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# OLIGONUCLEOTIDES SITE-SPECIFICALLY SPIN-LABELED AT 5′-TERMINAL OR INTERNUCLEOTIDE LINKAGE AND THEIR USE IN GENE ANALYSES

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Spin-labeled oligonucleotides (S-probes) were synthesized and examined as DNA probes to monitor hybrid formation. TEMPO was introduced either at the internucleotide linkage of 5′-terminus (Type 1) or at the 5′-terminal hydroxyl group (Type 2) and both types of S-probes were used in this study. The presence of target DNA was detected in solution by EPR spectroscopy for both types of S-probes. Hybridization of the S-probes resulted in notable broadening of EPR line width, accompanied by a decrease in the EPR signal height ratio for $I(-1)/I(0)$. $I(-1)/I(0)$ of S-probes having no spacer between oligonucleotide and TEMPO decreased more markedly than that of S-probes with a spacer, indicating that TEMPO should be introduced to an oligonucleotide directly to monitor hybrid formation. When M13mp8 single-stranded DNA with or without an EcoRI recognition site was selected as a target DNA, hybrid formation was detected only for DNA containing EcoRI site in solution using spin-labeled oligonucleotides.

KEY WORDS: DNA probe, Spin-labeled oligonucleotide, EPR, M13 phage DNA.

ABBREVIATIONS: EPR, electron paramagnetic resonance; 4-amino-TEMPO, 4-amino-2,2,6,6-tetramethylpiperidine-N-oxyl; 4-aminohexylamino-TEMPO, 4-aminohexylamino-2,2,6,6-tetramethylpiperidine-N-oxyl; 4-oxo-TEMPO, 2,2,6,6-tetrametylpiperidine-4-one-N-oxyl; $I(+1)$, height of low-field EPR line; $I(0)$, height of center-field EPR line; $I(-1)$, height of high-field EPR line.

## INTRODUCTION

Since the early demonstrations of the use of DNA probes in gene analysis on a solid phase by Southern (1), the technique has been employed for many research applications. Radioactively labeled probes have been utilized as DNA probes so far because of their high sensitivity. But the radiation emitted from the probes is potentially toxic to human. Additionally, the solid phase hybridization assay used in the conventional method consists of a multistep procedure and is time-consuming. Therefore, development of a new type of DNA probe method, which is a combination of non-radioactive probe and solution phase hybridization assay, has been strongly desired (2). For instance, fluorescent-labeled DNA probes, which are safe and stable compared to conventional radioactive DNA probes, which are safe and stable compared to conventional radioactive DNA probes, have been investigated[3,4]. As an alternative method, spin-labeled DNA probes have been

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developed[5-12]. EPR signals reflect changes of molecular motion of a spin-label upon hybridization. Several types of spin-labeled oligonucleotides have been developed recently and their behaviors in hybrid solution have been studied. In the previous papers[10,12], we reported the synthesis of spin-labeled oligonucleotides site specifically labeled at the internucleotide linkage of 5′ terminus with 4-amino-TEMPO and detection of specific base sequences in solution using spin-labeled oligonucleotides. In this study, two types of S-probes were prepared and examined for their abilities to monitor hybrid formation. The changes in EPR signal height ratio $I(+1)/I(0)$ and $I(-1)/I(0)$ were measured as index to monitor hybrid formation. Hybridization between S-probes and their complementary oligonucleotides could be best monitored by the change in the $I(-1)/I(0)$ values for all S-probes. Furthermore, M13mp8 single-stranded DNA was selected as target DNA and hybrid formation was detected in solution using spin-labeled oligonucleotides.

## MATERIALS AND METHODS

### *Materials*

Fully protected deoxyribonucleoside phosphoramidites were obtained from Milligen Biosearch (Burlington, MA, USA) and deoxyribonucleoside hydrogen phosphonate (triethylammonium salt) were from Sigma Chemical Company (St. Lowis, MO, USA). 4-amino-TEMPO and N,N′-carbonyldiimidazole (CDI) were purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI, USA). Pivaloyl chloride was obtained from Nakalai Tesque, Inc. (Kyoto, Japan). M13mp8 RF I DNA and T4 DNA ligase were products of Nippon Gene Co., Ltd. (Tokyo, Japan). EcoRI and S1 Nuclease were purchased from Takara Shuzo Co., Ltd. (Kyoto, Japan). Other reagent were from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and used without further purification.

