Acridine-bearing PNA for Efficient Protection of Designated Site of DNA from Nuclease S1

Binzhi Ren, Sheng Ye, Xingguo Liang, and Makoto Komiyama*
Research Center for Advanced Science and Technology, The University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo, 153-8904

(Received May 28, 2003; CL-030473)

By using peptide nucleic acid (PNA) bearing an acridine at its N-terminus, a predetermined portion of DNA substrate was efficiently protected from nuclease S1. Promotion of the protecting activity by the acridine is remarkable enough to provide the target DNA fragment in high selectivity and yield.

Site-selective scission of DNA is one of the most attractive and urgent themes.[1] If huge DNA of higher animals and higher plants is precisely cut at the target position, new biotechnology can be developed. Most of previous attempts involve conjugation of non-enzymatic catalysts to sequence-recognizing oligonucleotides.[2] Recently, we have proposed another strategy in which DNA digestion by nuclease S1 (specific to hydrolysis of single-stranded DNA) is restricted to predetermined position by using PNA as protecting agent.[3,4] The portions which are complementary with the PNA, as well as the phosphodiester linkages near these portions, were protected from the enzymatic digestion, and thus the scission occurred at the designated parts. These results indicated that still more eminent protecting agent, if available, can pave the way to site-selective DNA scission. Here we show that the PNA bearing an acridine is far superior to unmodified PNA in DNA protection from nuclease S1. By use of this modified PNA, desired fragments are selectively prepared from substrate DNA in high yields.

All the PNAs and DNAs are presented in Scheme 1.[5] In $PNA^{(Acr)}$, an acridine is bound to the N-terminus of $PNA^{(Un)}$ by a flexible linker (-NH-$(CH_2)_5$-C(O)-). The DNA substrates, labeled with fluorescein at the 5′-termini, were treated with nuclease S1 (from Life Technologies) in the presence of the PNA or DNA additives at pH 4.6 (acetate buffer) and 20 °C for 5 min. Then, the mixtures were analyzed by denaturing polyacrylamide gel electrophoresis and quantified with a Fuji Film FLA-3000G imaging analyzer.[6]

The typical gel electrophoresis patterns are presented in Figure 1. When $DNA^{(GG)}$ was treated with nuclease S1 in the presence of $PNA^{(Acr)}$, the 12-mer oligonucleotide (from A1 to G12) was selectively formed (lane 1 in Figure 1). Note that

PNA and DNA additives:

$PNA^{(Un)}$: H_2NOC-(Gly)-TCTTCGCGGA-NH_2
$PNA^{(Acr)}$: H_2NOC-(Gly)-TCTTCGCGGA-NHCO(Acr)
$DNA^{(Un)}$: 3'-TCTTCGCGGA-5'
$DNA^{(Acr)}$: 3'-TCTTCGCGGA(Acr)-5'

DNA Substrates:

	1	12	26
$DNA^{(GG)}$:	5'(FITC)-AGAAGCGCCTGGCAGTGTACCAGGCC-3'		
$DNA^{(GA)}$:	5'(FITC)-AGAAGCGCCTGACAGTGTACCAGGCC-3'		
$DNA^{(GT)}$:	5'(FITC)-AGAAGCGCCTGTCAGTGTACCAGGCC-3'		
$DNA^{(GC)}$:	5'(FITC)-AGAAGCGCCTGCCAGTGTACCAGGCC-3'		
$DNA^{(AG)}$:	5'(FITC)-AGAAGCGCCTAGCAGTGTACCAGGCC-3'		

Scheme 1. The PNA and DNA additives as well as the DNA substrates. The underlined parts of DNA substrates are complementary with the additives.

