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A carbon nanotubes based ATP apta-sensing platform and its application in cellular assay

Libing Zhang[a,b], Hui Wei[a], Jing Li[a], Tao Li[a], Dan Li[a], Yunhui Li[b], Erkang Wang[a,*]

[a] State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin 130022, PR China
[b] School of Chemistry and Environmental Engineering, Changchun University of Science and Technology, Changchun, Jilin 130022, PR China

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ABSTRACT

In this paper, a sensitive and selective fluorescent aptasensor for adenosine triphosphate (ATP) detection is constructed, based on the noncovalent assembly of dye-labeled ATP aptamer and single-walled carbon nanotubes (SWNTs). In the absence of ATP, the dye tethered to the ATP aptamer is close to SWNTs, which can effectively quench fluorescence of the dye. Upon adding ATP, the fluorophore keeps away from the quencher, since ATP specifically binds to the aptamer and competes with carbon nanotubes, resulting in an increase in the fluorescence intensity. This enables ATP to be detected down to 4.5 nM. To the best of our knowledge, this is the most sensitive fluorescent ATP aptasensor. In addition, prominent fluorescence signals were obtained in cellular ATP assays, thus the aptasensor could be used to detect ATP in real samples.

1. Introduction

The design and construction of chemo- and biosensors for recognizing biologically important small molecules and anions have received considerable attention in recent years (Li et al., 2005). The anionic adenosine triphosphate (ATP) is generally acknowledged as "energy currency" in most animate beings, which plays an important role in most enzymatic activities (Gourine et al., 2005). The concentration and dissipative rate of ATP have been found to be closely related to many diseases such as hypoxia, hypoglycemia, ischemia and Parkinson's disease (Greene et al., 1985; Bush et al., 2000; Dimonte, 1991; Harkness and Saugstad, 1997; Przedborski and Vila, 2001). Consequently, monitoring ATP and its consumption in a simple, highly selective, sensitive manner has attracted considerable attention. So far, although several approaches have been developed for detecting ATP such as gold nanoparticles based colorimetric assays (Wang et al., 2007), fluorometric sensors (Lee et al., 2004; Jhaveri et al., 2000) and an electrochemical detection method (Zuo et al., 2007), it remains important to find new approaches that could improve the simplicity, selectivity and sensitivity of ATP detection.

Recently, for analytical assays, many nanomaterials including nanoparticles, nanotubes and nanowires have been used to create new types of analytical tools for life science and biotechnology (Niemeyer, 2001). Specifically, carbon nanotubes have emerged as one of the most extensively studied nanomaterials due to their unique chemical, electrical, and mechanical properties (Ajayan, 1999; Tasis et al., 2006). The potential for application of carbon nanotubes ranges from molecular electronics to ultrasensitive biosensors. Several investigators have recently reported on covalent or noncovalent functionalization of single-walled carbon nanotubes (SWNTs) with nucleic acids (Tang et al., 2006; Wang et al., 2003; Zheng et al., 2003) and proteins (Kam and Dai, 2005; So et al., 2005). The ssDNA wrapped around individual SWNTs through π-stacking interactions between the nucleotide bases and the SWNT sidewalls on the formation of stable complex. Also, the examples of noncovalent interactions of SWNTs with organic or dye-labeled biomolecules have been reported (Boul et al., 2007; Nakayama-Ratchford et al., 2007; Yang et al., 2008a). Photophysical studies have indicated that SWNTs can act collectively as quenchers for dyes, which provides a basis for creating new sensing platform to detect small molecules via fluorescence signal.

Therefore, in this paper, we present a sensitive fluorescent aptasensor for ATP detection, which is based on the noncovalent assembly of SWNTs and dye-labeled ATP aptamer. In this approach, anti-ATP DNA aptamer is labeled with a fluorescein derivative, 6-carboxy-fluorescein (FAM). Fig. 1 shows the signaling scheme of this approach. Briefly, SWNTs exist in aggregate state in solution, and then, the binding of the dye-labeled aptamer with individual SWNTs disperses them into the solution. The activity results

* Corresponding author. Tel.: +86 431 85262003; fax: +86 431 85689711.
E-mail address: ekwang@ciac.jl.cn (E. Wang).

