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Synthesis and DNA Nicking Studies of a Novel Cyclic Peptide: Cyclo[Lys-Trp-Lys-Ahx-]

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Abstract—Two novel cyclic tetrapeptides: cyclo[Lys-Tyr-Lys-Ahx-] **7a** and cyclo[Lys-Trp-Lys-Ahx-] **7b** were synthesized by coupling protected amino acid in solution and the subsequent cyclization effected by the pentafluorophenyl ester method as described in previous papers. These cyclic peptides were designed and synthesized to study their interaction with DNA, based on previous reports that linear peptides Lys-Tyr-Lys and Lys-Trp-Lys could bind to various forms of DNA and cleaved supercoiled DNA at apurinic sites. Ethidium bromide displacement assay showed that the apparent DNA binding constant of linear Lys-Tyr-Lys and cyclic peptide **7a** are far below 1×10^3 M^{-1}, whereas those of cyclic peptide **7b** and linear Lys-Trp-Lys are 1.9×10^4 M^{-1} and 9.5×10^3 M^{-1}, respectively. Kinetic studies using agarose gel electrophoresis showed that cyclic peptide **7b** and Lys-Trp-Lys possessed DNA nicking activity on natural supercoiled φX174 DNA with nicking rate of 50.7 and 75.6 pM min^{-1} at 65 °C, respectively, whereas cyclic peptide **7a** and linear Lys-Tyr-Lys were devoid of the corresponding activity. The DNA nicking rate increased significantly with increase in reaction temperature. At reaction temperatures lower than 65 °C , the DNA nicking rate of cyclic peptide **7b** exceeded that of linear Lys-Trp-Lys. The addition of 1 μM ferrous ion did not give significant enhancement effect on the DNA nicking rate by the peptides. UV irradiation gave a marked rate enhancement on the DNA nicking rate of linear Lys-Trp-Lys and a moderate enhancement on the DNA nicking rate of cyclic peptide **7b**.

Introduction[1]

The studies of new DNA cleavage agents are of considerable importance in molecular biology and organic chemistry. A number of pioneering studies by Hélène and coworkers[2–6] showed that simple tripeptides Lys-X-Lys (where X is an aromatic residue) can bind to polydeoxyribonucleotides, native and UV-irradiated denatured DNA with the aromatic ring stacked between bases and the positively charged lysyl side chains interacted with the phosphates via ionic bonds. In addition, specific single strand cleavage of supercoiled plasmid DNA at apurinic sites was reported for Lys-Trp-Lys and Lys-Tyr-Lys tripeptides and β-elimination nicking of the apurinic DNA backbone was proposed.[7]

Our approach in the further investigation of Lys-X-Lys peptides in DNA binding and nicking process of natural supercoiled DNA is by a tentative design of cyclic peptides containing the Lys-X-Lys sequence, and on the kinetic studies of DNA nicking by the corresponding linear and cyclic peptides. We chose to synthesize conformationally restricted cyclic peptides cyclo[Lys-Tyr-Lys-Ahx-] **7a** and cyclo[Lys-Trp-Lys-Ahx-] **7b** by the following rationale (where Ahx is 6-aminohexanoic acid). We envisage that the stereochemical fit of small-ring-sized cyclic peptides to the minor groove of DNA should be superior to that of the corresponding linear peptides. In addition, cyclic peptides lack the C-terminal carboxylate function, and will be expected to have higher DNA binding affinity since the anionic carboxylate group in linear peptides will have electrostatic repulsion with the anionic phosphate backbone of DNA. Like the γ-aminobutyric acid (GABA) residue,[8] the Ahx residue can be used as a spacer group for the cyclopeptide ring since the additional methylene groups in this residue would ease the tension for ring closure and also provide additional hydrophobic interaction with DNA. During the course of this study we used agarose gel electrophoresis to monitor the rate of DNA nicking by linear Lys-X-Lys peptides and the corresponding cyclic peptides at various elevated temperatures, in the absence and presence of UV which causes denaturation of DNA.

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Experimental

All of the protected amino acid derivatives were purchased from Sigma chemical Co. (St. Louis, MO) and Schweizerhall Co. (South Plainfield, NJ) or synthesized in the laboratory according to published procedures. Supercoiled ϕX174 (RF-I) DNA was purchased from New England Biolabs, Inc., Beverly, MA. Melting points were determined on a Mel-Temp apparatus (Cambridge, MA) and were uncorrected. Medium pressure column chromatography was performed using Merck 230–400 mesh silica gel. TLC was performed on Merck silica gel 60 on aluminum sheets. A Sage Model 351 syringe pump was employed in all cyclization reactions. Optical rotations were determined on a Rudolph Autopol II instrument. HPLC analysis and purification of the cyclic peptides were performed on Vydac TP201 reversed-phase columns: (column 1, 10 μm, 0.4×25 cm; column 2, 10 μm, 1.0×25 cm), using a Beckman 110B solvent delivery module and equipped with a Soma S-3702 variable wavelength UV detector monitoring at 220 nm (analytical) or 245 nm (preparative). Elemental analyses were performed at the Chemistry Department, Cheng-Kung University.

