Nucleic Acids Symposium Series No. 50 **307–308**
doi:10.1093/nass/nrl153

Electrochemical detection of DNase I activity

Katsuya Fujita[1], Masanori Kanazawa[1,2], Kosuke Mukumoto[3], Takahiko Nojima,[3] Shinobu Sato[4], Hiroki Kondo[4], Michinori Waki[1] and Shigeori Takenaka[1]

[1]Department of Materials Science, Faculty of Engineering, Kyushu Institute of Technology, 1-1 Sensui-cyo, Tobata-ku, Kitakyushu-shi, Fukuoka 804-8550, Japan, [2]BioScience Division, Nihon Millipore K. K. Yokohama 240-0005, Japan, [3]Department of Applied Chemistry, Faculty of Engineering, Kyushu University, Motooka 819-0395 Japan and [4]Department of Bioscience and Bioinformatics, Faculty of Computer Science and Systems Engineering, Kyushu Institute of Technology, 680-4 Kawazu, Iizuka-shi, Fukuoka 820-8502, Japan

ABSTRACT

DNase I in one μl of the water could quantitate electrochemically with the detection limit of 0.01 units (ca. 20 pg) by using the ferrocenyl oligonucleotide-immobilized electrode prepared by thiolated oligonucleotide and ferrocenyl carbodiimide as a simple labeling reagent of redox unit.

INTRODUCTION

DNase I is a polymorphic enzyme which can digest nucleic acid and it is known as a candidate of the probe molecule for the endonucleolytic activity involved in apoptosis or programmed cell death[1] and involved in the initiation of systemic lupus erythematosus.[2] Therefore, it is important to develop the detecting technique of DNase I activity and the method utilized fluorescence resonance energy transfer (FRET) which called DNase Alert[3] or the single radial enzyme-diffusion (SRED) method[4] was developed. On the other hand, electrochemical DNase I assay is especially expected in the possibility of simple and rapid method. Hence, electrochemical nuclease S1 assay was reported by Hillier et al.[5] Electrochemical enzymatic DNA extension detection was successfully achieved as an example of telomerase by us.[6]

We succeeded to the synthesis of ferrocenylcarbodiimide **1** as a simple labeling reagent of nucleic acid.[7] Use of this reagent will lead to thiolated oligonucleotide carrying ferrocene parts to be immobilized on the gold electrode. The electrode carrying the ferrocenyl oligonucleotide should permit an electrochemical DNase I assay. The concept of the system presented here was shown in Fig. 1. After treatment of DNase I, the ferrocenyl oligonucleotide immobilized on the electrode should cleave and lead to the decrease of the amount of ferrocene molecules. This gave a decrease current based on the redox reaction of the ferrocene. Then electrochemical DNase I assay should achieve by the current change between before and after treatment with DNase I. In this system, we chosed the single stranded DNA for the substrate of DNase I to clear the digestion reaction. When double stranded DNA was used as substurate, the niking reaction of DNase I for the oposite strande immobilized on the electrode will not couse the current change. This paper showed the preparation of ferrocenyl oligonucleotide-immobilized electrode and its application for the electrochemical DNase I assay.

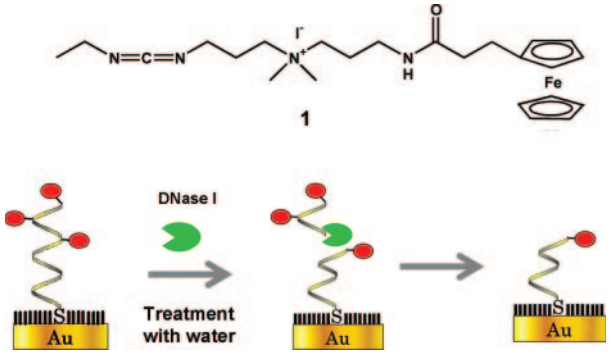

Fig. 1 (A) Chemical structure of ferrocenyl carbodiimide derivative **1** and (B) concept of the electrochemical DNase I assay by using ferrocenyl oligonucleotide-immobilized electrode. Thiolated oligonucleotide having ferrocene units was prepared by using **1** and immobilized on the gold electrode. The solution containing DNase I was dipped on this electrode. DNase I cleaved the DNA on the electrode and the ferrocene units com off from the electrode resulting in the decreased current based on the redox reaction of ferrocene.

RESULTS AND DISCUSSION

The reaction of 5'-protected thiolated oligonucleotide, 5'-HO$(CH_2)_6$-SS-$(CH_2)_6$-d(A_{10} ACA AAT AAC AAA TAT)-3' with excess amount of 1 in 20 mM borate buffer (pH 9.5) containing 30% DMSO for 9 h at 45 °C gave the thiolated oligonucleotide carrying the ferrocene parts. MALDI-TOF-MS measurement before and after DNase I treatment (incubation in 40 mM Tris-HCl (pH 7.5), 80 mM $MgCl_2$, and 50 mM DTT at room temeprature for 30 min) showed that the oligonucleotide thus obtained was modified by **1** at its three thymine sites. This result also shows that the thiolated oligonucelotide modified by ferrocene units can be digested with DNase I under the above condition, which is supprted by the previous work.[7]

The ferrocenyl oligonucleotide-immobilized electrode was prepared as follows: the protected group of the thiolated oligonucleotide carrying three ferrocene molecules was deprotected by the treatemnt with 0.1 M DTT over night and 1 μl of its aqueous solution containing 1 M NaCl was dipped on the pretreated gold electrode having 2.0 mm^2 in area and was kept at room temperature over night and 1 μl of 1 mM 6-mercapt-1-hexanol was subsequently dipped on the electrode and was kept for 1.5 h at 45 °C to mask the bare surface part of the electrode by the self assembly monolayer (SAM).