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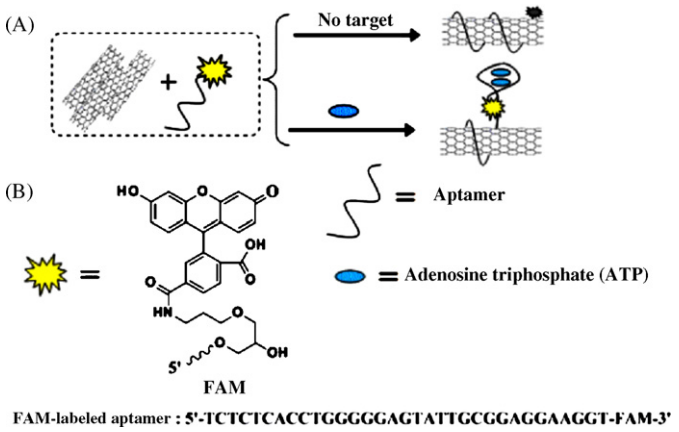

Fig. 1. (A) Scheme for signaling biomolecular interactions by the assembly of SWNTs and dye-labeled aptamer. (B) Structures of FAM, the FAM-labeled aptamer and ATP.

in the formation of stable aptamer/SWNTs complex. TEM images of the aptamer/SWNTs complex were shown in Fig. S1 (supplementary information). In the absence of ATP, the dye tethered to the ATP aptamer is close to SWNTs, which can effectively quench fluorescence of the dye. Upon adding ATP, the fluorophore keeps away from the quencher, since ATP specifically binds to the aptamer (Huizenga and Szostak, 1995) and competes with carbon nanotubes (Yang et al., 2008b; Wang et al., 2009). This results in an increase in the fluorescence intensity. This signaling mechanism makes it possible to detect target by fluorescence spectroscopy. It should be noted that the selectivity of this approach is mainly determined by the specific interaction between aptamer and target.

2. Experimental

2.1. Chemicals and materials

The FAM-labeled anti-ATP aptamer (5′-TCTCTCACCTGGGGGAGTATTGCGGAGGAAGGT-FAM-3′) was synthesized by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). The concentration of aptamer was determined using the 256 nm UV absorbance and the corresponding extinction coefficient. Single-walled carbon nanotubes were purchased from Shenzhen Nanotech Port Co. Ltd. (China). Adenosine triphosphate (ATP), guanosine triphosphate (GTP) and N,N-dimethylformamide, anhydrous, 99.8% (DMF) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Cytidine triphosphate (CTP) and uridine triphosphate (UTP) were purchased from Bio Basic Inc. (Markham Ontario, Canada). RPMI-1640 medium and fetal bovine serum (FBS) were obtained from Gibco (USA). All the other chemicals were of analytical reagent grade and were used as received without further purification. Solutions were prepared with deionized (DI) water processed with a Milli-Q ultra-high purity water system (Millipore, Bedford, MA, USA).

2.2. Apparatus

Fluorescent emission spectra were recorded on a PerkinElmer LS55 Luminescence Spectrometer (PerkinElmer Instruments, U.K.). UV–visible absorbance spectra were recorded on a Cary 500 scan UV–vis–NIR spectrophotometer (Varian, Harbor City, CA) at room temperature. A JASCO J-810 spectropolarimeter (Tokyo, Japan) was utilized to collect the circular dichroism (CD) spectra in the Tris–HCl buffer. The optical chamber (1 cm path length, 1 mL volume) was deoxygenated with dry purified nitrogen (99.99%) before use and kept in the nitrogen atmosphere during experiments. Three scans from 200 to 320 nm at 0.1 nm intervals were accumulated and averaged. The background of the buffer solution was subtracted from the CD data. Transmission electron microscopy (TEM) measurements were made on a TECNAI G2 (Philips, Holland) with an accelerating voltage of 200 kV.

2.3. Preparation of SWNTs

In the experiment, the SWNTs were treated by refluxing in 4.0 M HNO_3 for 24 h, subsequently, the 3:1 concentrated H_2SO_4:HNO_3 mixture was chosen as the oxidizing acid in this cutting operation. The SWNTs were sonicated for 8 h in ice bath. Finally, they were filtered with a 220 nm millipore size membrane with the aid of a pump and thoroughly washed with DI water to obtain a neutral state, finally, they were dried under vacuum at 60 °C overnight to obtain purified SWNTs.