Electrophoretic studies of DNA nicking

In each vial 1.5 μL of ϕX174 DNA (RF-I) was incubated with drugs in PBS in the presence of 1 μM Fe^{++} at various fixed temperatures for various intervals and then the reaction was quenched at 0 °C and 2 μL of 1200 mM mannitol was added. The addition of mannitol proved to be unnecessary and was not used in subsequent experiments. For UV irradiation experiments a UVP model UVGL-25 lamp (short wavelength) fitted with ten 0.2 mm plastic filters for the attenuation of UV intensity was used. 6X sampling dye (2 μL) and 0.5X TBE (2 μL) were added to each vial and the reactions were analyzed by electrophoresis on a Hybaid 12×14 cm horizontal submarine unit using 0.6% agarose gel in 0.5X TBE at 100 volt for about 2 h. The gel was stained with ethidium bromide (0.5 μg/mL) for 15–20 min, destained with buffer for 5 min, and photographed with polaroid 667 film under short wavelength UV light. The electrophoretic band volumes were analyzed by a PC computer installed with Vilber Lourmat BIO-1D analysis software (Marne La Vallee, Cedex 1, France).

DNA binding assay

DNA binding studies were carried out on λ-DNA according to the ethidium bromide displacement method of Morgan et al.,[9] as described previously.[8]

Boc-Ahx-OPa 1a. This compound was prepared according to published procedures[8,10] in a 76% yield. TLC, $CHCl_3$/MeOH (92:8), R_f 0.68; mp 73–74 °C. Anal. calcd for: $C_{19}H_{27}NO_5$: C 65.31, H 4.01, N 7.79. Found: C 65.19, H 4.01, N 7.82.

Boc-Lys(Z)-Ahx-OPa 2a. Boc-Ahx-OPa (2.0 g, 5.74 mmol) in CH_2Cl_2 (5.2 mL) was stirred with TFA (7.5 mL) for 50 min. and the solvents removed in vacuo. Boc-Lys(Z) (2.18 g, 5.74 mmol) in CH_2Cl_2 (70 mL) was treated with HOBt (0.85 g, 6.31 mmol) and DCC (1.78 g, 8.61 mmol) at 0 °C for 10 min and then at room temperature for 50 min and then added to the TFA salt (in CH_2Cl_2, 7 mL) prepared as stated above. DIEA was added to adjust the pH to neutrality. After 60 min the reaction mixture was filtered, diluted with CH_2Cl_2 (100 mL) and washed successively with citric acid (10%, 2×20 mL), saturated $NaHCO_3$ (2×20 mL), and saturated NaCl (2×20 mL), dried ($MgSO_4$) and evaporated to give a crude solid. Silica gel chromatography ($CHCl_3$) afforded the dipeptide **2a** as a white solid (3.28 g, 93%), TLC, $CHCl_3$/MeOH (96:4), R_f 0.53; mp 85–86 °C; $[\alpha]_D^{29}$ −3.73 (*c* 0.54, $CHCl_3$/MeOH, 4:1). Anal. calcd for $C_{33}H_{45}N_3O_8$: C 64.79, H 7.42, N 6.87. Found: C 64.79, H 7.37, N 6.88.

Boc-Tyr(OBzl)-Lys(Z)-Ahx-OPa 3a. Boc-Lys(Z)Ahx-OPa (1.48 g, 2.42 mmol) was deblocked with TFA/CH_2Cl_2. Boc-Tyr(OBzl)-OH (0.90 g, 2.42 mmol) in CH_2Cl_2 (30 mL) was treated with HOBt (0.36 g, 2.66 mmol) and DCC (0.75 g, 3.63 mmol) at 0 °C for 10 min and at room temperature for 50 min and then added to the TFA salt prepared as stated above in CH_2Cl_2 (13 mL). DIEA was used to adjust the pH to neutrality. After 1.5 h, the reaction mixture was chilled in an ice-bath, filtered, diluted with CH_2Cl_2, and washed successively with citric acid (10%), saturated $NaHCO_3$, and water, dried ($MgSO_4$), and evaporated to give a crude solid. Silica gel chromatography using stepwise elution ($CHCl_3$/MeOH; 99:1, 98:2, 97:3, 96:4) afforded a white solid (1.65 g, 79%), TLC, $CHCl_3$/MeOH (96:4), R_f 0.58; mp 124–125 °C, $[\alpha]_D^{29}$ −14.11 (*c* 0.46, $CHCl_3$/MeOH; 4:1). Anal. calcd for $C_{49}H_{60}N_4O_{10}$: C 67.34, H 7.03, N 6.41. Found: C 67.60, H 6.97, N 6.42.