### *Assay System*

A high performance liquid chromatography system (CCPM, Tosoh, Tokyo, Japan) equipped with a reversed phase column (Wakosil 5C 18-200 N, 4.6 × 150 mm, Wako) was used to purify labeled oligonucleotides with a linear gradient of acetonitrile (low: 5% acetonitrile in 100 mM triethylammonium acetate (TEAA, pH 7.0), high: 50% acetonitrile in 100 mM TEAA). UV-melting curves of oligonucleotides were obtained by a UV spectrometer (UV-260, Shimadzu Co., Kyoto, Japan) equipped with an automatic thermal controller. UV absorbance at 260 nm was monitored as a function of temperature from 0 to 70°C for the thermal denaturation of the DNA oligomers. The temperature for all UV measurement was increased at a rate of 0.3°C/min. All thermal denaturation solutions contained 100 mM phosphate buffer (pH 7.0). Each strand was present at 2.5 $\mu$M. EPR measurements were performed on a JEOL PE-3X ESR Spectrometer (JEOL, Tokyo) with X-band cavity resonator at room temperature using an aqueous quartz flat cell (outer dimensions being 45 × 10 × 2 mm, LABOTEC, Tokyo, Japan). Before EPR measurement, the sample solutions were heated to 85°C and then slowly cooled to room temperature. The EPR spectra were recorded at 9.56 GHz resonant frequencies and with 100 kHz field modulation of 0.08 mT amplitude, 3.0 sec of response time, 64 min of sweep time, and nonsaturating microwave power of 10 mW.

*Synthesis of 4-Aminohexylamino-TEMPO*

86 mg (0.5 mmol) of 4-oxo-TEMPO and 950 mg (5 mmol) of hexamethylenediamine dihydrochloride were dissolved in 30 mL of dry methanol, followed by the addition of 25 mg (0.4 mmol) of $NaBH_3CN$ and Molecular Sieves 3A. The mixture was then allowed to stand for 24 h at room temperature. The resulting mixture was filtered through a glass filter and evaporated to dryness under reduced pressure. The residue was dissolved in 30 mL of 1N HCl and extracted with 3 × 30 mL of chloroform. The aqueous layer was adjusted to pH 11 by adding NaOH, and extracted with 3 × 30 mL of chloroform. The organic layer was washed with water, then evaporated to dryness under reduced pressure, resulting in 98 mg (0.36 mmol) of 4-aminohexylamino-TEMPO (yield: 72%).

*Preparation of Spin-labeled Oligonucleotides*

*Type 1*

Spin-labeled oligonucleotide Type 1 was synthesized by a combination of the phosphoramidite[13] and the H-phosphonate[14,15] chemistry on controlled pore glass beads (CPG). 9 mer (dGGAATTCGT) complementary to the EcoRI recognition sequence in M13mp8 ssDNA was synthesized by the phosphoramidite chemistry on CPG. The resin was taken up in a gas-tight syringe and washed with $CH_3CN$, followed by drying *in vacuo*. DMTr-deoxyguanosine H-phosphonate (0.1 mL, 0.2 M in pyridine/$CH_3CN$ (1/1, v/v)) was added to the solid support and stirred for 10 sec, followed by the addition of pivaloyl chloride (0.1 mL, 1.0 M in pyridine/$CH_3CN$ (1/1, v/v)) for 3 min. This condensation step was repeated twice. The CPG was then washed well with $CH_3CN$/pyridine (1/1, v/v), followed by $CH_3CN$, and dried *in vacuo*. Introduction of 4-amino-TEMPO (Type 1 (A)) was carried out by the reported procedure[11]. Introduction of 4-aminohexylamino-TEMPO (Type 1 B)) was done under similar conditions as above. After finishing the oxidation reaction, the reagents were washed off sequentially with $CCl_4$ and $CH_3CN$ and the CPG was dried *in vacuo*. Subsequently, the resulting products were treated with concentrated $NH_4OH$ solution and purified by reversed-phase liquid chromatography (RPLC).

*Type 2*

10 mer (dGGGAATTCGT) was synthesized by the phosphoramidite chemistry on CPG. The resin was taken up in a gas-tight syringe and washed with $CH_3CN$ and dried *in vacuo*. 50 mg of N,N′-carbonyldiimidazole (CDI) was dissolved in 1 mL of dioxane and this solution was introduced into the gas-tight syringe. The activation was allowed to proceed for 1 h at room temperature with occasional shaking[16]. The solid support was washed well with dioxane and dried *in vacuo*. Introduction of 4-amino-TEMPO (Type 2(A)) was carried out as follows. The activated oligomer was allowed to react with 0.4 mL solution of 0.2 M 4-amino-TEMPO dissolved in DMSO for 24 h at 55°C. The solid support was washed off sequentially with DMSO, dioxane and methanol, and the CPG was dried *in vacuo*. Introduction of 4-aminohexylamino-TEMPO (Type 2(B)) was done under the similar conditions as described above. Both Type 2(A) and Type 2(B) were treated with concentrated $NH_4OH$ solution and then purified by RPLC.