2.4. Performance of ATP detection

The purified SWNTs were sonicated in DMF for 5 h to give a homogeneous black solution and stored for use. The working solution containing the FAM-labeled aptamer (referred as P_0, Fig. 1) was obtained by diluting the stock solution to a concentration of 50 nM using 20 mM Tris–HCl buffer (146 mM NaCl, 5.0 mM KCl, 1.0 mM $MgCl_2$, pH 7.4). For ATP assays, 25 μL of the P_0 stock solution, appropriate concentrations of ATP solution, and SWNTs solution as prepared were mixed and incubated for 2.0 h at 37 °C, then the fluorescence intensity was measured. Fluorescence intensity was recorded at 520 nm with an excitation wavelength of 480 nm.

2.5. Cellular ATP assay

Human adenocarcinoma HeLa cells were chosen for the experiment. The cells were grown in RPMI-1640 medium supplemented with 10% FBS in a 96-well plate and routinely cultured in a humidified 5% CO_2 incubator at 37 °C. HeLa cells were cultured to the ~70% confluency, and collected after trypsin digestion. The collected cells were washed twice by PBS and then suspended in Tris–HCl buffer. Cell lysis (approximately 200,000 cells/mL) was performed by repeated cycles of freezing and thawing, and then the lysed cells were ready for ATP assays. The ATP detection protocol was the same as above mentioned.

3. Results and discussion

3.1. UV–vis absorption spectra

The optical spectral properties of P_0 in the absence and presence of SWNTs were investigated, respectively. Fig. S2 (see supplementary information) shows the UV–vis absorption spectra of the free state of P_0 (curve a) and P_0–SWNTs (curve b). P_0 is characterized by absorption bands of the DNA sequence (256 nm) and FAM (495 nm). Whereas, the addition of SWNTs into the solution of P_0 caused a hypochromic-red shift of the 495 nm Soret band of FAM (the inset of Fig. S2, supplementary information), indicating that there is a notable electronic communication between the two π-systems of SWNTs and the dye in the ground state (Yang et al., 2008b).

3.2. Fluorescence quenching by SWNTs

The concentration effect of SWNTs on the fluorescence quenching of P_0 was investigated. Fig. S3 (see supplementary information) shows the fluorescence intensity of P_0 under different concentrations of SWNTs. With the increase in the concentration of SWNTs, the fluorescence intensity of P_0 decreased evidently. It is found that more than 70% of FAM's fluorescence was quenched with 10 μg/mL

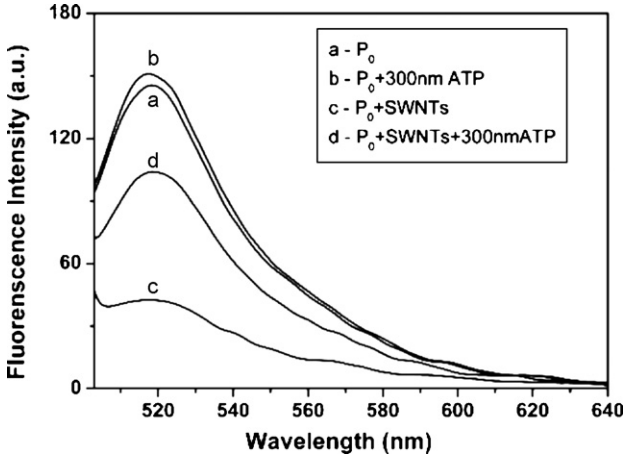

Fig. 2. Fluorescence emission spectra of P_0 (50 nM) at different conditions: (a) P_0 in Tris–HCl; (b) P_0 + 300 nM ATP; (c) P_0 + SWNTs; (d) P_0 + SWNTs + 300 nM ATP.

