THE MODIFICATION OF NUCLEOSIDES AND THEIR 5'-PHOSPHATES BY N^{α},N^{α},N^{ε},N^{ε}-TETRAMETHYLLYSINE HYDRAZIDE

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The specific 3'-terminal modification of high-molecular-weight ribonucleic acids is used both for terminal analysis and for the characterization of preparations of RNA and their heterogeneity [1-5].

The present paper describes the synthesis of N^{α}, N^{α}, N^{ε}, N^{ε}-tetramethyllysine hydrazide (TLH), a substance with a strongly pronounced basic function, and characterizes the chromatographic properties of its derivatives with nucleosides and their 5'-phosphates.

Periodate-oxidized nucleosides and their 5'-phosphates form condensation products with N^{α}, N^{α}, N^{ε}, N^{ε}-tetramethyllysine hydrazide, obviously in a ratio of 1 : 1, as is confirmed by the formation of products with the same chromatographic mobility of reaction with 1-, 2-, and 10-fold excesses of TLH. After reaction, the mixture shows a new spot on paper chromatography always accompanied solely by the excess of TLH but with no unchanged nucleoside or its dialdehyde derivative. The IR spectrum of TLH-modified adenosine (A-TLH) does not show the ν_{CO} band characteristic for a free aldehyde group, since there are no bands in the spectrum with frequencies above 1670 cm^{-1}. Apparently, the structure of the compounds formed is similar to that of the analogous products obtained by modification with isonicotinic acid hydrazide [3]. The stabilities of the derivatives also proved to be extremely similar. The nucleoside derivatives of TLH liberated the corresponding heterocyclic bases after incubation at pH 13 and, to a small extent, at pH 1.

The TLH-modified nucleoside 5'-phosphates liberate the corresponding bases partially at pH 1 and pH 9, while at pH 13 β elimination takes place to a considerable extent. Modification with TLH has a pronounced effect on the mobility of the nucleosides and nucleoside 5'-phosphates on paper chromatography.

The chromatographic mobilities of the products of the condensation of the oxidized nucleosides and their 5'-phosphates with N^{α}, N^{α}, N^{ε}, N^{ε}-tetramethyllysine hydrazide (the mobilities of the initial nucleosides and nucleotides taken as 1.00) are given below:

Compound	Relative mobility
System 1	
A-TLH	0.78
G-TLH	0.72
U-TLH	0.68
C-TLH	0.61
System 2	
pA-TLH	2.03
pG-TLH	2.94
pU-TLH	1.62
pC-TLH	2.23

The high mobilities of the derivatives are observed only in fairly acidic chromatographic systems, which can be used for their separation from nonmodified nucleotide material.

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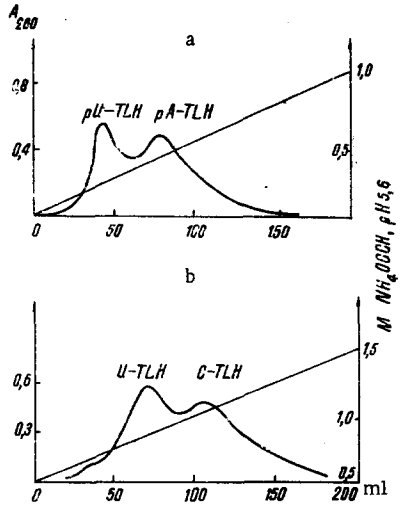

Fig. 1. Chromatography of the condensation products on Dowex-50 W × 4 cation-exchange resin (100/200, NH_4^+ form). Combined separation of the mixture of the products of the condensation of N^{α}, N^{α}, N^{ε}, N^{ε}-tetramethyllysine hydrazide with uridine 5'-phosphate (pU-TLH) and adenosine 5'-phosphate pA-TLH) in a 0.1-1 M gradient of ammonium formate (a) and of N^{α}, N^{α}, N^{ε}, N^{ε}-tetramethyllysine hydrazide with uridine (U-TLH) and cytidine (C-TLH) in a 0.5-1.5 M gradient (b).

