SYNTHESIS OF OLIGOPEPTIDES CONTAINING DL-β-(1-URACILYL)- AND DL-β-(9-ADENINYL)-α-ALANINE AND LYSINE RESIDUES AND STUDY OF THEIR REACTION WITH DNA

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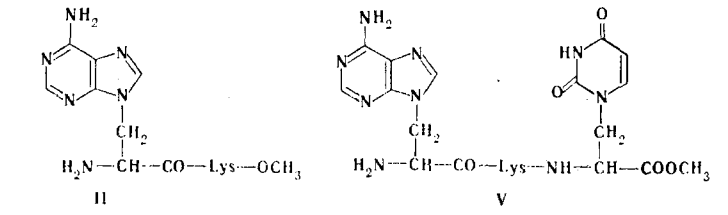
Nucleopeptides containing β-(1-uracilyl)- and β-(9-adeninyl)-α-alanine and lysine residues were obtained. The reaction of the bases in the nucleopeptides and the reaction of the nucleopeptides with DNA were investigated.

We and other authors have previously reported the synthesis of oligo- and polypeptides containing 1-pyrimidinyl- and 9-purinyl-α-amino acid (nucleopeptide) residues [1-11]. Little study has thus far been devoted to the biological properties of synthetic nucleopeptides and their principal components, viz., 1-pyrimidinyl- and 9-purinylamino acids, but one should note the pronounced properties of cytokinins among derivatives of 9-purinyl amino acids and the high capacity of DL-β-(9-adeninyl)-α-alanine to stimulate the action of adenylate cyclase [12, 13]. Little study has also been devoted to the reaction of synthetic nucleopeptides with nucleic acids.

The goal of the present research was to synthesize peptides that contain pyrimidine and purine residues in combination with amino acids with basic character (lysine, for example) and to study their reaction with DNA. One might have assumed that lysine molecules, on the basis of electrostatic interactions with the phosphate groups of nucleic acids, would promote the formation of stronger hydrogen bonds between the nitrogen bases of the nucleopeptide and the nucleic acid [14, 16].

To shed some light on this problem we undertook the synthesis of a number of di-, tri-, and tetrapeptides, viz., DL-β-(9-adeninyl)-α-alanyl-L-lysine methyl ester (II), DL-β-(1-uracilyl)-α-alanyl-L-lysyl-L-lysine methyl ester (VII), DL-β-(9-adeninyl)-α-alanyl-L-lysyl-DL-β-(1-uracilyl)-α-alanine methyl ester (V), and DL-β-(9-adeninyl)-α-alanyl-L-lysyl-L-lysyl-DL-β-(1-uracilyl)-α-alanine methyl ester (XI).



N^{α}-Benzyloxycarbonyl-DL-β-(9-adeninyl)-α-alanyl-N^{ε}-benzyloxycarbonyl-L-lysine methyl ester

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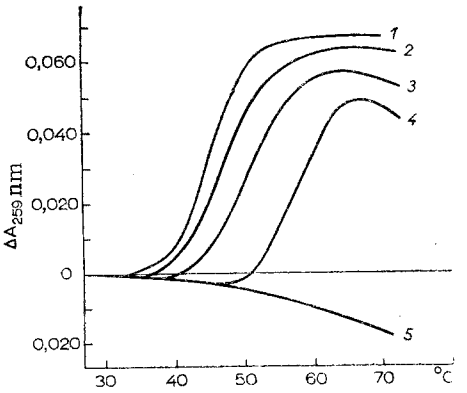

Fig. 1. Thermal denaturation of DNA and complexes of DNA with peptide VII in $2.5 \cdot 10^{-4}$ M EDTA at pH 7.0 and a DNA concentration (P^-) of $2.9 \cdot 10^{-5}$ M: 1) DNA; 2-4) complexes with DNA:peptide ratios of 0.3, 0.6, and 1.5, respectively; 5) dependence of the absorption of peptide VII on the temperature (the concentration of the sample corresponds to curve 4 in this figure).