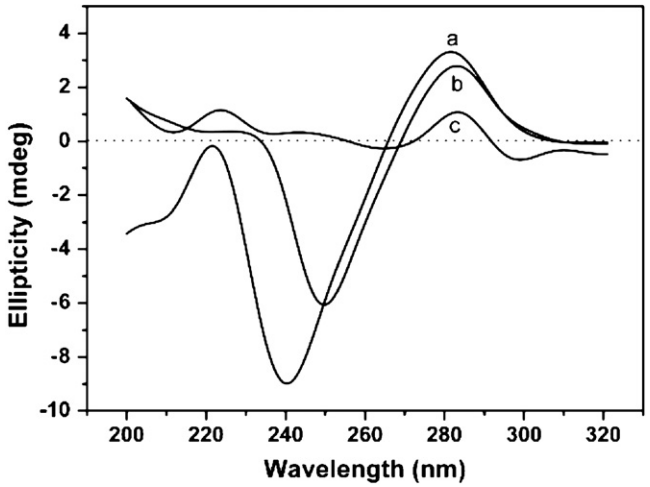

Fig. 3. CD spectra of P_0 (5.0 μM) at different conditions: (a) P_0 in Tris–HCl; (b) P_0 + SWNTs (2.0 mg/mL); (c) P_0 + SWNTs (2.0 mg/mL) + ATP (50 μM).

SWNTs in the solution. This result reveals that SWNTs can efficiently quench the fluorescence of FAM, which may be attributed to the electron or energy transfer from the fluorophore to the SWNTs (Boul et al., 2007; Yang et al., 2008a,b).

3.3. Fluorescence recovery by ATP

Fig. 2 shows the fluorescence emission spectra of P_0 at different conditions. Curve a was obtained in the absence of SWNTs. Upon excitation at maximal absorption wavelength (520 nm) of FAM, P_0 shows strong fluorescence emission by FAM. In the presence of SWNTs, the fluorescence emission is very weak, it is about 30% of the free P_0 (curve c). However, in the presence of ATP, then added the SWNTs to the solution, the fluorescence emission is about 71% of the free P_0 (curve d). This result indicates that the existence of ATP can recover the fluorescence intensity compared with that only in the presence of SWNTs. Curve b was measured in the presence of ATP, but the fluorescence intensity is higher than that of the free P_0. To check this phenomenon whether or not influences the sensitivity of detecting ATP, we investigate the fluorescence emission spectra of P_0 at different conditions of ATP, as shown in Fig. S4. No obvious emission change could be observed from free P_0 upon adding low concentration of ATP. Only P_0 shows fluorescence enhancement by high concentration of ATP due to the change of the microenvironment of FAM by the target complex. It can be seen that the fluorescence intensity changes (F/F_0) of P_0–ATP is smaller than that of P_0–ATP–SWNTs, where F_0 and F are FAM intensities at 520 nm in the absence and presence of ATP, respectively. The F/F_0 of P_0–ATP at 520 nm is 1.05 in the presence of 300 nM ATP, which is significantly lower than that observed from P_0–ATP–SWNTs (F/F_0 = 2.45) in the same concentration of ATP. The above-mentioned experimental results demonstrate that the aptamer/SWNTs approach could be used as a sensitive approach for ATP detection.

3.4. Studies on sensing mechanism

As mentioned, the proposed approach could work well for the detection of ATP. To further demonstrate how this sensing system works, CD measurement is utilized to monitor the conformation change of P_0 in different cases (Fig. 3). The CD spectrum of P_0 alone has a positive band near 280 nm and a negative band at about 240 nm (curve a). This is the typical characteristic of B-form DNA duplex (Baker and Bowers, 2007; Trantirek et al., 2000). Upon adding SWNTs, the positive and negative bands still appear, but there is an obvious decrease in the ellipticity (curve b). This change of CD spectra should be attributed to the interaction of P_0 with SWNTs. In this case, P_0 is thought to wrap around the SWNTs, resulting in the decrease in its ellipticity. While in the presence of SWNTs and ATP, P_0 has a positive band near 285 nm and a negative band at 265 nm in its CD spectrum (curve c). This indicates the formation of the antiparallel quadruplex structures induced by ATP (Huizenga and Szostak, 1995). It provides an evidence for the interaction of ATP with P_0 around the SWNTs.

However, a key question must be answered, i.e., how does P_0 interact with ATP in the SWNTs solution? The binding of ATP with P_0 could occur either in solution or at the surface of SWNTs. With the former case, the P_0 first got away from the nanotube surface and then ATP binding took place in solution. The dye molecule of P_0 removed from the nanotube, therefore, in such a way that it is no longer quenched. With the latter case, the P_0 was absorbed on the nanotube surface, and ATP complexation is formed on the nanotube surface. This is where the P_0 undergoes a change in conformation in response to interaction with ATP. The dye molecule of P_0 extended from the nanotube, therefore, in such a way that it may be still a little quenched, since the P_0 is still absorbed on the nanotube.

