DNA Cleavage

A Synthetic Block Copolymer Regulates S1 Nuclease Fragmentation of Supercoiled Plasmid DNA**

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The materials chemistry and physics of block copolymer supramolecular assembly continue to receive considerable attention in the construction of higher-ordered architectures with unique morphologies and functions.[1–10] Of particular

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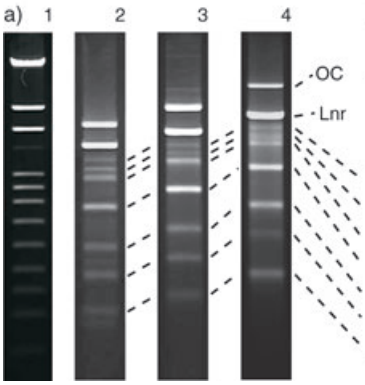
interest is the exploitation of the spatial order intrinsic to block copolymers for the amplification or modulation of molecular recognition processes by using integrated multisite interactions as well as a distinct spatial matching. Herein we report an unprecedented finding that DNA supramolecular assembly with the synthetic block copolymer poly(ethylene glycol)-*b*-poly(L-lysine) (PEG-PLL)[6,11–14] modulates supercoiled plasmid DNA into a particular structure. The incorporated DNA within the assembly is cleaved into seven distinct regular fragments by S1 nuclease, an enzyme known to cleave single-stranded DNA. The seven characteristic DNA fragments were of well-defined molecular sizes; in each case these were 10/12, 9/12, 8/12, 6/12, 4/12, 3/12, and 2/12 of the original plasmid length (Figure 1). It is notable that the cleaved sites



b)

Fragments

Length of the fragment [bp] / Size ratio to the native DNA [%]

no	pBR322 4363 bp	pGL3-Luc 5256 bp	ColE1 6646 bp	ratios in fraction
1	3690 / 84.6	4560 / 84.6	5630 / 84.8	10/12
2	3260 / 75.3	4100 / 76.1	5000 / 75.2	9/12
3	2940 / 67.4	3680 / 68.2	4490 / 67.6	8/12
4	2180 / 50.0	2740 / 50.8	3360 / 50.6	6/12
5	1460 / 33.5	1830 / 34.0	2240 / 33.7	4/12
6	1050 / 24.1	1340 / 24.9	1650 / 24.9	3/12
7	750 / 17.1	910 / 16.8	1090 / 16.4	2/12

Figure 1. Regulated fragmentation of plasmid DNAs complexed with PEG-PLL by the S1 nuclease: a) Gel electrophoresis results. Lane 1: marker DNA; lane 2: pBR322 (4363 bp); lane 3: pGL3-Luc (5256 bp); lane 4: ColE1 (6646 bp). OC = relaxed open circular DNA, Lnr = linearized DNA. b) Length of the obtained fragments and size ratio of them to the native DNA. The size ratio of each fragment from three plasmid DNAs is represented as a fraction in the right-hand column.

are located just outside of protein-coding regions, a fact which simultaneously indicates that the S1 nuclease cuts out genes from DNA.

The block copolymer used in this study comprises a cationic PLL segment, which is the plasmid-binding portion, and a nonionic PEG segment, which forms a hydrophilic and hydrated palisade surrounding the ion-paired complex between PLL and plasmid DNA (see the Supporting Information). This allows to obtain a water-soluble nanoscale assembly (100-nm size) without precipitation. The size-specific plasmid DNA cleavage by S1 nuclease was observed at an acidic pH value of 4.9 for the complex, particularly in a unit molar ratio (amino group/phosphate) of 1.0. As seen in lane 2 of Figure 1a, pBR322 DNA is cut into seven distinct fragments (as listed in Figure 1b). This regular fragmentation was not specific only for pBR322 but seems common for other plasmids. Surprisingly, the other plasmids, pGL3-Luc and ColE1, when complexed with PEG-PLL, were also cut into seven fragments of the same fractions when measured against each original plasmid (Figure 1a, lanes 3 and 4, and Figure 1b). The consistency of the fragmentation results clearly indicates the systematic cleavage of plasmid DNA at well-defined intervals. It should be noted that this regular cleavage of plasmid DNA complexed with PEG-PLL is only observed for the supercoiled DNA form. Relaxed open circular DNA, prepared by treatment with topoisomerase I, also formed stable complexes with PEG-PLL but exhibited no sensitivity to S1 nuclease (data not shown). Additionally, a complex with linear plasmid DNA, prepared by treatment with a restriction enzyme (EcoRI) having a unique recognition site on the plasmid, was smoothly degraded to oligo-DNA pieces in a nonspecific manner (data not shown). The observed differences in S1 nuclease sensitivity to plasmid isomer constructs suggest that topological features of the block copolymer–DNA supramolecular complex influence the enzymatic fragmentation process.