Boc-Lys(Z)-Tyr(OBzl)-Lys(Z)-Ahx-OPa 4a. Boc-Tyr(-OBzl)-Lys(Z)-Ahx-OPa (1.48 g, 1.71 mmol) was deblocked with TFA/CH_2Cl_2. Boc-Lys(Z)-OH (0.65 g, 1.71 mmol) in CH_2Cl_2 (21.1 mL) was treated with HOBt (0.25 g, 1.88 mmol) and DCC (0.53 g, 2.57 mmol) at 0 °C for 10 min and at room temperature for 50 min and then added to the TFA salt prepared as stated above in CH_2Cl_2 (9 mL). DIEA was used to adjust the pH to neutrality. After 1.5 h, the reaction mixture was chilled in an ice-bath, filtered, diluted with CH_2Cl_2 (100 mL), and washed successively with citric acid (10%), saturated $NaHCO_3$, and water, dried ($MgSO_4$), and evaporated to give a crude solid. Silica gel chromatography ($CHCl_3$, 100%) afforded a white solid (1.36 g, 71%), TLC, $CHCl_3$/MeOH (96:4), R_f 0.45; mp 154–155 °C, $[\alpha]_D^{29}$ −26.11 (*c* 0.5, $CHCl_3$/MeOH; 4:1). Anal. calcd for $C_{63}H_{78}N_6O_{13}$: C 67.12, H 6.97, N 7.46. Found: C 66.78, H 7.01, N 7.38.

Boc-Trp-Lys(Z)-Ahx-OPa 3b. Boc-Lys(Z)-Ahx-OPa (5 g, 8.17 mmol) was deblocked with TFA/CH_2Cl_2. Boc-Trp-OH (2.49 g, 8.17 mmol) in CH_2Cl_2 (100 mL), DMF (1.0 mL) was treated with HOBt (1.21 g, 8.99 mmol) and DCC (2.53 g, 12.26 mmol) at 0 °C for 10 min and at

room temperature for 50 min and then added to the TFA salt prepared as stated above in CH_2Cl_2 (50 mL). DIEA was used to adjust the pH to neutrality. After 1.5 h the reaction mixture was chilled in an ice-bath, filtered, diluted with CH_2Cl_2 (200 mL) , and washed successively with citric acid (10%), saturated $NaHCO_3$, and water, dried ($MgSO_4$), and evaporated to give a crude solid. Silica gel chromatography ($CHCl_3$, 100%) afforded a white solid (5.75 g, 88%), TLC, $CHCl_3$/MeOH (96:4), R_f 0.45; mp 62–63 °C, $[\alpha]_D^{29}$ −12.35 (*c* 0.49, $CHCl_3$/MeOH; 4:1). Anal. calcd for $C_{44}H_{55}N_5O_9.0.5\ H_2O$: C 65.49, H 6.99, N 8.68. Found: C 65.38, H 6.95, N 8.67.

Boc-Lys(Z)-Trp-Lys(Z)-Ahx-OPa 4b. Boc-Trp-Lys(Z)-Ahx-OPa (5.75 g, 7.21 mmol) was deblocked with TFA/CH_2Cl_2. Boc-Lys(Z)-OH (2.74 g, 7.21 mmol) in CH_2Cl_2 (90 mL) was treated with HOBt (1.07 g, 7.93 mmol) and DCC (2.23 g, 10.82 mmol) at 0 °C for 10 min and at room temperature for 50 min and then added to the TFA salt prepared as stated above in CH_2Cl_2 (39 mL). DIEA was used to adjust the pH to neutrality. After 1.5 h the reaction mixture was chilled in an ice-bath, filtered, diluted with CH_2Cl_2 (150 mL), and washed successively with citric acid (10%), saturated $NaHCO_3$, and water, dried ($MgSO_4$), and evaporated to give a crude solid. Silica gel chromatography using stepwise elution ($CHCl_3$/MeOH; 99:1, 98:2, 97:3) afforded a white solid (6.5 g, 85%), TLC, $CHCl_3$/MeOH (96:4), R_f 0.45; mp 120–121 °C, $[\alpha]_D^{29}$ −26.73 (*c* 0.39, $CHCl_3$/MeOH; 4:1). Anal. calcd for $C_{58}H_{73}N_7O_{12}.0.5\ H_2O$: C 65.15, H 6.98, N 9.17. Found: C 65.28, H 6.96, N 9.17.

