THE EFFECT OF POLYMERISM ON THE ANTIGENIC PROPERTIES OF PREPARATIONS OF DESOXYRIBONUCLEIC ACID

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As reported earlier [2], preparations of highly polymerized desoxyribonucleic acid (DNA) are antigens, i.e. in the immunization of animals they cause the formation of antibodies reacting specifically in the complement fixation test (CFT) with DNA. The aim of the present work was to clarify the question of the effect of polymerism on the antigenic properties of DNA preparations.

EXPERIMENTAL METHODS

DNA preparations were isolated from calf thymus and cow liver. Immediately after slaughtering the animal these organs were frozen with dry ice and the desoxyribonucleoprotein (DNP) isolated from them by the method of Mirsky and Pollister [4]. The nucleoprotein was extracted with 1 M NaCl and after centrifuging was precipitated by the addition of 6 volumes of distilled water to a final concentration of 0.14 M NaCl. The obtained fibers were dissolved in 1 M NaCl and the operation repeated 3-4 times. For obtaining pure DNA, free of protein, the DNP solution was treated with a 4 : 1 chloroform-butyl alcohol mixture [5]. The operation was repeated 18 to 34 times — till there was no qualitative reaction for protein (biuret reaction and trichloroacetic acid reaction). The clear DNA solution was precipitated with two volumes of 96 % ethyl alcohol and the profuse white fibers which settled out were dissolved in physiological saline. All the operations were carried out at a temperature +2 to +4°.

Phosphorus in the obtained preparations was determined by the Fiske-Subarrow method, nitrogen — by Conway's method. The molecular weight was calculated from the viscosity [1] which was measured in 0.2 M NaCl in an Ostwald viscometer.

The preparations had a high molecular weight — from 2,200,000 to 7,800,000. As already reported, such DNA preparations have antigenic properties.

Despite the fact that the DNA preparations after thorough and prolonged purification from protein gave negative results in the qualitative tests for protein, we are not justified in stating that the obtained preparations were devoid of protein, since these tests are not sensitive enough.

In order to clear up the question of the effect of protein impurity on the antigenic properties of the DNA preparation we immunized rabbits with DNA preparations of varied degree of purification from protein. For immunization we used the following preparations: preparation No. 1 ($N/P = 3.1$), No. 2 ($N/P = 2.0$) and No. 3 ($N/P = 1.66$).

The most protein-free preparation No. 3 had a molecular weight 5,000,000.

These preparations were injected intravenously into the rabbits 3 days in succession. Each rabbit received 9 to 10 mg of DNA in one injection. This dose was repeated in the following two weeks. 7-8 days after the last immunization the rabbits were starved for 4 hours, then blood was taken from them and sera prepared. In the 2-3 days previous to immunization we had taken blood from these rabbits and prepared sera which were employed as controls.

TABLE 1

Results of Experiments

Test antigen	Antiserum against preparation No. 1					Antiserum against preparation No. 2					Antiserum against preparation No. 3					Normal serum				
	Dilution																			
	1:10	1:20	1:40	1:80	1:160	1:10	1:20	1:40	1:80	1:160	1:10	1:20	1:40	1:80	1:160	1:10	1:20	1:40	1:80	1:160
Preparation № 2	++	+	H	H	H	+++	++	±	H	H	++++	++++	+++	++	±	H	H	H	H	H
№ 3	++	+	±	H	H	++	+	H	H	H	++++	+++	++	+	H	H	H	H	H	H

NOTE. Controls of antigen, antisera, normal serum and complement gave hemolysis.

TABLE 2

Complement Fixation Reaction in System of Serum Against Depolymerized DNA + DNA Preparation

Test-antigen	Antiserum against depolymerized DNA					Normal serum (control)				
	Dilution									
	1 : 10	1 : 20	1 : 40	1 : 80	1 : 160	1 : 10	1 : 20	1 : 40	1 : 80	1 : 160
Depolymerized DNA, preparation No. 1	H	H	H	H	H	H	H	H	H	H
The same, No. 2	H	H	H	H	H	H	H	H	H	H
Polymerized DNA	H	H	H	H	H	H	H	H	H	H

NOTE. Controls of antigens, antisera and normal serum gave hemolysis.

TABLE 3

Complement Fixation Reaction in System of Serum Against DNA Preparations Treated and Not-treated with DNA-ase + DNA Preparation

Test-antigen	Antisera against DNA treated with DNA-ase (Dilution 1:10)	Antisera against DNA treated with DNA-ase (Dilution 1:20)	Antisera against DNA treated with DNA-ase (Dilution 1:40)	Antisera against DNA treated with DNA-ase (Dilution 1:80)	Antisera against DNA treated with DNA-ase (Dilution 1:160)	Antisera against DNA before treatment with DNA-ase (Dilution 1:10)	Antisera against DNA before treatment with DNA-ase (Dilution 1:20)	Antisera against DNA before treatment with DNA-ase (Dilution 1:40)	Antisera against DNA before treatment with DNA-ase (Dilution 1:80)	Antisera against DNA before treatment with DNA-ase (Dilution 1:160)	Normal serum (Dilution 1:10)	Normal serum (Dilution 1:20)	Normal serum (Dilution 1:40)	Normal serum (Dilution 1:80)	Normal serum (Dilution 1:160)
DNA treated with DNA-ase; preparation No. 1	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
No. 2	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
DNA before treatment with DNA-ase; preparation No. 1	H	H	H	H	H	4+	4+	3+	2+	H	H	H	H	H	H
No. 2	H	H	H	H	H	4+	4+	2+	+	H	H	H	H	H	H

NOTE. Controls of antigens, antisera and normal serum gave hemolysis.

