SEROLOGICAL ACTIVITY OF HIGH-POLYMER DESOXYRIBONUCLEIC ACID

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The question of the serological activity of nucleic acids has been little studied and the literature contains contradictory data on this problem.

A. Menzel and M. Heidelberger [7], R. Pennel [9] and others consider bacterial nucleic acids to be serologically active and to give a specific precipitation reaction with antibacterial serums. According to data of D. Lackman, S. Mudd, J. Smolens, M. Sevag and M. Weiner [4], antiserums to nucleic acids do not give a precipitation reaction with homologous material.

Studies by Bernheimer and by Massamyne and Tokytomi [5] state that high-polymer ribonucleic acid (RNA) is serologically active, while Katsuko [3] and others report that polymeric RNA does not possess serological activity. U. Blix and C. Iland [2] assert that desoxyribonucleic acid (DNA) has serological activity.

The purpose of this study was further examination of the question of the serological activity of high-polymer DNA.

METHODS

DNA was isolated from calf thymus gland and beef liver. Thymus was frozen with dry ice immediately after slaughter and reached the laboratory in a frozen state; then it was homogenized for 1 minute in 0.14 M NaCl and 0.01 M sodium citrate to remove ribonucleoproteins according to the method of A. Mirsky and A. Pollister [8]. The precipitate was extracted in 1 M NaCl and after centrifugation reprecipitated by addition of 6 volumes of water to a concentration of 0.14 M NaCl.

The nucleoprotein threads obtained were dissolved in 1 M NaCl and the procedure was repeated several times. In order to obtain pure DNA without admixture of protein, the solution was treated with a chloroform-butanol mixture, 4:1 [10]. The procedure was repeated 18-34 times. The clear viscous DNA solution was precipitated with 2 volumes of 96% alcohol and the white threads which precipitated were dissolved in physiological solution.

All procedures were conducted at 2-4°. DNA preparations from beef liver were obtained in the same way. Phosphorus was determined in the preparations by the method of Fiske-Subbarow and nitrogen according to Conway. Molecular weight of preparations was calculated from viscosity by the method of D.M. Snitkovsky [1]. Viscosity was measured in 0.2 M NaCl in the Ostwald viscometer at 25°.

Data on chemical analysis of the DNA preparations are given below.

DNA Prepn.	Content in mg/ml		Ratio N/P	Molecular Wt.
	N	P		
No 1 (from thymus)	0.165	0.1	1.65	2 800 000
No 2 (from thymus)	0.4	0.24	1.66	5 000 000
No 3 (from thymus)	0.532	0.31	1.7	2 200 000
No 4 (from thymus)	0.0336	0.02	1 68	7 000 000
No 5 (from liver)	0.1344	0.082	1.64	6 000 000
No 6 (from liver)	0.168	0.1	1.68	7 800 000

Qualitative tests for protein were negative in all preparations.

DNA preparations upon reprecipitation with alcohol precipitated in the form of white threads readily soluble in 0.14 M NaCl, giving clear solutions of high viscosity.

The DNA preparations in 0.14 M NaCl were administered intravenously to rabbits in the amount 9-12 mg on 3 successive days. The administration was repeated during the course of 2 weeks.

Each rabbit received in all 80-110 mg DNA. Blood was taken 7-8 days after the last injection from rabbits fasted for 4 hours. The control was blood serum of the same rabbits obtained 2-3 days prior to immunization.

The serums obtained were analyzed by the complement-fixation reaction (CFR).

Antigen titer was determined in the presence of normal rabbit serum in order to eliminate anticomplementary action.

RESULTS

Experiments on the reaction of antiserums with the test antigen DNA gave the results presented in Table 1.

TABLE 1

Reaction of DNA with Antiserum (Normal Serum)

Antigen	Antiserum to DNA from thymus					Normal serum				
	1:10	1:20	1:40	1:80	1:160	1:10	1:20	1:40	1:80	1:160
DNA prepn. No.1	4+	4+	3+	2+	±	h	h	h	h	h
DNA prepn. No.2	4+	4+	3+	+	h	h	h	h	h	h

Notes. 1) The letter "h" in Tables 1-4 signifies hemolysis. 2) The control for antigens, antiserums, normal serum and complement gave hemolysis.

As seen from Table 1, DNA reacts with DNA antiserum in 1:80 dilution, giving 2+ and +, and does not react at all the normal serum. The results presented were obtained with 12 DNA antiserums from thymus and with 5 DNA antiserums from liver.

Since the DNA preparations, despite negative qualitative tests for protein, possibly contained a small admixture of protein, it was of interest to study the effect of protein on serological activity of DNA. With this purpose we checked the serological activity of preparations with respect to the extent of purification from protein.

Experimental results are given in Table 2.

Analogous results were obtained with 3 DNA preparations and with 6 antiserums to high-polymer DNA. From Table 2 it is seen that DNA with high protein content is serologically less active than DNA purified of protein.