Boc-Lys(Z)-Tyr(OBzl)-Lys(Z)-Ahx-OH 5a. The tetrapeptide **4a** (0.80 g, 0.71 mmol) was dissolved in glacial acetic acid (39.5 mL) and water (6.2 mL) added. Zn powder (4.4 g) was then added in small portions. After stirring overnight the mixture was filtered and the solvents evaporated in vacuo. The residue was washed successively with hexane (2×6 mL), EDTA-Na_2 (2.5%, 2×15 mL), and water (2×3 mL), dried over P_2O_5 in vacuo to afford a white solid which was used in subsequent steps without further purification.

Boc-Lys(Z)-Trp-Lys(Z)-Ahx-OH 5b. This free acid was prepared from the tetrapeptide **4b** by the same procedure as prepared for **5a**.

Cyclo[Lys(Z)-Tyr(OBzl)-Lys(Z)-Ahx-] 6a. The tetrapeptide **5a** obtained above was esterified with pentafluorophenol (0.38 g, 2.05 mmol) and DCC (0.28 g, 1.37 mmol) in CH_2Cl_2 (18 mL) for 5 h at room temperature. The reaction mixture was filtered and evaporated in vacuo. The solid obtained was treated with TFA/CH_2Cl_2 (1.2:1) for 50 min and the solvents removed in vacuo. The TFA salt obtained was dissolved in dioxane and injected via a syringe pump into a rapidly stirred solution of dioxane (1200 mL), pyridine (170 mL), and ethanol (21 mL) maintained at 80–82 °C for 8 h. The solvents were evaporated to give a crude solid. Silica gel chromatography using stepwise elution ($CHCl_3$/MeOH, 98:2, 97:3, 96:4, 95:5) afforded a white solid, 0.19 g, 30%. TLC ($CHCl_3$/MeOH, 92:8), R_f 0.40; mp 212–213 °C, $[\alpha]_D^{30}$ −45.29 (*c* 0.36, $CHCl_3$/MeOH, 4:1). MS: (FAB, Xe beam): calcd for $C_{50}H_{62}N_6O_9$: 890.48. Found: 889.45 $[MH]^-$. Anal. calcd for $C_{50}H_{62}N_6O_9.0.5\ H_2O$: C 66.72, H 7.06, N 9.34. Found: C 66.82, H 7.08, N 9.09.

Cyclo[Lys(Z)-Trp-Lys(Z)-Ahx-] 6b. This protected cyclic tetrapeptide was prepared from the tetrapeptide **5b** by a similar procedure as prepared for **6a** in a 32% yield. TLC ($CHCl_3$/MeOH, 92:8), R_f 0.40; mp 208–209 °C, $[\alpha]_D^{30}$ −35.0 (*c* 0.37, $CHCl_3$/MeOH, 4:1). MS: (FAB, Xe beam): calcd for $C_{45}H_{57}N_7O_8$: 823.41. Found: 822.45 $[MH]^-$. Anal. calcd for $C_{45}H_{57}N_7O_8.0.5\ H_2O$: C 64.89, H 7.02, N 11.77. Found: C 64.60, H 6.99, N 11.54.

Cyclo[Lys-Tyr-Lys-Ahx-] 7a. The protected cyclic peptide 6a (0.04 g, 44.92 mmol) was mixed with anisole (0.25 mL) and stirred with anhydrous HF (ca. 1 mL) at 0 °C for 30 min. The solvents were removed in vacuo, and the residue was dried in a vacuum dessicator over P_2O_5 for 2 h. The solid obtained was dissolved in a minimum amount of water and washed with ether, and the aqueous layer was lyophilized. The product was purified by semipreparative reversed-phase HPLC (column 2, 15% MeOH in 0.1% TFA increased to 70% MeOH in 0.1% TFA in 1 h, flow rate: 2 mL/min) to afford, after lyophilization, a hygroscopic powder (0.008 g, 33.5%), pure to HPLC (column 1, isocratic, 15% MeOH in 0.02 N AcOH, 1 mL/min, t_R 2.8 min), $[\alpha]_D^{29}$ −41.10 (*c* 0.39, H_2O/MeOH, 1:1). MS: (FAB, Ar beam): calcd for $C_{27}H_{44}N_6O_5$: 532.34. Found: 533.37 $[MH]^+$.

