DNA Hybridization by Peptide Nucleic Acids Attached to Controlled Pore Glass†

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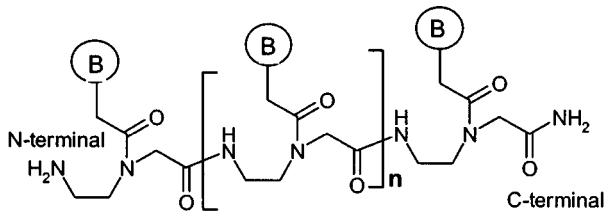
Sequence-specific hybridization between DNA in solution and a probe attached to solid support is used as a powerful tool for the detection and analysis of genetic materials.[1] DNAs are most often used as the probes fixed on the solid support.[1] RNAs, peptide nucleic acids (PNAs),[2] and pyrrole/imidazole-containing polyamides[3] are capable of sequence-specific hybridization with DNA. Pyrrole/imidazole-containing polyamides are at present useful for recognition of only short (hexamer or shorter) double-stranded DNAs. RNAs are much more difficult to handle and to synthesize compared with DNAs or PNAs. Pyrrole/imidazole-containing polyamides and RNAs are not, therefore, useful as the probes for DNA recognition.

PNAs have pseudo-peptide backbones of repeating *N*-(2-aminoethyl)glycine units onto which the nucleobases of DNA are attached.[2] PNAs form duplexes with nucleic acids, adopting Watson-Crick type base pairing and stacking patterns similar to those of DNA-DNA duplexes.[2] Since the negatively charged backbone of DNA is replaced by a neutral one in PNA, PNA-DNA duplexes are free from electrostatic repulsion between the two negatively charged backbones unlike DNA-DNA duplexes. Consequently, PNA-DNA duplexes have greater stability compared with the corresponding DNA-DNA duplexes at low salt concentrations. Although formation of PNA-DNA duplexes in solution has been extensively investigated, DNA hybridization by immobilized PNA has been rarely studied.

In the present study, we examined whether PNA attached to controlled pore glass (CPG) can form strong and specific complex with DNA dissolved in solution. CPG is silica glass with uniform and controlled pores. A glycerol-containing CPG derivative (CPG-glycerol) was oxidized with $NaIO_4$ to obtain an aldehyde-containing CPG derivative (CPG-CHO) in the present study. Then, the terminal amino group of a PNA molecule was linked to CPG-CHO by reductive amination with sodium cyanoborohydride. In Table 1, PNAs attached to CPG are summarized, together with DNAs used as substrates. HoPuPNA, HoPyPNA, and MSPNA are complementary to HoPyDNA, HoPuDNA, and MSDNA, respectively.



PNA (B = A, G, C, T)

When HoPyDNA, HoPuDNA, and MSDNA were incubated with the PNA-free CPG-glycerol under the conditions of hybridization conditions, the amount of the DNA adsorbed onto the resin was negligible. This suggests that adsorption of DNA onto the PNA-containing CPG (CPG-PNA) would occur mainly through interaction with the PNA moieties. The degree of DNA hybridization by CPG-PNA was measured at 25 °C and pH 7.50. The suspension of 20 mg CPG-PNA in 1 mL of DNA-containing buffer solution was incubated for 5 min at 70 °C and then for 2 hr at 25 °C in a Vortemp (400 rpm) and then centrifuged to separate the buffer solution. From the absorbance value of the buffer solution measured at 260 nm, the concentrations of DNA ($[DNA]_f$) dissolved in the solution and DNA ($[DNA]_b$: defined as DNA concentration attainable when the resin-DNA complex is assumed to be dissolved) bound to the resin are calculated. The data obtained for DNA hybridization by various CPG-PNA are summarized in Figure 1.

The data of Figure 1 were analyzed in terms of the scheme of eq 1 and the expression of eq 2 derived therefrom by assuming independent binding to each binding site, by analogy with Langmuir adsorption as described[4] previously. In eq 1 and 2, BS stands for the binding site on the resin available for DNA hybridization, BS_o for the total amount of the host site on the resin (expressed as the concentration attainable when the resin is dissolved), and K_f ($=k_{ad}/k_{de}$) for the formation constant of the BS-DNA complex. By non-linear regression, the data of Figure 1 produced the values of log K_f summarized in Table 2.

$$\text{BS} + \text{DNA} \underset{k_{de}}{\overset{k_{ad}}{\rightleftharpoons}} \text{BS-DNA} \qquad (1)$$

$$[\text{DNA}]_b = [\text{DNA}]_f[\text{BS}]_o/([\text{DNA}]_f + 1/K_f) \qquad (2)$$

The complementary DNAs are complexed by the CPG-PNA with appreciably higher formation constants compared with the mismatched DNAs. The log K_f value for complexes

†This paper is dedicated to Professor Sang Chul Shim for his distinguished achievements in chemistry.

