Poly (thymine)-Templated Formation of Copper Nanoparticles as Fluorescent Probe for Label-Free Detection of ATP

Wenping Zhu*, Xue Bai, Liyan Dai

School of Chemistry and Chemical Engineering, Zhoukou Normal University, Zhoukou 466001, China.

wenping315@163.com

Abstract

A simple, label-free fluorescent strategy for adenosine triphosphate (ATP) detection was developed using poly(thymine)-templated formation of copper nanoparticles (CuNPs) as fluorescent probe. In this method, poly(thymine) ssDNA (T40) can effectively template the formation of T40-CuNPs with excellent fluorescence. ATP is a good inhibitor of S1 nuclease that can protect T40 from the hydrolysis of S1 nuclease, which succeeds in the formation of the T40-CuNPs. The proposed method has a detection limit of 6 μM for ATP, which can be applied to the determination of samples in complex biological environments with satisfactory results.

Keywords

Copper Nanoparticles; ATP Detection; Poly (thymine); S1 Nuclease.

1. Introduction

Known as the major energy currency of the cell,adenosine triphosphate (ATP) plays an important role in regulation of cellular metabolism and biochemical pathways in cell physiology[1]. Moreover, ATP is also considered as an indicator of cell viability and cell damage[2], abnormal behaviour of ATP in biosystems always indicates disease, such as Parkinson's, chronic inflammatory conditions and several neurological and psychiatric disorders[3]. Therefore, the determination of ATP is essential in biochemical study as well as clinical diagnosis.

Traditional methods for ATP detection, such as luciferase-mediated bioluminescence and high performance liquid chromatography, require not only costly and unstable bioluminescence agents but also cumbersome sample preparation processes[4,5]. Moreover, several strategies have been developed for ATP detection based on synthetic host-guest receptors, peptides, conjugated polymers and ATP aptamer[6]. Although the aptamer-based strategies for the ATP assay have attracted much attention, these methods are still limited by expensive labelling, complex pretreatment process and time-consuming materials preparation[7]. Therefore, it is extremely valuable to develop a novel method for simple and sensitive detection of ATP with low cost.

poly(thymine) (poly-T) single-stranded DNA (ssDNA) can act as efficient template favoring the formation of fluorescent copper nanoparticles (CuNPs). The synthesis of CuNPs is highly efficient and can be accomplished within minutes, which holds great potential for signal transducing and has got wide applications in biochemical analysis[8]. In this study, a label-free fluorescent method for ATP detection was developed based on poly-T templated formation of CuNPs.

2. Experimental section

2.1 Chemicals and reagents

Oligonucleotide (T40) was synthesized and purified by Shanghai Sangon Biotech Co., Ltd. (Shanghai, China). ATP solution, sodium ascorbate, copper(II) sulfate ($CuSO_4$), S1 nuclease and goat serum were also obtained from Shanghai Sangon Biotech Co., Ltd. (Shanghai, China). Other reagents and chemicals were of analytical grade and used as received. The reaction buffer solutions employed in this work was S1 buffer (40 mM sodium acetate, pH 4.5, 0.3 M NaCl and 2 mM $ZnSO_4$).

2.2 Assay procedure

For a typical ATP detection experiment, 43 μL 0.1×S1 buffer, 2 μL ATP solution, and 5 μL S1 nuclease diluent were mixed, kept at room temperature for 15 min. Then, 3.8 μL of 40 μM T40 was added into the above mixture and incubated at 37°C for 30 min. Subsequently, 226.2 μL MOPS buffer, 10 μL of 70 mM sodium ascorbate, 10 μL of 30 mM copper(II) sulfate were added into the above mixture and incubated at room temperature for 10 min. Finally, the fluorescence measurements were performed on a FP-750 fluorescence spectrophotometer (JASCO, Japan) with an excitation wavelength (λ_{ex}) of 340 nm, and the fluorescence spectrum was recorded at 635 nm.

