ANTIGENICITY OF DNA OF BACTERIOPHAGES

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The literature devoted to the antigenicity of the desoxyribonucleic acids (DNA) is not very extensive, yet the information given is highly contradictory. According to some authors DNA is antigenic, but according to others it possesses no antigenic properties [6, 11, 13, 15, 17, 19-22].

The antigenicity of DNA isolated from phages T_2 and S_d of Escherichia coli was studied.*

EXPERIMENTAL METHOD

The experiments were carried out on phage T_2 of E. coli and phage S_d, causing lysis of the atypical strain E. coli SK. Cultivation, concentration, and purification of the phages were carried out as described earlier [10].

The DNA preparations were isolated either by the phenol method in mild conditions, as described fully earlier [8], or by a combination of the phenol and chloroform methods. In the last case the DNA preparation was subjected to an additional three or four deproteinizations with chloroform by Sevag's method in severe hydrodynamic conditions. The spectrophotometric and viscosimetric determination of DNA were carried out as described earlier [9]. The protein content was estimated by Lowry's method [18] with albumin as standard, and without introducing a correction for the reaction of the DNA itself with this reagent. The DNA was denatured by heating for 10 min at 100° followed by rapid cooling to 0°. Hydrolysis of the DNA by DNase took place at 37° for 2 and 40 h (100 mg enzyme to 1 mg DNA). For treatment of the DNA with viper (Vipera lebetina) venom [4], 200 μg of lyophilized venom was taken for each milligram DNA; incubation took place in acetate buffer, pH 8, +0.02 M $MgSO_4$ for 24 h at 37°. Sodium thiomerthiolate in a dilution of 1:10,000 was added to the DNA solution as a preservative.

The preparations of phage protein used as antigen in the precipitation reaction in agar were first treated with DNase and then subjected to a cycle of purification.

The conditions of immunization and of the immunological determination were as follows. After tests for sterility the DNA preparations were injected into rabbits intravenously or subcutaneously. The intravenous injections were given at intervals of 2-3 days in doses of 500 μg each, and the subcutaneous injections at intervals of 3-4 days, in the same doses. In both cases each rabbit received 6-8 mg DNA. Samples of the sera were taken on the 2nd, 4th, and 8th days after the last injection of antigen. The samples of the rabbit sera taken before and after immunization were tested in the precipitation reaction in agar [2] and in the complement fixation reaction in the cold [3]. The native and denatured DNA preparations of the phage tested in the precipitation reaction in agar as antigen were used in concentrations of 500, 250, 125, 62, 31, and 15 μg/ml. The immune sera were tested in dilutions of 1:0, 1:2, 1:4, 1:8, 1:16, and 1:32.

In the complement fixation reaction (CFR) the antigen was DNA solution in concentrations of 12 and 20 μg/ml [6], while the immune sera were diluted 1:10, 1:20, and 1:40 respectively. As complement, a mixture of guinea pigs' sera preserved by the method described above [1] was used. The unit of complement was determined by titration in the presence of antigen; 1.5-2 units of complement were taken. In the experiments to study the sensitizing properties of DNA, guinea pigs weighing 300-350 g were given a subcutaneous injection of 500-1000 μg DNA, and one month later they received an intravenous injection of the reacting dose of DNA, amounting to 200-1000 and 2000 μg.

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TABLE 1. Characteristics of DNA Preparations from Phages T_2 and S_d used for Immunization and as Antigens in the Precipitation Reaction in Agar and the Complement Fixation Reaction

Preparation	Phage	Method of isolation	Characteristic viscosity in length/g	Protein (in %)
I	T_2	Deproteinization with phenol	250	0.9
II	S_d	The same	170	0.7
III	T_2	Deproteinization with phenol and chloroform	120	Traces
IV	S_d	The same	110	"

TABLE 2. Results of Immune Sera of Rabbits in the Precipitation Reaction in Agar with Phage Protein and with DNA of Phage S_d before and after Treatment with DNase and Viper Venom

Serum No.	DNA, native	DNA + DNase, 2 h	DNA + DNase, 40 h	DNA + venom, 24 h	Phage S_d protein
15	+	+	+	–	+
15	+	+	+	–	+

Note: +) positive reaction; –) negative reaction.

EXPERIMENTAL RESULTS

The characteristics of the DNA preparations isolated from phages T_2 and S_d by a mild phenol method and a severe method of deproteinization with phenol and chloroform are shown in Table 1.

