Developments of Highly Sensitive DNA Sensors

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ABSTRACT

The large enhancements of optical properties of the dye-intercalated DNA lead us to apply the dye-intercalated DNA as various sensors with a high sensitivity to detect environmentally toxic gases such as dioxine, NOx or carbon monoxide. This paper retorts on DNA sensors for the further applications of DNA as materials. Also, bio-medical applications of DNA sensors such as a glucose sensor are reported.

1. INTRODUCTION

Pure DNA which is isolated from Salmon roe has a huge high molecular weight of over billion and can form a strong and uniform film and the double helical structure of DNA has a characteristic feature of intercalation of various optical dyes among stacked layers of nucleic acid bases to enhance optical properties of dyes , so that applications of DNA as materials are now possible in such areas as photonics, separation process or biomedical materials. Recent research results on DNA-lipid complexes have shown various attractive applications such as E/O or O/E devices, optical memories, switches and sensors[1-4]. It was reported[2] to study on possibility of basic optical characteristics, such as refractive indices, absorbance and fluorescence intensity, and photochromic properties, of spiropyran-doped DNA-cetyltrimethylammonium (CTMA) complex films, which were derived from DNA from Salmon, showed potential applications to optical switches[5, 6]. Although DNA-lipid complexes showed promising potentials for optical functional devices such as switching or signal processing devices, their response speeds were relatively slow to apply them to practical uses. It was shown[5, 6] that much faster response speed (switching times) could be attained by increasing the excitation light intensity. Thus, applications of DNA photonic devices have been widely studied in the world[7-10]

The large enhancements of optical properties of the dye-intercalated DNA lead us to apply the dye-intercalated DNA as various sensors with a high sensitivity to detect environmentally toxic gases such as dioxine, NOx or carbon monoxide. This paper retorts on DNA sensors for the further applications of DNA as materials. Also, bio-medical applications of DNA sensors such as a glucose sensor are reported.

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2. EXPERIMENTALS

2.1 Preparation of DNA-Lipid Complex Films

Figure 1 shows the preparative method of DNA-lipid complex films. Single-chain trimethylammonium type lipid (CTMA hereafter) was used to form DNA-lipid complexes. First, refined DNA was dissolved in distilled water. Lipid solution dissolved in distilled water was mixed with the DNA aqueous solution. Then, the DNA-lipid complex was washed in distilled water, followed by drying process in a vacuum oven for 24 hours at 40^0C. After drying process, the DNA-lipid complex was dissolved in mixed solution of EtOH:$CHCl_3$=1:4, together with optical dye compounds. Finally, the solution was poured onto a Teflon-coated dish, followed by evaporating the solvent to obtain films, as schematically shown as below:

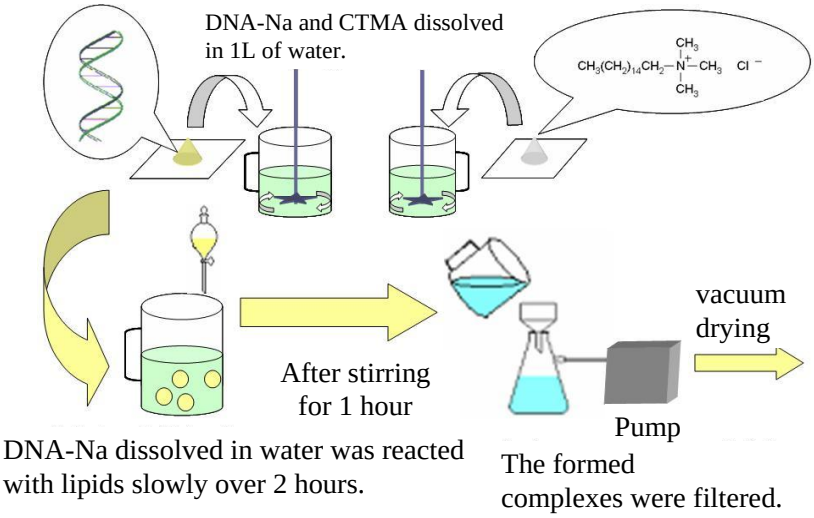


Figure 1 Preparative method of DNA-lipid complex films

The DNA-CTMA complex was dissolved in ethanol in an amount of 1g/100ml and the solution was cast on a Tefron-coated glass, followed by evaporating ethanol at 50^0C to obtain DNA-CTMA films.[1).]