3. Results and discussion

3.1 Working principle of the proposed method

The working principle of the proposed fluorescent method for ATP detection is schematically illustrated in Fig. 1. As shown in Fig. 1, in the absence of ATP, S1 nuclease degrades T40, releasing mono- or small oligonucleotide fragments and failure to acting as the template for the formation of fluorescent CuNPs. Moreover, ATP has been reported to be a good inhibitor of S1 nuclease, it can protect single-stranded nucleic acids from degradation by S1 nuclease. In the presence of ATP, it can interact with S1 nuclease to protect T40 from degradation, which results in an ideal template for CuNPs formation and the subsequent significantly enhanced fluorescence signal.

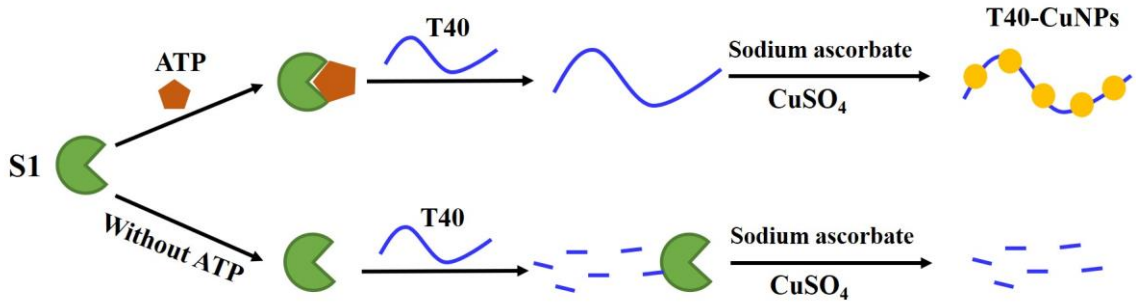

Fig. 1 Schematic illustration of the label-free method for ATP detection by using poly T-templated CuNPs as fluorescent probe

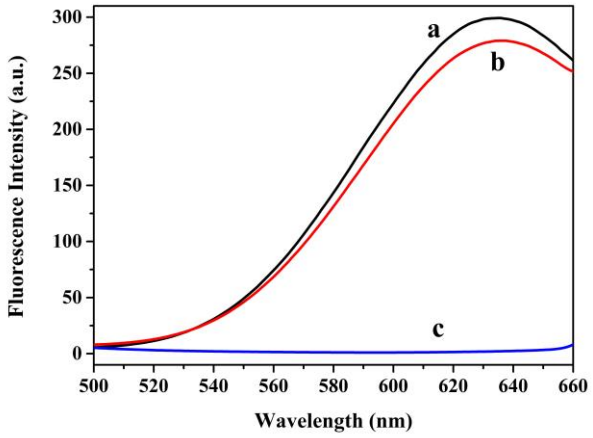

Fig. 2 Fluorescence emission spectra of CuNPs at different conditions

3.2 Verification the feasibility of the proposed method

In order to verify the feasibility of this method for ATP detection, fluorescence characterization was then investigated (see Fig.2). In the absence of ATP and S1 nuclease, T40 served as the template for

the formation of CuNPs with a significant fluorescence signal (curve a). When in the both presence of ATP and S1 nuclease, ATP was able to protect T40 against the cleavage of S1 nuclease, and T40 kept the original long state, resulting in the formation of fluorescent T40-CuNPs (see curve b). However, in the absence of ATP, T40 was degraded by S1 nuclease, thus failed to template the formation of fluorescent CuNPs, yielding a weak background signal (see curve c). The above results demonstrated that the proposed method for ATP detection was reasonable.

3.3 Optimization of experimental conditions

In order to obtain a better sensing performance for ATP, experimental conditions including the concentrations of S1 nuclease, sodium ascorbate and copper sulfate have been investigated respectively.

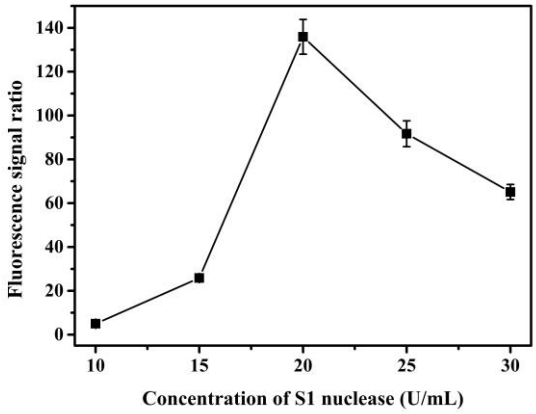

Fig. 3 Impact of S1 nuclease concentration on the fluorescence signal ratio of the ATP assay