It is clear from Table 1 that deproteinization in severe conditions was associated with the removal of the residual protein, but led to a considerable decrease in the characteristic viscosity as a result of the effect of hydrodynamic action, intensified many times over in the presence of the heavy phase of the organic solvent. Preparations I and II, isolated in mild conditions, consisted of one molecular type of DNA, because phages T_2 and S_d contain only one molecule of DNA [9]. The traces of protein contaminating the DNA preparations of this type evidently were mechanical in character and largely removed by chromatography on a protein adsorbent [7]. So far as the nature of these mechanical protein impurities is concerned, several components of the phage corpuscle (for example, the tail rods of phage T_2) are known to possess high stability and to be incapable of removal of DNA preparations during phenol deproteinization [14].

As a result of immunization of six rabbits with preparations of native and denatured DNA containing protein (preparation I and II), antibodies appeared in their serum precipitating homologous antigen, and also protein of the corresponding phages. A positive precipitation reaction was observed with sera diluted in the ratio of 1 : 4-1 : 8. The same sera, when tested in the precipitation reaction with DNA solution free from protein (preparations III and IV) gave negative results. Next, 24 rabbits were immunized with carefully deproteinized native and denatured phage DNA (preparation III for phage 2 and preparation IV for phage S_d). Not one of the 24 immune sera reacted in the precipitation reaction with either the DNA not containing protein or the DNA with protein impurities.

Preliminary denaturation of the DNA during the tests with sera obtained by immunization of rabbits with carefully deproteinized preparations did not demask any of the "latent" antigenic groups.

It should be noted that DNA preparations containing proteins possess toxic and allergic properties: in the process of intravenous and subcutaneous immunization, some of the rabbits died. In addition, during subcutaneous immunization, at the place of the third and subsequent injections of the preparation, necrotic areas developed in the rabbits. In the rabbits immunized intravenously or subcutaneously with carefully deproteinized DNA preparations, neither local nor general reactions were observed.

Some sera obtained as a result of immunization of rabbits with poorly purified DNA preparations and giving a positive precipitation reaction with homologous, badly purified antigen were subjected to further analysis. In this case not only native DNA, but also DNA after hydrolysis with DNase and viper venom, and also phage protein were tested as antigen.

Table 2 shows that precipitation was observed with native DNA, with DNA after hydrolysis by DNase, and with phage protein. Prolonged hydrolysis combined with a high concentration of the enzyme guaranteed that the polymer

DNA was completely split up into a mixture of oligo- and mononucleotides. It must be remembered that the DNA of phage S_d does not contain glucosidated hydroxymethylcytidyl nucleotides, which in phage T_2 may essentially interfere with the hydrolysis of the DNA. The ability of the preparation of phage DNA to react with homologous serum disappeared only after treatment of the DNA with viper venom, containing only phosphodiesterase and 5-nucleotidase of all the enzymes of nucleic acid metabolism [5], while for complete hydrolysis of the phage DNA by phosphodiesterase, without preliminary treatment with DNase, an exposure of many days was required. For this reason, the destruction of the antigenic activity of DNA after contact with viper venom was most probably associated with the action of the venom enzymes, not on the DNA, but on the protein or other contaminants left in the preparation after phenol deproteinization, and disappearing after treatment of the DNA with chloroform.

Since the results of the precipitation reaction in agar demonstrated the appearance of precipitating antibodies only when the animals were immunized with badly purified preparations, only the sera of rabbits immunized with DNA subjected to additional deproteinization with chloroform (preparations III and IV for DNA of phages T_2 and S_d respectively) were tested in the CFR.

Of the seven sera against DNA of phage T_2 and the 12 sera against the DNA of phage S_d so tested, only 3 gave a feebly positive CFR with native and denatured DNA of phage S_d in a dilution of 1 : 10. With higher dilutions the results were negative. The nonimmune sera did not react in the CFR.

Negative results were also obtained when a more sensitive reaction of immunity was used – the sensitization reaction. In these tests 33 guinea pigs received an injection of carefully deproteinized DNA (preparation IV of DNA of phage S_d).

It may be concluded from the results obtained that the more thorough deproteinization of phage DNA with a chloroform – octanol mixture removes the antigenic properties of the DNA usually observed in preparations subjected to mild phenol deproteinization.

SUMMARY

A study was carried out on the antigenicity of DNA of phages T_2 and S_d of E. coli with the aid of a precipitation reaction by diffusion in gel, complement fixation and sensitization tests.

Positive results were obtained only with inadequately purified DNA preparations deproteinized with phenol. Additional deproteinization after Sevag deprived DNA of its antigenic properties.

Sera of rabbits immunized with phage DNA gave a positive precipitation reaction with phase protein treated with desoxyribonuclease.

Denatured DNA did not show any "latent" antigenic groups.

Antibodies formed in the immune sera of animals are most likely the result of protein presence in phage DNA preparations.

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