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Interaction of ε-L-β-lysine as a Tail Analogy of Tallysomycin-A to a Double Helical DNA Oligonucletide d(CGCTTCGAAGCG)$_2$

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Abstract : During the screening of material which has the antimicrobial activity against aminoglycoside-resistant bacteria, A new material ε-(L-β-lysine) polypeptide from a culture medium of *Streptomyces* sp.(DWGS2) was isolated, and the structure and the physicochemical properties of the new material were elucidated. The new material was separated by column chromatography of the culture medium using Dowex1×2, Silica gel, and Sephadex LH20 etc. The structure and molecular weight were determined with the data of NMR, MALDI mass, and ESI mass experiments. And the monomer obtained by hydrolysis of the new material with 6N-HCl was identified as a L-β-lysine(T_2), which is a tail of bleomycin. As tail-region analogy, T_2(β-lysine derivatives from streptomyces) interactions with a self-complementary oligonucleotides, d(CGCTTCGAAGCG)$_2$, was investigated by NMR.
Keywords: NMR, β-lysine, DNA, Interaction

INTRODUCTION

Antitumor antibiotics, including bleomycin and tallysomycin are clinically used against certain malignant lymphomas, squamous cell carcinomas, and testicular carcinoma. This glycopeptide has a metal-binding moiety that cleaves the specific DNA sequence, sugar rings(glucose, mannose, tallose) and tail regions(bithiazole, sulfonium, lysine) that recognize and bind noncovalently to specific DNA base sequences. Screening of the culture broth of soil-derived microorganisms has been continued to provide an

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important source of novel natural products possessing potentially useful biological activities.[1,2] During the course of a screening program to discover new fermentation-derived anti-microbial compounds, especially having the activity against aminoglycoside-resistant bacteria,[3] the new material, ε-(L-β-lysine) polypeptide (m.w. 2300) was isolated from a culture medium of *Streptomyces* sp.(DWGS2). This report describes the complete structure elucidation, physicochemical properties and isolating procedure of ε-(L-β-lysine) polypeptide. As tail-region analogy, T_2(β-lysine derivatives from streptomyces) interactions with a self-complementary oligonucleotides, d(CGCTTCGAAGCG)$_2$, was monitored and studied by NMR.

EXPERIMENTAL

Materials

Culture media for fermentation and bio-assay were purchased from Difco and all kinds of eluting reagent for column chromatography was used of analytical reagent grade. Precoated kieselgel 60 F254(No. 5715) for TLC plate and silica gel for column chromatography were purchased from Merck and Dowex 1×2(No. 44290, Cl- form strong basic; 50-100 mesh) for ion exchange resin from Fluka and Sephadex LH-20(No. 17-0090) for Gel filtration from Pharmacia. All other chemicals used were of analytical reagent grade. 200 ODU of single-stranded, d(CGCTTCGAAGCG) was purchased from Biosynthesis Co. and was purified with by reverse-phase HPLC and desalted Sephadex G25 column. The double-stranded d(CGCTTCGAAGCG)$_2$ was then prepared by annealing and cooling the single-stranded DNA sample up to 80 ℃ for NMR experiment.

Isolation and purification

The isolation procedure of the active compound from the culture broth of *streptomyces sp.* DWGS2 is schematically shown in Fig. 1. Culture broth(10 L) was boiled in order to be sterilized for 10 min. and filtered with 0.2 um membrane filter. Three times(30L) of methanol in volume was added in this clear filtered broth and then stood alone overnight at 4 ℃ and centrifuged to give the active precipitate. This precipitate was

dissolved in 1 L of water and applied to Dowex-1×2 column(40 × 200 mm) which is anion exchange(-OH form) to remove coloring and anionic impurities and subsequently washed with 1L of water. The eluate gathered was concentrated in vacuum to give active residue, which was applied to silica gel column(Silica gel 60, MERK 70 ~ 230 mesh, ASTM 60 × 800 mm) and eluted with a solution which was composed with chloroform/methanol/28% ammonia water (2:6:3). Eluted fraction was collected by fraction collector(EYELA DC 12000) and for each fraction TLC(Rf : 0.35) and bioassay was performed simultaneously to find out the active fraction. Silica plate was developed with chloroform/methanol/28% ammonia water (2:6:3) and sprayed by dragendorff reagent[4,5](purple color, for determining of nitrogen containing compounds, basic bismuth nitrate 0.64 mg, potassium iodide 1.6 g, acetic acid 20 mL dissolved in 80 mL of water.). Bioassay was performed by cylinder plate method. 20 uL of test sample was loaded on the medium made of nutrient agar containing 1.0%(w/v) peptone, 0.3%(w/v) beef extract, 0.2%(w/v) sodium chloride, which was inoculated with *Bacillus subtilis* ATCC6633(incubated at 37℃ for 12hrs), *Serratia marcescens* Sma 62(incubated at 30℃ for 12hrs) and *S. marcescens* AG4410(incubated at 30℃ for 12hrs). Antibacterial activity was revealed as a clear zone of inhibition of growth. The active fraction was concentrated in vacuum. The aqueous residue applied to Sephadex LH-20 column and eluted with water and for each fraction TLC and bioassay was performed again to find out the active fraction. The active fraction was dried in vacuum to obtain the active compound(pale brown, amorphous solid, 80 ㎎).

