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Abstract

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SYNTHESIS OF PEPTIDES AND POLYPHOSPHATES OF ADENYLIC ACID FROM N-(ADENOSINEPHOSPHO)-PHENYLALANINE

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Data have appeared in the literature ([1],[2]) making it possible to propose the presence in the organism of such compounds in which peptides are linked with nucleotides by means of a P—N bond. Questions of the chemical lability of such compounds, their ability to interact with amino acids, phosphoric acid, and other substances present in the organism remain unclear.

Table 1

Rate constants of hydrolysis of compounds A and B under the action of acid and alkali (37°).

Hydrolyzing agent	$K_{[A]}$	$K_{[B]}$
1 *N* HCl	Not hydrolyzed	$5.6 \cdot 10^{-1}$
0.1 *N* HCl	Not hydrolyzed	$1.5 \cdot 10^{-2}$
1 *N* NaOH	$2 \cdot 10^{-2}$	$6.1 \cdot 10^{-2}$

Using N-adenosinephosphoric derivatives of phenylalanine as an example, we attempted to determine the possibility of the participation of these compounds in the synthesis of peptides, nucleoside polyphosphates, and nucleoside phosphosulfate carried out *in vitro*. The methyl esters of N-(2′:3′-isopropylideneadenosine-5′-benzylphospho)-phenylalanine (A) and N-(2′:3′-isopropylideneadenosine-5′-phospho)-phenylalanine (B) were investigated.

(A) $R = C_6H_5CH_2$,
(B) $R = H$

It proved that the P—N bond in substance B is labile. If this substance is boiled with carbobenzoxy (Cbz)-amino acids (glycine, valine, tyrosine) in absolute dioxane, the formation of dipeptides (I) can be detected. It should be noted that with compound A the peptide-formation reaction does not proceed.

Fig. 1

Figure 1: Fig. 1

From substance B it is fairly easy to pass to the di- (II) and triphosphates of adenosine (III) if it is treated with phosphoric or pyrophosphoric acids in pyridine. This process is analogous to that carried out in the laboratories of Todd ([3]) and Khorana ([4]) with amides of phosphoric acid and nucleotides. Substance B is also characterized by reactions with other anions of strong acids. Thus, upon interaction of substance B with sulfuric acid in pyridine, adenosine phosphosulfate (IV) is formed.

$$\mathrm{A{-}O{-}P(OH)(O){-}NH{-}CH(CH_2C_6H_5){-}COOCH_3}$$

$$\xrightarrow{H_2PO_4^-} \mathrm{A{-}O{-}P(OH)(O){-}O{-}P(OH)(O){-}OH} \qquad \text{(II)}$$

$$\xrightarrow{H_2P_2O_7^-} \mathrm{A{-}O{-}P(OH)(O){-}O{-}P(OH)(O){-}O{-}P(OH)(O){-}OH} \qquad \text{(III)}$$

$$\xrightarrow{HSO_4^-} \mathrm{A{-}O{-}P(OH)(O){-}O{-}S(OH)(O){=}O} \qquad \text{(IV)}$$

$$\mathrm{Cbz{-}NH{-}CH(R){-}COOH} \longrightarrow \mathrm{Cbz{-}NH{-}CH(R){-}CO{-}NH{-}CH(CH_2C_6H_5){-}COOCH_3} \qquad \text{(I)}$$

where *A* is isopropylideneadenosine.

The hydrolysis of A and B by alkali and acid of different concentrations at 37° was studied. The amount of liberated amino acid was determined by means of the ninhydrin reaction. On the basis of the hydrolysis data (Fig. 1), the rate constants for the hydrolysis of A and B under various conditions were calculated (see Table 1).

As follows from Fig. 1 and Table 1, the phosphoamide bond in compound A, in which the amino-acid residue is attached to the diesterified phosphate group of the nucleotide, is considerably more resistant to the action of hydrolyzing agents than in compound B, where the same amino-acid residue is attached to the monoesterified phosphate group of the nucleotide. It turned out that the rate constants for acid hydrolysis of compound B coincide with the corresponding literature data for simple phosphoamino acids and peptides ([5]), which, as is known, belong to macroergic, i.e., energy-rich, compounds. This makes it possible to assume that in compounds of type B the phosphoamide bond is also energy-rich.

The data presented above indicate the high reactivity of compound B (a model of a *P*-amino-acid derivative of a mononucleotide), which makes it possible to suppose that compounds of this type participate in intracellular processes.

Fig. 1

Visible labels in Fig. 1: $1N$ HCl (B); $1N$ NaOH (B); $1N$ NaOH (A); $0.1N$ HCl (B); $1N$ HCl (A); $0.01N$ HCl (B). The vertical axis is marked in percent, and the horizontal axis in minutes.

Experimental Part

2′ : 3′-Isopropylideneadenosine ([6]) was obtained from adenosine and acetone in the presence of *p*-toluenesulfonic acid ([7]). The yield of chromatographically pure 2′ : 3′-isopropylideneadenosine was 80%, m.p. 220°.

Substance A was obtained from 2′ : 3′-isopropylideneadenosine by the procedure described earlier ([8]). M.p. 80° (decomp.). UV absorption in 50% ethanol: $\lambda_{\max}$260 mμ (ε11100). R_f0.87 in the system *n*-butanol saturated with water (system 1). The substance was dried in vacuo over P_2O_5 at 37° for 48 h.

