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PEPTIDES OF AMINONUCLEIC ACIDS

POLYPEPTIDES OF β-(1-PYRIMIDYL)-α-AMINO ACIDS

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Polyamino acids with degrees of polymerization on the order of 9-10 were obtained by polymerization of p-nitrophenyl esters of β-(1-pyrimidyl)-α-alanines. Their hybridization with RNA is demonstrated.

In order to create polymeric physiologically active substances capable of forming associates with nucleic acids we developed methods for the synthesis of polypeptides of β-(1-pyrimidyl)-α-amino acids and studied their properties.

To synthesize the poly[β-(1-uracilyl)-α-alanines] we chose polymerization of their p-nitrophenyl esters. It is well known that the method of activated esters, which makes it possible to obtain polypeptides of regular structure, has found broad application in recent years in peptide chemistry [1]. We have previously described the preparation of N-carbobenzoxy-DL-Willardiine [β-(1-uracilyl)-α-alanine] p-nitrophenyl ester [2]. A similar method was used to obtain N-carbobenzoxy-β-(1-thyminyl)-α-alanine p-nitrophenyl ester. The carbobenzoxy group of the compounds is split out with 33% HBr in glacial acetic acid. The hydrobromides of the p-nitrophenyl esters of (1-uracilyl)amino acids are extremely hygroscopic compounds, and they are therefore used in the polymerization reaction immediately after their preparation. Small amounts of acetic acid impurity do not interfere with the polymerization and catalyze the aminolysis of the p-nitrophenyl esters. The polymerization was carried out in 5 days at room temperature in dimethylformamide (DMF) solution (with the addition of a definite amount of triethylamine) by the methods in [3, 4]. The polypeptides were precipitated from solution by the addition of methylene chloride. In an investigation of the effect of different amounts of triethylamine on the polymerization and on the molecular weight of the polymer obtained it was established that it is not possible to change the degree of polymerization substantially in this way.

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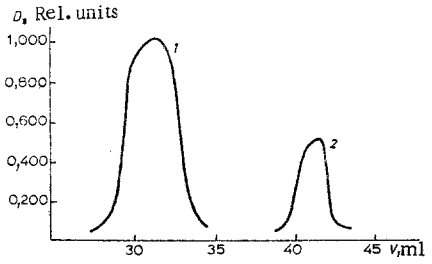

Fig. 1

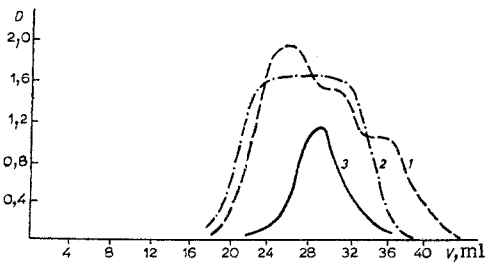

Fig. 2

Fig. 1. Gel filtration of the polypeptides obtained from β-(1-uracilyl)-α-alanine (1) and angiotensin (mol. wt. 1033) (2) with a column (35.0 by 1.0 cm) filled with Sephadex G-15.

Fig. 2. Fractionation of the polypeptides obtained from β-(1-uracilyl)-α-alanine (1), β-(1-thyminyl)-α-alanine (2), and angiotensin (mol. wt. 1033) (3) with a column (53.0 by 1.0 cm) filled with Sephadex G-25.

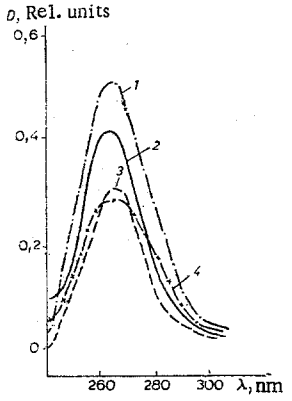

Fig. 3

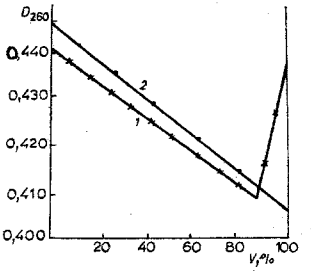

Fig. 4

Fig. 3. UV spectra of β-(1-uracilyl)-α-alanine [1) in water; 2) in 0.1 N HCl] and poly[β-(1-uracilyl)-α-alanine] [3) in water; 4) in 0.1 N HCl].