NMR

All spectra were measured on Varian unity 300 (300 MHz), JEOL Lambda 400 (400 MHz) and Bruker DRX (800 MHz, Univ. of Maryland). ^{1}H NMR and ^{13}C NMR chemical shifts are reported in ppm with the chemical shift of the residual protons of the solvent used as internal standard. NMR experiments for the active compound were carried out under 10% D_2O solvent (90% H_2O) at 5℃). DNA-T_2 complex was prepared by microtitration of T_2 into DNA duplex. Various 2D NMR experiments with WATERGATE pulse sequence for water suppression, including COSY, TOCSY, NOESY

experiments, were performed. NMR experiments for the hydrolyzed product were carried out under the condition of pH 4.5 (buffered with phosphate) and 22℃.

MALDI-TOF-MS and ESI-MS

Mass spectra were measured on Jeol JMS SX102A and HX110/110A for EI and FAB, and VG Quattro ESI-MS for ESI-ms, Kratos Kompact MALDI 2 for MALDI(Matrix Assisted Laser Desorption Ionization) spectrum. Any mass data was not able to be obtained with EI and FAB mass but the meaningful data for the active compound was obtained with MALDI-MS using 2,5-dihydroxybenzoic acid(DHB)[6] as a matrix. A part of the active compound was dissolved in 50 part in weight of 50% acetonitrile solution made of DHB saturated water and put on polished stainless steel probe and let be dried. MALDI-MS spectrum of the active compound shows the peaks, m/z of 1819.8, 1946.2, 2074.1, 2202.2, 2329.8, 2457.4, 2585.2, 2712.1, 2842.9, 2966.8, 3100.6. For ESI-MS, a part of the active compound and hydrolyzed product was dissolved in around 500 part in weight of water respectively and injected into ESI-MS(positive). ESI-MS spectrum for hydrolyzed product shows that the molecular weight is 147.

Acid hydrolysis of ε-(L-β-lysine) polypeptide

In order to obtain the hydrolyzed product of the active ε-(L-β-lysine), 50mg of the polypeptide was put into 2mL of 6N HCl solution and boiled for 24 hours. For these reactant, TLC(Rf : 0.35) was performed to find out whether the active material underwent hydrolysis well. Silica plate was developed with chloroform/methanol/28% ammonia water (2:6:3) and sprayed by Ninhydrine reagent.[7,8] There was a single violet colored spot (Rf 0.4) on TLC plate. This hydrolyzed product was concentrated in vacuum and dissolved in small quantity of water and then in order to desalt and purify the hydrolyzed product, it was applied to Sephadex LH 20 Column(φ 50 × 700 mm) and eluted with water. The eluate was collected by fraction collector and TLC was performed for each fraction in order to find which fraction contained the hydrolyzed product. The eluate containing hydrolyzed product was gathered and concentrated in vacuum. Recrystallization in methanol gives white needle-shaped crystal powder.

