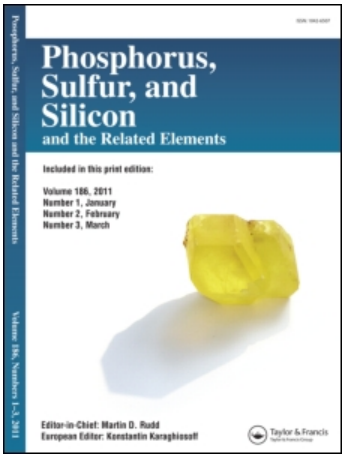

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Hydrolysis of DNA by N-Phosphoryl Branched Peptide

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A N-phosphoryl branched peptide (DIPP-Leu)$_2$-Lys-OCH$_3$ was found to efficiently cleave double-strand DNA such as PUC19 and Lambda DNA at pH 8.5 in 100 mM Tris-HCl buffer. The T4 ligase experiment implied that the DNA cleavage occurs via a hydrolytic path.

Keywords Branched peptide; DNA cleavage; hydrolysis; n-phosphorylation

The development of the nuclease mimics is of increasing importance in biotechnology and medicine. During the last decade, extensive studies on artificial nucleases have been carried out. However, most of them concentrated on metal complexes, there is only a few research results on peptides.[1–3] Toulme and Helene et al. found that Lys-Trp-Lys can recognize and cleave DNA at apurinic sites.[4–6] The basic polypeptides have been reported to accelerate the hydrolysis of ribonucleic acids.[7,8]

Likewise, our previous studies have demonstrated that the dipeptide seryl-histidine and related oligopeptides can cleave DNA, protein,

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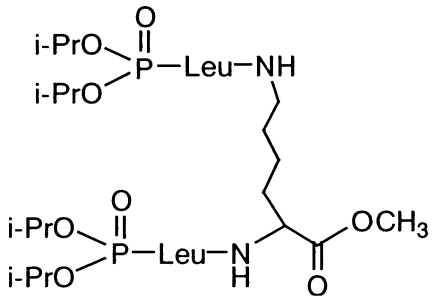
and carboxyl ester.[9,10] It is noted that the dipeptide is the shortest peptide for cleavage agents ever reported. The studies in our lab have also shown that introducing phosphoryl groups to amino acids stimulated many interesting chemical properties.[11,12] One typical example of this was that the N-phosphoryl branched peptide $(DIPP\text{-}Leu)_2\text{-}Lys\text{-}OCH_3$ which was recently synthesized exhibited anti-virus activities, as it can inhibit the growth of both K562 and A2780 cells in cell biological tests.[13] Simultaneously, in the present, work we found that the $(DIPP\text{-}Leu)_2\text{-}Lys\text{-}OCH_3$ can efficiently hydrolyze DNA also.

RESULTS AND DISCUSSION

The chemical structure of the N-phosphoryl branched peptide $(DIPP\text{-}Leu)_2\text{-}Lys\text{-}OCH_3$ is illustrated in Scheme 1. Hydrolysis properties of PUC19 plasmid DNA were studied at different pHs from 7.1 to 8.9 in a Tris-HCl buffer by agarose gel electrophoresis. The result shows that the DNA cleavage by $(DIPP\text{-}Leu)_2\text{-}Lys\text{-}OCH_3$ is pH-dependent and the optimum pH is about 8.5 in Tri-HCl buffer (Figure 1). Similar results were obtained in the same experiment using Lambda DNA in stead of PUC19 DNA.



SCHEME 1 The chemical structure of the N-phosphoryl branched peptide $(DIPP\text{-}Leu)_2\text{-}Lys\text{-}OCH_3$.