One microliter of the aqueous solution containing different amount of DNase I in 40 mM Tris-HCl (pH 7.5), 80 mM $MgCl_2$, and 50 mM DTT, where the ferrocenyl oligonucelotide can be digested with DNase I, was dipped on this electrode and kept for 30 min at room temperature. Square wave voltammography (SWV) measurement was carried out in 10 mM $NaHPO_4/NaH_2PO_4$ buffer (pH 7.0) containing 0.1 M $NaClO_4$ (SWV was carried out in the cell furnished with three electrode of the ferrocenyl oligonucleotide-immobilized electrode as a working electode, Ag/AgCl as a reference electrode, and Pt wire as a counter electrode with an amplitude of 50 mV, applied potential of 10 mV, and ferquency of 10 Hz). Peak current at 0.2 V vs. Ag/AgCl based on the redox reaction of the ferrocene was observed in the SWV measurement of this electrode as shown in Fig. 2A(a). After treatment with the aqueous solution containing DNase I, this peak was diminished depending on its activity. Figure 2A(b) showed the case of 1 unit of DNase I. This result showed that the electrode can be used for the electrochemical DNase I assay. However, the intensity of the peak current was scattered with the individual electrode suggesting to the variation of the immobilized amount of the ferrocenyl oligonucleotide.

To resolve this problem, the plot between the peak currents i_o and i before and after treatment of DNase I. Figure 2B shows the good linear correlation in the plot between i_o and i in the case of 0.1 unit DNase I. The slope of 0.94 was observed when treatment with the solution no containing DNase I and the slope in the case of Fig. 2B showed the much lower slope than that without DNase I. The slopes in the different amount of DNase I were measured and plotted against DNase I concentration. The result thus obtained showed that the good linear correlation with 0.01 – 1.0 units of DNase I with the detection limit of 0.01 units or 20 pg (Fig. 3). When this electrochemical DNase I assay was carried out at 37 °C, the detection limit was inproved 100-times. However, the correlation between DNase I concentration and the electrochemical response was scattered. This suggested that too strong activity of DNase I for the amount of the ferrocenyl oligonucelotide on the electrode.

In conclusion the ferrocenyl oligonucelotide-immobilized electrode could be used for the electrochemical DNase I assay as a estimation of the slope in the i_o-i plot. This technique might be extended for the electrochemical nuclease assay for the different nuclease enzyme.

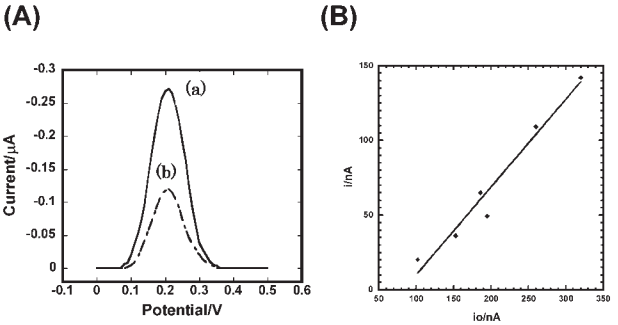

Fig. 2 (A) Square wave voltammogram of the ferrocenyl oligonucleotide-immobilized electrode before (a) and after (b) treatment of the aqueous solution containing 0.1 units of DNase I. (B) Plot of the peak current i_o against i in the case of 1.0 μl of 0.1 units DNase I.

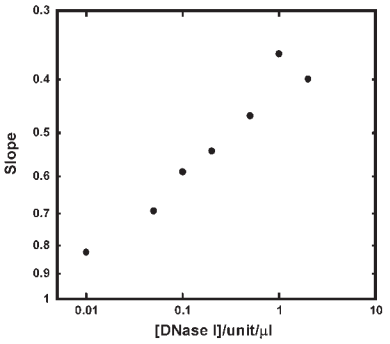

Fig. 3 Plot of the slope obtained from the i_o-i plot against DNase I concentration in the aqueous solution.

ACKNOWLEDGEMENTS

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan. S. S. is also grateful for the financial support from the Japan Society for the Promotion of Science.

REFERENCES

1. Nadano, D., Yauda, T., Kishi, K. (1993) *Chin. Chem.*, **39**, 448-452.
2. Yasuda, T., Takeshita, H., Nakazato, E., Nakajima, T. (1998) *Anal. Biochem.*, **255**, 274-276.
3. http://www.ambion.com/
4. Takeshita, H., Nakajima, T., Mogi, K., Kaneko, Y., Yasuda, T., Iida, R., Kishi, K. (2003) *Clin. Chem.*, **50**, 446-448.
5. Hillier, S. C., Frost, C. G., Jenkins, A. T. A; Braven, H., Keay, R. W., Flower, S. E.; Clarkson, J. M. (2004) *Bioelectrochemistry*, **63**, 307-310.
6. Ssto, S., Kondo, H., Nojima, T., Takenaka, S. (2005) *Anal. Chem.*, **22**, 7304-7309.
7. Mukumoto, K., Nojima, T., Takenaka, S. (2005) *Tetrahederon*, **61**, 11705-11715.
8. Matsuda, M., Ogoshi, H. (1966) *J. Biochem.* (*Tokyo*), **59**, 230- 237.