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1965

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Abstract

Full Text

CHEMISTRY

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SYNTHESIS OF URIDYLYL-(5′→N)-PHENYLALANYL-3′(2′)-O-ADENOSINE

(Presented by Academician A. N. Nesmeyanov, 25 VII 1964)

Continuing the study of the properties of O-aminoacyl derivatives of nucleosides as the simplest models of possible participants in protein biosynthesis at the ribosomal level, uridylyl-(5′→N)-phenylalanyl-3′(2′)-O-adenosine (I) was synthesized. This compound contains not only the "active" ester bond between the carboxyl group of the amino acid and the hydroxyl of adenosine, characteristic of O-aminoacyl derivatives of sRNA, but also a phosphoamide bond between the amino acid and the nucleotide (uridylic-(5′)-acid).

[[chemical structural formula]]
A = adenine y = uracil (I)

The study of the properties of compounds of type (I) is of considerable interest if peptide synthesis in ribosomes is regarded as a sequential chain of reactions of aminoacylation of amino groups entering the ribosome in the form of O-aminoacyl-sRNA. In this case compound (I) may be considered as the structure with which this chain of peptide-synthesis reactions begins, and the amino group of whose amino acid is blocked by a nucleotide (possibly by the terminal nucleotide of ribosomal RNA) and therefore cannot undergo aminoacylation. The participation of structures of type (I) as donors of N-terminal amino acids in peptide biosynthesis may also be assumed from the fact that, for the chemical synthesis of peptides, blocking of the amino group of the N-terminal amino acid is necessary.

It should be noted that after alkaline hydrolysis of the polynucleotide chain of ribosomal RNA, nucleotide peptides with a phosphoamide bond between the fragments were isolated ([1], [2]). On the synthesized model we propose to study both the influence of the ester bond on the character of the phosphoamide bond and, most importantly, the degree of activation of the carboxyl group, which according to preliminary data ([3]) may be sufficient for peptide synthesis without the participation of other activating structures. The synthesis of (I) was carried out from uridylic-(5′) acid and 3′(2′)-O-phenylalanyladenosine ([3]) (III) as a result of the formation of a phosphoamide bond between these fragments. Since 3′(2′)-O-phenylalanyladenosine is an unstable compound, it was necessary to carry out the synthesis of (I) in the absence of moisture. The active derivative

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of uridylic acid capable of serving as a phosphorylating agent in the synthesis of nucleotide peptides of the phosphoamide type, as we showed earlier ([4]), is—with the readily available [(5)] pyrophosphate derivative of uridine (II), which is readily soluble in some organic solvents.

uridine pyrophosphate derivative (II) + phenylalanyl-3′(2′)-*O*-adenosine (III) $\longrightarrow$ uridyl-(5′ $\rightarrow$ *N*)-phenylalan

The reaction between (II) and (III) was carried out in absolute dioxane (20°, 40 h). Compound (I) was isolated by preparative paper chromatography in the system 96% ethanol—ammonium acetate (5:2) (for R_f in this and other systems see Table 1). The yield of (I) was 15% based on the uridylic acid introduced into the reaction. The low yield of (I) is apparently associated with its lability in the presence of moisture, as a result of which, during preparative chromatography, some amount of the substance decomposes. A possible side process occurring between (II) and (III) is phosphorylation of the amino group of adenine, which is part of (III), since on the chromatograms a compound was detected that was developed readily by ninhydrin (5 min, 70°) and by the phosphorus reagent, indicating the presence in the compound of a free amino group of an amino acid and a phosphorus-containing residue.

Table 1

Comparative characteristics of uridyl-(5′ $\rightarrow$ *N*)**-phenylalanyl-**3′(2′)**-O-adenosine (I)**

No.	Compounds	Yield, %	R_f in systems* 1	R_f in systems* 2	R_f in systems* 3	R_f in systems* 4	R_f in systems* 5	Ratio A : U : Phe**
1	Uridyl-(5′ $\rightarrow$ *N*)-phenylalanyl-3′(2′)-O-adenosine (I)	15	0.48	0.39	0.22	0.37	0.47	1.00 : 0.96 : 0.80

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No.	Compounds	Yield, %	R_f in systems* 1	R_f in systems* 2	R_f in systems* 3	R_f in systems* 4	R_f in systems* 5	Ratio A : U : Phe**
2	Methyl ester of uridyl-(5′ → N)-phenylalanine (5)		0.73	0.34		0.54		
3	Uridyl-(5′ → N)-phenylalanine (5)		0.36			0.31		
4	Uridylic-(5′) acid		0.20	0.06		0.09	0.85	
5	Adenosine		0.74	0.38	0.12	0.47	0.55	

* For paper-chromatography systems see the experimental part. The chromatograms were examined in UV light and sprayed with ninhydrin, followed by heating at 70° for 40 min.
** Here A is adenosine, U is uridylic-(5′) acid, Phe is phenylalanine.

Compound (I) is a white crystalline substance, highly hygroscopic and decomposing on standing in air. The UV absorption spectrum of (I) (λ_{max}218 and 216 mμ and λ_{min}229 mμ) is analogous to the spectrum of a mixture of equimolar amounts of adenosine and uridylic acid. However, in the region of 220 mμ a new absorption maximum of greater intensity appears, which possibly arises from the presence of an amino acid in (I). Compound (I) is not developed by ninhydrin under conditions in which amino acids with free amino groups are detected (5 min, 70°), but upon heating for 40 min at 70° a coloration typical of ninhydrin complexes of amino acids begins to develop. This difference in the ability to give a colored compound with ninhydrin was used for the identification of (I).