2.2 DNA sensors

(1) NOx sensor

Porphyrin was intercalated into DNA-CTMA by mixing 1g porphyrin and 1g of DNA-CTMA complex in 100ml of ethanol, followed by casting on a Teflon-coated glass to obtain the porphyrin-intercalated DNA-CTMA films. The porphyrin-intercalated DNA-CTMA films were subjected to expose in air containing various amounts of NOx. Visible spectra of the NOx-exposed DNA-CTMA film intercalated with porphyrin were measured.

(2) Glucose sensor

Various dyes were intercalated into DNA-CTMA film before or after the intercalation of dyes which was carried out by dipping the DNA-CTMA film into water-ethanol (1/1 in volume) of dyes.. Following dyes were used. These DNA-CTMA films intercalated with these dyes were dipped into aqueous solution of glucose for 1 hr, followed by drying and exposing with green light of 350nm to measure fluorescence light changes.

Intercalated dyes

1. aromatic boronic acids
2. Rhodamin 6G
3. Tetraphenylporphyrin
4. Ethidium bromide
5. Cholorofile
6. Raser-emitting dyes

3. RESULTS

3.1 NOx gas sensor

Tetramethylporphyrin (TMPy) or tetraphenylporphyrin (TPPy) were doped into the DNA-CTMA Films. Visible spectra before and after exposing the TPPy-doped film in the air containing 5ppm NOx gas are shown in Figure 1, which showed absorption shift from 424nm to 442nm.

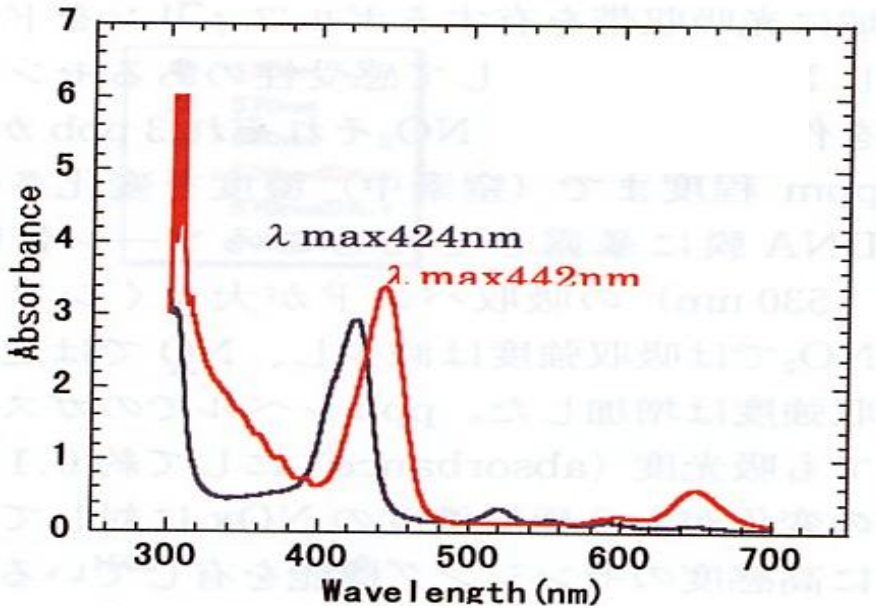


Figure 1 Spectrum change of DNA−CTMA film doped with tetraphenylporfirin (TPPy) In the presence of NOx gas

The DNA-CTMA ethanol solution (1g in 100ml) was cast on the surface of quartz plate of QCM in order to measure weight differences before and after exposing in the air containing 3ppb NOx gas and results are summarized in Figure 2.