To determine whether the target interaction occurs on the surface of the carbon nanotube or not, the fluorescence intensity of pure P_0 and P_0–SWNTs in the presence of ATP (300 nM) was measured, respectively. As shown in Fig. 2, the fluorescence intensity of P_0–SWNTs in the presence of ATP (curve d) is not reach the value of the fluorescence intensity of pure P_0 (curve a), there is still a little quenched. This result indicates that the complexation of P_0 and ATP might occur on the nanotube surface by forming a ternary complex among P_0, SWNTs, and ATP. To further confirm this notion, we isolated ATP complex of P_0–SWNTs by dialysis against Tris–HCl buffer with a membrane (molecular weight cutoff 8000–14,000) and measured the fluorescence intensity of the dialysis product and the nanotube complex. Specifically, it was found that, the nanotube complex inside the membrane was highly fluorescent, however the product outside the membrane was nonfluorescent (Fig. S5). Collectively, these results demonstrate that the interaction of P_0 and ATP indeed occurs on the nanotube surface but not in the solution.

3.5. The kinetic behavior

In addition, the kinetic behaviors of P_0 and SWNTs as well as P_0–ATP with SWNTs were also studied. Fig. S6 (see supplementary information) shows fluorescence quenching of P_0 as a function of incubation time. In the absence of ATP, the curve exhibits a rapid reduction in the first 0.5 h and a slow decrease over a 1.5-

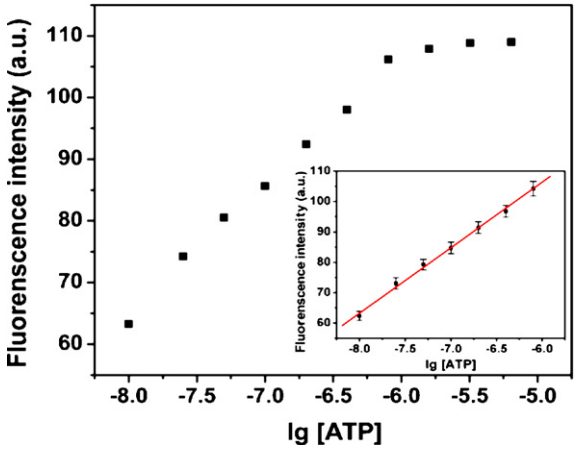

Fig. 4. Fluorescence intensity of P_0–SWNTs in the presence of increasing amounts of ATP from 10 nM to 6.4 μM against the logarithm of the concentration of ATP. The inset is the linear relationship between the fluorescence intensity and the logarithm the concentration of ATP. Fluorescence intensity was recorded at 520 nm with an excitation wavelength of 480 nm.

h period. It is hypothesized that the surface effect of SWNTs and the charge properties of aptamer should be the main reason for the low adsorption space (Jeng et al., 2006, 2007; Vogel et al., 2007). In the presence of ATP, a fluorescence decrease of P_0 is also observed. However, the formation of ATP-aptamer reduces the absorbance of P_0 onto the SWNTs and thus fluorescence quenching efficiency. This, in turn, results in an overall fluorescence increase, which displays fluorescence enhancement compared to that without ATP. The experimental results demonstrate that the aptamer/SWNTs approach could be used as a sensitive approach for ATP detection in aqueous solution.

3.6. Sensitivity and selectivity

For the sensitivity study, a series of ATP solution from 10 nM to 6.4 μM were investigated. As shown in Fig. 4, with the increase in the concentration of ATP, the fluorescence intensity was enhanced obviously. However, when the concentration of ATP reached a high value, the fluorescence intensity would not change any more. The linear range is from 10 to 800 nM (R^2 = 0.996), and the detection limit is estimated to be 4.5 nM (three times the standard deviation of the blank solution). To the best of our knowledge, the present aptasensor is the most sensitive fluorescent ATP aptasensor so far and the sensitivity of the present aptasensor is much better than