In a study of the chromatography of the products on a cation-exchange resin, it was found that the substances are not eluted by the system developed previously [6] and possess well-defined basic properties. The elution of the products takes place only at high concentrations of the salt gradient in agreement with the increase in the total positive charge of the substances (Fig. 1). Derivatives of the nucleoside 5'-phosphates are eluted at 4°C from a column 0.5 × 9 cm previously equilibrated with 0.05 M ammonium phosphate buffer solution at pH 5.6 by comparatively high concentrations of formate: pU-TLH 0.30 M; pA-TLH 0.40 M; pC-TLH 0.50 M; pG-TLH 0.55 M; 0.75-1 M ammonium formate buffer solution is necessary to elute the nucleoside derivatives. In our opinion, this property broadens the possibility of analyzing the 3'-terminal nucleosides of RNA, since the modified compounds formed by condensation with TLH – A-TLH, G-TLH, U-TLH, and C-TLH – can be separated selectively from a large excess of ballast nucleotide material which is not sorbed onto a cation-exchange resin under such conditions.

In determining the 3'-terminal fragments in an RNase hydrolyzate of RNA modified with the [^{14}C]hydrazide of isonicotinic acid, it was found that a considerable part of the terminal label is liberated in the form of nonnucleotide material [7]. The splitting off of the modifying agent from RNA on the electrophoresis of such an RNA in polyacrylamide gel has also been reported [8]. This shows the instability of the bond of the hydrazides with the 3'-terminal oxidized nucleoside in the composition of the RNA. It is obvious that it is more effective to use modification with hydrazides to separate and identify the 3'-terminal structures of RNA after the enzymatic hydrolysis of the RNA. In some cases, particularly in the investigation of dilute solutions, N^{α}, N^{α}, N^{ε}, N^{ε}-tetramethyllysine hydrazide may be the most suitable 3'-terminal modifying agent. The selective introduction of a strong cationic grouping into the 3'-terminal nucleoside or oligonucleotide of an RNase hydrolyzate of RNA must facilitate its isolation and analysis.

EXPERIMENTAL

To synthesize N^{α}, N^{α}, N^{ε}, N^{ε}-tetramethyllysine hydrazide we used L-lysine monohydrochloride (pure-for-analysis grade) (Reanal, Hungary). The course of the synthesis was checked by thin-layer chromatography on KSK silica gel (sieve 0.09) in the butan-1-ol–pyridine–acetic acid–water (5:2:2:2) system. Paper chromatography was performed on washed Whatman 1 paper in the phenol–water (4:1) system [9]. The chromogenic agent was a 0.5% solution of ninhydrin in ethanol. The spots of the L-lysine, hydrazine hydrate, the hydrazide, and the products of partial methylation were shown up at 110°C and those of tetramethyllysine and its ester appeared when the plate was subsequently heated at 150-160°C. On paper, the methylated derivatives could be seen as yellow spots on a red background after spraying with a 0.04% solution of Thymol Blue in butan-1-ol–ethanol (1:1) with the addition of H_2SO_4 to 0.01 N. The IR spectra* of the compounds were recorded on a UR-20 instrument using KBr tablets for the solid materials.

N^{α}, N^{α}, N^{ε}, N^{ε}-Tetramethyllysine. This was obtained basically by Ikutani's method [10]. A mixture of 12.4 g of Lys·HCl, 50 ml of formalin (40%), and 7 g of Pd/C catalyst (10%) in 250 ml of ethanol–water (10:1) was hydrogenated at 40 atm and 30°C until the absorption of hydrogen ceased completely (about 2 h). The catalyst was separated off by filtration, the solution was repeatedly evaporated with ethanol, and the syrupy substance was dried over P_2O_5 and activated carbon. Thin-layer chromatography showed one spot with R_f 0.30 (R_f value of Lys·HCl 0.60), and paper chromatography showed a ninhydrin-negative spot with R_f 0.79 (R_f Ala 0.55) [9]. The decomposition temperature of the N^{α}, N^{α}, N^{ε}, N^{ε}-

*The IR spectra were taken by I. Dipan.

tetramethyllysine hydrochloride was 203°C, which corresponds to literature information [11]. IR spectrum, cm^{-1}: 1620 (COOH); in the initial Lys·HCl: 1615 (COOH).