When the DNA fragments cleaved by the N-phosphoryl branched pepetide were incubated with DNA T4 ligase for several hours, there was obvious upward shift in the electrophoresis image (Figure 2). The increase in size of cleavage fragments indicated that they were religated by the T4 ligase. It is known that the ligation by the T4 ligase requires free 3′-hydroxyls and 5′-phosphates for ligation. Hence, it suggests that the cleavage process of DNA by $(DIPP\text{-}Leu)_2\text{-}Lys\text{-}OCH_3$ is via a hydrolytic path. In addition, the conclusion was also supported by

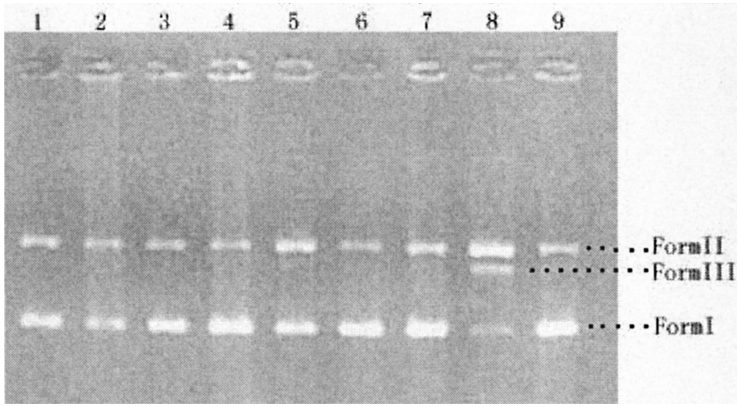


FIGURE 1 Agarose gel electrophoresis of PUC19 DNA cleavaged by 5 mM $(DIPP\text{-}Leu)_2\text{-}Lys\text{-}OCH_3$ for 24 h at 50°C in a 100-mM different pH Tris-HCl buffer. Lane 1: control without cleavage agent, pH = 7.0; Lanes 2–9: with 5 mM cleavage agent, pH = 7.1, 7.3, 7.5, 7.7, 8.0, 8.3, 8.5, and 8.9, respectively.

the fact that the cleavage occured in the presence of DMSO as a radical scavenger.

It should be noted that mechanistic details of DNA hydrolysis by $(DIPP\text{-}Leu)_2\text{-}Lys\text{-}OCH_3$ might show some difference in seryl-histidine.

FIGURE 2 Ligation of DNA cleavage fragments by T4 ligase. PUC19 plasmid DNA was incubated with or without $(DIPP\text{-}Leu)_2\text{-}Lys\text{-}OCH_3$ for 24 h at 50°C in pH 8.5 Tris-HCl buffer, then it was incubated with or without T4 ligase at 37°C for 6 h. Lane1: without cleavage agent and T4 ligase; lane 2: with cleavage agent and no T4 ligase; lane 3: with T4 ligase and no cleavage agent; lane 4: with cleavage agent and T4 ligase.

There is no hydroxyl of serine to serve as a nucleophilic group in the former compared with the latter. Further studies to elucidate the mechanism are in progress.

EXPERIMENTAL

Preparation of (DIPP-Leu)$_2$-Lys-OCH$_3$

The object product was synthesized according to the method in our previous report and was characterized by ESI-MS, ^{1}H-NMR, ^{31}P-NMR, and elemental analysis.[14]

Cleavage Reactions and Cleavage Products Analyses

0.5 μL PUC19 DNA (1 μg/μL),0.25 μL (DIPP-Leu)$_2$-Lys-OCH$_3$ (0.5M DMSO solution) and 24.25 μL Tris-HCl buffer (100 mM) in the pH range 7.1–8.9 were added into PCR Tubes, sealed, and incubated at 50°C for appropriate periods of time. After incubation, a portion of the incubated solution was removed and analyzed by 1% agarose gel electrophoresis at 50 V for 1–1.5 h. Then the gel stained by 0.5 μg/mL ethidium bromide was imaged.

T4 ligase reaction

The reaction solution is the same as started in the previous paragraph to generate DNA cleavage fragments. After incubation for 24 h, 10-μ-L incubated solution was removed, one half of the solution was reserved at −20°C, and the residual solution was incubated with 1 μL T4 ligase and 2 μL ligation buffer containing ATP for 6 h at 37°C. Afterwards, all of them were analyzed by 1% agarose gel electrophoresis at 50 V for 1–1.5 h. Then the gel stained by 0.5 μg/mL ethidium bromide was imaged.

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