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Abstract

Full Text

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ON THE HYDRAZINOLYSIS OF PYRIMIDINE NUCLEOSIDES AND DNA

For the study of the primary structure of nucleic acids, chemical methods for the modification of nucleic bases are of great importance ([1]). It is well known that hydrazine cleaves pyrimidine nucleosides without affecting purine nucleosides ([2–4]). Hydrazine has been used to obtain apyrimidinic nucleic acids ([5,6]). However, the conditions used for apyrimidinization (anhydrous hydrazine, 60–100°, 1–3 hr) also lead to considerable hydrolysis of phosphodiester bonds and, consequently, to destruction of the biopolymer. In this connection, the known methods of hydrazinolysis are unsuitable for determining the primary structure, although they can be applied to the statistical study of polypurine sequences ([7]).

In continuation of the work being carried out in our laboratory on the study of specific methods of chemical modification of nucleic acids, in particular the action of nitrogenous bases, we investigated in greater detail the reaction of nucleosides with aqueous hydrazine solutions as a function of reagent concentration, temperature, and pH. It was shown that considerably milder conditions can be used for the cleavage of pyrimidine nucleosides by hydrazine.

It turned out that the action of aqueous hydrazine solutions also leads to cleavage of uridine, cytidine, and thymidine, but does not affect adenosine and guanosine.

The reaction rate depends substantially on pH. At pH 5.5 (7 M hydrazine, 37°) the reaction does not proceed; at pH 7.0 uridine and cytidine are cleaved slowly; at pH 9.5 uridine, cytidine, and thymidine are completely cleaved in 24–48 hr. The reaction rate decreases in the series uridine > cytidine > thymidine (cf. ([7])). When the temperature is lowered to 0° (pH 9.5; 7 M hydrazine), complete cleavage of uridine and cytidine requires 10 days, and of thymidine, 16 days.

The structure of the products of the reaction of hydrazine with pyrimidine nucleosides does not depend on the reaction conditions. At 0, 37, and 100° (7 M hydrazine solution, pH 9.5), as well as at 80° with hydrazine hydrate, uridine, cytidine, and thymidine are cleaved with formation, respectively, of 3-oxypyrazole, 3-aminopyrazole, and 4-methyl-3-oxypyrazole (cf. ([4])).

The results of the reaction of nucleosides with hydrazine under mild conditions

Fig. 1. Sedimentation diagram of phage T_2 DNA. Solid line—the starting substance; dashed line—after apyrimidinization.

Figure 1: Fig. 1. Sedimentation diagram of phage T_2 DNA. Solid line—the starting substance; dashed line—after apyrimidinization.

made it possible to propose a new method for obtaining apyrimidinic DNA, which practically excludes spontaneous destruction of the polymer. As is known, under the conditions of apyrimidinization according to Takemura ([5]), substantial nonspecific cleavage of the phosphodiester bond occurs, decreasing when the temperature is lowered. Naturally, cleavage of pyrimidines at low temperature proceeds more slowly; however, carrying out the hydrazinolysis of phage T_2 DNA at 0° (7 M hydrazine, pH 9.5) proved quite sufficient for complete cleavage even of thymidine and 5-oxymethylcytosine within the polymer in 10 days. After hydrolysis of the apyrimidinic DNA thus obtained with 72% hydrochloric acid, no pyrimidine bases were detected on a paper chromatogram.

Thus, hydrazinolysis proceeds quantitatively under milder conditions than had previously been described ([5,6]).

To determine the extent of spontaneous degradation of the polymer during hydrazinolysis under the conditions described above, the results obtained were compared—

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Fig. 1. Sedimentation diagram of phage T_2 DNA. The solid line is the starting substance; the dashed line is after apyrimidinization.

···obtained by centrifugation in a sucrose gradient for phage T_2 DNA before and after hydrazinolysis. If nonspecific hydrolysis of the phosphodiester bond had proceeded to a significant extent, the apyrimidine DNA obtained should have given a broad zone with an average value of S considerably smaller than that of the starting DNA. However, as can be seen from Fig. 1, apyrimidine DNA gives a narrow zone with an S value differing little from the sedimentation constant of the starting substance.

These data indicate that the new method for obtaining apyrimidine DNA is not inferior to the known methods in the completeness of modification. At the same time, the sharp decrease in degradation of the polymer in this case makes the method considerably more suitable for studies of nucleic acids, in particular for studying primary structure.

Experimental Part

To obtain a 7 M solution of hydrazine, 7 ml of freshly distilled hydrazine were dissolved in 13 ml of water. The solution was acidified to the required pH value with concentrated hydrochloric acid. The nucleosides were chromatographically pure preparations from the firms Reanal and Calbiochem; phage T_2 DNA was kindly provided to us by L. A. Osterman (Institute of Radiation and Physicochemical Biology, Academy of Sciences of the USSR). Centrifugation in a sucrose gradient was carried out in a Spinco centrifuge, model L, rotor SW39.

