Talanta 104 (2013) 198–203

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

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

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ARTICLE INFO

Article history:
Received 1 July 2012
Received in revised form
7 November 2012
Accepted 8 November 2012
Available online 17 November 2012

Keywords:
Guanosine triphosphate
Zinc ion
Guanosine 3′-diphosphate-5′-di(tri)-phosphate
Fluorescence

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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http://dx.doi.org/10.1016/j.talanta.2012.11.010

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

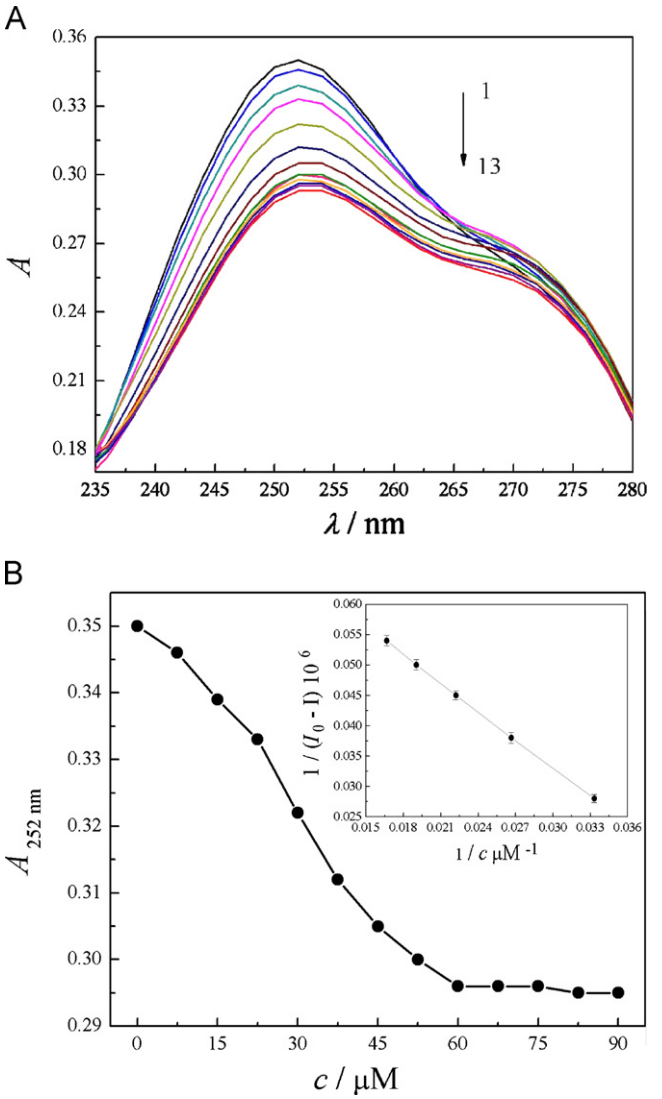

Fig. 1. Absorption decrease of GTP induced by Zn(II). (A) Absorption spectra of GTP upon the addition of Zn(II); (B) A dynamic response of the absorption intensity at 252 nm against the molar ratios of Zn(II) and GTP. Inset shows Benesi–Hildebrand fit for absorption decrease of GTP by Zn(II). Concentrations: 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 5.56×10^4 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