The sera obtained were analyzed in the Bordet-Gengou complement fixation test with DNA.

The results of the experiments are given on Table 1.

It can be seen from Table 1 that preparations No. 2 and 3 as test-antigens react with antiserum against high-protein DNA (N/P = 3.1) in dilution 1 : 20 with +, and with antiserum against low-protein DNA (N/P = 2.0)in dilution 1 : 20 with ++ and +, while with antiserum against purified DNA (N/P = = 1.66) in dilution 1 : 80 they react with ++ and +.

Thus, the antigenic properties of DNA preparations do not diminish on their being freed from protein but, on the contrary, increase.

Next we investigated the effect of depolymerization of DNA on the antigenic properties of the DNA preparations.

For this purpose we used DNA preparations depolymerized in the extraction process. In all we used 5 preparations which were either not re-precipitated with alcohol or in their re-precipitation produced small fibers which did not give viscous solutions on dilution with physiological saline.

We give the results of chemical analysis of the DNA preparations

Preparation	N mg/ml	P mg/ml	N/P
No. 1 (from thymus)	0.05	0.03	1.67
No. 2 (from thymus)	0.166	0.1	1.66
No. 3 (from thymus)	0.135	0.08	1.69
No. 4 (from liver)	0.103	0.06	1.72
No. 5 (from liver)	0.198	0.12	1.65

Qualitiative tests for protein were negative.

Immunization of rabbits with these preparations was done in the manner described above. Each rabbit received 90 to 100 mg of DNA. The antisera obtained were assayed in the CFT. As antigens we used a polymerized DNA preparation of molecular weight 6,000,000 and the depolymerized DNA preparations.

The results of the experiments are given in Table 2.

It can be seen from Table 2 that antiserum against depolymerized DNA neither reacts with polymerized, nor with depolymerized DNA. These results were obtained with 8 antisera against thymus DNA preparations and with 6 antisera against liver DNA preparations.

Thus, as a result of depolymerization the DNA preparations lose their antigenic properties. We may assume then that the antigenic properties of DNA preparations depend primarily on the polymeric state of the DNA itself. For a further clarification of this question we carried out immunization of rabbits with preparations of DNA treated with DNA-ase.

The DNA-ase was isolated according to N. McCarty [3] and purified from protease impurities, the activity of which was tested by the cleavage of 2 % hemoglobin at 37° in 24 hours, measured by a determination of tyrosine. The DNA preparations were subjected to DNA-ase for 1 hour at temperature 37° and pH = 6.27.

The action of the enzyme was estimated from the decrease in viscosity of the DNA solutions.

Preparation	Before treatment with DNA-ase	Viscosity	After treatment with DNA-ase
No. 1	50		10
No. 2	40		9

The enzyme-treated preparations were used for immunization of rabbits in the way described above. Each rabbit received 90 to 100 mg of DNA preparation. As control we carried out immunization with the same DNA preparations, not treated with the enzyme. The obtained antisera were assayed in the CFT.

The results are given in Table 3.

It can be seen from Table 3 that antisera against DNA preparations treated with DNA-ase do not contain antibodies to DNA, while antisera against polymerized DNA do contain such antibodies. These results were obtained with 6 antisera against 2 depolymerized DNA preparations.

50 days after the last immunization with depolymerized DNA blood was again taken from the animals and the antisera obtained were analyzed for content of antibodies to DNA. Since the antisera did not contain antibodies to DNA, we re-immunized the animals with highly polymerized DNA preparations in the same way as for the immunization with depolymerized DNA. Each rabbit received 90-100 mg of DNA. As a result of immunization with the polymerized DNA, antibodies to DNA formed in the blood of the animals.

On the basis of these results we may assume that the antigenic properties of DNA preparations are determined by the state of polymerization, since preparations depolymerized in extraction or depolymerized by DNA-ase lose their antigenic properties.

SUMMARY

Preparations of highly polymeric DNA are antigens, i.e. in immunization of rabbits they induce antibody formation, which specifically fixate the complement with homologous DNA preparations. The antigenic properties of DNA preparations are evidently determined by the polymeric condition of DNA and not by their protein admixtures. DNA preparations lose their antigenic properties in depolymerization.

LITERATURE CITED

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