Cyclo[Lys-Trp-Lys-Ahx-] 7b. The protected cyclic peptide **6b** (0.03 g, 36.41 mmol) in MeOH (3 mL), dioxane (5 mL), and glacial acetic acid (4 mL) was hydrogenated over Pd-C (10%, 0.01 g) for 2 h. The mixture was filtered over Celite, evaporated in vacuo, and purified by semipreparative reversed-phase HPLC (column 2, 15% MeOH in 0.1% TFA increased to 70% MeOH in 0.1% TFA in 1 h, flow rate: 2 mL/min) to afford, after lyophilization, a hygroscopic powder (0.01 g, 49.5%), pure to HPLC (column 1, isocratic, 15% MeOH in 0.02 N AcOH, 1 mL/min, t_R 2.8 min), $[\alpha]_D^{29}$ −28.89 (c 0.45, H_2O/MeOH, 1:1), MS: (FAB, Ar beam): calcd for $C_{29}H_{45}N_7O_4$: 555.35. Found: 556.34 $[MH]^+$.

Results and Discussion

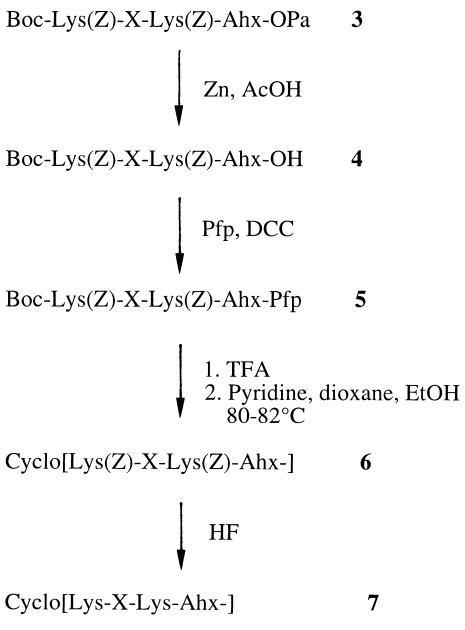
The strategy for the synthesis of the cyclic tetrapeptides **7a** and **7b** is shown in Figure 1. The Lys ε-amino group was protected by the benzyloxycarbonyl (Z) group and the phenolic group of Tyr was protected by the OBzl group. The *C*-terminal carboxyl group was protected by the phenacyl group (OPa) since it is very stable to trifluoroacetic acid cleavage of the Boc group prior to the coupling reactions.[10] The OPa group can be selectively removed by Zn/acetic acid before the esterification of the *C*-terminal carboxyl group with pentafluorophenol and *N,N′*-dicyclohexylcarbodiimide (DCC). The nonchiral 6-aminohexanoic acid residue was chosen as the C-terminal residue in order to minimize racemization during the cyclization process.

The linear tetrapeptides were synthesized by stepwise coupling of protected amino acid residues in solution using the DCC/HOBt method[11] (Fig. 1). Selective removal of the phenacyl groups of the linear peptides by Zn/AcOH gave the free acids, which were esterified by DCC/pentafluorophenol to give the active esters. Cyclization of the linear peptides was effected by the elevated temperature procedure[12–15] in dioxane/pyridine/ethanol at 80–82 °C to afford the protected cyclic peptides in moderate yields. The monomeric structures of the protected cyclic peptides were established by fast atom bombardment–mass spectrometry (FAB–MS). The side chain protecting groups were then removed by either HF or catalytic hydrogenation on palladium charcoal and the crude products were purified by reversed-phase HPLC to afford the target cyclic peptides **7a** and **7b** in homogeneous form. The molecular weights of the cyclic peptides **7a** and **7b** were determined by mass spectrometry using the FAB mode.

Ethidium bromide displacement assay[8,9] showed that whereas the apparent DNA binding constant of cyclo [Lys-Tyr-Lys-Ahx-] and linear Lys-Tyr-Lys are far below 1×10^3 M^{-1}, those of cyclo[Lys-Trp-Lys-Ahx-] and Lys-Trp-Lys are 1.9×10^4 and 9.5×10^3 M^{-1}, respectively. Because of the rather low DNA binding constant of cyclo[Lys-Trp-Lys-Ahx-], it did not produce footprints on a 253-mer DNA fragment and thus the sequence specificity of the cyclic peptides are not yet known.[16] Our approach in this study was to use agarose gel electrophoresis for monitoring the nicking rate of ϕX174 DNA by linear Lys-X-Lys peptides compared to the corresponding cyclic peptides at various elevated temperatures, in the absence and presence of UV which causes the denaturation of DNA.

