A Method for SNP Genotyping Using MoS₂ and a DNA Intercalating Dye

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Abstract. The genotype is an association with aerobic capacity in the human sports. Therefore, a simple and rapid method for detecting SNPs was developed in this study. SG (SYBR Green I), DNA sequences, and molybdenum disulfide (MoS₂) were used in this sensing system. MoS₂ quenched the fluorescence from SG intercalated into DNA duplex. The SG fluorescence signals from the matched and mismatched DNA duplex were significant different after the addition of MoS₂. This method can clearly distinguish SNPs in DNA sequences.

Introduction

Single nucleotide polymorphisms (SNPs) are important markers genetic studies. Some studies have reported an association between genotype and human aerobic capacity [1]. Therefore, SNPs can be used to powerful tools in the study of human sports. Up to now, a variety of methods have been used for SNPs determination including nucleotide incorporation [2], sequence cleavage [3], molecular beacon [4, 5], direct sequencing [6], and fluorescence energy transfer [7]. However, some methods are limited by requirements for complicated procedures, time-consuming or expensive reagents. Therefore, developing a simple and rapid detection method for SNPs is one of the promising goals for a wide variety of applications. Recently, MoS₂ emerging two-dimensional-layered materials analogous to graphene has been attracting much attention because of its unique physical and chemical properties, such as unusual optical properties, and energy harvesting properties [8, 9]. At present, most applications based on MoS₂ are fluorescence-based methods. In this study, we developed a label-free, simple and rapid method for SNPs discrimination at room temperature based on MoS₂.

Materials and Methods

Materials and Chemicals

ssDNA probes, as well as the matched and mismatched DNA targets were synthesized by Sangon (Shanghai, China) and were employed to evaluate the SNPs discrimination. Their sequences were listed in Table 1. Other chemicals were purchased from Sigma (Shanghai, China). Ultrapure water (18.2 MV cm) was used in the experiments. As illustrated in Scheme 1, SG (SYBR Green I) intercalated well into perfectly matched dsDNA (P2T) and resulted in high fluorescence.
Table 1. DNA probes and other oligonucleotides used in the experiments.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence (5’ to 3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>A GCA TCT TAT CCG GGT</td>
</tr>
<tr>
<td>P</td>
<td>ACC CGG ATA AGA TGC T</td>
</tr>
<tr>
<td>M1</td>
<td>ACC GGG ATA AGA TGC T</td>
</tr>
<tr>
<td>M2</td>
<td>ACT GGG ATA AGA TGC T</td>
</tr>
<tr>
<td>M3</td>
<td>ACT GGG ATA AGA TCC T</td>
</tr>
</tbody>
</table>

Sensor Preparation

MoS$_2$ nanosheets prepared by chemical exfoliation according to the method reported by Eda$^{[10]}$. MoS$_2$ powder was dissolved in Milli-Q ultrapure water and then sonicated for 30 min (1000 W). Then a homogeneous MoS$_2$ aqueous dispersion was obtained for use. The solution containing 10 nM DNA was obtained by using PBS buffer to dilute the stock solution.

Fluorescence Spectroscopy Analysis

A fluorescence spectrometer (F-4600, Hitachi Co. Ltd., Japan) with a Xenon lamp excitation source was employed to record fluorescence spectra. The fluorescence measurements were carried out using a Cary Eclipse spectrophotometer. The optical path length of a quartz fluorescence cell was 1.0 cm. The excitation was set at 497 nm and the emission was monitored at 525 nm. The fitting of the experimental data was accomplished with the software Origin 8.0.

Results and Discussion

Design Strategy for SNPs Detection

The sensing was performed in solutions using single-stranded DNA (ssDNA) probes, SYGreen I (SG), and MoS$_2$. The matched (T) or non-matched DNA targets (M1, M2, M3) can hybridize with ssDNA (P) and form DNA duplex. The SG molecules intercalated into the DNA duplex. MoS$_2$ unwinded the mismatched DNA duplex through the interaction between MoS$_2$ and ssDNA. The matched DNA duplex had the weaker MoS$_2$/DNA binding than mismatched DNA duplex. MoS$_2$ quenched the fluorescence of SG. Therefore, the SG from matched DNA duplex had higher fluorescence intensity after the addition of MoS$_2$. Then SNPs can be detected by using this method.

Optimization of MoS$_2$ Concentration for this Sensor

The different fluorescence intensities of SG intercalated into matched DNA duplex with the change of MoS$_2$ concentrations was showed in Fig.2. The fluorescence intensity of SG
trended to a minimum value at 2.5µg/mL and 80% of the fluorescence was quenched. Higher concentration of MoS\textsubscript{2} than 2.5µg/mL was not only redundancy. Therefore, 2.5µg/mL MoS\textsubscript{2} was used for the following experiments.