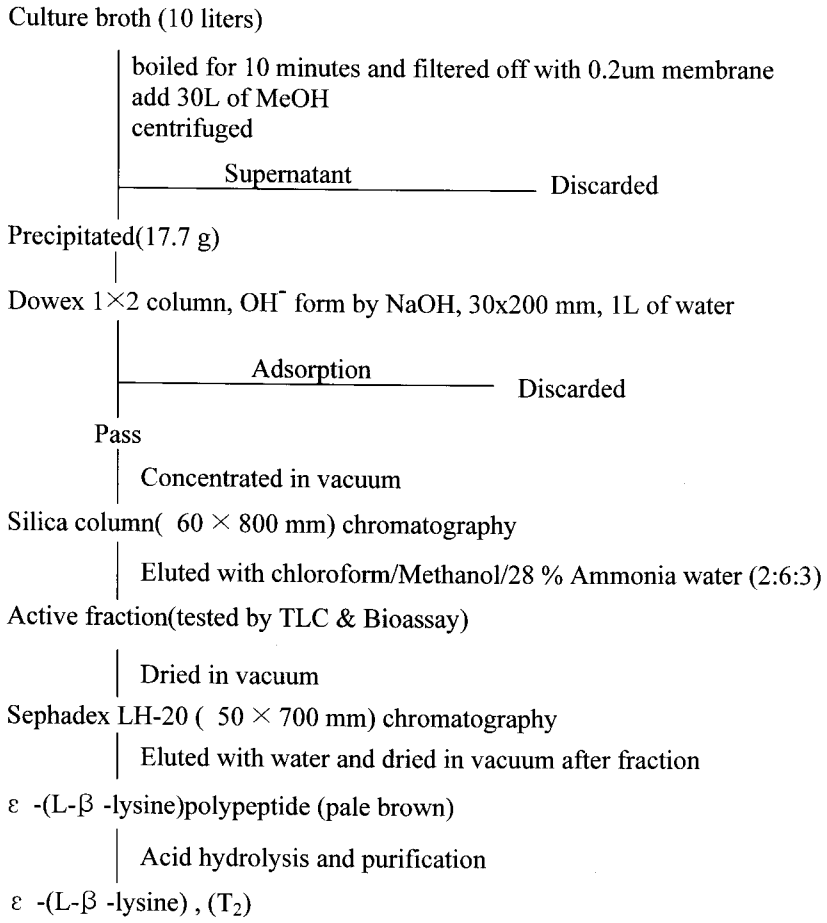

Fig.1. Preparation of acid hydrolysis product (T_2) from ε -(L-β -lysine)polypeptide.

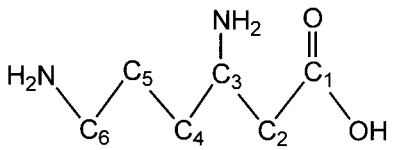

Fig.2. Molecular structure of T_2. The ε -(L-β -lysine) is an analog of the tail region of DNA-binding bleomycin.

RESULTS AND DISCUSSION

In the isolating process we found that nitrogen was contained in the compound because the active compound was colored to purple by dragendorff reagent and the hydrolyzed product was colored to violet by Ninhydrine reagent. MALDI-MS spectrum of the active compound shows the peaks, 1819.8(n=13), 1946.2(n=14), 2074.1(n=15), 2202.2(n=16), 2329.8(n=17), 2457.4(n=18), 2585.2(n=19), 2712.1(n=20), 2842.9(n=21), 2966.8(n=22), 3100.6(n=23) and these peaks were explained follow formula.

$$\text{Molecular ion} = [\text{DHB}(154) - \text{H2O}(18) + \text{M}(128 \times n + 18) + \text{H}]^+$$

These peaks tell us that active compound is a polymer composed of the monomer with molecular weight 128. The mass spectrum of hydrolyzed product shows that the molecular weight of the monomer is 146 and from NMR data of the monomer it is determined that the monomer is L-β-lysine which is a dextro isomer having an optical rotation, $[\alpha]^{24}D$ = + 50.6 (c=1%,H2O). H.E. Carter,[9] and D. Keirs[10] repoted that the specific rotation of L-β-lysine was +24 and +18 respectively. Our the specific rotation value is bigger than that of H.E. Carter, and D. Keirs, which is thought like that our L-β-lysine is purer than theirs. Anyway we can see that this is dextro isomer. So it is determined that the active compound is ε-(L-β-lysine) polypeptide. In the ESI-MS spectrum we were not able to assign all peaks because the spectrum is very complicated, but assigned some peaks to 2194.29(128 × 17 + 18), 2451.72(128 × 19 + 18), 2577.69(128 × 20 + 18). These data also support that the active compound is ε-(L-β-lysine) polypeptide.

The antimicrobial activity of ε-(L-β-lysine) polypeptide is not only better than equal to the activity of known aminoglycoside type of antibiotics(MIC=3.125 - 6.25ug/mL) but also effective against aminoglycoside-resistant bacteria and fungi. ε-(L-β-lysine) polypeptide can be utilized for the treatment of diseases caused by aminoglycoside-resistant bacteria, and if the mechanism of antimicrobial activity against aminoglycoside-resistant bacteria is figured out, it will make great contribution to the development of new antibiotics.