Table 1. Structure of PNAs attached to CPG and DNA substrates[a]

PNA	DNA
HoPuPNA: ^{C}GAGAAGGAAAAAG^{N}	HoPuDNA: $^{5'}$GAAAAAGGAAGAG$^{3'}$
HoPyPNA: ^{C}CTTTTTCCTTCTC^{N}	HoPyDNA: $^{5'}$CTCTTCCTTTTTC$^{3'}$
MSPNA: ^{C}CTCTAGGTCAAGCTA^{N}	MSDNA: $^{5'}$GAGATCCAGTTCGAT$^{3'}$

[a]Superscripts N, C, 5', and 3' denote amino, carboxy, 5', and 3' termini, respectively.

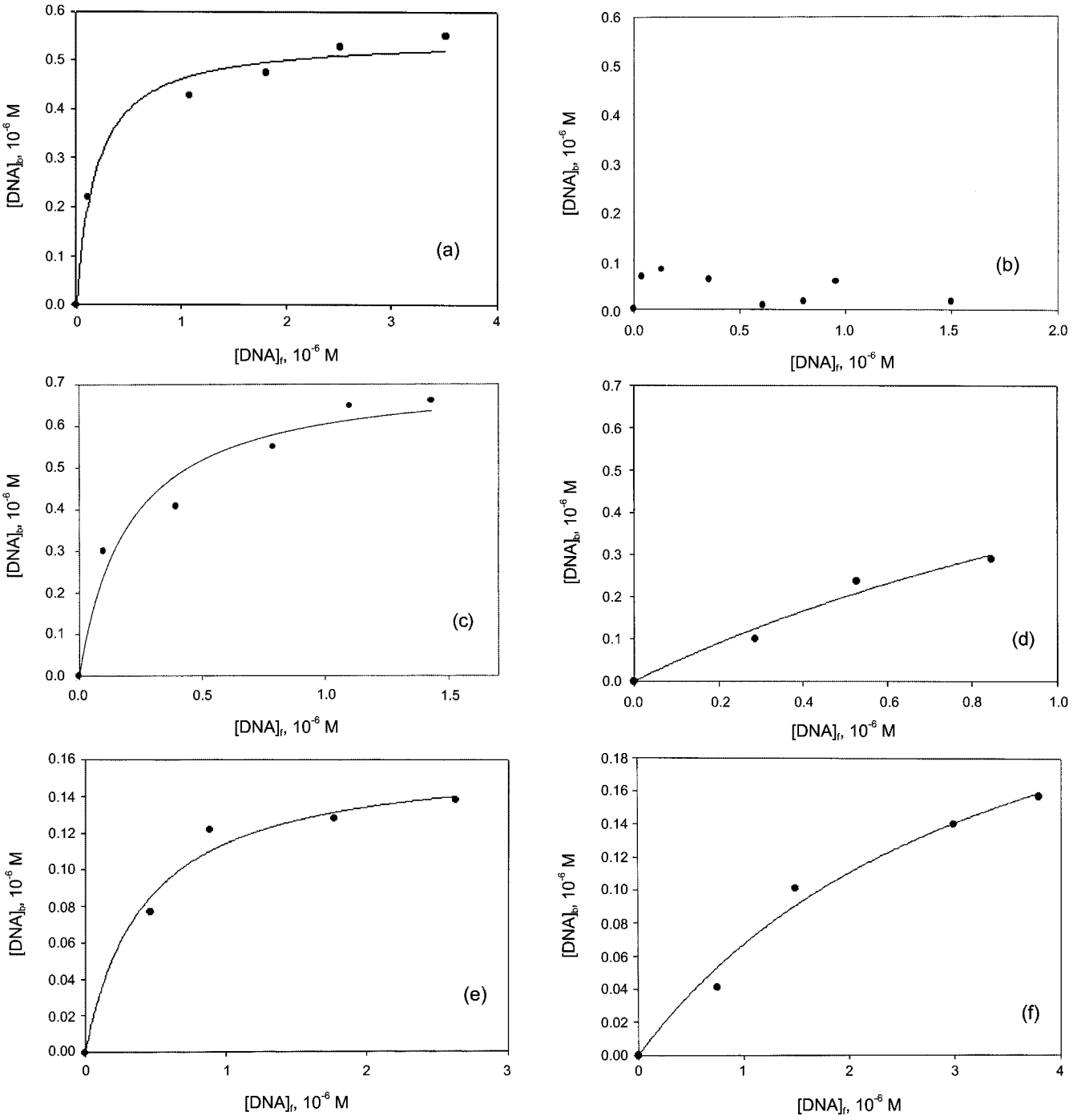


Figure 1. Plot of $[DNA]_b$ against $[DNA]_f$ measured for DNA hybridization with various CPG-PNAs at pH 7.50 and 25 °C: (a) hybridization of HoPyDNA with CPG-HoPuPNA, (b) hybridization of HoPuDNA with CPG-HoPuPNA, (c) hybridization of HoPuDNA with CPG-HoPyPNA, (d) hybridization of HoPyDNA with CPG-HoPyPNA, (e) hybridization of MSDNA with CPG-MSPNA, and (f) hybridization of HoPyDNA with CPG-MSPNA.

formed between 13-15 mer DNA with the complementary PNA is estimated to be greater than 13 at 25 °C in water.[5] The log K_f values for complexation of the three CPG-PNA resins with the respective complementary DNAs are measured in the present study as 6.4-6.7. This suggests that the specific base pairing for the A/T and G/C pairs through hydrogen bonding is much weaker on the CPG surface compared with the bulk water.

The CPG-PNA resins showed widely different affinity for the mismatched DNAs. Complexation of HoPuDNA by CPG-HoPuPNA was negligible. On the other hand, binding of HoPyDNA by CPG-HoPyPNA or HoPyDNA by CPG-MSPNA was quite strong. Mismatched DNAs may be bound to CPG-PNAs *via* nonspecific interactions such as aggregation of hydrophobic residues.

In conclusion, specific hydrogen-bonding between A/T and G/C pairs becomes much weaker on attachment of PNA to CPG. Non-specific interaction between DNA and PNA can be quite strong on the CPG surface depending on DNA and PNA used. Thus, PNA attached to CPG according to the method described here is not suitable for sequence-specific recognition of DNA. If long spacers are inserted between PNA and CPG, selective binding of complementary DNA to PNA might be improved.[6]