Since the concentration of S1 nuclease was an important factor influencing the concentration of T40 template, the impact of S1 nuclease concentration on the ATP assay was first investigated (see Fig.3). From Fig.3, it was found that fluorescence signal ratio increased dramatically upon adding S1 nuclease concentration at first, then declined rapidly like a parabolic trend, and the maxmium was obsreved at 20 U/mL of S1 nuclease. Thus, this concentration of S1 nuclease was used in all further experiments.

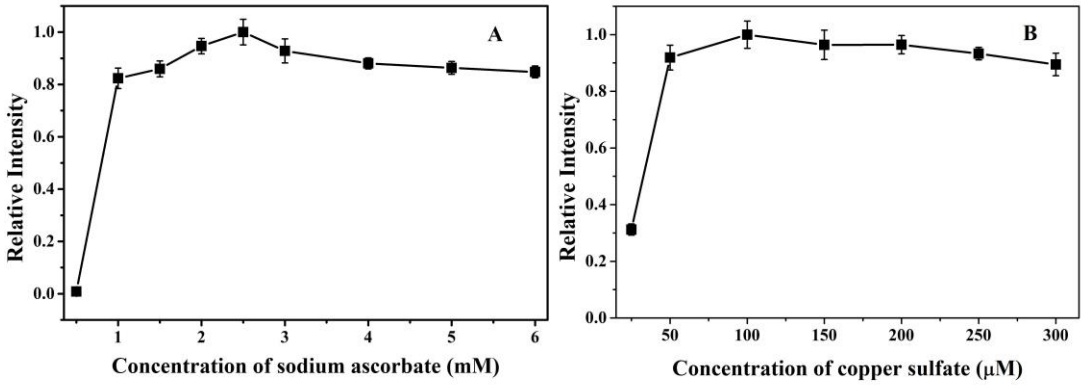

Fig. 4 Impact of concentration of sodium ascorbate and copper sulfate on the relative intensity

In order to find optimal conditions for the formation of T40-CuNPs, impact of concentration of sodium ascorbate and copper sulfate were systematically investigated. Firstly, the concentration of S1 nuclease and ATP were fixed at 20 U/mL and 1 mM, respectively. As shown in Fig.4A, the

maximu of relative intensity was obtained at 2.5 mM sodium ascorbate, thus this concentration was chosen to be the optimal and used in all further experiments. Furthermore, from Fig.4B, it was found that the highest relative intensity of the ATP assay was obtained at 100 mM of copper sulfate. Therefore, the optimal concentration of copper sulfate was chosen to be 100 mM and used in the further formation of T40-CuNPs.

3.4 Sensitivity of the fluorescent ATP detection

Under optimal conditions, we investigated the sensitivity of the proposed fluorescent method towards ATP. From Fig. 5A, it was observed that the fluorescence intensity was continually increased as the ATP concentration range from 0 to 5.0 mM, indicating that the cleavage of T40 was gradually prohibited. As shown in Fig. 5B, the fluorescence intensity exhibited a good linear correlation with ATP concentration in the dynamic range of 0.01-1.0 mM, and the regression equation was $Y=267.9X+0.489$ ($R^2=0.9914$), the limit of detection (LOD) for ATP was estimated to be 6 M according to the 3σ rule. This LOD is comparable to those of many reported ATP aptamer-based strategies.