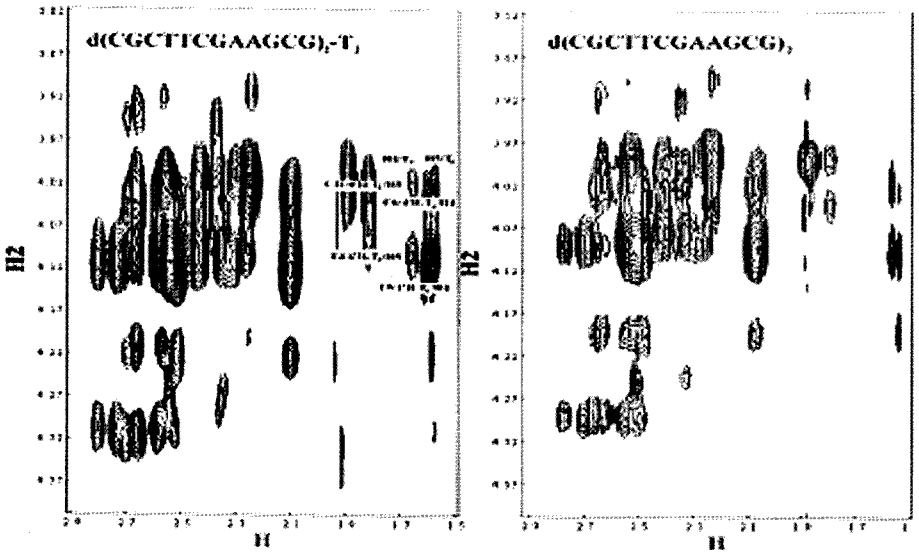

Fig. 3. The direct NOE interactions of d(CGCTTCGAAGCG)$_2$-T_2 complex.

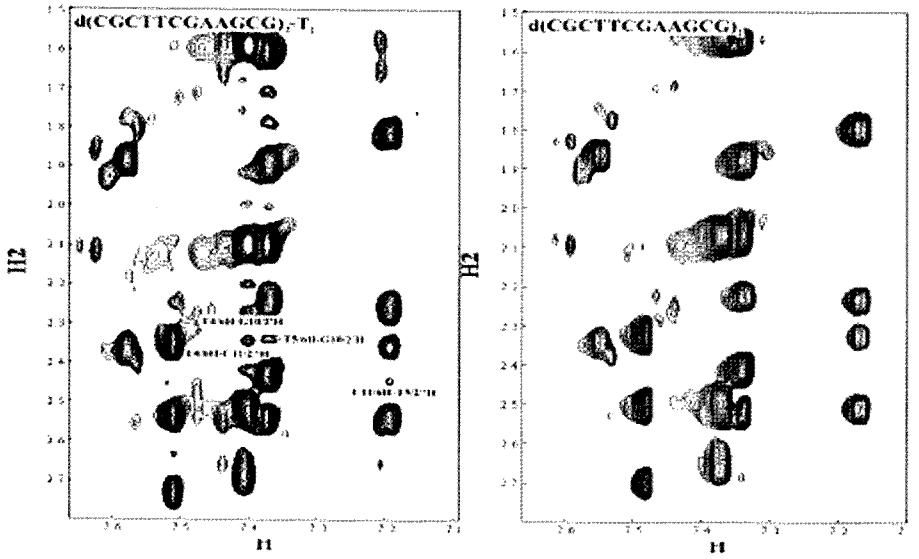

Fig.4. The residual NOE interactions of d(CGCTTCGAAGCG)$_2$- T_2 complex.

By acid hydrolyzing the ε-(L-β-lysine) polypeptide, a monomer T_2 which is an analog of the tail region of DNA-binding bleomycin was obtained. The DNA-T_2 complex was prepared by the addition of T_2 into a solution containing a double-stranded

oligonucleotide d(CGCTTCGAAGCG)$_2$ which is easily found in tumor cell. Important NOE interaction between DNA and T_2 were observed associated with the sequences (T4, T5, C6) and complementary sequences (G10, C11). As shown in Fig. 3, four important NOE interactions C11/4'H-T2/5H, C6/4'H-T2/4H, T4/4'H-T2/5H and T5/4'H-T2/4H were observed. In addition, the addition of T_2 into DNA resulted in the structural modification by exhibiting several intraresidual NOE interactions, including T4/6H-G10/2'H, T4/6H-C11/2"H, T5/6H-G10/2'H and T5/2"H-C11/6H interactions as shown in Fig. 4. Results exhibit that ε-(L-β-lysine) may be placed near the sites of sequence T4, T5 and C6 in a double-stranded oligonucleotide d(CGCTTCGAAGCG)$_2$. The used double-stranded oligonucleotide d(CGCTTCGAAGCG)$_2$ exhibits a typical B-type helix and further structural refinement of DNA-T_2 complex will be carried out.

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