SHORT COMMUNICATION

Chungang Wang · Zhanfang Ma

Colorimetric detection of oligonucleotides using a polydiacetylene vesicle sensor

Abstract A new system for the colorimetric detection of oligonucleotides was developed using polydiacetylene vesicles, which play the dual role of an indicator of color transition and an amplification tag. The results are of significance in understanding the mechanism of color transition of biological recognition in polydiacetylene systems and in designing new biosensors.

Keywords Polydiacetylene vesicles · Oligonucleotide detection

Introduction

As the number of diseases caused by genetic defects continues to grow, there have been increasing demands for simple, rapid, and reliable methods for the detection of oligonucleotides. Many DNA detection assays have been developed using radioactive labels, molecular fluorophores, chemiluminescence schemes, electrochemical tags, nanostructure-based labels [1–5], and conjugated polymers [6, 7]. Conjugated polymers such as polydiacetylene and polythiophene display a remarkable array of color changes that arise from thermal transitions, mechanical stress, and ion binding. Accordingly, the development of efficient sensors utilizing conjugated polymers, especially polydiacetylene, as sensing matrices has received much attention from researchers [8, 9]. The polydiacetylene functions as the signaling component of the sensor, while selective recognition is provided by including lipids that have been modified with a receptor for the analyte of interest. The cross-linked polydiacetylene vesicles have a deep blue color as a result of the extensively conjugated polymer backbone. Binding of the analyte to the embedded receptor causes the vesicles to change color from blue to red. In this work, we therefore report a new colorimetric method for the detection of oligonucleotides using polydiacetylene vesicles functionalized with probe oligonucleotides which play the dual role of color transition indicator and amplification tag. The sequences of oligonucleotides used in this work are as follows:

Probe 1: 5′-TCTCAACTCGTATTTTTT-$(CH_2)_3$-cholesteryl-3′
Probe 2: 5′-cholesteryl-$(CH_2)_3$-TTTTTTCGCATT-CAGGAT-3′
Target DNA: 5′-TACGAGTTGAGAATCCTG-AATGCG-3′
Mismatched DNA: 5′-GCGTAACTCCTAAGAGT-TGAGCTA-3′

Experimental

Preparation of polydiacetylene vesicles

10,12-Tricosadiynoic acid (TCDA) was purchased from Fluka (Switzerland) and was used without further purification. The mixtures of TCDA (70%), dimyristoylphosphatidylcholine (DMPC) (29 mol %), and probe oligonucleotides (probe 1 or 2, 1%) were dried using a rotary evaporator, followed by addition of 10 mM PBS buffer solution (pH 7.0) and probe sonication at ca. 70°C. The vesicle solution obtained was cooled and kept at 4°C overnight and then polymerized using UV light irradiation at 254 nm for 10 min. The resulting polydiacetylene vesicle solution exhibits an intense blue appearance.

Quantitative assay

The color change of the mixed vesicles from blue to red was quantified by calculating the colorimetric response (CR) according to following equation as previously reported [18]. The CR is derived from the change in the

C. Wang · Z. Ma (✉)
Chemistry Department, Northeast Normal University,
Changchun 130024, P. R. China
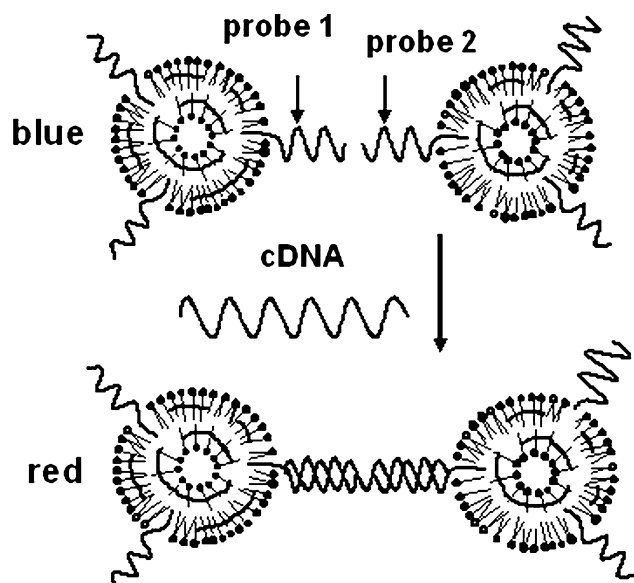E-mail: maf097@nenu.edu.cn

ratio of absorbances at 630 and 540 nm in the absence (B_0) and presence (B_1) of target oligonucleotide molecules.