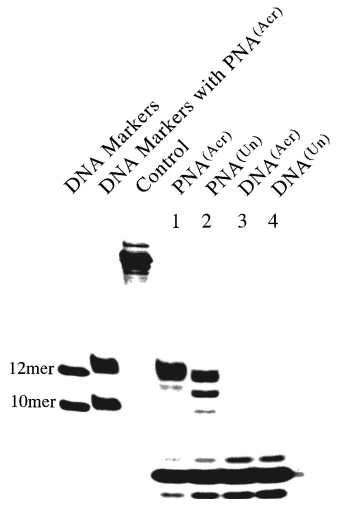

Figure 1. Polyacrylamide gel electrophoresis patterns for the nuclease S1 digestion of $DNA^{(GG)}$ in the presence of various PNA and DNA additives. Reaction conditions: $[DNA^{(GG)}] = 5.0$ and [the additive] = 10.0 μmol/L, [NaCl] = 45 mmol/L, and [nuclease S1] = 1.5 unit/μL at pH 4.6 and 20 °C for 5.0 min (L = dm^3). The bands in the bottom of gel are associated with the FITC derivatives that are formed by enzymatic digestion near the 5′-end of the DNA substrate.

$PNA^{(Acr)}$ is complementary with A1-T10 of $DNA^{(GG)}$. Under the conditions employed, the yield of this 12-mer fragment was 29 mol%. By-product was only the 11-mer (A1-G11), and its yield was marginal (<2 mol%). The 12-mer fragment (as well as the 11-mer) was completely characterized by MALDI-TOF MS. The observed mass number was 4233.6, which fairly agreed with the theoretical value (4231.2). The enzymatic digestion occurred at the phosphodiester linkage between G12 and C13 (and also at all the linkages in its 3′-side). However, the linkage between G11 and G12 was hardly digested. Apparently, the nuclease S1/$PNA^{(Acr)}$ combination strictly differentiates the second unpaired nucleotide (G12) from the first one (G11), and digests only the linkage in the 3′-side of the former. In lanes 2-4 in Figure 2, G12 in $DNA^{(GG)}$ was replaced by another nucleotide (A, T, or C), and the DNA was similarly treated with the nuclease S1/$PNA^{(Acr)}$ combination. The scission was also selective at the linkage between the nucleotide-12 and C13, as further confirmed by MS.[6] Replacement of G11 to A did not much affect the site-selective scission (lane 5 in Figure 2).

When unmodified PNA ($PNA^{(Un)}$) was used in place of

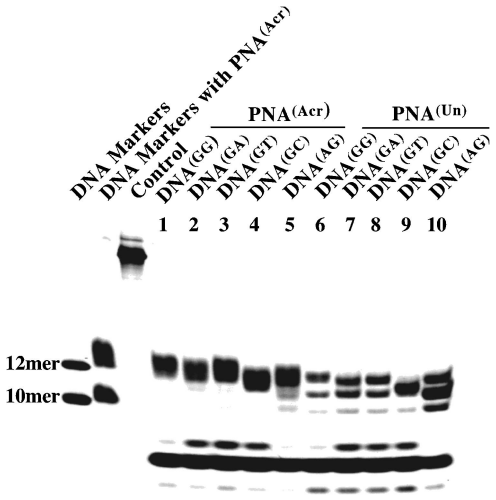

Figure 2. Electrophoresis patterns for the nuclease S1 digestion of various DNA substrates in the presence of $PNA^{(Acr)}$ (lanes 1-5) or $PNA^{(Un)}$ (lanes 6-10). Reaction conditions are the same as described in Figure 1.

$PNA^{(Acr)}$, however, both 10-mer (A1-T10) and 11-mer (A1-G11) fragments were formed from $DNA^{(GG)}$ in considerable amounts, in addition to the 12-mer fragment (lane 2 in Figure 1). The yields of the 10-mer, 11-mer, and 12-mer fragments were 2, 7, and 12 mol%, respectively. The acridine in $PNA^{(Acr)}$ is essential for the efficient and selective formation of the 12-mer fragment. This remarkable effect of the acridine is also evident for the scission of other DNA substrates in Figure 2 (compare lanes 1-5 with lanes 6-10).[6]

Use of the PNA derivative is essential for the present site-selective scission. Upon using DNA additives ($DNA^{(Acr)}$ or $DNA^{(Un)}$) under the same reaction conditions, $DNA^{(GG)}$ was completely cleaved and no oligonucleotides were obtained (lanes 3 and 4 in Figure 1). Here, both $DNA^{(Acr)}$ and $DNA^{(Un)}$ are susceptible to the digestion by nuclease S1. Furthermore, DNA/DNA duplexes are less stable than the corresponding DNA/PNA duplexes.[4] These two factors make the protection less effective.