In aqueous solutions (pH 5), compound (I) is completely hydrolyzed within 2 hours (20°), with formation of uridylic-5′ acid, adenosine, and phenylalanine. However, the cleavage of phenylalanine under these conditions is apparently accompanied by formation of products of interaction of the amino acid with the initial compound (I), as a result of which phenylalanine peptides are formed; these were detected on chromatograms of hydrolysate I (R_f 0.3—0.5 in the

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system butanol—acetic acid—water (4 : 5 : 1)). Therefore, for the quantitative analysis of phenylalanine in (I), when determining the ratio adenosine : uridylic acid : phenylalanine, hydrolysis of (I) was carried out in 6 N HCl (3 hours). Nevertheless, the amount of phenylalanine, in comparison with adenosine and uridylic acid, was low.

The ease of hydrolysis of (I) at pH 5 makes it possible to suppose that, at pH values close to 7, the activity of the high-energy ester bond in this compound will be sufficient for aminoacylation reactions involving it. The study of the properties of compound (I) is continuing.

Experimental Part

Systems for paper chromatography. System 1: 96% ethanol—ammonium acetate (5 : 2). System 2: butanol—acetic acid—water (4 : 5 : 1). System 3: butanol—formic acid—water (77 : 10 : 13). System 4: isopropanol—ammonia—water (7 : 1 : 2). System 5: 5% disodium sodium phosphate—isoamyl alcohol (2 : 1).

For detection of amino-acid derivatives of nucleosides and nucleotides, the chromatograms were examined in UV light.

For detection of amino acids, chromatograms were sprayed with a 0.2% solution of ninhydrin in acetone. In quantitative chromatography of amino acids, a solution of 0.5 g of ninhydrin in 95 ml of absolute acetone with addition of 5 ml of distilled water and 1 ml of glacial acetic acid was used.

For elution of the ninhydrin complex of phenylalanine, 80% ethanol with addition of 1 drop of a 1% solution of nickel salt was used.

Synthesis of uridylyl-(5′→N)-phenylalanyl-3′(2′)-adenosine (I). To a solution of 64.5 mg (0.1 mmol) of uridylic-(5′) acid (obtained by passing its barium salt through Dowex-50) in 5 ml of water, 0.1 ml (0.22 mmol) of trioctylamine was added, and the mixture was evaporated to dryness. Residual moisture was removed by distillation with abs. dioxane (3 × 5 ml) at 40°. The residue was dissolved in 10 ml of abs. dioxane, filtered, and the dioxane was distilled off in vacuum (30°) with addition of abs. benzene (3 × 5 ml) and abs. toluene (4 × 5 ml). To the residue, 1 ml of abs. dioxane was added, and, with stirring over 5 min, 0.08 ml (0.4 mmol) of diphenyl chlorophosphate and a thoroughly dried solution of 0.12 ml (0.5 mmol) of tributylamine in 0.5 ml of abs. dioxane were introduced. The reaction mixture was shaken and left at 20° (3 hours). The clear solution was evaporated at 30° to a volume of 0.3 ml, 25 ml of cooled abs. ether was added, the mixture was shaken and left at −20° for 30 min. The ether was decanted, and oil (II) was dissolved in 5 ml of abs. dioxane and evaporated in vacuum to a volume of 2 ml (25°). To the yellowish solution II, a solution of 150 mg (0.4 mmol) of freshly prepared 3′(2′)-O-phenylalanyladenosine (3) in 8 ml of abs. dioxane was added dropwise. The reaction mixture was shaken, left for 40 hours (20°), filtered, evaporated in vacuum to a volume

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of 2 ml, and subjected to preparative chromatography in system I. After development of the chromatograms, the zone with R_f 0.48—0.50 was cut out, and compound (I) was eluted with abs. methyl alcohol (by flow-through). The eluate was evaporated to a volume of 1 ml, poured from a capillary into abs. ether, and left at −20° (2 hours). The precipitate (I) was separated by centrifugation and dried over P_2O_5. Yield of (I): 25 mg (15%).

Determination of the composition of (I).

The ratio of the components in (I) was determined as follows. 0.5 mg of the substance was dissolved in a 100-ml volumetric flask in distilled water (pH 5.0), and the solution was divided into 2 portions of 50 ml each. One portion was left at room temperature for 2 hours, after which the solution was evaporated to a volume of 0.5 ml and applied to a chromatogram in system 5. The chromatogram was examined under UV light, and the spots corresponding to uridylic acid (R_f 0.85) and adenosine (R_f 0.55) were cut out. The uridylic acid and adenosine were eluted with distilled water in flow-through mode; the volumes of the eluates were brought to 10 ml, after which the optical density of the solutions was measured on an SF-4 at λ 261 and 290 mμ for uridylic acid and at λ 260 and 290 mμ for adenosine. The amounts of uridylic acid and adenosine [6] were, respectively, 0.112 and 0.118 μmol.

The second portion of the solution was evaporated in vacuo to dryness and boiled with 2 ml of 6 N hydrochloric acid for 3 hours. The hydrolysate was chromatographed in system 2; after development of the chromatogram with ninhydrin, the spot with R_f 0.53, corresponding to phenylalanine, was cut out. The colored complex was eluted, and its optical density was measured on an SF-4 at λ 570 mμ. The phenylalanine content, calculated from a calibration curve, was 0.87 μmol.

Thus, in compound (I) the ratio adenosine : uridylic acid : phenylalanine is 0.118 : 0.112 : 0.87 (1 : 0.95 : 0.8), which confirms the proposed structure of the product.

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Received
20 VII 1964

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