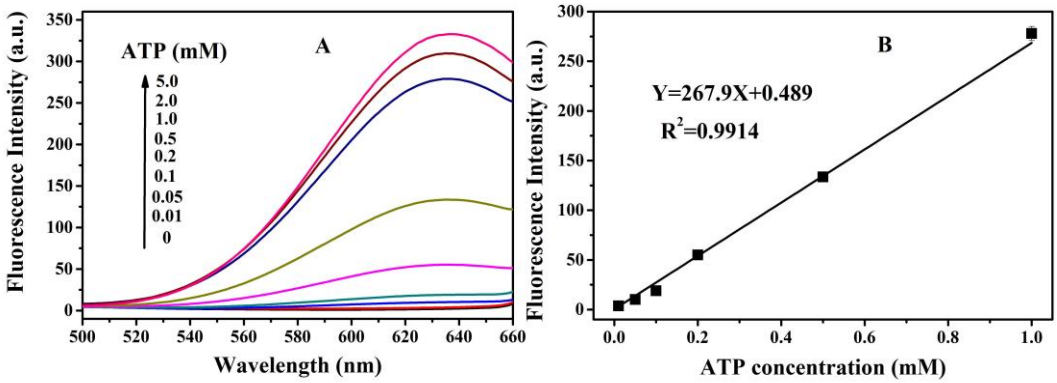

Fig. 5 (A) Fluorescent spectra of T40-CuNPs with different concentrations of ATP; (B) The linear relationship of the fluorescence intensity of T40-CuNPs and ATP concentration

3.5 Real sample analysis

To investigate the feasibility of this method in complex biological media, the proposed method was applied to detect ATP in diluted goat serum (1%) for recovery tests, and the experimental results are shown in Table 1. As shown in Table 1, three different concentrations of ATP were spiked into the complex fluids through a standard addition method and measured. The recoveries of the samples were satisfactory, demonstrating the practical applicability for ATP detection in real complex samples.

Table 1. Determination of ATP in diluted goat serum

Samples	Added (mM)	Found (mM)	Recovery (100%)	RSD (%, n=3)
1	0.5	0.476	95.2	3.43
2	0.2	0.211	105.5	4.62
3	0.1	0.0944	94.4	3.96

4. Conclusion

In summary, label-free fluorescent determination of ATP was realized by integrating poly-T templated CuNPs and inhibition of S1 nuclease. This method has achieved several advantages. First, there is only one DNA probe (T40) used throughout experiments, which is easy to operate and cost-effective. Second, the proposed method may hold great potential for detecting other inhibitors of S1 nuclease. Finally, this method showed an outstanding recovery for ATP detection in complex

biological media. In view of these advantages, the proposed method may be helpful for the further exploration of DNA-templated CuNPs in practical applications.

Acknowledgements

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References

[1] J. Wang, Y.X. Jiang, C.S. Zhou, et al. Aptamer-based ATP assay using a luminescent light switching complex, Analytical Chemistry, vol.77 (2005) 3542-3546.

[2] T.T Zhang, Y. Peng, R. Yuan, et al. Target-catalyzed assembly formation of metal-ion dependent DNAzymes for non-enzymatic and label-free amplified ATP detection, Sensors and Actuators B: Chemical, vol.273 (2018) 70-75.

[3] H.Y. Huang, S. Fu, Y. Huang, et al. Free-labelled fluorescent method for ATP detection assisted by T4 DNA ligase, Analytical Methods, vol.9 (2017) 1046-1049.

[4] C.S. Lin, Z.X. Cai, Y.R.Wang, et al. Label-free fluorescence strategy for sensitive detection of adenosine triphosphate using a loop DNA probe with low background noise, Analytical Chemistry, vol.86 (2014) 6758-6762.

[5] N.M. Danesh, M. Ramezani, A.S. Emrani, et al. A novel electrochemical aptasensor based on arch-shape structure of aptamer-complimentary strand conjugate and exonuclease I for sensitive detection of streptomycin, Biosensors & Bioelectronics. vol.75 (2016) 123-128.

[6] W.P. Zhu, Z.W. Zhao, Z. Li, et al. A ligation-triggered highly sensitive fluorescent assay of adenosine triphosphate based on graphene oxide, Analyst, vol.137 (2012) 5506-5509.

[7] Q.W. Song, R.H. Wang, F.F. Sun, et al. A nuclease-assisted label-free aptasensor for fluorescence turn-on detection of ATP based on the in situ formation of copper nanoparticles, Biosensors & Bioelectronics, vol.87 (2017) 760-763.

[8] Z.G. Mao, Z.H. Qing, T.P. Qing, et al. Poly(thymine)-templated copper nanoparticles as a fluorescent indicator for hydrogen peroxide and oxidase-based biosensing, Analytical Chemistry, vol.87 (2015) 7454-7460.