![Figure 2](image)

Figure 2. The fluorescence intensity of SG intercalated into DNA (10 nM) in the presence of various concentrations of MoS\textsubscript{2} (0.33, 0.66, 1, 1.5, 1.83, 2.17, 2.5, 2.83, 3.17, 3.5µg/mL).

**Kinetic Behavior of MoS\textsubscript{2}-DNA based Sensor**

The fluorescence quenching kinetics of the MoS\textsubscript{2}-DNA based sensor for the detection of SNPs was studied in the presence of MoS\textsubscript{2}. Fig.3 showed the intensity of fluorescence was rapidly decreased after the DNA duplex was adsorbed on the surface of MoS\textsubscript{2} at room temperature (RT). The fluorescence intensity decreased with prolonged time and reached equilibrium in 20 min (Fig.3). About 80% fluorescence of P2T was quenched by MoS\textsubscript{2}. Therefore, we performed the following experiments in 20 min.

![Figure 3](image)

Figure 3. Kinetic behavior of MoS\textsubscript{2}-DNA based sensor.

**Temperature Investigation of MoS\textsubscript{2}-DNA based Sensor**

As shown in Fig 4, the detection temperature was also investigated. The fluorescence intensity of P2T decreased with temperature increasing. However, that of P1MT remained at a low level. The difference between P2T and P1MT by this MoS\textsubscript{2}-based sensor can be found
from 25 to 55 °C. Therefore, temperature control was not necessary for SNPs detection in this method.

![Graph showing fluorescence intensity vs. temperature](image)

**Figure 4.** The fluorescence intensity of P2T and P1MT in the presence of MoS$_2$ at different temperatures (25, 30, 35, 40, 45, 50, 55°C).

**Detection Performance**

P1MT, P2MT and P3MT were respectively 1, 2 and 3 bases mismatched. P1MT had weaker binding to MoS$_2$ than P2MT and P3MT. In this study, MoS$_2$ was introduced to improve the single base mismatch discrimination. As shown in Fig.4a, the fluorescence intensity of P2T was much higher than that of T and PMT after the addition of MoS$_2$. Therefore, P1MT had higher fluorescence intensity than P2MT and P3MT. The enlarged fluorescence difference between P2T and PMT showed great improvement of S/N (Figure 5b). The signal ratios for matched targets were much higher than that for mismatched.

![Graph showing fluorescence intensity vs. wavelength](image)

**Figure 5.** (a) The fluorescence intensity of SG/DNA complexes in the presence of MoS$_2$. (b) $F_{P2T}/F_{PMT}$ and $F_{P2T}/F_T$ in the absence and presence of MoS$_2$. P, T, and M were 10 nM, respectively. 2.5µg/mL MoS$_2$ together with 0.5×SG were used.

All procedures were performed at RT.

It is also noted that the whole reaction process was completed within 20 min for all the DNA targets, which was much faster than most of previous methods for SNPs detection (see Table 2). This showed this method was rapid for SNPs detection.
Table 2. Comparison of Some Methods for SNPs Detection.

<table>
<thead>
<tr>
<th>Sensing system</th>
<th>Detection signal</th>
<th>Detection time</th>
<th>Ref</th>
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</thead>
<tbody>
<tr>
<td>ligase</td>
<td>absorbance</td>
<td>10 min</td>
<td>2</td>
</tr>
<tr>
<td>exonuclease III</td>
<td>fluorescence</td>
<td>30 min</td>
<td>3</td>
</tr>
<tr>
<td>molecular beacon</td>
<td>fluorescence</td>
<td>15 min</td>
<td>4</td>
</tr>
<tr>
<td>molecular beacon</td>
<td>fluorescence</td>
<td>&gt;2 h</td>
<td>5</td>
</tr>
<tr>
<td>triple-stem probes</td>
<td>fluorescence</td>
<td>30 min</td>
<td>11</td>
</tr>
<tr>
<td>peptide nucleic acid</td>
<td>absorbance</td>
<td>35 min</td>
<td>12</td>
</tr>
<tr>
<td>silver nanoclusters</td>
<td>fluorescence</td>
<td>1h</td>
<td>7</td>
</tr>
<tr>
<td>toehold exchange reaction</td>
<td>optical resonance</td>
<td>40 min</td>
<td>13</td>
</tr>
<tr>
<td>DNA intercalating dye/MoS$_2$</td>
<td>fluorescence</td>
<td>20 min</td>
<td>The present work</td>
</tr>
</tbody>
</table>

Summary

We developed a simple method based on MoS$_2$ for rapid and reliable detection of SNPs in DNA sequence by employing superquencher (MoS$_2$) and DNA intercalator (SG). The mismatched DNA sequences could be differentiated from the matched DNA targets based on the signal changes. MoS$_2$ improved the S/N through lowering the fluorescence response of ssDNA. This method was a label-free approach and provides insights into improving SNPs detection. Therefore, it was a promising technique and may inspire application in human sports.

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References