(I) was obtained by condensation of N^{ε}-benzyloxycarbonyl-L-lysine methyl ester with N^{α}-benzyloxycarbonyl-DL-β-(9-adeninyl)-α-alanine, the synthesis of which was described in [2]. After removal of the ester protective group, dipeptide I was subjected to reaction with DL-β-(1-uracilyl)-α-alanine methyl ester [1] to give N^{α}-benzyloxycarbonyl-DL-β-(9-adeninyl)-α-alanyl-N^{ε}-benzyloxycarbonyl-L-lysyl-DL-β-(1-uracilyl)-α-alanine methyl ester (IV). N^{α}-Benzyloxycarbonyl-DL-β-(1-uracilyl)-α-alanyl-N^{ε}-benzyloxycarbonyl-L-lysyl-N^{ε}-benzyloxycarbonyl-L-lysine methyl ester (VI) was synthesized by the reaction of N^{α}-benzyloxycarbonyl-DL-β-(1-uracilyl)-α-alanine [1] with N^{ε}-benzyloxycarbonyl-L-lysyl-N^{ε}-benzyloxycarbonyl-L-lysine methyl ester. The synthesis of N^{α}-tert-butoxycarbonyl-N^{ε}-benzyloxycarbonyl-L-lysyl-DL-β-(1-uracilyl)-α-alanine methyl ester (VIII) was carried out by condensation of DL-β-(1-uracilyl)-α-alanine methyl ester with N^{ε}-benzyloxycarbonyl-N^{α}-tert-butoxycarbonyl-L-lysine methyl ester. N^{α}-Benzyloxycarbonyl-DL-β-(9-adeninyl)-α-alanyl-N^{ε}-benzyloxycarbonyl-L-lysyl-N^{ε}-benzyloxycarbonyl-L-lysyl-DL-β-(1-uracilyl)-α-alanine methyl ester (IX) was obtained after removal of the butoxycarbonyl protective group and condensation of the dipeptide with N^{α}-benzyloxycarbonyl-DL-β-(9-adeninyl)-α-alanyl-N^{ε}-benzyloxycarbonyl-L-lysine (VII). The benzyloxycarbonyl protective group was removed in all cases with a solution of hydrogen bromide in glacial acetic acid, the tert-butoxycarbonyl protective group was removed with trifluoroacetic acid, and the ester protective group was removed by alkaline hydrolysis.

A comparison of the UV spectra of peptides V and IX and their hydrolysates showed that, like dinucleotide monophosphates, peptides V and IX display a hypochromic effect, the magnitude of which amounts to 9.0 and 11.5%, respectively. The dependence of the absorption spectra of the nucleopeptides on the temperature was investigated. The positive and negative maxima of the differential spectra are approximately equal for nucleopeptides that contain only one uracil or adenine residue. A small hyperchromic effect is observed for the peptides that contain two nucleo amino acid residues. The form of the differential spectra and the magnitude of the hyperchromism are close to the form and magnitude observed for nucleosides, nucleotides, oligonucleotides, and their analogs [17, 18]. The results constitute evidence for weak interaction of the bases in the composition of the nucleopeptide.

The reaction of nucleopeptides II, V, VII, and IX with DNA was investigated by thermal denaturation. All of the investigated nucleopeptides stabilize the double helix of DNA. Peptide VII is the most effective peptide in this regard (Fig. 1). It is known [19] that the lysyltyrosyllysyl peptide interacts with DNA considerably more strongly than lysyllysine or lysylalanyllysine; this is explained by the effect of the aromatic function of tyrosine. However, nucleopeptides stabilize the double helix of DNA to a smaller extent than lysyllysine.

EXPERIMENTAL

Thin-layer chromatography (TLC) was carried out on Silufol UV-254 plates in the following systems: A) isopropyl alcohol–water–ammonia (7:2:1); B) butanol–acetic acid–water (9:1:2). The substances were detected on the chromatograms from the absorption of UV rays; the compounds that contained an amino group were developed with ninhydrin. The UV spectra of solutions of the compounds in water (pH 7.0) and 0.1 N HCl were recorded with a Spectromom-204 spectrophotometer. Deoxyribonucleic acid from calf's spleen (a product of the Biokhimreaktiv Nongovernmental Organization) in $2.5 \cdot 10^{-4}$ M EDTA (pH 7.0) was used. The changes in the absorption spectra with the temperature were recorded at 259 nm with a Specord UV-vis spectrophotometer (Zeiss, German Democratic Republic) equipped with a thermostattable cuvette holder. The temperature in the cuvettes was raised continuously at a rate of 1°C/min by means of an ultrathermostat. In the recording of the spectra the samples were maintained at a predesignated temperature for 10 min. The melting curves were recorded by means of a PDS-021 two-coordinate recorder. The hypochromism of nucleopeptides that contain two or more amino acids was determined after hydrolysis of the peptides with 57% perchloric acid at 130°C [4].