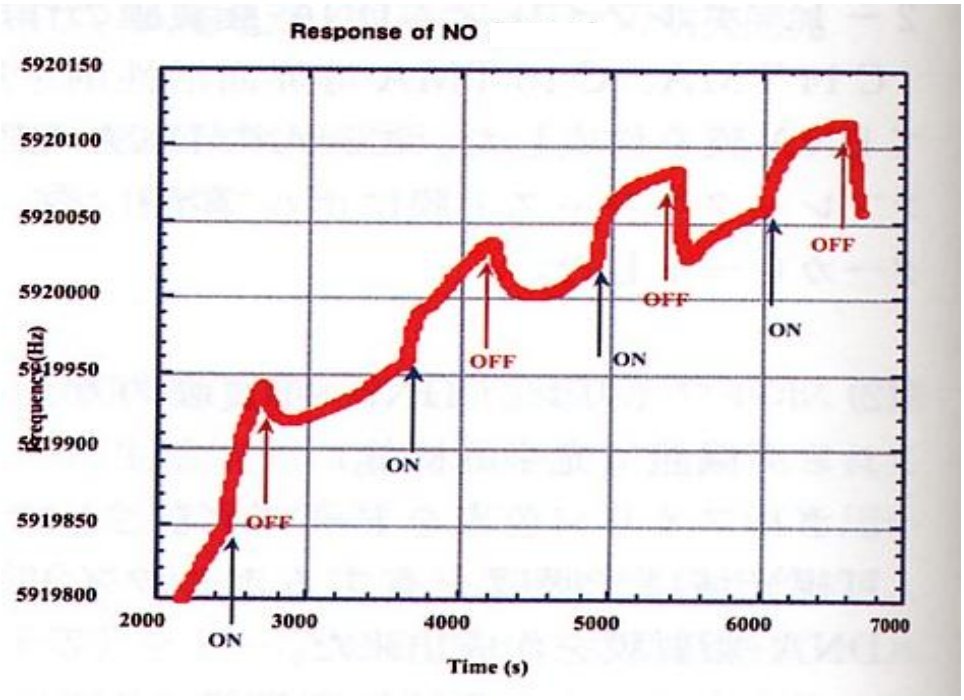


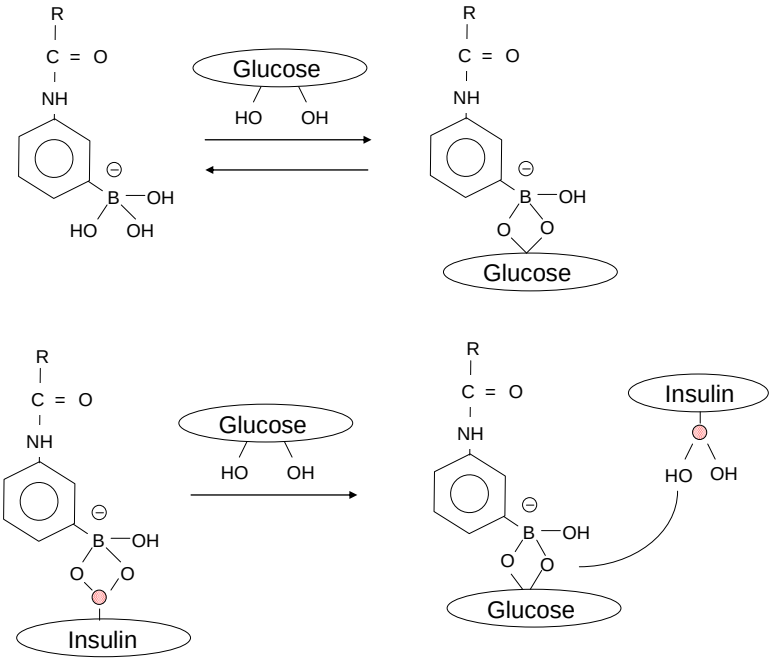
Figure 2 Weight differences before and after exposing in the air containing 5ppb NO gas.

Figure 2 shows rapid responses of weight changes by repeating the exposure in NO-containing air (5ppb) and clean air, so that these results support that the DNA-CTMA film containing TPPy can be applied to a highly sensitive NOx gas sensor.

3.2 Glucose sensor

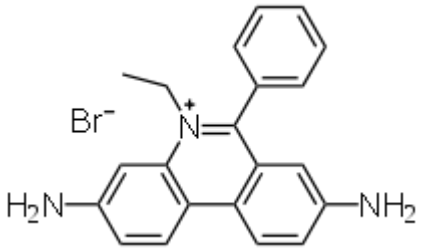
Various dyes as shown in experimental section were intercalated into DNA-CTMA as 1/1 molar ratio to obtain DNA-CTMA films. These dye-intercalated DNA-CTMA films were irradiated by a raser of 350nm light in order to measure fluorescence light intensity. It is known that aromatic boronic acids can form a selective hydrogen-bonding with glucose as shown below:

Aromatic boronic acid combines selectively with glucose.