*Preparation of M13mp8 Single-Stranded DNA Lacking EcoRI Site*

EcoRI recognition site of M13mp8 RF DNA was deleted as follows. The polycloning site of the bacteriophage M13mp8 RF DNA (3 $\mu$g) was cleaved with a restriction endonuclease EcoRI (28 units) and the resulting protruding termini of linear DNA was removed with nuclease S1 (24 units), producing blunt ended DNA. The blunt ended DNA was then ligated by bacteriophage T4 DNA ligase (1,000 units). The enzymatic reactions were monitored by agarose gel electrophoresis. Competent *E. coli* cells (JM 109) were transformed by the ligated DNA and plated on H-plates containing IPTG and X-gal. Preparation of single-stranded recombinant of M13 DNA was carried out by using a resulting single white plaque as seed according to a standard procedure[17]. Resultant M13mp8 ssDNA lacking EcoRI site is referred to M13mp8M ssDNA.

*Endonuclease Assay*

Both S-probe/M13mp8 ssDNA and S-probe/M13mp8M ssDNA hybrids were tested as substrates for the EcoRI endonuclease. 1 $\mu$L of S-probe/M13 DNA complexes (each strand: 1 $\mu$M) were added to 1.5 mL Eppendorf tubes, dissolved in 10 $\mu$L of distilled water, warmed to 70°C for 3 min, and cooled slowly to 30°C. Above mixtures were then dissolved in 2 $\mu$L of reaction buffer (1 M Tris-HCl, pH 7.5, 500 mM NaCl, 70 mM 2-Mercaptoethanol) and kept at 30°C for 15 min before reactions were started. Reactions were initiated by adding 7 $\mu$L of EcoRI (98 units), and incubated at 30°C for 1 h. Final concentration of S-probe/M13 DNA complexes was present at 50 nM (each strand). Reactions were stopped by addition of 2 $\mu$L of 0.5 M EDTA at 0°C. Aliquots were withdrawn and analyzed by electrophoresis on 0.8% agarose gels in 90 mM Tris-borate (pH 8.0), 2 mM EDTA, followed by staining with ethidium bromide and visualization under UV light.

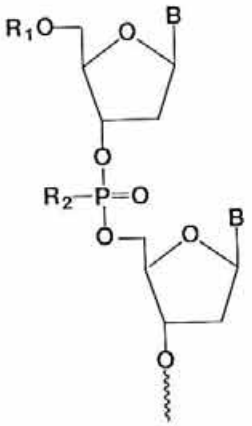
## RESULTS

*Preparation of Spin-labeled Oligonucleotides*

Two types of spin-labeled oligonucleotides (S-probes) were prepared by a combination of an automated DNA synthesizer and a gas-tight syringe method for the introduction of spin labels. Structures of the S-probes are shown in Figure 1. Yields of the introduction of 4-amino-TEMPO and 4-aminohexylamino-TEMPO were 62% and 70%, respectively for the synthesis of Type 1. For Type 1, a pair of diastereoisomers were produced with respect to the resulting phosphoramidate linkage. These isomers were separated by RPLC and the isolated isomers were referred to isomer 1 and 2 according to the order of the elution. Yields of the introduction of 4-amino-TEMPO and 4-aminohexylamino-TEMPO were 70% and 88%, respectively for the synthesis of Type 2.

*Spectroscopic Behavior of the Spin-labeled Oligonucleotides in Solution*

In order to examine the ability of the S-probes to form hybrids with their complementary oligonucleotides, UV melting curves were measured. The melting temperatures (Tm) of the duplexes between the S-probes and their complementary 20 mers (37–39°C) were almost identical with that of the duplex formed by the



Type 1(A) : $R_1$=H, $R_2$=TEMPO-NH-
Type 1(B) : $R_1$=H, $R_2$=TEMPO-NH-$(CH_2)_6$-NH-
Type 2(A) : $R_1$=TEMPO-NH-CO-, $R_2$=$O^-$-
Type 2(B) : $R_1$=TEMPO-NH-$(CH_2)_6$-NH-CO-, R2=$O^-$-

FIGURE 1 Structures of S-probes.

corresponding oligonucleotide carrying no spin label (dGGGAATTCGT) and its complementary 10 mer (40°C) (Table I). These results indicate that the S-probes synthesized in this study could be applied as hybridization probes in solution.

Figures 2 and 3 show EPR spectra of the S-probes in the absence and presence of their complementary 20 mers. Two values for the signal height ratios, I(+1)/I(0) and I(11)/I(0), were calculated and used to evaluate hybrid formation.