Fig. 4. Optical densities of mixtures of poly[β-(1-uracilyl)-α-alanine (1) and β-(1-uracilyl)-α-alanine (2) with RNA in a 0.1 M solution of Na_2HPO_4.

We used gel filtration on Sephadex G-25 and G-15 as in [5] to purify the polymers, fractionate them, and simultaneously determine their molecular weights. A buffer with pH 8.6 (an 8 M solution of urea and a 0.5 M solution of ammonium bicarbonate) was used as the eluent for destruction of the presumed hydrogen bonds between the nucleopeptide and the Sephadex. Angiotensin and Blue Dextran 2000 were used as the standards.

As seen from the chromatograms presented in Figs. 1 and 2, the molecular weights of the polypeptides average ~2000 and range from 1500 to 2500. The molecular weight of poly-DL-Willardiine was also determined by ultracentrifugation and was found to be 2170.

The polymerization of the p-nitrophenyl esters of the amino acids and peptides makes it possible to obtain polymers with relatively low molecular weights. It is known that N-carboxy anhydrides of amino acids form polymers with fixed molecular weights, the magnitude of which may vary over a wide range [1]. Unfortunately, we were unable to synthesize N-carboxy anhydrides of β-(1-pyrimidyl)- and β-(9-purinyl)-α-alanines. Various synthetic methods were checked (liquid and gaseous phosgene, PCl_5, and $SOCl_2$ were used as the reagents [3]), but they did not give positive results. The corresponding amino-nucleic acid was re-isolated in all cases.

In order to obtain polypeptides with higher molecular weights we also investigated other polycondensation methods. Blout and Roches [6] used dicyclohexylcarbodiimide for the intermolecular condensa-

tion of low-molecular-weight polypeptides. Using this method we obtained a high-molecular-weight product, which is currently under investigation, by treatment of the polypeptide of β-(1-uracilyl)-α-alanine (molecular weight 2000) with dicyclohexylcarbodiimide.

A decrease in the molar absorption coefficient, as has also been noted for some other synthetic analogs of polynucleotides [7, 8], is observed in the UV spectrum of poly[β-(1-uracilyl)-α-alanine] as compared with the starting monomer in water and 0.1 N hydrochloric acid (Fig. 3).

During a spectrophotometric study of the reaction of the polypeptides obtained in this study with nucleic acids we found that poly[β-(1-uracilyl)-α-alanine] (mol. wt. 2000) in a 0.1 M solution of Na_2HPO_4 gives a hypochromic effect with RNA of ~7%; this indicates the formation of a complex between this polymer and RNA (Fig. 4). Complexing is characteristic only for the polymer, inasmuch as it was established in subsequent experiments that the starting amino acid, β-(1-uracilyl)-α-alanine, does not display hypochromism on reaction with nucleic acids.

EXPERIMENTAL

The polypeptide was purified on Sephadex G-15 with elution with a 1% solution of ammonia in water. The molecular weight of the polypeptide was established on Sephadex G-25 with a buffer with pH 8.6 (8 M urea and 0.5 M ammonium bicarbonate) and with a Spinko E ultracentrifuge at 56,000 rpm.

N-Carbobenzoxy-β-(1-thyminyl)-α-alanine (I). A 3.5-g (19 mmole) sample of carbobenzoxy chloride and 25 ml of 0.1 N sodium hydroxide solution were added dropwise simultaneously with stirring to a cooled (to 0°) solution of 3.6 g (17 mmole) of β-(1-thyminyl)-α-alanine in 30 ml of 0.1 N sodium hydroxide solution, after which the suspension was stirred at 0° for 3 h and allowed to stand at room temperature for 12 h. The solution was extracted with ether, and the aqueous layer was separated, cooled, and acidified to pH 3 with concentrated hydrochloric acid. The resulting precipitate was removed by filtration and washed with acetone and ether to give 13.7 g (72%) of a product with mp 205-206° (absolute ethanol). Found: C 55.36; H 4.98; N 12.01%. $C_{16}H_{17}N_3O_6$. Calculated: C 55.33; H 4.98; N 12.10%.