No fluorescence enhancements were observed when the DNA-CTMA films were doped with phenyl or naphthalene boronic acids, presumably no intercalation occurred. On the other hand, when 2-anthraceneboronic acid (2-ABA)was doped in the DNA-CTMA film, strong enhancements of fluorescence light were observed by irradiating the doped films with 350nm raser light, as clearly seen in Figure 3. However, no change of the fluorescence intensity occurred when the 2-ABA-doped DNA-CTMA film was immersed in aqueous solution of glucose of 1/1 molar ratio for 1hr, followed by measuring fluorescence light. Therefore, 2-ABA doped DNA-CTMA film is not applicable for the glucose sensor.

Various dyes were intercalated into DNA-CTMA films to measure fluorescence intensity changes and results are summarized in Table 1. It was found that only ethidiumu bromide (EtBr) which was known as a typical intercalater for DNA showed spectrum changes in the presence of glucose in aqueous solution, as shown in Figure 4. The chemical structure of EtBr is shown as below:



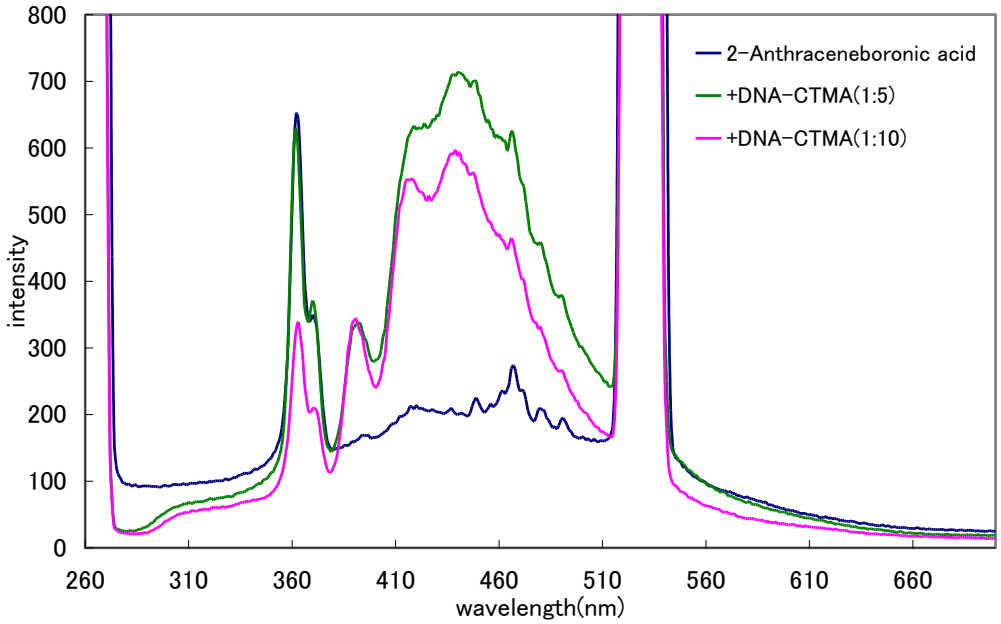


Figure 3 Fluorescence spectrum of DNA-CTMA film, doped with 2-anthracene boronic acid

Table 1 Effect of dye intercalation of dyes intercalated into DNA-CTMA

Intercaraters	Detection of glucose	Fluorescence change
1. Aromatic boronic acids		
Phenyl boronic acid	x	no change
Naphthalene boronic acid	△	Quenching
Anthracene boronic acid	○	Luminescence
2. Chlorofil a	x	no change
3. Tetraphenylporphyrin	△	Luminescence
4. Ethidium bromide	◎	Luminescence