$$CR = [(B_0 - B_1)/B_0] \times 100\%$$

where $B = A_{blue}/(A_{blue} + A_{red})$; A is the absorbance at either the "blue" component in the UV–Vis spectrum (ca. 630 nm) or "red" component (ca. 540 nm); B_0 is the red/blue ratio of the control sample; and B_1 is the value obtained after addition of target DNA and the other vesicle solution.
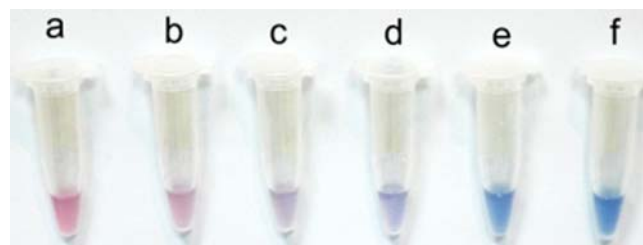
Results and discussion

The schematic diagram of the method used in this study is shown in Fig. 1. Since polydiacetylenic structures can change color with environmental perturbations such as mechanical stress (mechanochromism) [10, 11], temperature (thermochromism) [12–14], biological recognition (affinochromism/biochromism) [15–20], and changes in chemical environment [21], commonly used methods to functionalize polydiyacetylene-based sensors include chemically connecting a functional group to the diacetylene molecule and inserting the obtained probe into a diacetylene matrix monolayer or vesicle, or directly inserting functional molecules into a polydiacetylene matrix monolayer or vesicle through non-covalent bonding. For either of these methods, the recognition between functional molecule and corresponding receptor with larger molecular weight and volume may easily cause perturbation of the conjugated polydiacetylene backbone, resulting in the color change from blue to red during the recognition. For oligonucleotides with linear structures, however, the recognition between probe oligonucleotides and target DNA molecules may be insufficient to cause perturbation of the ene–yne alternating polydiacetylene chain if no amplification tag is used. Thus, we proposed a new oligonucleotide recognition model using polydiacetylene vesicles as the indicator and amplification tag at the same time during the recognition, as shown in Fig. 1. Probe oligonucleotide 1 (probe 1) and probe 2 are partially complementary to opposite ends of the target DNA. The recognition took place between probe 1 inserted into polydiacetylene vesicles (vesicle 1) and target DNA molecule and this then was amplified with other polydiacetylene vesicles containing probe 2 (vesicle 2) through the hybridization of probe 2 and target DNA. In this case, the force imposed on the conjugated backbones of vesicle 1 was remarkably enhanced by vesicle 2. At the same time, the similar force imposed on vesicle 2 was also enhanced by vesicle 1 through the interaction between target DNA and probe 1 in vesicle 1. Vesicles 1 and 2 therefore both play the roles of color change indicator and amplification tag in the detection of oligonucleotides.

The polymerized vesicles obtained appeared deeply blue (Fig. 2) and showed an absorption maximum at 640 nm (Fig. 3, curve a). After 20 μM target DNA molecules was added to the mixture of the vesicles 1 and 2 at room temperature under stirring, the color of the mixed system turned from deeply blue to red as shown in Fig. 2. The absorption maximum shifted from 640 to 540 nm (Fig. 3, curve b) and the corresponding CR was ca. 34% (Fig. 4a). With the decrease of the concentration of target DNA, the tendency of the color change from blue to red weakened and the corresponding CRs gradually decreased as shown in Fig. 4. For 2 nM target DNA, no remarkable color change was observed with the naked eye; the corresponding CR is ca. 2.5%. For the control experiment, no color change was observed with the naked eye when the PBS buffer (pH 7.0) was added instead of target DNA to the mixed polydiacetylene vesicle solution, and the corresponding CR was less than 0.5%. These results illustrate that this method is able to detect oligonucleotides at the nanomolar level. For the mismatched oligonucleotides with 20 μM, no color changes were observed, and the corresponding CR was less than 4% as shown in Fig. 4g.

Fig. 1 Schematic diagram of the colorimetric detection of DNA using polydiacetylene vesicles functionalized with probe DNA