N-Carbobenzoxy-β-(1-thyminyl)-α-alanine p-Nitrophenyl Ester (II). A 1-g (7 mmole) sample of p-nitrophenol and 1.35 g (7 mmole) of dicyclohexylcarbodiimide were added with vigorous stirring at 12° to 2.43 g (7 mmole) of I in 220 ml of dry dioxane, after which the mixture was stirred at 12° for 3 h and allowed to stand at room temperature for 24 h. The precipitated dicyclohexylurea was separated, and the filtrate was vacuum evaporated. The resulting oil crystallized on trituration with dry methanol to give 3.0 g (62%) of a product with mp 208-209° (nitromethane). Found: C 56.27; H 4.39; N 11.50%. $C_{22}H_{20}N_4O_8$. Calculated: C 56.50; H 4.07; N 11.98%.

β-(1-Uracilyl)-α-alanine p-Nitrophenyl Ester Hydrobromide (III). A 2.27-g (5 mmole) sample of N-carbobenzoxy-β-(1-uracilyl)-α-alanine p-nitrophenyl ester was suspended in 16 ml of glacial acetic acid, after which 16 ml of a 33% solution of hydrobromic acid in glacial acetic acid was added. The solid material gradually dissolved, and 200 ml of absolute ether was added to the solution after 1 h. The resulting precipitate was removed by filtration, washed with ether, and vacuum dried to give 0.9 g (57%) of a product with mp 181-182°.

β-(1-Thyminyl)-α-alanine p-Nitrophenyl Ester Hydrobromide (IV). This compound was obtained as in the preceding experiment from II. The yield of product with mp 175-178° was 0.63 g (62%).

Poly[β-(1-uracilyl)-α-alanine] (V). A 1.0-g (2.5 mmole) sample of III was dissolved in 1.2 ml of absolute DMF, after which 2.2 ml (2.6 mmole) of triethylamine was added, and the mixture was allowed to stand at room temperature for 5 days. The precipitated triethylammonium bromide was removed by filtration, and 20 ml of methylene chloride was added to the filtrate. The resulting precipitate was removed by filtration, washed successively with methylene chloride and ether, and vacuum dried to give 0.56 g (65%) of a fraction with mol. wt. 2000 and mp 170-185°.

Poly[β-(1-thyminyl)-α-alanine] (VI). This polymer, with mp 168-173° (the fraction with mol. wt. 2000) was similarly obtained in 63% yield from IV.

Hydrolysis of Poly[β-(1-uracilyl)-α-alanine]. A 5-mg sample of V was heated on a boiling-water bath with 1 ml of 6 N hydrochloric acid for 24 h. The development of amino acids was proved by means of paper chromatography and the ninhydrin reaction.

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SYNTHESIS ON THE BASIS OF 3-CARBOXYMETHYLMERCAPTO-4,5-DIPHENYL-1,2,4-TRIAZOLE HYDRAZIDE

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3-Carboxymethylmercapto-4,5-diphenyl-1,2,4-triazole hydrazide was synthesized, and some of its chemical properties were studied.

It is known [1, 2] that 1,2,4-triazole-3-thiones react successively with esters of chloro-substituted acids and hydrazine hydrate to give acid hydrazides. Some acid hydrazides of substituted 1,2,4-triazole-3-thiones and their derivatives have potential antitubercular activity [3, 4]. The synthesis of 3-carboxymethylmercapto-4,5-diphenyl-1,2,4-triazole hydrazide (I) is described in the present paper. Some of its chemical properties were studied. Thus hydrazones Vc-e, g were obtained by reaction of I with aldehyde (RCHO), and hydrazo compounds IVa, b were obtained by the action of acid anhydrides [$(RCO)_2O$] on III.

The reactions of III with isothiocyanates (allyl and phenyl) give acyl thiosemicarbazides (VIc, l), which form 1,2,4-triazole-5-thione derivatives (VIIc, l) on refluxing in 2 N sodium hydroxide solution and undergo cyclization to thiazoline derivative VIII on treatment with bromine in carbon tetrachloride.

Condensation of acetoacetic ester with hydrazide III gives IX, which undergoes hydrazinolysis to give starting hydrazide III on heating with a twofold excess of hydrazine hydrate in methanol. Compounds Xc, h-k, which are also obtained by condensation of the products of diazo coupling of acetoacetic esters XIh, i with hydrazide III, are formed by the action of arenediazonium salts on IX (see Table 1).

EXPERIMENTAL

The UV spectra of ethanol solutions of the compounds were recorded with an SF-4 spectrophotometer. Compounds I and VII were obtained by the method in [5], while XI was obtained by the method in [6].

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