Methyl Ester of N^{α}, N^{α}, N^{ε}, N^{ε}-Tetramethyllysine. The tetramethyllysine was dissolved in 100 ml of absolute methanol, and a current of dry hydrogen chloride was passed while the reaction mixture was cooled in an ice bath. The reaction lasted 25-30 h, after which the ester with R_f 0.50 had been formed completely. The solution was evaporated and the residue was recrystallized from absolute n-butanol. This gave the dihydrochloride of the methyl ester of N^{α}, N^{α}, N^{ε}, N^{ε}-tetramethyllysine with the composition $C_{11}H_{26}ON_2Cl_2$, mp 162-164°C. IR spectrum, cm^{-1}: 1750 ($COOCH_3$) as compared with 1740 cm^{-1} for Lys·OCH_3 and 1750 cm^{-1} for Ala·OCH_3.

N^{α}, N^{α}, N^{ε}, N^{ε}-Tetramethyllysine Hydrazide. A solution of the ester obtained (8.7 g) in 100 ml of absolute n-butanol was treated with 47 ml of hydrazine hydrate (84%) and the mixture was boiled for 30 h. The formation of the hydrazide was monitored by thin-layer chromatography (R_f of the hydrazide 0.38; R_f of the excess of hydrazine hydrate 0.80). After the cooling of the mixture, the bulk of the hydrazine hydrate (lower phase) was separated off, the butanol solution was evaporated to dryness, and the residue was dried in a desiccator over H_2SO_4. Then it was dissolved with heating in 30 ml of absolute methanol, the solution was cooled, and the addition of 70 ml of absolute ether precipitated hydrazine hydrochloride with a small loss of the main substance. The solution was filtered and evaporated. The colorless oily substance obtained was treated with an excess of hydrochloric acid, and the mixture was evaporated with ethanol and dried over NaOH. Recrystallization from absolute ethanol gave 3.6 g (39%) of N^{α}, N^{α}, N^{ε}, N^{ε}-tetramethyllysine hydrazide trihydrochloride with mp 176-178°C. The formation of a trihydrochloride with the composition $C_{10}H_{27}ON_4Cl_3$ was confirmed by titration [12]. On paper it gave one spot with R_f 0.5 (system 1) and 0.1 (system 2). IR spectrum, cm^{-1}: 1350 w, 1380 sh., 1405 w, 1440 sh., 1465 m, 1480 m, 1540 sh., 1570 m, 1630 w, 1710 s ($CONHNH_2$).

The products of the condensation of the nucleosides and their 5'-phosphates with N^{α}, N^{α}, N^{ε}, N^{ε}-tetramethyllysine hydrazide (TLH) were obtained as described previously [6]. IR spectrum of A-TLH, cm^{-1}: 1305 m, 1335 m, 1350 w, 1370 w, 1390 w, 1415 m, 1475 m, 1575 m, 1610 s, and 1670 s, as compared with adenosine – 1578 m, 1610 m, 1670 s.

Descending chromatography of the nucleoside derivatives was performed on FN II paper (GDR) in system 1) tert-butanol–formic acid–water (70:15:15), and the derivatives of the nucleoside 5'-phosphates were chromatographed in system 2) butan-1-ol–6 N HCl (7:3). In a study of the stability of the condensation products, to show the presence of the purine bases we used the system butan-1-ol–acetic acid–water (4:1:5). The stability of the substances was determined by incubating them at pH 1, 3, 7, 9, and 13 at 37°C for 18 h followed by paper chromatography (the 0.1 N KOH was neutralized with $HClO_4$).

SUMMARY

N^{α}, N^{α}, N^{ε}, N^{ε}-Tetramethyllysine hydrazide, its derivatives with oxidized nucleosides, and their 5'-phosphates have been synthesized. The stabilities of the modification products and the influence of the introduction of the hydrazide on the chromatographic properties of model compounds have been characterized. The possibility is discussed of using N^{α}, N^{α}, N^{ε}, N^{ε}-tetramethyllysine hydrazide as a specific 3'-terminal modifying agent for RNA.

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