In order to shed light on the role of acridine of $PNA^{(Acr)}$, the melting temperatures T_m of the duplexes of DNA substrates with $PNA^{(Acr)}$ or $PNA^{(Un)}$ were measured (Table 1).[7] The T_m values for $PNA^{(Acr)}$ are higher than those for $PNA^{(Un)}$. However, the difference in T_m is quite small, in spite of the significant difference in the DNA-protecting activity. It is also noteworthy that all the T_m values, even with $PNA^{(Un)}$, are much higher than the reaction temperature. The main role of the acridine is not to stabilize the whole part of DNA/PNA duplexes. Assumedly, the acridine intercalates between the base-pairs near the termini of hetero-duplexes and suppresses the breathing motion there. As the result, the phosphodiester linkages near the termini are sufficiently protected from nuclease S1, and only the 3′-side of the second nucleotide from the termini is susceptible to the enzymatic digestion. Alternatively, the acridine could directly interact with the nucleobase near the termini.

Table 1. Melting temperatures (in °C) of DNA/PNA duplexes

DNA	PNA	
	$PNA^{(Acr)}$	$PNA^{(Un)}$
$DNA^{(GG)}$	61.6	57.8
$DNA^{(GC)}$	63.3	57.7
$DNA^{(GT)}$	59.5	58.0

In conclusion, PNA bearing an acridine is eminent for site-selective protection of DNA from nuclease S1, and provides the target DNA fragments in high yields. The applications of these findings, as well as mechanistic study on the role of acridine, are currently under way in our laboratory.

We should like to thank Prof. Hisakazu Mihara and Dr. Akinori Kuzuya for kind assistance in preparing PNAs. This work was partially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, Culture and Technology, Japan.

References and Notes

1 Reviews: a) M. Komiyama and J. Sumaoka, *Curr. Opin. Chem. Biol.*, **2**, 751 (1998). b) E. L. Hegg and J. N. Burstyn, *Coord. Chem. Rev.*, **173**, 133 (1998). c) A. Sreedjara and J. A. Cowan, *J. Biol. Inorg. Chem.*, **6**, 337 (2001).

2 Y. Kitamura and M. Komiyama, *Nucleic Acids Res.*, **30**, e102 (2002), and references cited therein.

3 a) S. Ye, X. G. Liang, Y. Yamamoto, and M. Komiyama, *Chem. Lett.*, **32**, 10 (2003). b) M. Komiyama, S. Ye, X. G. Liang, Y. Yamamoto, T. Tomita, J. M. Zhou, and H. Aburatani, *J. Am. Chem. Soc.*, **125**, 3758 (2003).

4 A review on PNA: P. E. Nielsen, *Curr. Opin. Struct. Biol.*, **9**, 353 (1999).

5 $PNA^{(Un)}$ was synthesized according to the literature (T. Takahashi, K. Hamasaki, A. Ueno, and H. Mihara, *Bioorg. Med. Chem.*, **9**, 991 (2001)), and a glycine residue was attached to its C-terminus. This PNA was converted to $PNA^{(Acr)}$ by the reaction with 9-carboxyhexylamino-6-chloro-2-methoxyacridine synthesized from 6-amino-hexylcarboxylic acid and 6,9-dichloro-2-methoxyacridine.

6 The mobility of some fragments in PAGE is considerably affected by coexisting $PNA^{(Acr)}$ or $PNA^{(Un)}$. Accordingly, all the fragments reported in this paper have been concretely characterized by MALDI-TOF MS.

7 The T_m values were determined by using the absorbance at 260 nm under the conditions that [DNA] = 2.0 and [$PNA^{(Acr)}$ or $PNA^{(Un)}$] = 4.0 μmol/L at pH 4.6 ([NaCl] = 45 mmol/L) .

Published on the web (Advance View) July 14, 2003; DOI 10.1246/cl.2003.714