TABLE I
Melting temperatures of hybrids of spin-labeled oligonucleotides and complementary oligonucleotides.

| Sample | Tm(°C) |
|---|---|
| Type 1(A) isomer 1 + C20 | 38 |
| Type 1(A) isomer 2 + C20 | 39 |
| Type 1(B) isomer 1 + C20 | 37 |
| Type 1(B) isomer 2 + C20 | 38 |
| Type 2(A) + C20 | 37 |
| Type 2(B) + C20 | 39 |
| Natural Type + C10 | 40 |

Type 1: (A) GpGGAATTCGT; (B) GpGGAATTCGT
Ⓢ |.....Ⓢ

Type 2: (A) Ⓢ-GGGAATTCGT; (B) Ⓢ.....-GGGAATTCGT

Natural Type: 5′-GGGAATTCGT
C20: 5′-TGATTACGAATTCCCGGGGA
C10: 5′-ACGAATTCCC

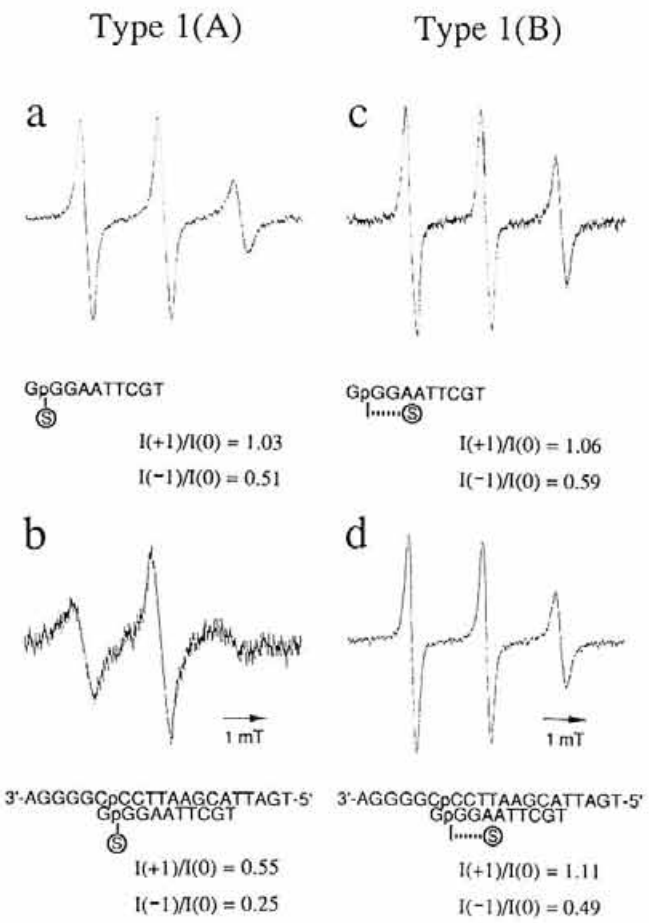



FIGURE 2 EPR spectra of Type 1 S-probes labeled at the internucleotide linkage of 5′ terminus with either 4-amino-TEMPO (a and b) or 4-aminohexylamino-TENTO (c and d) in the absence and presence of their complementary 20 mers. The single-stranded DNA (a and c) were present at 5 $\mu$M in 100 mM phosphate buffer (pH 7.0). For the S-probe/C20 mer complexes (b and d), each strand was present at 5 $\mu$M in 100 mM phosphate buffer (pH 7.0). For Type 1, isomer 1 are shown here. EPR settings were as follows: modulation amplitude, 0.08 mT; receiver gain, 2.5 × 1000 for a and d, 2.8 × 1000 for c, and 5.6 × 1000 for b; response time, 3 sec; sweep time, 64 min.

Hybridization of the Type 1(A) and Type 2(A) S-probes with their complementary 20 mers resulted in broadening of EPR line width, accompanied by appreciable decrease in $I(-1)/I(0)$. $I(+1)/I(0)$ for Type 2(A), however, was almost identical with that of probe alone, although there was appreciable reduction of $I(+1)/I(0)$ for Type 1(A). When the Type 1(B) and Type 2(B) S-probes hybridized with their complementary 20 mers, EPR line width was slightly broadened and $I(-1)/I(0)$ decreased. However, $I(+1)/I(0)$ for both Type 1(B) and Type 2(B) was almost identical with that of the probe alone. These results indicate that the change in the $I(-1)/I(0)$ ratio is more sensitive to hybrid formation than that in the $I(+1)/I(0)$. To examine whether $I(-1)/I(0)$ is dependent on the sizes of hybridized complexes,