Fig. 2 Images of DMPC/TCDA vesicles containing probes 1 and 2 used to detect target DNA. Concentrations of target DNA: **a** 20 μM; **b** 2 μM; **c** 200 nM; **d** 20 nM; **e** 2 nM; **f** control
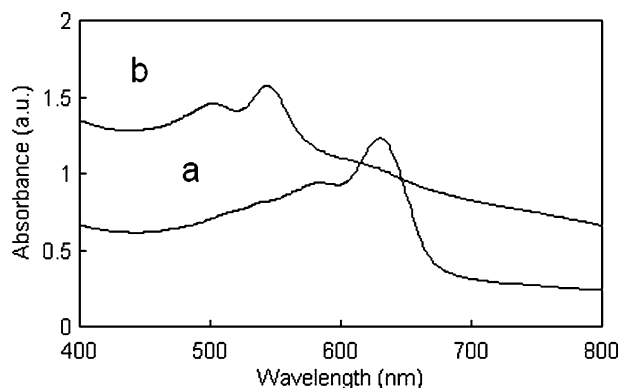
Fig. 3 Representative visible spectra of the mixture of vesicle suspensions containing probes 1 and 2 without target DNA (**a**) and after 20 μM target DNA addition (**b**)
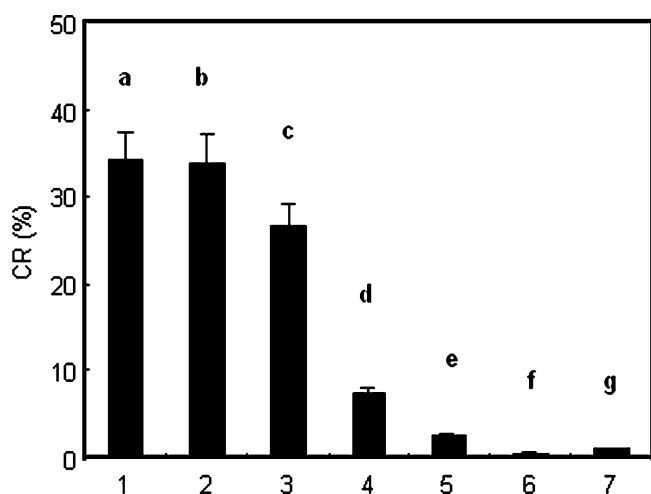
Fig. 4 CR values obtained upon addition of target DNA and mismatched DNA to the vesicles 1 and 2 (with volume ratio 1:5). Concentrations of target DNA: **a** 20 μM; **b** 2 μM; **c** 200 nM; **d** 20 nM; **e** 2 nM; **f** control; **g** 20 μM mDNA

To address the effect of non-specific interaction between vesicles and target DNA, vesicles without probe oligonucleotides were prepared. These vesicles did not change color after exposure to target DNA solution even at high concentration (20 μM), and the corresponding CR is ca. 3%. When 20 μM target DNA was added into vesicle 1 without vesicle 2, no color change was observed. Similar results were obtained when 20 μM target DNA was added into vesicle 2 without vesicle 1. These results indicated that vesicles 1 and 2 play the dual roles of color change indicator and amplification tag during the detection of oligonucleotide for the system used.

Conclusion

We have developed a new colorimetric method to detect oligonucleotides using polydiacetylene vesicles. In this system, polydiacetylene vesicles play the dual role of indicator of color transition and amplification tag. The results illustrate a new approach to the detection of oligonucleotide molecules and are of significance in understanding the mechanism of color transition of biological recognition for polydiacetylene systems and in designing new biosensors.

References

1. Nicewarner-Pena SR, Freeman RG, Reiss BD, He L, Pena DJ, Walton ID, Cromer R, Keating CD, Natan MJ (2001) Science 294:137–141
2. Zhao XJ, Tapec-Dytioco R, Tan WH (2003) J Am Chem Soc 125:11474–11475
3. Yu CJ, Wan YJ, Yowanto H, Li J, Tao CL, James MD, Tan CL, Blackburn GF, Meade TJ (2001) J Am Chem Soc 123:11155–11161
4. Park S-J, Taton TA, Mirkin CA (2002) Science 295:1503–1506
5. Ma ZF, Li JR, Jiang L, Yang M, Sui SF (2002) Chem Lett 3:328–329
6. Leclerc M (1999) Adv Mater 11:1491–1498
7. Ho HA, Boissinot M, Bergeron MG, Corbeil G, Dore K, Boudreau D, Leclerc M (2002) Angew Chem Int Ed 41:1548–1551
8. Okada S, Peng S, Spevak W, Charych DH (1998) Acc Chem Res 31:229–239
9. Jelinek R, Kolusheva S (2001) Biotechnol Adv 19:109–118
10. Nallicheri RA, Runer MF (1991) Macromolecules 24:517–525
11. Carpick RW, Sasaki DY, Burns AR (2000) Langmuir 16:1270–1278
12. Carpick RW, Mayer TM, Sasaki DY, Burns AR (2000) Langmuir 16:4639–4647
13. Lee DC, Sahoo SK, Cholli AL, Sandman DJ (2002) Macromolecules 35:4347–4355
14. Deckert AA, Fallon L, Kiernan L, Cashin C, Perrone A, Encalarde T (1994) Langmuir 10:1948–1954
15. Ma ZF, Li JR, Liu MH, Cao J, Zou ZY, Tu J, Jiang L (1998) J Am Chem Soc 120:12678–12679
16. Ma ZF, Li JR, Jiang L, Cao J, Boullanger P (2000) Langmuir 16:7801–7804
17. Gill I, Ballesteros A (2003) Angew Chem Int Ed 42:3264–3267
18. Reichert A, Nagy JO, Spevak W, Charych DH (1995) J Am Chem Soc 117:829–830
19. Pan JJ, Charych DH (1997) Langmuir 13:1365–1367
20. Kolusheva S, Shahal T, Jelinek R (2000) J Am Chem Soc 122:776–780
21. Jonas U, Shah K, Norvez S, Charych DH (1999) J Am Chem Soc 121:4580–4588