Chromatography was carried out on Goznak paper in the following systems: I—ethanol—water (3 : 1); II—satd. ammonium sulfate solution—isopropanol—water (79 : 2 : 19); III—isopropanol—water (7 : 3); IV—ethanol—1 M ammonium acetate solution (5 : 2); V—isopropanol—1% formic acid—10% ammonia (50 : 25 : 1); VI—0.05 M borate buffer, pH 9.5; VII—*n*-butanol—ethanol—water (5 : 1 : 1); VIII—isopropanol—hydrochloric acid—water (170 : 49 to 250 M).

Action of aqueous hydrazine solutions on nucleosides. The nucleoside (1-10 mg/ml) was dissolved in 7 M aqueous hydrazine solution of the appropriate pH value and kept at 0, 37, or 100°. At definite intervals, aliquots (~10 µl) were taken for paper chromatography and, after appropriate dilution, for spectral determinations (dilution 1/400 with 0.05 M tris-HCl buffer solutions of pH 2.0, 7.0, and 10.0).

Hydrazine solutions kept under the same conditions as the reaction mixture were used as controls for spectrophotometry and paper chromatography.

The course of the reaction was judged from the change in the spectrum of the reaction mixture and the change in the amounts of the corresponding pyrazole and nucleoside after separation by paper chromatography.

Action of hydrazine hydrate on nucleosides. The nucleoside (10 mg/ml) was dissolved in hydrazine hydrate, kept at 80°, the hydrazine was evaporated in vacuo, and the residue was dissolved in water and applied to the chromatogram. The results of these experiments are summarized in Table 1.

Action of an aqueous hydrazine solution on DNA. 1.9 mg of phage T_2 DNA was dissolved, with careful stirring, in 1.0 ml of 7 M aqueous hydrazine (pH 9.5) and kept at 0 +4°.

Table 1

Conditions and results of the reaction of nucleosides with hydrazine

Nucleoside	pH	Reaction temp., °C	Reaction duration, h	Reaction result	I	II	III	IV	V
Uridine	—	—	—	—	0.72	0.62	0.61	0.63	0.46

Nucleoside	pH	temp., °C	duration, h	Reaction result	I	II	III	IV	V
»	***	80	2	The reaction proceeded completely	0.85	0.78	0.79	0.77	0.65
»	9.5	100	2	The same					
»	9.5	37	24	The same					
»	9.5	0	240	The same					
»	7.0	37	170	Incomplete					
»	5.5	37		No changes					
Cytidine	–	–	–	–	0.78	0.73		0.89*	0.41
»	***	80	2	The reaction proceeded completely	0.64	0.64		0.60*	0.65
»	9.5	100	2	The same					
»	9.5	37	24	» »					
»	9.5	0	240	» »					
»	7.0	37	170	Incomplete					
»	5.5	37		No changes					
Thymidine	–	–	–	–		0.47**			
»	***	80	3	The reaction proceeded completely		0.53**			

Nucleoside	pH	temp., °C	duration, h	Reaction result	I	II	III	IV	V
»	9.5	37	48	The same					
»	9.5	0	400	» »					
»	7.0	37		The reaction does not proceed					
»	5.0	37		» » »					

* In system VI.
** In system VII.
*** In hydrazine hydrate.

Aliquot samples were taken every 48 h, lyophilized, and hydrolyzed with 72% hydrochloric acid. On the tenth day the hydrolysate of the sample no longer contained pyrimidine bases.

After 11 days the polymer was separated from low-molecular-weight compounds on a Sephadex G-25 column (medium, 2.5 × 40 cm) equilibrated with 0.01 M NaCl. The polymer fraction (40 ml, OD_{260} = 0.490) was divided into two samples.

A control DNA sample was passed similarly through the same column (1.1 mg; 50 ml of solution with OD_{260} = 0.250 was obtained).

I. Samples of solutions of DNA (25 ml) and apyrimidinic DNA (20 ml) after gel filtration were dialyzed against water (48 h, 0°). The residues in the dialysis chambers (26.5 ml, OD_{260} = 0.265 and 21 ml, OD_{260} = 0.490, respectively) were lyophilized and hydrolyzed with 72% hydrochloric acid (0.1 ml, 110°, 6 h). After chromatography in system VIII, guanine (R_f 0.32), adenine (R_f 0.58), 5-hydroxymethylcytosine (R_f 0.68), and thymine (R_f 0.81) were detected and identified in the DNA hydrolysate. In the hydrolysate of apyrimidinic DNA, only adenine and guanine were detected.

II. Samples of DNA and apyrimidinic DNA after gel filtration were lyophilized, dissolved in 0.5 ml of water, and layered into a tube with a sucrose gradient (5% → 20% in 0.05 M Tris-HCl buffer, pH 7.2). The profiles obtained after centrifugation at 35,000 rpm for 165 min are shown in Fig. 1.

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