Rapid Communications

A RuO$_2$ nanosheet as a novel quencher-free platform for the detection of nucleic acids in a homogeneous solution

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Abstract

A fluorescent dye-labeled DNA probe was adsorbed and quenched on the monolayer of RuO₂ nanosheets. Significant fluorescent recovery was observed by the addition of the complementary DNA due to the desorption of the probe from the surface of the RuO₂ nanosheet through the duplex formation. The efficiency of fluorescence recovery was higher than that for graphene oxide, which was known as a quencher-free platform for the detection of nucleic acids in a homogeneous solution.

Keywords: Nucleic acids sensor, RuO₂ nanosheet, quencher-free analytical platform, homogeneous assay.
In recent years, growing attention has been paid to atomically thin two-dimensional (2D) nanosheets as promising platforms for separation,\(^1\) catalysis,\(^2\) chemo/biosensing,\(^3\) and energy production,\(^4\) due to their large surface area and intriguing physical properties derived from a quantum size effect associated with their ultra-thin thickness. These properties endow them with high molecular adsorption capacities, and light harvesting and electroconductive abilities.\(^5\) To date, their applications to biosensor were typically based on the simple working principle: quenching of the fluorescent dye-modified molecular probes adsorbed on these 2D materials by fluorescence energy transfer (FRET) and the following restoration of fluorescent signal through the desorption of these probes from their surface by the addition of target biomolecules.\(^3d\)

Graphene oxide (GO) is one of the most famous 2D nanosheets used as a FRET acceptor (fluorescence quencher) for homogeneous bioanalysis.\(^3d\) Recently, monolayer transition metal dichalcogenides (TMDCs) such as WS\(_2\),\(^7\) and MoS\(_2\),\(^8\) and transition metal oxide nanosheets such as MnO\(_2\),\(^9\) have emerged as another class of 2D platforms. Transition metal oxide nanosheets have distinct differences compared with GO and other 2D nanosheets in their potential to be used as insulators, semiconductors, and conductors, depending on their chemical composition and structures of the parent layered compounds.\(^10\) However, most transition metal oxide 2D nanosheets – except for MnO\(_2\), a redox active material – have not yet been studied for application to bioanalysis. The development of new transition metal oxide nanosheets applicable to bioanalysis is expected to lead to the fabrication of composite materials with sophisticated functionalities by simultaneous use of several nanosheets.\(^11\) For these reasons, it is very important to explore new functional transition metal oxide nanosheets that can be applied to biosensing.

A Ruthenium dioxide (RuO\(_2\)) nanosheet, known as a redox active and conductive material, consists of edge-shared RuO\(_2\) octahedral units with ultrathin thickness on the order of nanometers and an expanded surface area ranging from submicrometers to micrometers. It
shows intense and broad absorption of light derived from the d-d transition of Ru ions in the ligand field of RuO₆ octahedra (Fig. S1). Although it has shown promising prospects in the field of supercapacitors, rechargeable batteries, and oxygen evolution reaction electrocatalysts, until now research interest in this unique material have been limited to the areas of sustainable energy and electricity.¹² Here, as shown in Fig. 1a, we assessed the fundamental properties of RuO₂ nanosheet, such as adsorption and desorption of DNA, to demonstrate its potential as a new platform for the detection of biomolecules.

The DNAs used in this study (Fig. 1b) were purchased from Japan Bio Services Co., LTD. The probe was a 13-mer DNA modified with 6-FAM (6-carboxyfluorescein) at its 5´-end. RuO₂ nanosheet was prepared according to a procedure previously reported, followed by exfoliation in aqueous solution containing tetrabutyl ammonium cation (TBA⁺) (see ESI for detail).¹²b, ¹³ The single layer RuO₂ was recovered as the non-sedimented supernatant after a 30 min centrifugation at 10000 rpm. The obtained RuO₂ nanosheet was characterized by AFM (Fig. S2). The nanosheets were a few micron in lateral size and almost completely exfoliated into monolayers. Prior to use, RuO₂ dispersed solution was centrifuged for 10 min at 15000 rpm, and then supernatant was taken off, followed by the resuspension of RuO₂ in water. A GO nanosheet was prepared by Hummer’s oxidation of graphite followed by exfoliation in aqueous solution¹⁴ and used for comparison with RuO₂. GO, that contains nanometer-scale graphene-like sp² domains in an amorphous sp³ carbon matrix, can strongly adsorb single-stranded DNA via hydrophobic and π-stacking interactions with the exposed nucleobases. On the other hand, the affinity for double-stranded DNA is much lower because the nucleobases are shielded inside the hydrophilic double helix backbone.¹⁵