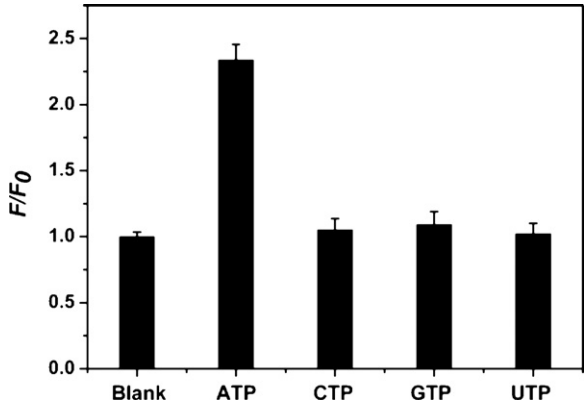

Fig. 5. Fluorescence intensity changes (F/F_0) of P_0–SWNTs in the presence of ATP (600 nM), GTP (1.2 μM), CTP (1.2 μM) and UTP (1.2 μM), respectively. Fluorescence intensity was recorded at 520 nm with an excitation wavelength of 480 nm.

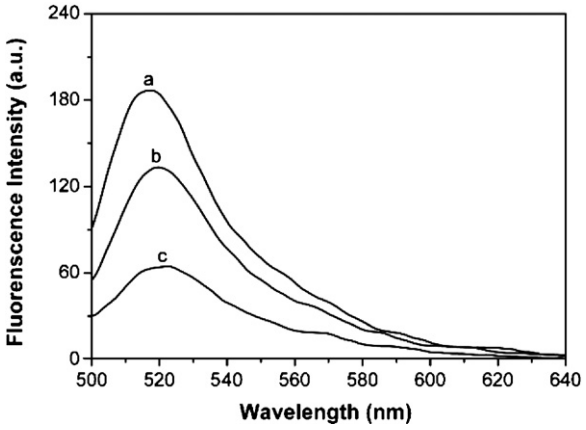

Fig. 6. Fluorescence emission spectra of (a) P_0 in the presence of freshly lysed cells (ATP present), (b) P_0–SWNTs in the presence of freshly lysed cells (ATP present) and (c) P_0–SWNTs in the presence of lysed cells after 24-h aging (ATP absent), excitation was at 480 nm.

other ATP aptasensors reported in the literatures, including colorimetrical (Chen et al., 2008; Wang et al., 2007), fluorescent (Lee et al., 2004; Zhao et al., 2009) and electrochemical (Shen et al., 2007) aptasensors. The result indicates that this aptasensor indeed possess the potential application of detecting ATP sensitively.

Subsequently, we carried out the selectivity tests of the current aptasensor. GTP, CTP and UTP, analogues of ATP, were chosen to testify the selectivity of this approach. The concentration of ATP was 600 nM, while the concentration of GTP, CTP and UTP were all chosen at 1.2 μM. Under the same experimental conditions, the fluorescence intensity was measured. It is found that the fluorescence intensity change (F/F_0) in the presence of ATP is much larger than that of in the presence of GTP, CTP and UTP (Fig. 5), respectively, implying that the three analogues could not interact with P_0 and thus not interfere with the detection of ATP. This result indicates that the interaction of P_0 with ATP is selective and specific.

3.7. Detection of ATP in heLa cell

In order to perform ATP assays in real samples, we employed this aptasensor to directly detect cellular ATP. We obtained a prominent fluorescence signal for freshly lysed cells (ATP present, curve b). In contrast, only a small residual signal was observed after storing cell lysates for 24 h (ATP absent, curve c) (Fig. 6). This clearly shows the aptasensor could be used to detect ATP in real samples sensitively and specifically and might become a promising probe for cellular ATP assays.

4. Conclusions

In conclusion, a simple, selective and sensitive fluorescent aptasensor for ATP detection by using a dye-labeled aptamer and SWNTs was developed. This design is based on the noncovalent interaction of aptamer with SWNTs by π-stacking between the nucleotide bases and the SWNT sidewalls. As to ATP, a sensitive detection limit of 4.5 nM was obtained. In addition, the aptasensor can be used to detect ATP in real samples since prominent fluorescence signal were obtained in cellular ATP assays. Therefore, this method can be used to detect ATP simply, selectively and sensitively. Generally, this work is significant and should have a broad interest due to: (1) ATP is a very important molecule in biological system and plays important role in many enzymatic reactions; (2) ATP is a small organic molecule, and there is not so many ligands can bind it. But aptamer can recognize ATP specifically and thus provide a promising method for its detection. Furthermore, aptamer

only needs one dye labeled to show high quenching efficiency by SWNTs; (3) more importantly, this method could be applied to cellular ATP assay, which might provide a promising method for cellular ATP assay in vivo in the future.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2010.01.002.

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