STUDIES ON THE FRACTIONATION OF TRANSFORMING DEOXYRIBONUCLEIC ACID OF PNEUMOCOCCUS

I. RECOVERY AND DOSE-DESPONSE RELATIONSHIPS*

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SUMMARY

Evidence is presented that pneumococcal transforming DNA is not degraded by chromatography on columns of the cellulose derivative anion-exchanger ECTEOLA. Essentially all of the mannitol- and penicillin-transforming activities added to the column were recovered in the column eluates, although more streptomycin-transforming activity was recovered than was apparent in the original, unfractionated DNA.

The results obtained by determining the transforming activity-dose response curves of selected fractions have been interpreted as indicating that DNA-induced transformation to streptomycin-resistance in pneumococci can be a property of DNA molecules which vary intrinsically in relative transforming ability.

The transforming activities were not restricted to any one chromatographic peak or region, but were present in several DNA fractions which had been obtained with eluting solutions of widely varying ionic strength and pH.

INTRODUCTION

The finding that deoxyribonucleic acid (DNA) isolated from many single sources is heterogeneous (for references see[1]) prompted attempts to fractionate the biologically-active DNA of pneumococcus (the transforming principle). EPHRUSSI-TAYLOR[2], fractionated streptomycin-transforming DNA of pneumococcus on histone-Kieselguhr columns[3] and obtained fractions which were as much as five times as active, per unit weight, as the original, unfractionated preparation. Similar results have also been obtained[1] with pneumococcal DNA by chromatography on columns of ECTEOLA a modified cellulose anion-exchanger developed by PETERSON AND SOBER[4].

This report deals with the fractionation of pneumococcal transforming DNA on ECTEOLA, and is concerned primarily with aspects of the recovery of transforming

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References p. 502.

activity from the column and with the transforming activity-dose response relationships of some of the column fractions.

MATERIALS AND METHODS

All experiments were performed with DNA prepared from a strain of pneumococcus resistant to streptomycin, penicillin, and sulfanilamide, and capable of fermenting mannitol. This strain was obtained from Dr. ROLLIN D. HOTCHKISS of the Rockefeller Institute, and has been described by MARMUR AND FLUKE[5].

DNA was prepared by lysing the cells with deoxycholate, and deproteinizing with chloroform and amyl alcohol, and precipitating with ethanol[6]. The preparation was also treated with ribonuclease and subsequently dialysed first against 2 *M* NaCl and then against 0.01 *M* phosphate buffer.

The receptor strain of pneumococcus, RA3, was also obtained from Dr. HOTCHKISS. The procedures for the transforming system[8] and the method of quantitation using anti-R serum[7] were employed as described.

Fractionation procedure: Continuous elution (Expt. 1): The double-mixing chamber procedure[9] was followed. A column containing 0.5 g ECTEOLA (5.1 × 0.8 cm; 0.20 mequiv./g; 0.51 % N) was used to fractionate 3.2 mg DNA at 0–3°.

Discontinuous elution (Expt. 2): The column and quantities were essentially the same as those used for continuous elution. The following eluents were used successively:

62 ml 0.01 *M* phosphate buffer pH 7; 145 ml 0.5 *M* NaCl, 0.01 *M* phosphate buffer; 73 ml 0.5 *M* NaCl, 0.01 *M* phosphate buffer, 0.1 *M* NH_3; 87 ml 0.5 *M* NaCl, 0.01 *M* phosphate buffer, 1 *M* NH_3; 114 ml 2 *M* NaCl, 0.01 *M* phosphate buffer, 1 *M* NH_3; 58 ml 0.5 *M* NaOH.

RESULTS

In each experiment, all of the DNA as well as all of the measurable transforming activities were removed by the exchanger when the nucleic acid solution was passed through the column.

The chromatographic profile of 3.2 mg of DNA isolated from pneumococci resistant to streptomycin, penicillin, and sulfanilamide and which could utilize mannitol is given in Fig. 1. The gradient elution system used is indicated below the fraction number. The profile shows five major peaks.

Selected fractions, identified by fraction number and molarity and pH of the eluting solution were assayed for their streptomycin-transforming activity. The results are given in Table I. The number of transformed cells was determined for each column fraction on aliquots diluted with 0.9 % saline to give a concentration (about 0.04 μg/ml) at which the number of cells transformed was proportional to the amount of DNA added. The specific activity was calculated as the number of cells transformed per μg DNA, and the relative activity determined by dividing the specific activity of the fraction by the specific activity of the original, unfractionated material. The data within each column were determined simultaneously with aliquots of the same competent culture. The three columns thus represent determinations at three different times.

References p. 502.