Fluorescence spectra of 10 nM probe solution, prepared in 5 mM sodium phosphate buffer containing 500 mM NaCl and 2 mM MgCl₂ (pH 7.0), was acquired for increasing amounts of RuO₂ or GO. As shown in Fig. 2a, fluorescence of the probe was significantly decreased by
increasing the amount of GO. Amount of GO required for complete fluorescence quenching was 1.2 μg/mL. Gradual quenching behavior was observed in the case of RuO\(_2\) (Fig. 2b). Although complete quenching was not observed until the concentration of RuO\(_2\) reached 50 μg/mL, where \(ca.\) 41 % of fluorescence was quenched, large quenching by the addition of RuO\(_2\) seemed to indicate that the probe DNA was adsorbed and quenched on RuO\(_2\) nanosheet as in the case of GO.

Desorption behavior of the probes from RuO\(_2\) was verified by the fluorescence recovery with the addition of the complementary (fmDNA) or the non-complementary scrambled DNA (scDNA), and the results were compared with those of the GO-based system. After the addition of each aliquot of fmDNA or scDNA to these probe-adsorbed nanosheets, the solutions were subjected to the fluorescence measurements. To assess the desorption efficiency, we evaluated the rates of released probes by normalizing the emission intensities based on the intensities before and after probe adsorption. Time dependencies of probe release are shown in Fig. 3a and the percentages of probe release at 60 min are summarized in Fig. 3b. The addition of scDNA hardly caused probe release for both RuO\(_2\) and the GO-based system. In the case of the GO-based system, the released probe was only \(ca.\) 21 % after the incubation with fmDNA for 60 min. The interaction between GO and nucleobases of DNA would be too strong to form the duplex efficiently even in the presence of fully complementary DNA. In contrast, the DNA adsorbed on RuO\(_2\) was rapidly released by duplex formation; fmDNA liberated \(ca.\) 87 % of adsorbed probe from RuO\(_2\) by its hybridization. This value was \(ca.\) 44 times larger than in the presence of scDNA, while the contrast between fmDNA and scDNA was only \(ca.\) 4 for the GO-based system. This was attributed to an appropriate affinity between the single-stranded DNA and RuO\(_2\) arising from a balance between the attractive interaction and electrostatic repulsion of negatively charged RuO\(_2\) with phosphate groups on DNA backbone in a given experimental condition. At the higher MgCl\(_2\) concentration (10 mM), higher quenching
efficiency (ca. 82 %) and lower release efficiency of the probe (ca. 14.1 %) were observed in the RuO₂ system (data not shown). These results might be attributed to the enhancement of screen effect (neutralization) on the negative charge of the RuO₂ surface. Similar behavior was reported in the GO-based system.¹⁶ Thus, the adsorption and desorption of DNA in the RuO₂-based system are governed by simple and general rules, and can be regarded as the binding and dissociation of anion polymers with negatively-charged polyelectrolyte nanosheet like those in a GO-based biosensing system.