TABLE 2

Effect of Protein on Serological Activity of DNA

Antigen	Ratio N/P	Antiserum to DNA					Normal serum				
		1:10	1:20	1:40	1:80	1:160	1:10	1:20	1:40	1:80	1:160
DNA	2	4+	3+	+	±	h	h	h	h	h	h
same	1.9	4+	3+	2+	+	h	h	h	h	h	h
»	1.73	4+	4+	3+	+	h	h	h	h	h	h
»	1.67	4+	4+	3+	+	±	h	h	h	h	h

Note. Control for antigens, antiserums, normal serum and complement gave hemolysis.

The fact that serological activity of DNA preparations does not decrease in proportion to purification from protein gives reason to assume that the serological activity is not determined by the protein. The serological reaction was conducted in a number of experiments with DNA preparations treated with crystalline trypsin. Two high-polymer DNA preparations of molecular weight 6 and 7 million were treated with trypsin for 1 hour at 37°, pH = = 8.04. In order to determine whether protein remains in DNA preparations after trypsin treatment, 15-20 mg of DNA preparations was dialyzed, the DNA precipitates after precipitation with alcohol were hydrolyzed with 6 M HCl for 28 hours with boiling and subjected to chromatographic analysis.

On chromatograms of hydrolyzates of these DNA preparations we found 4 amino acids: glycine, proline, tyrosine and leucine. Probably these are hydrolysis products of peptides and not of protein.

Results of determination of serological activity of these DNA preparations are given in Table 3.

TABLE 3

Serological Activity of DNA Preparations Treated with Trypsin

Antigen	Antiserum to DNA					Normal serum				
	1 : 10	1 : 20	1 : 40	1 : 80	1 : 160	1 : 10	1 : 20	1 : 40	1 : 80	1 : 160
DNA before trypsin treatment No. 1	4+	4+	3+	+	h	h	h	h	h	h
Same No. 2	4+	4+	2+	±	h	h	h	h	h	h
DNA after trypsin treatment No. 1	4+	4+	3+	±	h	h	h	h	h	h
Same No. 2	4+	4+	2+	±	h	h	h	h	h	h
Trypsin solution	h	h	h	h	h	h	h	h	h	h

Note. Control for antigens, antiserums, normal serum and complement gave hemolysis.

It can be seen from Table 3 that DNA treated with trypsin reacts with antiserum in 1:40 dilution, giving 3+ and 2+, i.e., exactly like the initial preparation not subjected to trypsin action. These results were obtained with 6 antiserums to DNA.

The fact that serological activity of DNA preparations does not change after trypsin treatment warrants the assumption that the serological activity is determined by the DNA itself. For further clarification of this question we conducted experiments on the effect of desoxyribonuclease (DNA-ase) on serological activity of DNA.

DNA-ase was isolated from pancreas [6] and purified from proteolytic enzymes, whose activity was determined by hemoglobin cleavage at 37° for 24 hours with subsequent determination of tyrosine at pH = 4.0, 6.22 and 8.04. The amounts of DNA-ase we used contained no proteases. The action of DNA-ase on DNA was estimated by change in viscosity of DNA solutions:

Prepn.	Viscosity	
	Before Enzyme Treatment	After Enzyme Treatment
No 1	50	10
No 2	43	11

DNA preparations not treated with enzyme after an hour in the thermostat had the same viscosity and upon reprecipitation with alcohol precipitated in the form of white threatds. Preparations treated with enzyme were not precipitated by alcohol, i.e., treatment with DNA-ase led to breakdown of their structure.

Experimental results on the effect of DNA-ase on serological activity of DNA preparations are given in Table 4.

TABLE 4

Effect of DNA-ase on Serological Activity of DNA Preparations

Antigen	Antiserum to DNA					Normal serum				
	1 : 10	1 : 20	1 : 40	1 : 80	1 : 160	1 : 10	1 : 20	1 : 40	1 : 80	1 : 160
DNA before enzyme treatment No.1	4+	4+	3+	1+	±	h	h	h	h	h
Same No.2	4+	4+	3+	2+	±	h	h	h	h	h
DNA treated with enzyme No. 1	h	h	h	h	h	h	h	h	h	h
Same No.2	h	h	h	h	h	h	h	h	h	h

Note. Control for antigens, antiserums, normal serum and complement gave hemolysis.

From Table 4 it can be seen that serological activity of DNA disappears completely after 1-hour treatment with DNA-ase.

Similar results were obtained with 6 antiserums to DNA. Consequently DNA-ase, by destroying the structure of DNA, deprives it of serological activity.

It can be concluded on the basis of the material presented that high-polymer DNA possesses serological activity, giving a specific complement-fixation reaction with homologous antiserum. These properties are inherent in the DNA itself and not in possible protein admixtures, since serological activity of DNA preparations is not altered in proportion to their purification from protein or after trypsin treatment.

The serological activity is determined by the polymeric state of the DNA molecule, since depolymerization of DNA by DNA-ase deprives it of serological activity.

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SUMMARY

Preparations of highly polymeric DNA are serologically active and give a specific reaction of complement fixation with homologous antiserums. Serological activity is determined by DNA itself and not by the protein and treatment by trypsin has no effect on its serological activity. Serological activity of DNA is determined by its polymeric condition.

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