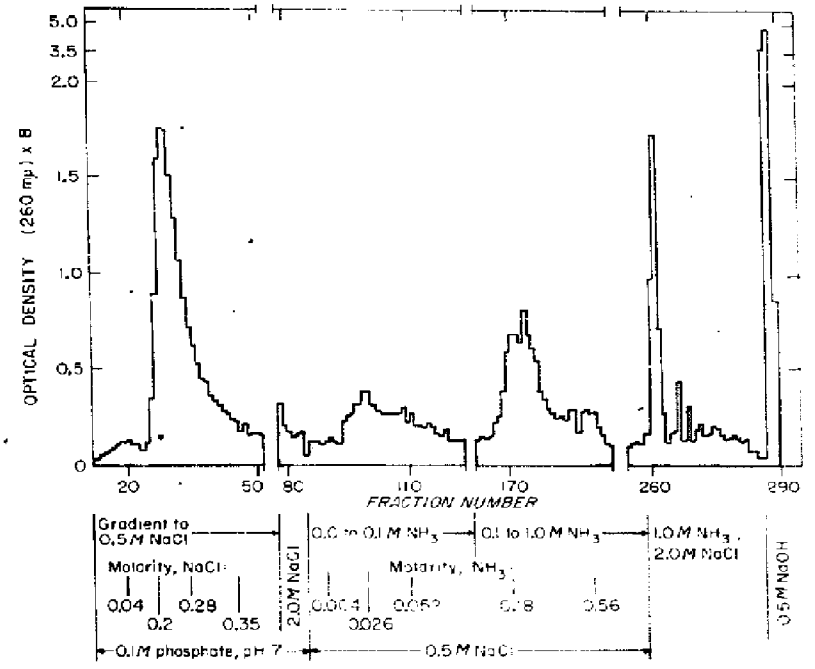

Fig. 1. Chromatographic fractionation of 3.2 mg of four-marker pneumococcal DNA on a column containing 0.5 g of ECTEOLA (5.1 × 0.8 cm; ECTEOLA-SF-1, 0.20 mequiv./g, 0.51 % N); flow rate 8 ml/h.

TABLE I

RELATIVE STREPTOMYCIN-TRANSFORMING ACTIVITY OF SELECTED CHROMATOGRAPHIC FRACTIONS OF PNEUMOCOCCAL DNA*

Fraction number	*Composition of eluent*		*pH*	*Relative transforming activity*		
	Molarity, NaCl	*Molarity, NH_3*				
Original			7.0	1.0	1.0	1.0
45	0.17		7.0	0.7	0.8	0.7
47	0.19		7.0	0.2	0.4	0.2
29	0.21		7.0	0.5	0.6	0.4
32	0.25		7.0	0.4	0.1	0.04
35	0.28		7.0	1.2	0.8	1.0
40	0.32		7.0	0.9	0.7	1.2
67	0.45		7.0	0	0.1	0.06
78	0.47		7.0	0	0.1	0.03
99	0.50	0.02	8.4	1.9	1.8	2.0
173	0.50	0.22	9.5		5.1	4.2
190	0.50	0.56	10.4	1.1	1.0	
260	2.00	1.0	11.0		5.1	4.8

* Experiment 1.

The relative activities of the fractions show considerable variation, *e.g.* fractions 173 and 260 possess at least fifty times the activity of others (*e.g.*, No. 67), and about five times the activity of the original material. As an aid in determining the cause of these variations in activity, the dose-response curves for fractions 173 and 260 (high relative activity), fraction 32 (low relative activity), and the original, unfractionated preparation were determined (Fig. 2).

References p. 502.

It should be noted (Fig. 1) that fractions 32, 173, and 260 occurred in completely separate regions of the chromatogram, and consequently had been exposed during elution to very different environments of ionic strength and pH. Despite this, the data of Fig. 2 demonstrate that each of these fractions and the unfractionated DNA transformed the same total number of cells provided enough DNA was added.

Since it would be impractical to determine the recovery of DNA and of transforming activity by analyzing separately the 300-odd fractions obtained by gradient elution, a second fractionation experiment was carried out in which discontinuous elution was used to obtain five gross fractions, each of which contained the corre-

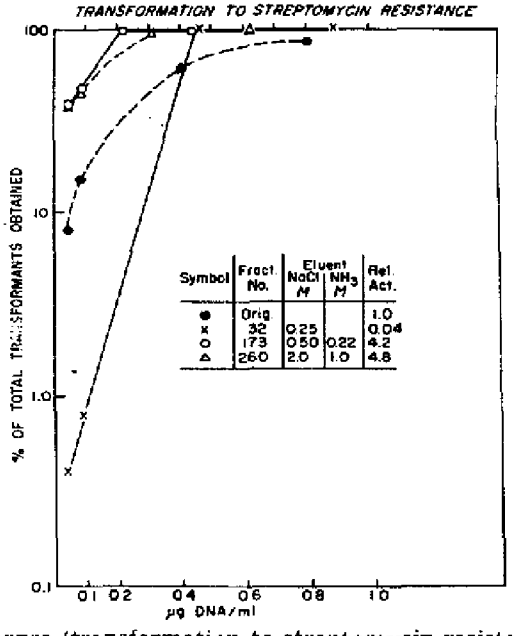

Fig. 2. Activity-dose response (transformation to streptomycin resistance) relationship of pneumococcal DNA and chromatographic fractions thereof.