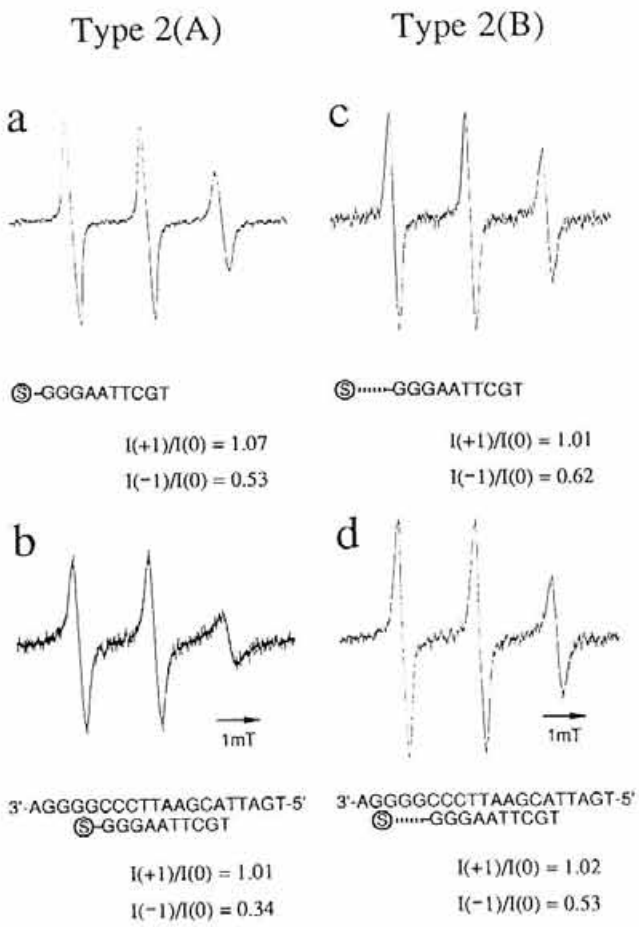



FIGURE 3 EPR spectra of Type 2 S-probes labeled at the 5′ terminal hydroxyl group with either 4-amino-TEMPO (a and b) or 4-aminohexylamino-TEMPO (c and d) in the absence and presence of their complementary 20 mers. The single-stranded DNA (a and c) were present at 5 $\mu$M in 100 mM phosphate buffer (pH 7.0). For the S-probe/C20 mer complexes (b and d), each strand was present at 5 $\mu$M in 100 mM phosphate buffer (pH 7.0). EPR settings were as follows: modulation amplitude, 0.08 mT; receiver gain, 2.8 × 1000 for a, 3.2 × 1000 for c and d, and 5.6 × 1000 for b; response time, 3 sec; sweep time, 64 min.

relative $I(-1)/I(0)$ were plotted against molecular weights of the probe/target hybrids (Figure 4). Relative $I(-1)/I(0)$ decreased with an increase in the molecular weights of the probe/target hybrids for all S-probes used. It should be noted that $I(-1)/I(0)$ of S-probes having no spacer molecule (Type 1(A), Type 2(A)) decreased more markedly upon annealing than that of S-probes with a spacer molecule (Type 1(B), Type 2(B)).

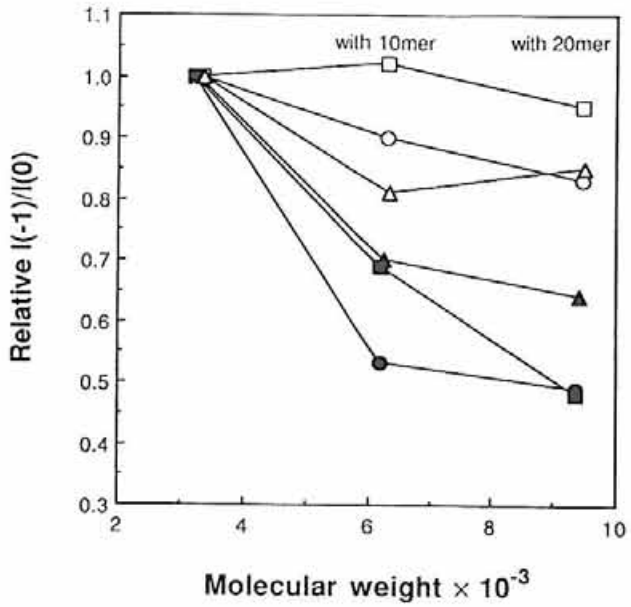



FIGURE 4 Plots of relative I(−1)/I(0) *vs* molecular weights of the probe/target hybrids. Relative I(−1)/I(0) were calculated based on I(−1)/I(0) of each S-probe alone. Symbols are as follows: (●), Type 1(A) isomer 1 with complementary oligonucleotides; (■), Type 1(A) isomer 2 with complementary oligonucleotides; (○), Type 1(B) isomer 1 with complementary oligonucleotides; (□), Type 1(B) isomer 2 with complementary oligonucleotides; (▲), Type 2(A) with complementary oligonucleotides; (△), Type 2(B) with complementary oligonucleotides. The base sequences are shown in Table I.