A previous study by Pierre and Laval[7] reported that both linear Lys-Trp-Lys and Lys-Tyr-Lys peptides were capable of nicking apurinic supercoiled DNA at AP sites. In this work, kinetic studies using agarose gel electrophoresis showed that linear Lys-Tyr-Lys and cyclo[Lys-Tyr-Lys-Ahx-] did not manifest DNA nicking activity on natural supercoiled ϕX174 DNA or supercoiled pBluescript SKII DNA (data not shown). On the other hand, cyclo[Lys-Trp-Lys-Ahx-] and linear Lys-Trp-Lys were shown to possess notable DNA nicking ability toward natural supercoiled DNA at temperature between 55 and 65 °C. The rates of DNA cleavage by cyclo[Lys-Trp-Lys-Ahx-] and Lys-Trp-Lys on ϕX174 DNA at different reaction temperatures are shown in Figures 2–4 and Table 1.

The DNA nicking rates were observed to increase as a linear function of the concentrations of cyclo[Lys-Trp-Lys-Ahx-] and linear Lys-Trp-Lys (Fig. 5), suggesting that the reaction is first order with respect to drug concentration. We have been using a low concentration of ferrous ion (1 μM) in the nicking experiments since it appeared to give more consistent nicking rates although no enhancement in the magnitude of nicking was observed in the presence of 1 μM of iron (II) (Fig. 6), suggesting that Fenton reaction[17] was not involved in the DNA nicking possess. The DNA nicking rates of cyclo[Lys-Trp-Lys-Ahx-] and linear Lys-Trp-Lys were also shown to increase as a linear function of reaction temperature (Fig. 4 and Table 1). At temperatures of



3a, 4a, 5a, 6a, 7a, X = Trp;

3b, 4b, 5b, 6b, X = Tyr(OBzl); 7b, X = Tyr

Figure 1. Scheme for the synthesis of cyclo[Lys-Trp-Lys-Ahx-] and cyclo[Lys-Tyr-Lys-Ahx-].

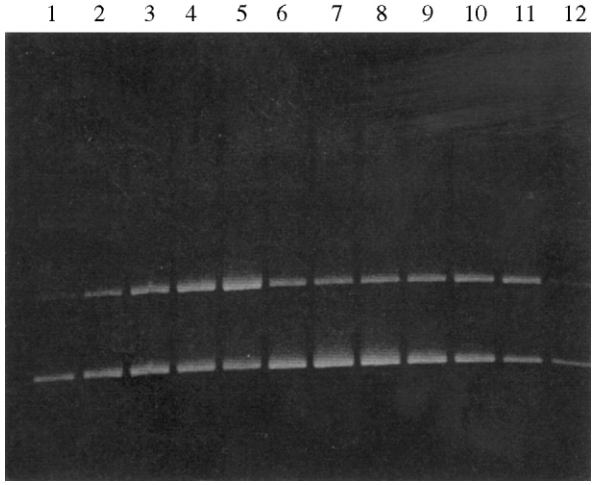


Figure 2. Nicking rate studies of ϕX174 DNA monitored by agarose electrophoresis. Vials containing 0.38 μL of DNA were incubated with cyclo[Lys-Trp-Lys-Ahx-] or Lys-Trp-Lys at a concentration of 200 μM in PBS at 60 °C and pH 7.54 at various intervals. The reaction was quenched in ice bath with 2 μL of 1200 mM mannitol added and the reaction mixture loaded immediatly to agarose wells. The upper band represents the open-circular form, and the lower band, the supercoiled form. Lanes 6 and 12, ϕX174 DNA as received. Lanes 1–5, DNA incubated with cyclo[Lys-Trp-Lys-Ahx-] at intervals of 2, 7, 17, 37, adn 67 min, respectively. Lanes 7–11, DNA incubated with Lys-Trp-Lys at intervals of 2, 7, 17, 37, and 67 min, respectively. The gel was stained with ethidium bromide, photographed, and the band volumes analyzed by a PC computer equipped with Vilber Lourmat BIO-1D analysis software (Marne La Vallee, France).

55 °C or below, the DNA nicking rate of Lys-Trp-Lys was very low but increased rather abruptly at 60 °C or above. The DNA nicking rate of cyclo[Lys-Trp-Lys-Ahx-] was notable at 55 °C and increased less abruptly at 60 °C or above. Irradiation with a laboratory UV lamp (short wave) gave only a small enhancement in the DNA nicking rate of cyclo[Lys-Trp-Lys-Ahx-] but produced a significant increase of DNA nicking rate with Lys-Trp-Lys (Table 1).