TABLE II

RECOVERY OF TRANSFORMING ACTIVITIES*

Columns A refer to values on ammonia-containing eluents; those in the B columns are values obtained after removal of ammonia.

Fraction number	*Eluent*		*DNA recovered***	*Total transformants % of original*					
	M, NaCl	*M, NH_3*	%	*Strep.****		*Pen.§*		*Mann.§*	
				A	*B*	*A*	*B*	*A*	*B*
1			4.5	0	0	0	0	0	0
2	0.5		25.0	89	89	25	25	47	47
3	0.5	0.1	12.5	47	47	20	20	49	49
4	0.5	1.0	19.5	5	33	3	38	0	0
5	2.0	1.0	18.5	0	4	0	0	0	0
6	0.5 *N* NaOH		7.0	0	0	0	0	0	0
Total received			87.0	141	173	48	83	96	96

* Experiment 2.
** Recovery based on determination of the CERIOTTI color value[16].
*** Average of 6 determinations.
§ Average of 3 determinations.

References p. 502.

sponding major peak found in the gradient elution profile. The recovery data are summarized in Table II. The fractions obtained with an ammonia-containing eluent were assayed for transforming activity both immediately following elution from the ECTEOLA (columns A, Table II) and after being placed *in vacuo* for at least three days followed by restoration of the original volume with sterile distilled water (columns B, Table II). Preliminary experiments with solutions of unfractionated DNA showed that although the presence of ammonia resulted in lower relative activities, the original activity could be quantitatively restored after *in vacuo* removal of the ammonia. By carrying out the assays in the linear response portion of the curve and knowing the total volume of each fraction, the per cent of the original activity contained in each of these fractions was calculated. As indicated in Table II, the recoveries of DNA and penicillin and mannitol-transforming activities were essentially quantitative. However, more total streptomycin transforming activity was found in the fractions than was apparent in the original unfractionated material.

DISCUSSION

This investigation was designed primarily to determine whether the transforming DNA is damaged by chromatography on ECTEOLA. The recovery of the transforming activities (Table II) indicates that if any degradation or denaturation does occur, it is not reflected in a loss of biological activity. In fact, there is an apparent gain in the streptomycin transforming activity, more being found in the fractions eluted from the column than was present in the original preparation. Since it has been shown that non-transforming DNA may inhibit transformation[6,10], the increase in streptomycin-transforming activity could result from a fractionation of non-transforming or inhibiting DNA present in the original sample. That is, in the original sample all of the DNA effecting transformation to streptomycin-resistance competes with inhibiting DNA present whereas after fractionation the inhibiting DNA may not be equally distributed or may not be equally effective in all of the fractions. This would result from an alteration in the ratio of inhibiting to transforming DNA. If this hypothesis should prove to be valid, the results also suggest that inhibition of transformation by DNA may be specific since the mannitol- and penicillin-transforming treactions did not show the increase found with streptomycin-transformation (Table II).

The data in Table II also apparently demonstrate some separation of transforming activities. For example, Fraction 4 contained 19.5 % of the original DNA, 33 % of the streptomycin-transforming activity, but no demonstrable mannitol-transforming activity. However, it is possible that this result, as well as the recovery data discussed above, may be due to fractionation of inhibiting DNA. Accordingly, these results alone do not constitute conclusive evidence for a separation of transforming factors.

The dose-response curves (Fig. 2) are of interest in that the original, unfractionated DNA and each of the fractions studied, when present in excess, transform the same number of cells within experimental error. HOTCHKISS has stated[10] that the yield of transformants in the plateau region (excess DNA) is "the most significant test for the quality of any new DNA fraction or preparation of transforming DNA". By this criterion, then, the three fractions were of the same quality as the original, and were not damaged as a result of chromatography on ECTEOLA.

References p. 502.

The results in Fig. 2 may also be interpreted as indicating that the differences in relative activity are not due to differences in the proportion of active to inactive DNA molecules in each fraction. If this were the case, fractions 173 and 260 should reach a higher plateau than the original, which in turn, should reach a higher plateau than fraction 32. It would appear, therefore, that streptomycin-transforming activity may reside in different DNA molecules with intrinsic differences in relative activity. It has not yet been possible to determine whether these variations are due to differences in ability to penetrate the cell, or to variations in incorporation into the genetic apparatus of the cell, or possibly to a combination of the two. Studies in progress using appropriately mixed fractions of the total DNA may offer answers to such questions.

These experiments indicate that transforming DNA behaves like other DNA's in that it, too is heterogeneous when examined by the anion-exchange technique. Detailed studies on DNA from other species have indicated that individual fractions from single sources differ in base composition[9], physico-chemical behavior[11,12], response to chemical or physical agents[11,13], and ability to serve as primer[14] in the enzymic synthesis of DNA[15]. It may therefore be inferred that the differences in biological activity exhibited by the pneumococcal DNA fractions arise from differences in their chemical and physical properties. It is believed that experiments with these and similar fractions may furnish some clues to the chemical basis of their genetic activity.

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