Polyion complex formation between DNA and cationic compounds is known to induce a coil–globule transition, thereby resulting in condensed complexes with ordered morphologies, mainly in toroidal or rod-like forms.[14–18] Consistent with this, charge-neutralizing DNA complexation with PEG-PLL produces a detectable transition from an expanded DNA superhelix into a compact state, as confirmed by static and dynamic light scattering[13] as well as by direct observation with fluorescence microscopy.[18] Although the structural details of the condensed plasmid in this complex have not yet been clarified, significant structural features are probably present in the supercoiled double-stranded DNA upon polyion-induced condensation. Constrained structural order (or regular disorder) in the double-stranded DNA structure might be regularly repeated in this polyion-induced condensation process. This would permit the DNA to adopt particular structures that compensate for the structural constraints accompanying conformation transitions during complexation. Consequently, these specific disordered sites in the condensed DNA strands may preferentially promote S1 nuclease attack, thereby resulting in the observed regular DNA fragmentation.

Supercoiled DNA associated with poly(L-lysine) homopolymer with degrees of polymerization (DP) of 19 and 260 exhibited only nonspecific S1 nuclease degradation without any ordered cleavage, as shown by a smeared electrophoresis gel stain (data not shown). This indicates that polyion complexation alone is not sufficient and that the PEG segment in the block copolymer plays a crucial role in regulating the nuclease sensitivity in addition to promoting the complex solution stability. Inter- or intramolecular steric repulsion of hydrated PEG segments may contribute to the modulated structure of plasmid DNA.

The sensitivity of single-strand-cleavage endonucleases like S1 nuclease against naked plasmid DNAs has been investigated since the 1980s.[19–22] The naked DNAs are first nicked and then linearized. The cleavage sites are cruciform loops, which are adopted by short inverted repeat (palindrome) sequences in the topologically stressed double helix.

Formation of the hairpin-loop structure relieves the topological strain in double-stranded circular DNA molecules. These inverted repeats, separated by nonrepetitious sections of DNA, are specifically cleaved by single-strand-specific nucleases at the center of each hairpin loop. Torsional stress of negative supercoiling of plasmid DNA occasionally induces a kink in the DNA strand with an acute angle. The high susceptibility of palindrome sequences to S1 nuclease digestion led us to assume that the regular fragmentation seen here in the system with the PEG-PLL/plasmid DNA (pDNA) complex might be a result of selective digestion at the cruciform loop regularly induced in the condensed DNA strands. To get an insight into this assumed mechanism, inverted repeats larger than five continuous sequences are picked up from three plasmids (pBR322, pGL3-Luc, and ColE1) by using computer analysis.[23] Neighboring smaller palindromes separated by one or two nonrepetitious sections of DNA were also taken into consideration. It is to be expected that hairpin stability will be directly proportional to stem length but inversely proportional to loop length. The inverted sequences were marked from four to one according to the hairpin stability, as summarized in Figure 2a as a function of base number. Obviously, there are quite a few palindrome sequences. Denaturation of the DNA double strand is necessary for transformation from the normal interstrand base pairing to the intrastrand base pairing of the cruciform structure. The binding energy of the A–T (adenine–thymine) pair is lower than that of the G–C (guanine–cytosine) pair, because two hydrogen bonds are contributing to the pairing between A and T while three contribute between G and C. Thus, the composition of A–T pairs within the palindrome sequences was also examined (Figure 2b). Refinement according to palindrome size and A–T composition allowed the extraction of several sites as candidates for S1 nuclease recognition; these are indicated as dotted lines in Figure 2a and b and as slashes in the pDNA maps (Figure 2d). By use of these refined sites, combinations satisfying the regular fragments were surveyed by consideration of fragment lengths, since two sites on the circular plasmid DNA must be cleaved (for example, 3/12+9/12 = 12/12). Consequently, combinations of cleavage sites yielding the seven DNA sizes were acquired for all three plasmid DNAs