It was proposed that the nicking of the DNA backbone with apurinic DNA may occur by β-elimination.[7] For natural supercoiled DNA, since both cyclo[Lys-Tyr-Lys-Ahx-] and Lys-Tyr-Lys did not possess DNA nicking activities at 65 °C whereas cyclo[Lys-Trp-Lys-Ahx-] and Lys-Trp-Lys were shown to nick DNA at a temperature of 55–65 °C, it is apparent that the presence of a Trp residue in both linear and cyclic peptides is required for the nicking process with natural supercoiled DNA. It follows that the intercalation of the indole ring of the tryptophan side chain between the base pairs of DNA is a major driving force of the nicking process. This view is supported by the fact that the apparent DNA binding constants of cyclo[Lys-Trp-Lys-Ahx-] and Lys-Trp-Lys are considerably greater than those of cyclo[Lys-Tyr-Lys-Ahx-] and Lys-Tyr-Lys.

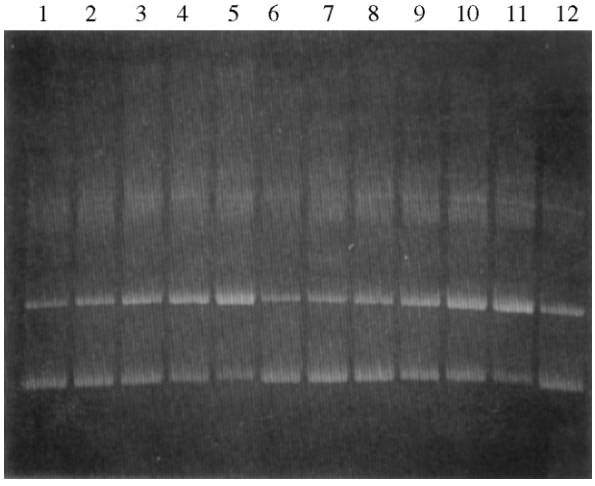


Figure 3. Nicking rate studies of ϕX174 DNA monitored by agarose electrophoresis. Vials containing 0.38 μL of DNA were incubated with cyclo[Lys-Trp-Lys-Ahx-] at a concentration of 200 μM in PBS at 65 °C and pH 7.54 at various intervals. The reaction procedure is similar to that described for Figure 2. Lane 6, ϕX174 DNA as received; lane 12, DNA irradiated by a laboratory UV lamp (short wave) with 10 0.2-mm thick plastic filters for 67 min. Lanes 1–5, DNA incubated with cyclo[Lys-Trp-Lys-Ahx-] at intervals of 2, 7, 17, and 67 min, respectively. Lanes 7–11, DNA incubated with cyclo[Lys-Trp-Lys-Ahx-] at intervals of 2, 7, 17, 37, and 67 min, respectively, and each vial was irradiated by a laboratory UV lamp as described.

At temperatures lower than 65 °C, the rate of DNA nicking by cyclo[Lys-Trp-Lys-Ahx-] is greater than Lys-Trp-Lys. This is explained by the fact that the apparent DNA binding constant of the cyclic peptide is higher than that of the corresponding linear peptide, possibly due to the absence of a negatively charged C-terminal group in the cyclic peptide. At temperatures lower than 55 °C, the nicking rate by linear Lys-Trp-Lys is very slow, suggesting that adequate energy must be provided for the attach of the peptide molecules upon DNA and also for the distortion of the DNA strand required for the nicking process. At temperatures higher than 65 °C, the flexible linear Lys-Trp-Lys may gain sufficient vibrational energy to adopt a more favorable conformation for intercalation into the DNA, resulting in higher nicking rates. UV irradiation experiments (Fig. 3 and Table 1) were performed using a laboratory UV lamp and the UV intensity was attenuated by ten 0.2-mm plastic filters to minimize the formation of thymine dimers and other photo products. UV irradiation may simply provide more vibrational energy to both the DNA and the flexible linear Lys-Trp-Lys, adopting more favorable conformations in the transitional peptide-DNA complex and resulting in higher nicking rates than cyclo[Lys-Trp-Lys-Ahx-]. At present, there is no evidence to indicate the binding of these peptides to the minor groove of DNA.

The present results show that the cyclic tetrapeptide cyclo[Lys-Trp-Lys-Ahx-] is a novel DNA nicking agent with a higher apparent DNA binding constant and also a higher nicking rate than the parent linear peptide at temperatures below 60 °C. This work also provides insights into the DNA nicking mechanism of the Lys-X-Lys series of peptides relevant to the design of new DNA cleavage agents.