For further discussion of the adsorption and dissociation behavior, the probe was indirectly immobilized on RuO₂ via capture DNA with the 6-FAM moiety (5’-end) of the probe placed near RuO₂ (Fig. S3). The probe itself was not directly adsorbed on the nanosheet surface in this system. This is the well-designed structural format for GO-based DNA sensing that gave signals with higher contrast than that of direct adsorption. The capture DNA consists of an A₂₀ tail that serves as an anchor to the RuO₂ and a partially complementary sequence with the probe for its capturing. The captured probe has a five nucleotide-long single-stranded dangling region serving as a toehold for strand exchange with fmDNA. Here, we designed the length of the toehold, which is normally seven or more nucleotides long, to be relatively short in order to make the strand exchange reaction slow to monitor it easily. At first, the capture/probe duplexes were adsorbed on RuO₂ (Fig. S4a) or GO (Fig. S4b). By the addition of fmDNA, as expected, gradual fluorescence recovery was observed as a result of the toehold-mediated strand exchange to form a stable probe/fmDNA duplex leading to the probe release from nanosheet surfaces. The fluorescence recovery at 60 min was ca. 27 % (Fig. S5a) for RuO₂. This value was comparable to that for the corresponding GO-based system (Fig. S5b). This implies that the probe release was simply governed by the toehold-mediated strand exchange reaction of the DNA probes indirectly immobilized on both RuO₂ and GO-based systems, because the interaction between the probe and the surface of the nanosheets was minimized by the indirect immobilization.
In conclusion, we investigated the fundamental properties of RuO$_2$ nanosheet for the application of bioanalysis using fluorescent dye-labeled DNA as an example of the constituents of the sensors. This unique nanosheet was able to quench fluorescent signals like GO. Although the binding of single-stranded DNA seems to be weaker than GO, the nature of reversible desorption of DNA made the signal contrast higher than GO-based relevant DNA sensor under certain conditions. The mechanism of adsorption and desorption of DNAs can be explained by the general knowledge of the interaction between negatively-charged polyelectrolytes. The simple principle would make it a versatile platform for biosensing. The emergence of new functional nanosheets with bio-applicable properties will lead to the construction of a sophisticated system for bioassay in combination with other functional material.

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Supporting Information

Detailed information on the synthesis and characterization of RuO$_2$ nanosheet. Adsorption and desorption behavior of the probe in a higher Mg$^{2+}$ concentration. Adsorption behavior of probe/capture duplex on GO or RuO$_2$. Desorption behavior of probe from probe/capture/RuO$_2$ or probe/capture/GO. These materials are available free of charge on the Web at http://www.jsac.or.jp/analsci/.

References
1. G. Liu, W. Jin, and N. Xu, *Angew. Chem. Int. Ed.*, **2016**, 55, 13384.

2. S. Navalon, A. Dhakshinamoorthy, M. Alvaro, and H. Garcia, *Coord. Chem. Rev.*, **2016**, 312, 99.

3. (a) T. Miyahata, Y. Kitamura, A. Futamura, H. Matsuura, K. Hatakeyama, M. Koinuma, Y. Matsumoto, and T. Ihara, *Chem. Commun.*, **2013**, 49, 10139. (b) Y. Kitamura, T. Miyahata, H. Matsuura, K. Hatakeyama, T. Taniguchi, M. Koinuma, Y. Matsumoto, and T. Ihara, *Chem. Lett.*, **2015**, 44, 1353. (c) L. Mo, J. Li, Q. Liu, L. Qiu, and W. Tan, *Biosens. Bioelectron.*, **2017**, 89, 201. (d) F. Tian, J. Lyu, J. Shi, and M. Yang, *Biosens. Bioelectron.*, **2017**, 89, 123.

4. (a) H. S. Lee, S.-W. Min, Y.-G. Chang, M. K. Park, T. Nam, H. Kim, J. H. Kim, S. Ryu, and S. Im, *Nano Lett.*, **2012**, 12, 3695. (b) Q. Lu, Y. Yu, Q. Ma, B. Chen, and H. Zhang, *Adv. Mater.*, **2016**, 28, 1917. (c) X. Wang, S. Kajiyama, H. Iinuma, E. Hosono, S. Oro, I. Moriguchi, M. Okubo, and A. Yamada, *Nat. Commun.*, **2015**, 6, 6544.

5. (a) V. Georgakilas, M. Otyepka, A. B. Bourlinos, V. Chandra, N. Kim, K. C. Kemp, P. Hobza, R. Zboril, and K. S. Kim, *Chem. Rev.*, **2012**, 112, 6156. (b) M. Xu, T. Liang, M. Shi, and H. Chen, *Chem. Rev.*, **2013**, 113, 3766.