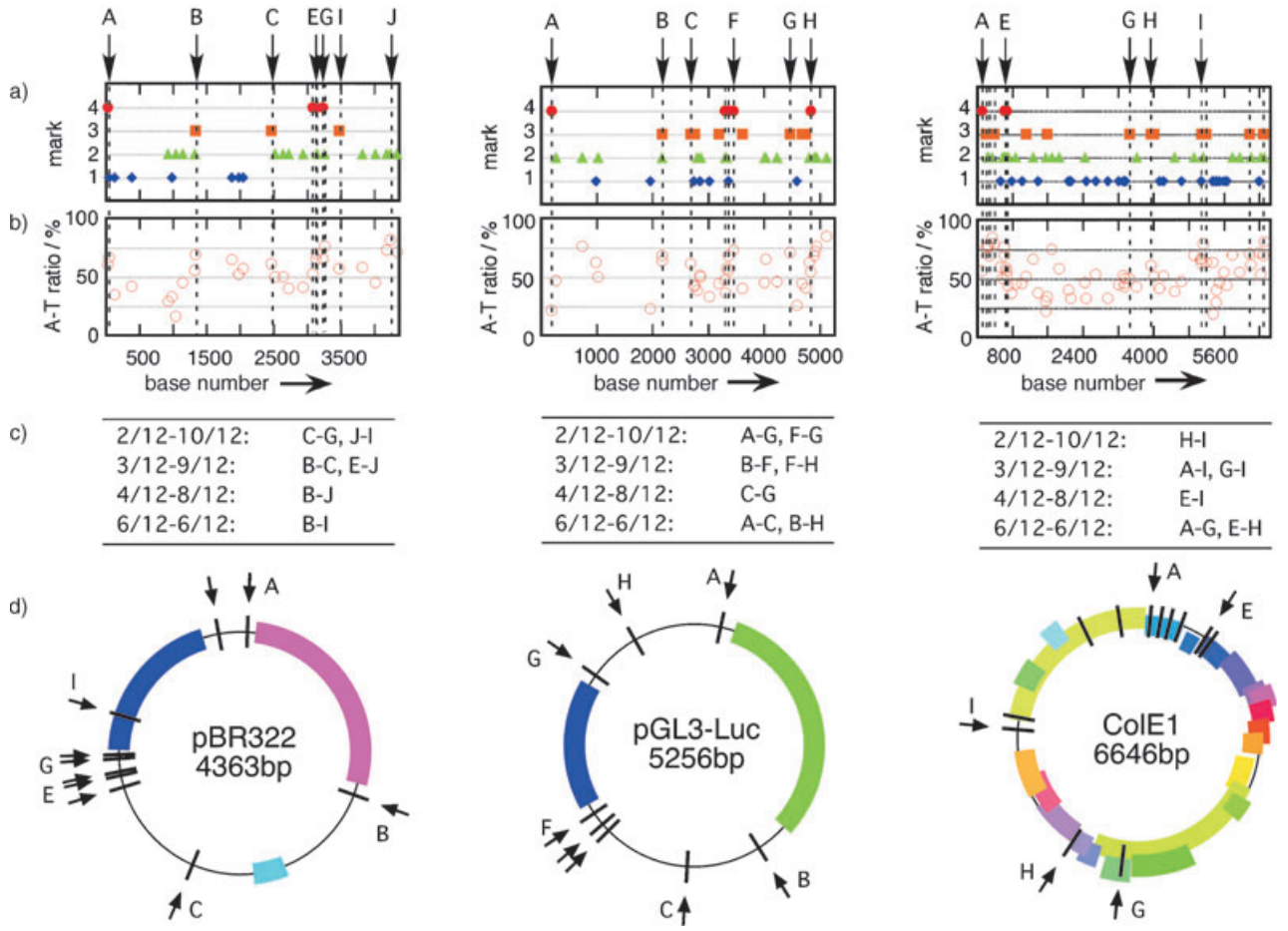


Figure 2. Palindrome maps and protein codes of the three plasmid DNAs pBR322 (left), pGL3-Luc (middle), and ColE1 (right). a) Cruciform stability by the size of inverted repeats. Higher mark numbers indicate higher cruciform stability. b) Percentage A–T composition in the considered palindrome sequences as a function of base number in the plasmid DNAs. Here, base 1 was defined as the recognition site of the EcoR1 restriction enzyme. The dotted lines in panels (a) and (b) indicate refined digestion sites as evaluated from the panels. The combinations of cleavage sites that satisfy the seven-piece fragmentation pattern are summarized in (c). Those evaluated cleavage sites are shown as arrows with capital letters in panels (a) and (d); in addition, correlation between the protein-coding regions and proposed cleavage sites is shown in (d).

(listed in Figure 2c) and those sites are indicated as arrows in Figure 2a and d. The regular fragmentation can be explained by assuming that the cruciform structures regularly induced in the DNA by complexation with the PEG-PLL block copolymer are digested by the single-strand-recognition endonuclease.

Obviously, development of the extruded cruciform structure requires definite stress. The cruciform formation in the naked pDNA is known to be driven by the torsional stress of negative supercoiling.[22] In the complex of pDNA with PEG-PLL, the stress of condensation by the polycation further amplified the torsional stress of the supercoiled conformation, so that the cruciform may be developed in multiple sites. It should be noted that the charge ratio of 1.0 is indeed a critical point in the pDNA transition from extended state to condensed state. The ethidium bromide exclusion assay showed an abrupt decrease in the relative fluorescence, which indicates condensation of DNA molecules, at a charge ratio range of around 1.0 (see the Supporting Information). Additionally, the transition of DNA shape is confirmed by direct observation with atomic force microscopy (Figure 3). Apparently, complex shapes at the charge ratio of 1.0 comprise preferentially condensed rod and toroid conformations.