### *Detection of M13 mp8 ssDNA*

Detection of target sequence in natural DNA was performed using the Type 1(A) and Type 2(A) S-probes. For this purpose, M13mp8 ssDNA was chosen as a target DNA and a deletion mutant (M13mp8M ssDNA) was used in a control experiment (Figure 5). Annealing of the S-probes to M13mp8 ssDNA resulted in broadening of EPR line width and I(−1)/1(0) decreased compared to those obtained from S-probe/M13mp8M ssDNA complex (Figure 6). The extent of broadening upon annealing was similar for both diastereoisomers of the Type 1 S-probes. Subsequently, probe/M13mp8 ssDNA and probe/M13mp8M ssDNA complexes were treated with a restriction endonuclease EcoRI. It is apparent in Figure 7 that probe/M13mp8 ssDNA complex was a substrate of EcoRI, but probe/M13mp8M ssDNA complex was not recognized as a substrate. These results clearly indicate that the S-probes used in this study hybridized with M13mp8 ssDNA in a sequence specific manner in solution.

## DISCUSSION

In DNA probe methods, it is essential that the reporter group attached to an oligonucleotide should only minimally perturb the double-stranded conformation to maintain the specificity of hybridization. Therefore, the site where a label

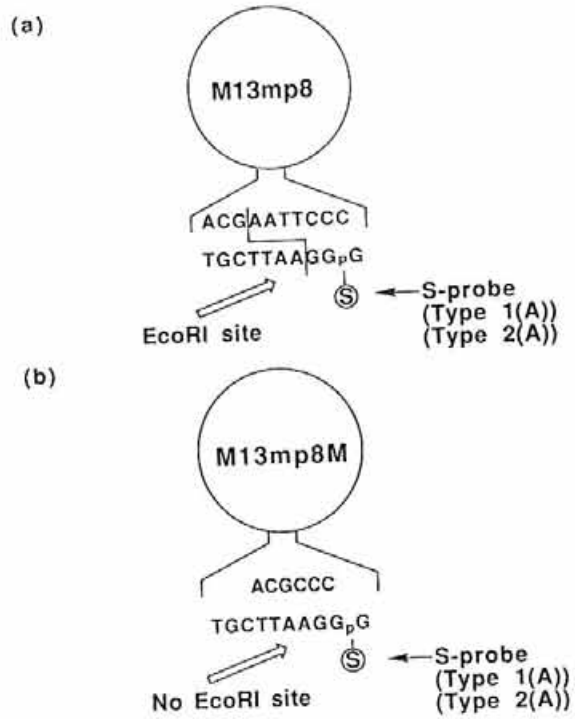



FIGURE 5 Schematic representation for detection of M13mp8 ssDNA using Type 1(A) and Type 2(A) S-probes.

molecule is introduced to an oligonucleotide is of major concern for a reliable assay. Several types of spin-labeled oligonucleotides, which do not appreciably disrupt double-stranded DNA conformation, have been developed recently[5-12]. In this study, TEMPO was introduced either at the internucleotide linkage of 5′ terminus or at the 5′ terminal hydroxyl group in order to satisfy the above requirement. Since both the internucleotide linkage of 5′ terminus and 5′ terminal hydroxyl group are not involved in Watson-Crick base pairing, we consider both sites are logical choice as modification sites. Introduction of TEMPO was a simple procedure and proceeded without producing any noticeable by-product. The melting temperature of the duplexes formed by S-probes and their complementary sequence was almost identical with that of the duplex formed by the corresponding oligonucleotide carrying no spin-label and its complementary oligonucleotide, indicating that the reporter group (TEMPO) does not interfere hybrid formation. The signal height ratios $I(+1)/I(0)$ and $I(-1)/I(0)$ were used to monitor hybrid formation. $I(-1)/I(0)$ was more sensitive to hybrid formation than $I(+1)/I(0)$ for all S-probes used in this study. Thus, our results indicate that $I(-1)/I(0)$ is the most appropriate index to monitor hybrid formation. Our results further demonstrated that spin-labeled oligonucleotides synthesized in this study can detect EcoRI recognition site in M13mp8 ssDNA, and is useful for the base sequence analysis.

An ideal label for a DNA probe should have the following properties: It (a) can be easily attached to DNA, (b) can be detectable at very low concentrations using