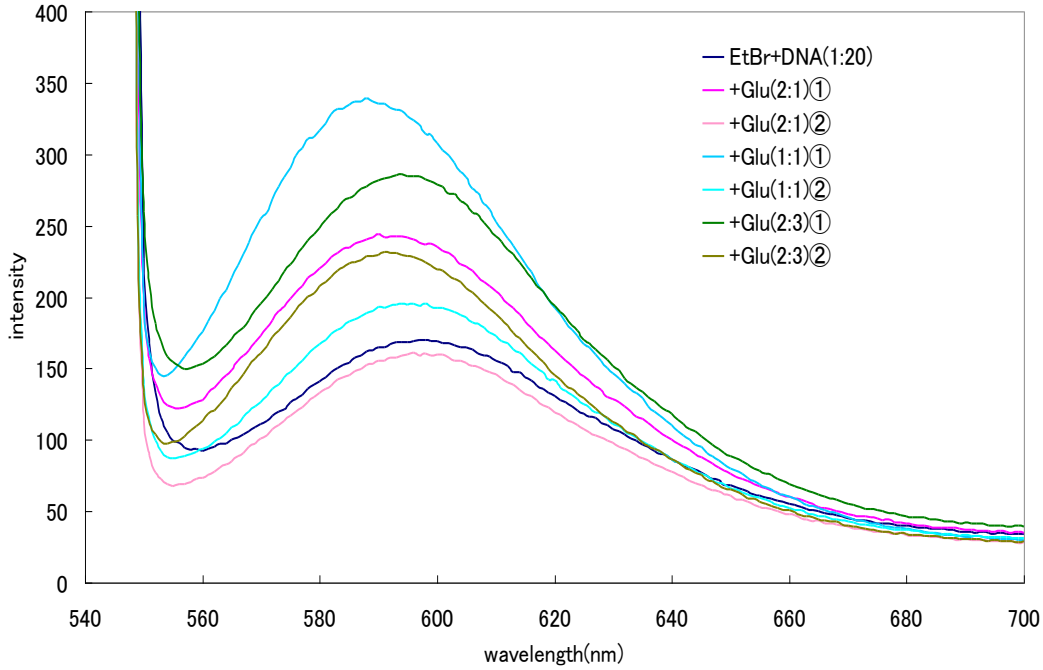


Figure 4 Fluorescence spectra of DNA film, doped with ethidium bromide (EtBr) in the presence of glucose (Glucose concentration corresponded to 100, molar ratios to DNA base pairs)

Absorption intensities at 590nm fluorescence light increased as function of glucose concentration, as shown in Figure 5. Thus, EtBr-intercalated DNA-CTMA films can be applied to detect glucose in aqueous solutions by measuring 590nm fluorescence intensity changes.

The reason why the fluorescence intensities at 590nm of the EtBr-intercalated DNA-CTMA occurred is not clear yet. It may be assumed that strong hydrogen bonding between EtBr with glucose may interact with the intercalation of EtBr into nucleic acid base pair layers of DNA double helix, so that this interaction of glucose may enhance the fluorescence intensity as a results. Further investigations of these phenomena are required to clarify the glucose interactions with EtBr.

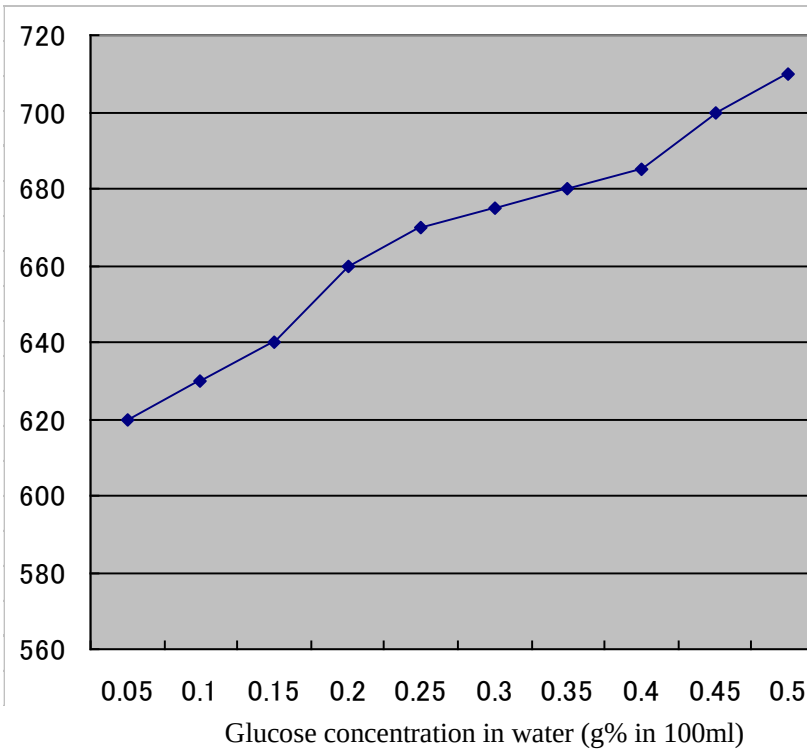


Figure 5 Fluorescence intensity changes as function of glucose concentration

4. Conclusion

It was found in this paper that the DNA-CTMA which was intercalated with various dyes could be applied to highly sensitive sensors to detect NOx gas in the air and also to measure glucose concentration in blood by measuring fluorescence intensity changes of the dye-intercalated DNA-CTMA films.

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