N^{α}-Benzyloxycarbonyl-DL-β-(9-adeninyl)-α-alanyl-N^{ε}-benzyloxycarbonyl-L-lysine Methyl Ester (I). A 0.2-ml sample of triethylamine was added to a solution of 0.66 g (2 mmole) of N^{ε}-benzyloxycarbonyl-L-lysine methyl ester hydrochloride in 30 ml of tetrahydrofuran (THF), the mixture was cooled to 0°C, and 0.45 g (2 mmole) of dicyclohexylcarbodiimide was added in portions with stirring. The mixture was stirred for 10 min, and a solution of 0.6 g (2 mmole) of N^{α}-benzyloxycarbonyl-DL-β-(9-adeninyl)-α-alanine [2] in 60 ml of THF was added. The mixture was stirred for another 3 h and allowed to stand at 20°C for 3 days. A 0.1-ml sample of glacial acetic acid was added, and the precipitated dicyclohexylurea was removed by filtration. The filtrate was evaporated *in vacuo*, and the precipitate was recrystallized from ethyl acetate–petroleum ether to give 0.6 g (55%) of a product with mp 70-72°C and R_f 0.94 (A) and 0.61 (B). Found: C 57.6; H 6.5; N 17.0%. $C_{31}H_{36}N_8O_7 \cdot H_2O$. Calculated: C 57.2; H 5.9; N 17.2%.

DL-β-(9-Adeninyl)-α-alanyl-L-lysine Methyl Ester (II) Dihydrobromide. A 4.6-ml sample of a 33% solution of HBr in acetic acid was added to a solution of 0.3 g (4.6 mmole) of peptide I in 1.2 ml of acetic acid, and the mixture was allowed to stand for 50 min. It was then cooled, and peptide II was precipitated by adding 50 ml of absolute ether. The precipitate was removed by filtration and washed with ether to give 0.2 g (81%) of a product with mp 193-194°C and R_f 0.22 (A) and 0.06 (B). Found: C 32.5; H 5.6; N 19.6%. $C_{15}H_{24}N_8O_7 \cdot 2H_2O \cdot 2HBr$. Calculated: C 32.0; H 5.4; N 19.9%. UV spectrum, λ_{max} ($\varepsilon \cdot 10^3$): 268 nm (13.2); water (pH 7.0): 259 nm (15.2), 0.1 N HCl.

N^{α}-Benzyloxycarbonyl-DL-β-(9-adeninyl)-α-alanyl-N^{ε}-benzyloxycarbonyl-L-lysine (III). A solution of 1.3 g (2 mmole) of peptide I in 30 ml of methanol was cooled to 10°C, 5.4 ml of 1 N sodium hydroxide was added in small portions, and the mixture was maintained at 20°C for 24 h. The methanol was removed by vacuum distillation, and the residual aqueous solution was acidified to pH 2 with 1 N hydrochloric acid and maintained at 4°C for 24 h. The precipitated III was removed by filtration and washed with water to give 0.9 g (75%) of a product with mp 148-150°C and R_f 0.87 (A) and 0.73 (B). Found: C 57.3; H 5.8; N 17.9%. $C_{30}H_{34}N_8O_7$. Calculated: C 56.8; H 5.4; N 17.7%.

N^{α}-Benzyloxycarbonyl-DL-β-(9-adeninyl)-α-alanyl-N^{ε}-benzyloxycarbonyl-L-lysyl-DL-β-(1-uracilyl)-α-alanine Methyl Ester (IV). This compound was obtained by the reaction of III with DL-β-(1-uracilyl)-α-alanine methyl ester [1] by a procedure similar to that used to prepare peptide I. Workup gave a product with mp 103-105°C (from ethyl acetate–petroleum ether) in 69% yield. Found: C 55.9; H 5.7; N 18.2%. $C_{38}H_{43}N_{11}O_{10}$. Calculated: C 56.1; H 5.3; N 18.9%.

DL-β-(9-Adeninyl)-α-alanyl-L-lysyl-DL-β-(1-uracilyl)-α-alanine Methyl Ester (V) Dihydrobromide. This compound, with mp 218-221°C, was obtained in 76% yield from peptide IV by a method similar to that used to prepare II. UV spectrum, λ_{max} ($\varepsilon \cdot 10^3$): 262 (19.6), water (pH 7.0); 262 nm (18.8), 0.1 N HCl. Found: C 35.9; H 4.6; N 20.2%. $C_{22}H_{31}N_{11}O_6 \cdot 2H_2O \cdot 2HBr$. Calculated: C 35.5; H 5.0; N 20.7%.