Found, %:	C 56.71; H 5.99; P 4.50
$C_{30}H_{35}O_8N_6P$. Calculated, %:	C 56.20; H 5.59; P 4.90

Substance B. 50 mg of substance A are dissolved in 50 ml of absolute methanol. To the solution are added 50 ml of water, and it is subjected to hydrogenolysis over a Pd catalyst ([9]) (10 mg PdO). Hydrogenolysis is carried out for 30 min. The catalyst is then removed by centrifugation, the solution is evaporated in vacuo at 30°, and the residue is dissolved in 3-5 ml of chloroform. The chloroform solution is poured from a capillary into dry petroleum ether. An amorphous precipitate B is isolated. Reprecipitation is carried out two more times. Yield 29 mg (67%), m.p. 116-118° (decomp.). UV absorption in 95% ethanol: $\lambda_{\max}$260 mμ. R_f0.50 in system 1. The substance is dried for 24 h over P_2O_5 in vacuo.

Found, %:	C 48.91; H 5.78; N 15.48
$C_{23}H_{29}O_8N_6P \cdot H_2O$. Calculated, %:	C 48.90; H 5.51; N 15.90

On standing for several days, substance B decomposes with the formation of new compounds with R_f0.00, R_f0.06, and R_f0.12 in system 1. The same substances are formed on heating B with 50% ethanol or upon more prolonged hydrogenolysis of A. All three substances give a positive reaction for phosphorus and have a UV absorption maximum (in 50% ethanol) at 260 mμ. After evaporation, the eluates of the spots with 50% ethanol were subjected to acid hydrolysis. Under the conditions of hydrolysis of B (Fig. 1), no amino acid appeared. Chromatographically, the methyl ester of phenylalanine was detected after boiling the eluates of all three spots with 1 N HCl (30 min).

Reaction of (B) with carbobenzyloxy amino acids. a) To a solution of 23 mg of freshly prepared substance B in 50 ml of absolute dioxane are added

46 mg of carbobenzyloxyglycine. The mixture is boiled for 2 h. The precipitate that separates (9 mg) is filtered off. The filtrate is evaporated in vacuo to dryness, the residue is dissolved in 50 ml of chloroform and washed with 0.1 *N* HCl and 0.1 *N* NaOH. The solution is evaporated in vacuo, and the residue is dissolved in 50 ml of 50% aqueous ethanol and subjected to hydrogenolysis over a Pd catalyst. The catalyst is removed by centrifugation, the centrifugate is evaporated to a volume of 1-2 mm and chromatographed in system 1. On the chromatogram the methyl ester of glycylphenylalanine is detected (R_f0.42), coinciding with a control sample. b) Under analogous conditions, the reaction of B with carbobenzyloxyvaline and carbobenzyloxytyrosine was carried out. In the reaction mixture, by paper chromatography, the methyl esters of valylphenylalanine (R_f0.69 in system 1) and tyrosylphenylalanine (R_f0.65 in the system n-C_4H_9OH—H_2O—CH_3COOH (4:1:1)) were detected. Substance A does not react with amino acids under the conditions described.

Reactions of (B) with phosphoric and pyrophosphoric acids. To 0.93 ml of 59% H_3PO_4 are added 2.3 ml of dry pyridine, and the mixture is evaporated to dryness in vacuo. The glassy mass is dissolved with heating in dry pyridine, and the solution is added to 18 mg of freshly prepared substance B dissolved in 1 ml of dry pyridine. The mixture is left at room temperature in the dark for 75 h. On paper electrophoresis (acetate buffer pH 4.8; 5 V/cm; 6.5 h) with cooling, tetrachlor-

with activated carbon in the reaction mixture two substances were detected that coincided with authentic ADP and ATP.

Reaction of (B) with sulfuric acid. To 450 mg of cooled 40% oleum are added 4 ml of dry pyridine and 20 mg of freshly prepared substance (B) in 1 ml of pyridine. The mixture is left for 90 h at room temperature. The precipitate is filtered off, the filtrate is evaporated to a small volume and subjected to electrophoresis in acetate buffer (pH 4.8; 5 V/cm, for 6.5 h). In addition to the starting substance (B), an intense spot IV, absorbing in the UV ($\lambda_{\max}$ 260 mμ) and coinciding with an authentic sample of ADP, was found on the electropherogram. Sulfur was detected qualitatively in the eluate from the spot. The yield of IV was determined in the usual manner from the optical density of the eluates of IV and (B) at 260 mμ and amounted to 61%.

Study of the hydrolytic stability of the phosphoamino bond in N-adenosinephosphoric derivatives of phenylalanine (A and B). Ten mg of the substance under investigation are dissolved in 1-2 drops of absolute ethanol, and 1 ml of a titrated solution of hydrochloric acid or sodium hydroxide is added. The mixture is placed in a thermostat (37°) for specified intervals of time. The solution is neutralized, and first 1 ml of citrate buffer (pH 5) is added, then 1 ml of a solution of ninhydrin in absolute ethanol (20 mg/ml), and finally 1 ml of absolute ethanol. The mixture is heated for 20 min on a boiling water bath, transferred to a volumetric flask, and diluted to 25 ml with 50% ethanol. Fifteen to sixty minutes after dilution, the optical density of the solutions is determined with an FEK-M photoelectric colorimeter. The measured optical-

density values are compared with a calibration curve obtained by measuring the optical densities of a series of standard solutions of phenylalanine methyl ester in ethanol. The results of the hydrolysis are given in Fig. 1.

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Note: Figure translations are in progress. See original paper for figures.