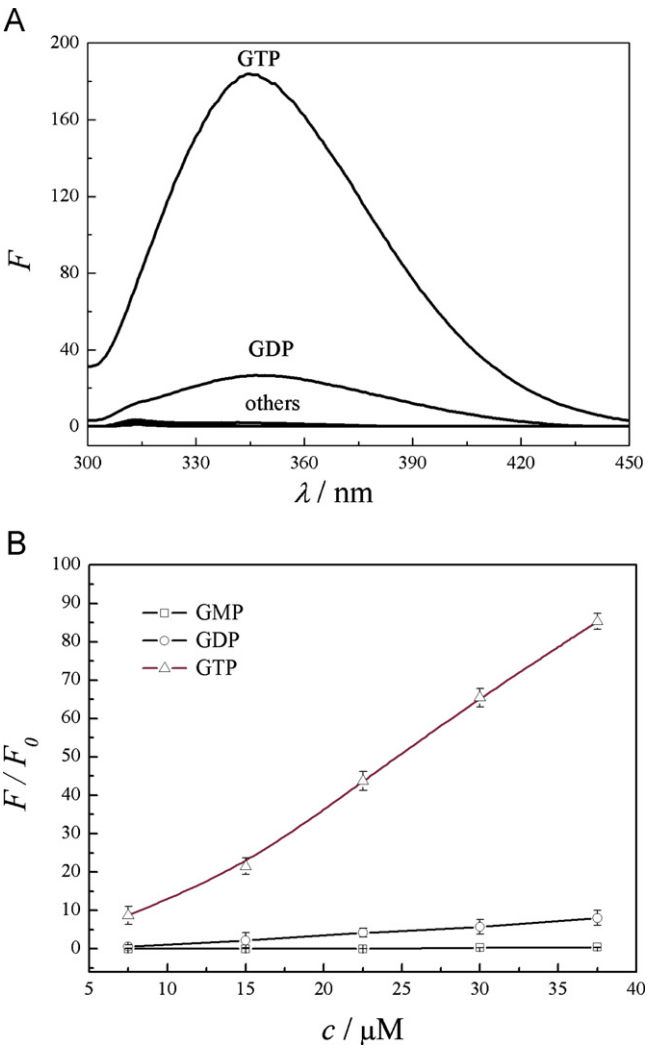

Fig. 5. Unique fluorescence response of GTP with Zn(II). (A) Fluorescence spectra of various nucleotides in the presence of 60 μmol L^{-1} Zn(II) (others including GMP, ATP, CTP and UTP). (B) Concentration-dependent F/F_0 with the addition of Zn(II) into 60 μM GMP, GDP and GTP. λ_{em}, 346 nm.

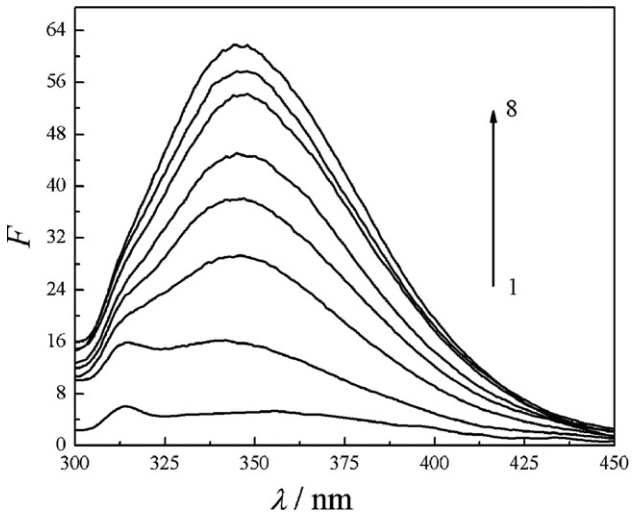

Fig. 6. Fluorescence enhancement of ppGpp induced by Zn(II). Concentrations: ppGpp, 105 μM; Zn(II), 0, 15, 30, 45, 60, 75, 90 and 105 μM (from 1 to 8).

emission of GTP and GDP newly appeared at 346 nm, which are different from other nucleotides including GMP, ATP, CTP and UTP. The degree of fluorescence increase of GTP and GDP with Zn(II) could be further demonstrated by comparing the relative fluorescence increase (F/F_0) at 346 nm (Fig. 5(B)). The concentration-dependent F/F_0 curves show that the fluorescence enhancement of GTP is much larger than that of GDP, which is larger enough to discriminate GTP from GDP. This could be interpreted by the stronger electrostatic interaction of GTP with the cationic Zn(II) center, leading to the higher affinity of GTP towards Zn(II). Therefore, the unique fluorescence response of GTP with Zn(II) has been proved, which could discriminate GTP from other structurally similar nucleotides successfully. Furthermore, the distinct response of other nucleotides with zinc ions was probably related to the different chemical structure of nucleic bases as well as the different binding effect between nucleotides and metal ions.

3.5. *Fluorescence response of stringent alarmone with zinc ions*

Considering the fluorescence emission of both GTP and GDP could be enhanced by Zn(II), we next investigated the fluorescence response of their derivative, the stringent alarmone {guanosine 3′-diphosphate-5′-di(tri)-phosphate [(p)ppGpp]}, with Zn(II) [42]. As a crucial global regulator of the gene expression in some bacteria and plants [42,43], (p)ppGpp, generated under condition of nutritional deprivation [44,45], influences many bacterial survival mechanisms [46]. Fluorescence examination shows that ppGpp is hardly fluorescent at the same condition, while the fluorescence of ppGpp could be largely recovered and enhanced when added with Zn(II) (Fig. 6). Because the negative charge in 5′-pyrophosphate of ppGpp was lower, the degree of fluorescence enhancement of ppGpp induced by Zn(II) was less than that of GTP. Based on the fluorescence increase induced by Zn(II), ppGpp could be easily detected at aqueous solution.

4. Conclusions

In summary, the unique fluorescence recovery and enhancement of GTP with Zn(II) have been studied by the spectrofluorometry method at the alkaline condition. This binding of GTP with Zn(II) displays extreme specificity, which effectively avoided the interference of other metal ions and nucleotides such as GDP, GMP and ATP. Consequently, the highly selective recognition of GTP could be established based on Zn(II), which is not having the disadvantages of organic artificial sensors such as complicated synthetic process, poor solubility or bad selectivity. Besides, the binding property of ppGpp with Zn(II) was similar to that of GTP, demonstrating that zinc ions has a potential to be one vital member of the fluorescent probes for ppGpp.

Acknowledgments

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2012.11.010.

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