N^{α}-Benzyloxycarbonyl-DL-β-(1-uracilyl)-α-alanyl-N^{ε}-benzyloxycarbonyl-L-lysyl-N^{ε}-benzyloxycarbonyl-L-lysine Methyl Ester (VI). This compound, with mp 149-150°C, was obtained in 69% yield by condensation of N^{α}-benzyloxycarbonyl-DL-β-(1-uracilyl)-α-alanine [1] with

N^{α},N^{ε}-bis(benzyloxycarbonyl)-L-lysyl-L-lysine trifluoroacetate by a procedure similar to that used to prepare I. Found: C 59.8; H 6.3; N 10.7%. $C_{14}H_{53}N_7O_{12}$. Calculated: C 59.4; H 6.2; N 11.0%.

DL-β-(1-Uracilyl)-α-alanyl-L-lysyl-L-lysine Methyl Ester (VII) Trihydrobromide. This compound, with mp 290-292°C and R_f 0.33 (A) and 0.08 (B), was obtained in 70% yield from peptide VI by a procedure similar to that used to prepare II. Found: C 31.7; H 5.4; N 12.6%. $C_{20}H_{35}N_7O_{12}\cdot 3H_2O\cdot 3HBr$. Calculated: C 31.3; H 5.7; N 12.8%.

N^{α}-tert-Butoxycarbonyl-N^{ε}-benzyloxycarbonyl-L-lysyl-DL-β-(1-uracilyl)-α-alanine Methyl Ester (VIII). This compound was obtained in dimethylformamide from DL-β-(1-uracilyl)-α-alanine methyl ester [1] and N^{ε}-benzyloxycarbonyl-N^{α}-tert-butoxycarbonyl-L-lysine by a procedure similar to that used to prepare peptide I. Compound VIII was isolated in the form of an oil with R_f 0.82 (A) and 0.57 (B).

N^{ε}-Benzyloxycarbonyl-L-lysyl-DL-β-(1-uracilyl)-α-alanine Methyl Ester (IX). A solution of 8.6 g (13 mmole) of peptide VIII in 5.5 ml of absolute trifluoroacetic acid was maintained at 20°C for 45 min, after which 10 ml of absolute benzene was added, and the mixture was evaporated *in vacuo*. The residue was reprecipitated from ethanol by means of absolute ether to give 6.8 g (77%) of a product with mp 208-210°C and R_f 0.76 (A) and 0.68 (B). Found: C 49.2; H 5.6; N 12.4%. $C_{22}H_{29}N_5O_7\cdot CF_3COOH$. Calculated: C 48.9; H 5.1; N 11.9%.

N^{α}-Benzyloxycarbonyl-DL-β-(9-adeninyl)-α-alanyl-N^{ε}-benzyloxycarbonyl-L-lysyl-N^{ε}-benzyloxycarbonyl-L-lysyl-DL-β-(1-uracilyl)-α-alanine Methyl Ester (X). This compound, with yield 71% and mp 110-112°C (ethanol—petroleum ether) and R_f 0.93 (A) and 0.65 (B), was obtained by reaction of peptides IX and III in dimethylformamide by a procedure similar to that used to prepare I. Found: C 57.7; H 6.0; N 16.5%. $C_{52}H_{61}N_{13}O_{13}$. Calculated: C 58.0; H 5.7; N 16.9%.

DL-β-(9-Adeninyl)-α-alanyl-L-lysyl-L-lysyl-DL-β-(1-uracilyl)-α-alanine Methyl Ester (XI) Trihydrobromide. This compound, with mp 178-180°C and R_f 0.64 (A) and 0.06 (B), was obtained in 70% yield from X by a procedure similar to that used to prepare peptide VII. UV spectrum, λ_{max} ($\varepsilon\cdot 10^3$): 262 (13.4), water (pH 7.0); 264 nm (15.1), 0.1 N HCl. Found: C 37.5; H 5.8; N 11.0%. $C_{28}H_{43}N_7O_7\cdot 3H_2O\cdot 3HBr$. Calculated: C 37.9; H 5.9; N 11.0%.

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