7. (a) L. Chen, J. Liu, X. Gu, H. Gong, X. Shi, T. Liu, C. Wang, X. Wang, G. Liu, H. Xing, W. Bu, B. Sun, and Z. Liu, *Adv. Mater.*, **2014**, 26, 1886. (b) Q. Xi, D.-M. Zhou, Y.-Y. Kan, J. Ge, Z.-K. Wu, R.-Q. Yu, and J.-H. Jiang, *Anal. Chem.*, **2014**, 86, 1361.

8. (a) L. Yan, H. Shi, X. Shi, Z. Deng, and L. Gao, *RSC Adv.*, **2017**, 7, 23573. (b) S. Su, W. Cao, W. Liu, Z. Lu, D. Zhu, J. Chao, L. Weng, L. Wang, C. Fan, and L. Wang, *Biosens. Bioelectron.*, **2017**, 94, 552.

9. (a) Z. Zhao, H. Fan, G. Zhou, H. Bai, H. Liang, R. Wang, X. Zhang, and W. Tan, *J. Am. Chem. Soc.*, **2014**, 136, 11220. (b) Y. Yuan, S. Wu, F. Shu, and Z. Liu, *Chem. Commun.*, **2014**, 50, 1095.
10. T. Nakato, J. Kawamata, and S. Takagi, “Inorganic Nanosheets and Nanosheet-Based Materials: Fundamentals and Applications of Two-Dimensional Systems”, 2017, Springer Japan KK, Tokyo, Japan.

11. Q. Lu, Y. Yu, Q. Ma, B. Chen, and H. Zhang, Adv. Mater., 2016, 28, 1917.

12. (a) J. Lim, J. M. Lee, C. Kim, S.-J. Hwang, J. Lee, and W. Choi, Environ. Sci.: Nano, 2019, 6, 2084. (b) W. Sugimoto, H. Iwata, Y. Yasunaga, Y. Murakami, and Y. Takasu, Angew. Chem. Int. Ed., 2003, 42, 4092.

13. (a) K. Fukuda, H. Kato, J. Sato, W. Sugimoto, and Y. Takasu, J. Solid State Chem., 2009, 182, 2997. (b) W. Sugimoto, K. Yokoshima, K. Ohuchi, Y. Murakami, and Y. Takasu, J. Electrochem. Soc., 2006, 153, A255.

14. W. S. Hummers, and R. E. Offeman, J. Am. Chem. Soc., 1958, 80, 1339.

15. S. He, B. Song, D. Li, C. Zhu, W. Qi, Y. Wen, L. Wang, S. Song, H. Fang, and C. Fan, Adv. Funct. Mater., 2010, 20, 453.

16. C. Lu, P.-J. J. Huang, B. Liu, Y. Ying, and J. Liu, Langmuir, 2016, 32, 10776.
Figure Captions

Fig. 1 (a) RuO$_2$-based DNA sensing system using adsorption–desorption of the fluorescent DNA probe. (b) Sequences of DNA used in this study.

Fig. 2 Fluorescence intensity changes ($\lambda = 517$ nm) of probe as a function of (a) GO and (b) RuO$_2$ concentration. The excitation wavelength was 490 nm. The measurements were carried out in a 5 mM sodium phosphate buffer (pH 7.0) containing 500 mM NaCl and 2 mM MgCl$_2$ at 5 °C. Concentration of probe was 10 nM.

Fig. 3 (a) Time dependencies of probe release from RuO$_2$ (filled circle) and GO (open circle) in the presence of 20 nM fmDNA (solid curve) or scDNA (dotted curve). The rates of released probe were calculated by normalizing the emission intensities based on the intensities before and after probe adsorption. Concentration of probe was 10 nM. Concentrations of RuO$_2$ and GO were 50 μg/mL and 1.2 μg/mL, respectively. (b) Percentages of probe released from RuO$_2$ and GO after the incubation with fmDNA or scDNA for 60 min. Measurements were carried out in a 5 mM sodium phosphate buffer (pH 7.0) containing 500 mM NaCl and 2 mM MgCl$_2$ at 5 °C.
Figures

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