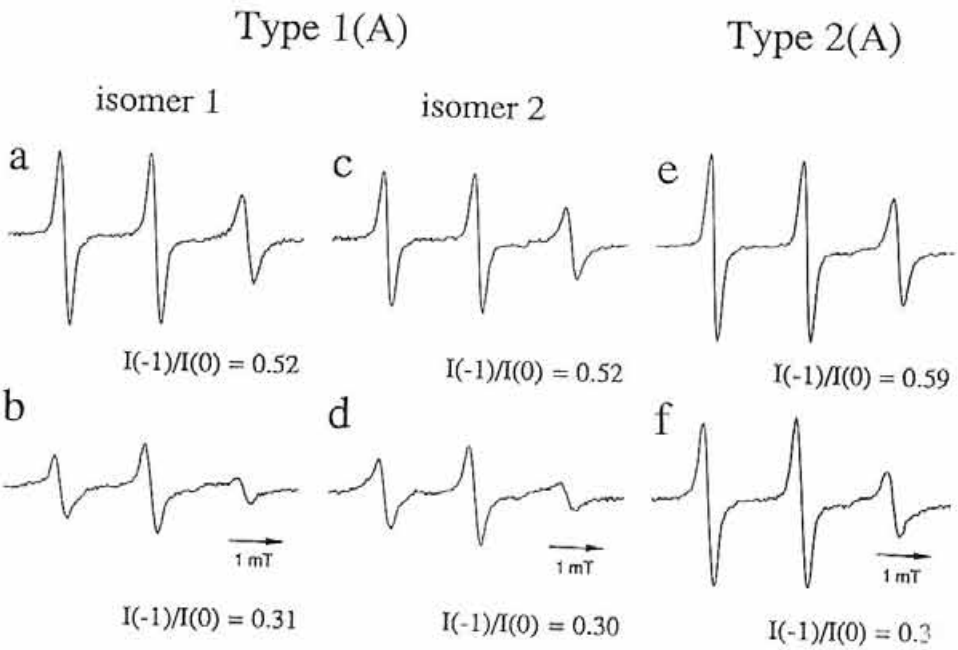



FIGURE 6 EPR spectra for S-probe/M13mp8 ssDNA (b, d and f) and S-probe/M13mp8M ssDNA (a, c and e) complexes. Each strand was present at 1 μM in 100 mM phosphate buffer (pH 7.0). EPR settings were as follows: modulation amplitude, 0.08 mT; receiver gain, 3.2 × 1000 for a, b, c, d and e, 2.5 × 1000 for f, response time, 3 sec; sweep time, 64 min.

simple instrumentation, (c) can produce a signal which is modulated when the labeled DNA probe hybridizes with its complementary DNA sequence (thus facilitating the development of solution-phase DNA probe assays), and (d) is stable at the elevated temperatures used in hybridization. It is considered that spin-labeled DNA probes fulfill these criteria except the sensitivity (b). But in view of the recent development of the amplification method of target sequences by polymerase chain reaction (PCR), where a target DNA is amplified by a factor of about 1,000,000-fold, the need for ultrasensitivity may not be an absolute requirement for DNA probe methods.

In conclusion, we have demonstrated the possibility of direct target detection without bound-free separation in aqueous solution and the present S-probe method is potentially applied for automated analysis of genes.

## *Acknowledgements*

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## *References*

1. E.M. Southern (1975) Detection of specific sequences among DNA fragments separated by electrophoresis. *Journal of Molecular Biology*, **98**, 503–517.