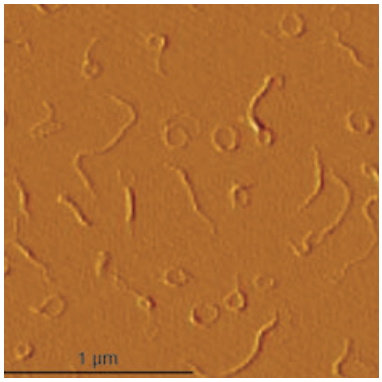


Figure 3. AFM image of plasmid DNA (pGL3-Luc) complexed with the PEG-PLL block copolymer at a charge ratio of 1.0. The image was taken in the amplitude mode for the complex after equilibration in solution for 24 hours. A scale bar is included in the figure.

Furthermore, the potential cleavage site (palindrome site) and protein-coding region have a very interesting correlation, in that the former is located just outside of the latter, as can be clearly seen in Figure 2d. It is worth mentioning that this is commonly observed among the three examined plasmids. This correlation is most distinctive in the ColE1 plasmid, which has various protein codes (Figure 2d). The fact that the selected inverted repeats are located just in the gap or at the terminus of various coding regions suggests that the cruciform structure may substantially contribute to the regulation of gene expression[21] and is most likely to be a landmark for DNA-binding proteins. It should be noted that hypersensitivity of structured DNA to S1 nuclease has been observed in regions of transcriptionally active genes in chromatin.[24] DNA breakage by S1 nuclease is observed in apoptotic cells[25] and also more in mitotic chromosomes than chromosomes in interphase cells.[26] This may be a reflection of the differences of stress under such circumstances. The cruciform structural motifs must be to regulate the binding of proteins, nucleases, promoters, or transcription factors; the full function remains to be determined. Yet, the potential for designing synthetic polymer constructs that reliably alter or mimic these supramolecular structures or for imparting new features of molecular recognition by using the materials of unconventional biopolymer complexes, as demonstrated here, is exciting.