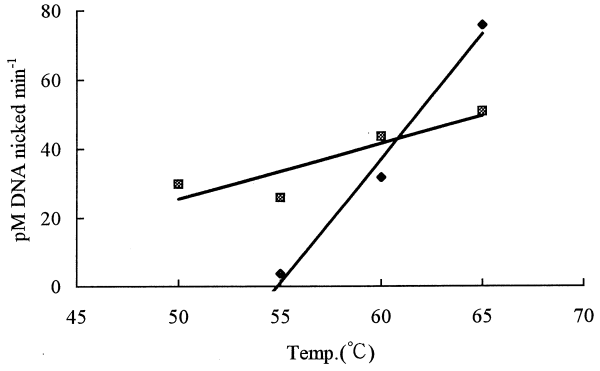


Figure 4. Nicking rate of ϕX174 DNA by cyclo[Lys-Trp-Lys-Ahx-] (200 μM) and Lys-Trp-Lys (200 μM) as a function of reaction temperature. ■, cyclo[Lys-Trp-Lys-Ahx-]; ◆, Lys-Trp-Lys.

Table 1. Nicking rate of ϕX174 DNA by cyclo[Lys-Trp-Lys-Ahx-] (200 μM) and Lys-Trp-Lys (200 μM)

Temperature (°C)	cyclo[Lys-Trp-Lys-Ahx-] v_1 (pM min^{-1})	cyclo[Lys-Trp-Lys-Ahx-] v_2 (pM min^{-1})	Lys-Trp-Lys v_1 (pM min^{-1})	Lys-Trp-Lys v_2 (pM min^{-1})
55	26	33	4	37
60	43	—	32	—
65	51	62	76	96

V_1, DNA nicking rate in the absence of UV.V_2, DNA nicking rate in the presence of short wave UV (laboratory UV lamp equipped with 10 thin plastic filters).

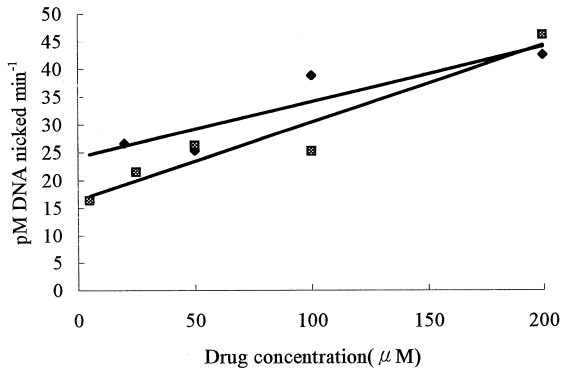


Figure 5. Nicking rate of φX174 DNA by cyclo[Lys-Trp-Lys-Ahx-] and Lys-Trp-Lys as a function of peptide concentration at 65 °C. ■, cyclo[Lys-Trp-Lys-Ahx-]; ◆, Lys-Trp-Lys.

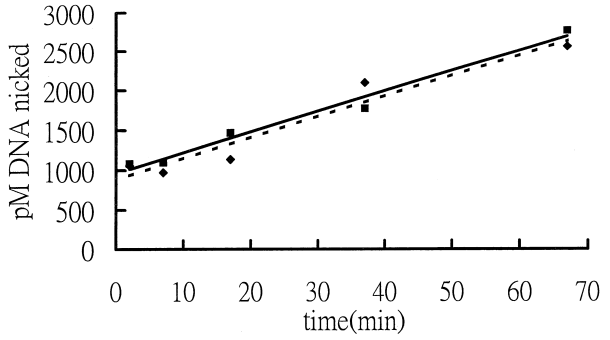


Figure 6. Nicking rate of φX174 DNA by Lys-Trp-Lys (50 μM) in the presence (dotted line) and absence (solid line) of ferrous sulfate (1 μM) at 65 °C.

Acknowledgements

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References and Notes

1. Abbreviations generally follow the IUPAC-IUB recommendation as published in *J. Biol. Chem.* **1989**, *264,* 668–673. Other abbreviations: Ahx, 6-aminohexanoic acid; Boc, *tert*-butyloxycarbonyl; Bzl, benzyl; Z, benzyloxycarbonyl; DCC, 1,3-dicyclohexylcarbodiimide; DIEA, *N,N*-diisopropylethylamine; HOBt, 1-hydroxybenzotriazole; OPa, phenacyl; Pfp, pentafluorophenol or pentafluorophenyl ester; TFA, trifluoroacetic acid.
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