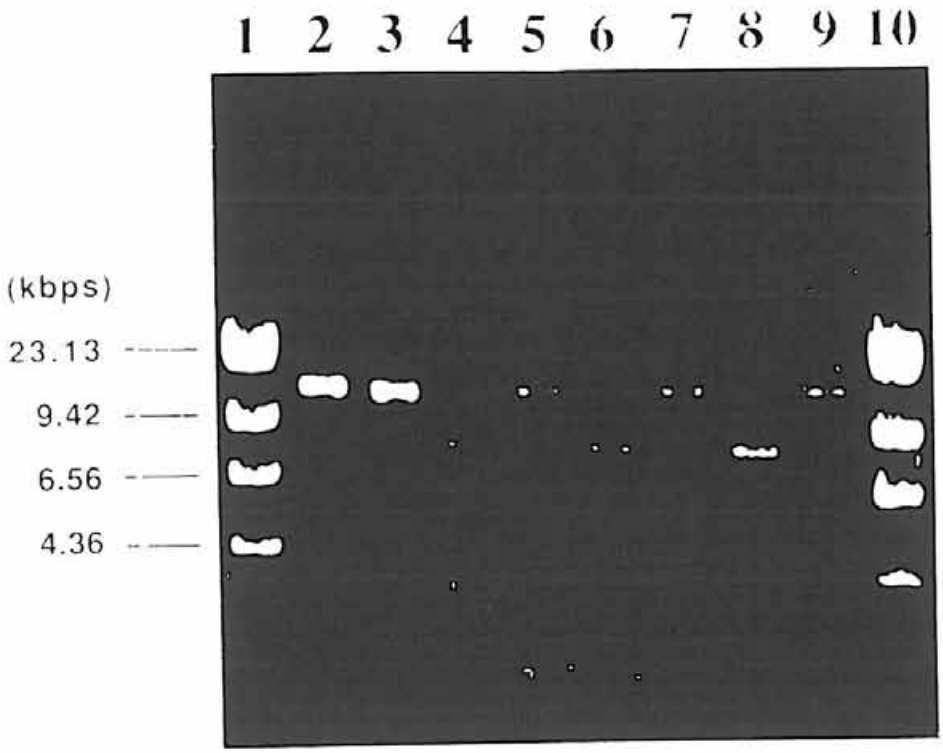


FIGURE 7 Product analysis of S-probe/M13mp8 ssDNA and S-probe/M13mp8M ssDNA complexes treated with EcoRI by agarose gel electrophoresis. The conditions of enzymatic reactions are described in MATERIALS AND METHODS. lane 1 and 10, phage DNA/Hind III digest; lane 2, M13mp8 ssDNA; lane 3, M13mp8M ssDNA; lane 4, [Type 1(A) isomer 1/M13mp8 ssDNA]/EcoRI digest; lane 5, [Type 1(A) isomer 1/M13mp8M ssDNA]/EcoRI digest; lane 6, [Type 1(A) isomer 2/M13mp8 ssDNA]/EcoRI digest; lane 7, [Type 1(A) isomer 2/M13mp8M ssDNA]/EcoRI digest; lane 8, [Type 2(A)/M13mp8 ssDNA]/EcoRI digest; lane 9, [Type 2(A)/M13mp8M ssDNA]/EcoRI digest. The bands between 6.56 and 9.42 kbps found in lane 4, 6 and 8 correspond to resultant linearized M13mp8 ssDNA.

2. J.A. Matthews and L.J. Kricka (1988) Analytical strategies for the use of DNA probes. *Analytical Biochemistry*, **169**, 1–25.
3. L.E. Morrison, T.C. Halder and L.M. Stols (1989) Solution-phase detection of polynucleotides using interacting fluorescent labels and competitive hybridization. *Analytical Biochemistry*, **183**, 231–244.
4. A. Murakami, M. Nakaura, Y. Nakatsuji, S. Nagahara, Q. Tran-Cong and K. Makino (1991) Fluorescent-labeled oligonucleotide probes: detection of hybrid formation in solution by fluorescence polarization spectroscopy. *Nucleic Acids Research*, **19**, 4097–4102.
5. S.-C. Kao and A.M. Bobst (1985) Local base dynamics and local structural features in RNA and DNA duplexes. *Biochemistry*, **24**, 5465–5469.
6. O.K. Strober, R.S. Keyes and A.M. Bobst (1990) An electron paramagnetic resonance probe to detect local Z-DNA conformations. *Biochemical and Biophysical Research Communications*, **166**, 1435–1440.
7. O.K. Strober, R.S. Keyes and A.M. Bobst (1990) Base dynamics of local Z-DNA conformations as detected by electron paramagnetic resonance with spin-labeled deoxycytidine analogues. *Biochemistry*, **29**, 8522–8528.
8. A. Spaltenstein, B.H. Robinson and P.B. Hopkins (1988) A rigid and nonperturbing probe for duplex DNA motion. *Journal of the American Chemical Society*, **110**, 1299–1301.
9. A. Spaltenstein, B.H. Robinson and P.B. Hopkins (1989) Sequence- and structure-dependent DNA

base dynamics: Synthesis, structure, and dynamics of site and sequence specifically spin-labeled DNA. *Biochemistry*, **28**, 9484-9495.
10. K. Makino, A. Murakami, S. Nagahara, Y. Nakatsuji and T. Takeuchi (1989) A study on spin-labelled oligonucleotide synthesis and its electron spin resonance behavior in solution. *Free Radical Research Communications*, **6**, 311-316.
11. O.K. Strober, D.D. Kryak, E.V. Bobst and A.M. Bobst (1991) Preparation and characterization of spin-labeled oligonucleotides for DNA hybridization. *Bioconjugate Chemistry*, **2**, 89-95.
12. S. Nagahara, A. Murakami and K. Makino (1992) *Nucleosides & Nucleotides*, **11**, 889-901.
13. M.H. Caruthers (1985) Gene synthesis machines: DNA chemistry and its uses. *Science*, **230**, 281-285.
14. B.C. Froehler and M.D. Matteucci (1986) Nucleoside H-phosphonates: valuable intermediates in the synthesis of deoxyolgonucleotides. *Tetrahedron Letters*, **27**, 469-472.
15. B.C. Froehler, P.G. Ng and M.D. Matteucci (1986) Synthesis of DNA *via* deoxynucleoside H-phosphonate intermediates. *Nucleic Acids Research*, **14**, 5399-5407.
16. L. Wachter, J. Jablonski and K.L. Ramachandran (1986) A simple and efficient procedure for the synthesis of 5'-aminoalkyl oligodeoxynucleotides. *Nucleic Acids Research*, **14**, 7985-7994.
17. T. Maniatis, E.F. Fritsch and J. Sambrook (Molecular cloning, a laboratory manual. (1982) Cold Spring Habor Laboratory. Cold Spring Habor, NY.