In conclusion, we have demonstrated that the size-specific cleavage of plasmid DNA by S1 nuclease can be mediated by condensed complexes of plasmids with synthetic block copolymers without any sequence-specific binding. The recognition site is probably the cruciform structure induced by DNA complexation with the block copolymer. This means that DNA inherently retains sequences that can transform its secondary structure by certain stimuli and the synthetic polymer reveals the functional structure by complexation. The observed unique sensitivity of PEG-PLL/pDNA complexes to S1 nuclease should provide an insight into the mechanisms of endogenous protein-induced modification of DNA and into the design of artificial restriction enzymes and gene-exploring systems through supramolecular assembly of synthetic macromolecular materials.

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[1] J.-M. Lehn, *Science* **2002**, *295*, 2400 – 2403.
[2] G. M. Whitesides, B. Grzybowski, *Science* **2002**, *295*, 2418 – 2421.
[3] J. D. Hartgerink, E. Beniash, S. I. Stupp, *Science* **2001**, *294*, 1684 – 1688.
[4] D. E. Discher, A. Eisenberg, *Science* **2002**, *297*, 967 – 973.
[5] S. Jain, F. S. Bates, *Science* **2003**, *300*, 460 – 464.
[6] A. Harada, K. Kataoka, *Science* **1999**, *283*, 65 – 67.
[7] J. N. Cha, G. D. Stucky, D. E. Morse, T. J. Deming, *Nature* **2000**, *403*, 289 – 292.
[8] J. A. Hubbell, *Science* **2003**, *300*, 595 – 596.
[9] R. Savic, L. Luo, A. Eisenberg, D. Maysinger, *Science* **2003**, *300*, 615 – 618.
[10] A. V. Kabanov, V. A. Kabanov, *Adv. Drug Delivery Rev.* **1998**, *30*, 49.
[11] S. Katayose, K. Kataoka, *Bioconjugate Chem.* **1997**, *8*, 702 – 707.
[12] S. Katayose, K. Kataoka, *J. Pharm. Sci.* **1998**, *87*, 160 – 163.
[13] K. Itaka, K. Yamauchi, A. Harada, K. Nakamura, H. Kawaguchi, K. Kataoka, *Biomaterials* **2003**, *24*, 4495 – 4506.
[14] K. Miyata, Y. Kakizawa, N. Nishiyama, A. Harada, Y. Yamasaki, H. Koyama, K. Kataoka, *J. Am. Chem. Soc.* **2004**, *126*, 2355 – 2361.
[15] U. K. Laemmli, *Proc. Natl. Acad. Sci. USA* **1975**, *72*, 4288 – 4292.
[16] S. M. Mel'nikov, V. G. Sergeyev, K. Yoshikawa, *J. Am. Chem. Soc.* **1995**, *117*, 9951 – 9956.
[17] J. Rackstraw, A. L. Martin, S. Stolnik, C. J. Roberts, M. C. Garnett, M. C. Davies, S. J. B. Tendler, *Langmuir* **2001**, *17*, 3185 – 3193.
[18] Y. Yamasaki, S. Katayose, K. Kataoka, K. Yoshikawa, *Macromolecules* **2003**, *36*, 6276 – 6279.

[19] D. M. J. Lilley, *Nucleic Acids Res.* **1981**, *9*, 1271 – 1289.
[20] N. Panayotatos, R. D. Wells, *Nature* **1981**, *289*, 466 – 470.
[21] U. R. Mueller, W. M. Fitch, *Nature* **1982**, *298*, 582 – 585.
[22] M. Gellert, M. H. O'Dea, K. Mizuuchi, *Proc. Natl. Acad. Sci. USA* **1983**, *80*, 5545 – 5549.
[23] Palindrome sequence analyses were performed on a Macintosh iBook 400 MHz computer by using the FROG-Mac program (written by N. Miki at Osaka University and available from http://pharma1.med.osaka-u.ac.jp/freeware.html).
[24] A. Larsen, H. Weintraub, *Cell* **1982**, *29*, 609 – 622.
[25] M. C. Peitsch, C. Mueller, J. Tschopp, *Nucleic Acids Res.* **1993**, *21*, 4206 – 4209.
[26] G. Juan, W. Pan, Z. Darzynkiewicz, *Exp. Cell Res.* **1996**, *227*, 197 – 202.