Table 2. Values of log K_f estimated for DNA-hybridization by various CPG-PNAs at pH 7.50 and 25 °C

CPG-PNA	DNA	log K_f
CPG-HoPuPNA	HoPyDNA	6.73 ± 0.12
CPG-HoPuPNA	HoPuDNA[a]	small
CPG-HoPyPNA	HoPuDNA	6.70 ± 0.10
CPG-HoPyPNA	HoPyDNA[a]	5.66 ± 0.58
CPG-MSPNA	MSDNA	6.38 ± 0.14
CPG-MSPNA	HoPyDNA[a]	5.19 ± 0.45

[a]Mismatched DNA

Experimental Section

DNA and PNA. DNAs purified with polyacrylamide gel electrophoresis were purchased from Bioneer Corp. PNAs were synthesized by automated synthetic procedures using an Expedite Model 8909 Nucleic Acid Synthesis System. Purity of PNA was confirmed by MALDI-TOF MS analysis using a Voyager-DE Biospectrometry Workstation.

PNA-containing CPG. $NaIO_4$ (90 mg, 0.421 mmol) was dissolved in 100 mL H_2O and 2 g CPG-glycerol (purchased from CPG Inc.) was suspended in the resulting solution. After the mixture was stirred at room temperature for 2 hours, CPG-CHO was collected by filtration, washed three times with distilled 30 mL H_2O and 30 mL methanol, and then dried in vacuo for 1 day. PNA (0.1 mole) and sodium cyanoborohydride (10 mg) were added to a 10 mL buffer solution (10 mM phosphate, pH 7.5), and CPG-CHO (1 g) was suspended in the resulting solution. This mixture was stirred for 12 hours at room temperature, and resulting CPG-PNA was collected by filtration, washed three times with distilled 30 mL H_2O and 30 mL methanol, and then dried in vacuo for 1 day. The progress of attachment of PNA to the resin was followed by the change in the UV-spectrum of the PNA solution.

Measurements. DNA hybridization with CPG derivatives was carried out at pH 7.50 (10 mM phosphate) and 25 °C. UV-Vis spectra were recorded with a Beckman DU 68 spectrophotometer.

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