FRACTIONATION OF PNEUMOCOCCAL DNA FOLLOWING SELECTIVE HEAT DENATURATION: ENRICHMENT OF TRANSFORMING ACTIVITY FOR AMINOPTERIN RESISTANCE*

By Muriel Roger

The different genetic markers of pneumococcal transforming DNA are inactivated in solution at characteristic temperatures, analogous to melting points. These temperatures are spaced sufficiently to permit inactivation of the lower melting markers while the others are preserved intact. This partially inactivated DNA has been considered to be a mixture of native and denatured molecules. If this is so, it should be possible to separate the native and denatured fractions because of their different physical properties and thus obtain a native fraction which is enriched for those molecules carrying the surviving markers. The experiments described below show, in fact, that a native DNA fraction is recoverable from selectively heat-inactivated DNA, and that both the specific and plateau transforming activities of the preserved markers are considerably increased.

Results and Discussion.—Elution of native and denatured DNA from methylated albumin columns: Heat denaturation brings about a change in a number of the physical properties of native DNA which can be interpreted as arising from the increased flexibility of the molecules due to disruption of the native hydrogen-bonded structure. Of interest for this work is the fact that denatured DNA is more tightly bound than native to weakly basic adsorbents, such as methylated albumin-coated kieselguhr (MAK). The stronger binding of denatured DNA to MAK columns was first reported by Mandell and Hershey who used continuous salt gradient elution of T2 phage DNA. Sueoka and Cheng described stepwise elution of E. coli DNA from MAK columns and recovered native DNA from a prepared mixture of native and denatured. Stepwise salt elution was selected for the fractionation of partially denatured DNA, since it provides several well-defined fractions of moderately high concentration. The greater resolving power of continuous gradient elution is not required when fraction separation is to be based upon native versus denatured DNA. To illustrate this point, a pair of stepwise elution profiles with native and completely heat-denatured pneumococcal DNA are shown in Figure 1. With this particular column material, the major portion of the native DNA was eluted at 0.73 M NaCl, while denatured DNA was not eluted until a salt concentration of 0.9 M was reached. Thus, no denatured DNA is released at a salt concentration just sufficient to elute native DNA. No differences in the ratios of specific transforming activities for any of the genetic markers could be detected in the MAK column fractions obtained from native DNA.

Different preparations of MAK varied somewhat with respect both to the absolute molarity of NaCl necessary to elute the DNA fractions and to the total recovery of material. However, the salt concentration required to elute denatured DNA was always about 0.2 molarity units higher than that which eluted native DNA.
The individual activities of pneumococcal DNA are characteristic for a solution of DNA which contains 0.14 M NaCl, 0.02 M KPO₄ buffer, pH 6.8. By choosing an inactivation temperature either between 89.5 and 90.0°C or between 91 and 92°C, it is possible selectively to inactive different groups of these markers. These results have previously been taken to indicate that each marker is associated with a small, homogeneous fraction within the entire molecular population. The DNA partially denatured at an intermediate temperature is then expected to consist of a mixture of native, active molecules and denatured, inactive molecules. By use of the MAK columns described above, the native molecules should be recoverable, thus achieving the isolation of that active fraction of the DNA which is denatured only at higher temperatures. This fraction should be more homogeneous than the original native DNA, and the specific activity of its surviving markers should be increased. The amount, degree of homogeneity, and increased activity of such surviving DNA should vary with the temperature of heating. While certain inevitable complications distort this oversimplified picture of heat inactivation and prevent quantitative fraction separation, the results presented below show the extent to which the predictions have been achieved.

The choice of the denaturation temperature likely to give the largest marker enrichment is determined by the five pneumococcal markers available for quantitative assay. At present, the aminopterin marker is alone in its measurable survival above 90.5°C. Unfortunately, it is also the only one studied whose complete re-
tention of activity below critical inactivation is sensitive to other factors (some known, others as yet unknown). Nevertheless, the advantage of heating at the highest temperature possible, in order to obtain a relatively small fraction of the total DNA, outweighs the handicap introduced by the incomplete predictability of the aminopterin marker.

The experiment represented by the bar graph in Figure 2 shows the elution pattern of a DNA sample which was heated for 10 min at 91.3°C. In this instance, the specific activity of the aminopterin marker decreased to 40 per cent of that in the original native DNA. In this, as in all other cases, the plateau activity for this marker remained unchanged. The streptomycin activity decreased to 1.7 per cent. (The details of the column fractionation procedures are described in the legends to the figures.) The optical density profile shows that 5.5 per cent of the DNA is eluted from MAK at 0.70 M NaCl, where native DNA is eluted, and that the major portion of the DNA is released where denatured DNA is expected. The profile for specific aminopterin transforming activity shows the highest activity where the native fraction is expected and very little activity where denatured DNA is expected.

Based on the activity of the native, unheated DNA, the specific activity of the aminopterin marker in the 0.70 M salt fraction has been increased 2.4-fold. Based on the activity of the partially heated DNA which was actually applied to the column, the specific activity of the marker has been increased 6-fold. These results constitute a significant increase in activity of the aminopterin marker.
From the percentage recovery of DNA in the various fractions, it is estimated that 10–20 per cent of pneumococcal DNA survives the temperature of 91.3°C. On this basis, the maximum expected enrichment in aminopterin activity for the heated DNA is between 5- and 10-fold. The 6-fold increase observed lies within this range.

The streptomycin resistance activities were also determined in this experiment, but are not included in Figure 2 because they are all too low to be shown on the same scale. The ratio of aminopterin to streptomycin activity in the 0.70 M salt fraction is 50:1. The details of the residual streptomycin activity in the various fractions will be shown in an experiment on renaturation.

Separation of native and denatured DNA fractions after denaturation at 90.0°C: Denaturation at temperatures lower than 91°C is expected to leave a larger fraction of the DNA intact, and therefore show a smaller activity increase in the salvaged native fraction. A denaturation temperature of 90.0°C was the next choice for study, because at this temperature the micrococin marker is inactivated, while both streptomycin and aminopterin activities remain intact. (Both streptomycin and aminopterin activities were preserved completely intact in this experiment.) A comparison of optical density and specific transforming activity of the native and heated DNA solutions with the MAK column fractions is shown in Figure 3.

The predicted differences from the 91.3°C experiment are apparent. After denaturation at the lower temperature, the optical density in the column fractions is almost evenly distributed among the 0.80, 0.90, and 1.0 molar NaCl eluates. About 15 per cent of the DNA is eluted where native DNA is expected (0.80 M NaCl for this particular MAK), and 30 per cent is eluted as denatured DNA. Again, the highest activity appears in the native fraction, with transformants to both streptomycin and aminopterin resistance increased to 1.5 times that of native DNA. Thus,
it is shown that the specific marker which is enriched is not determined by the MAK column, which merely distinguishes native from denatured DNA, but depends upon the denaturation temperature. The extent of enrichment is identical for the markers which survive. From the column behavior in this experiment, it is tentatively estimated that at 90.0°C, 60 per cent of pneumococcal DNA is denatured and 40 per cent remains intact.

*Increase in both specific and plateau activities of native MAK column fractions:* Specific activity measurements could be reliably obtained with the column fractions described above on the basis of optical density. If the column fractions with enhanced specific activity contain an increased proportion of the heat-resistant DNA molecules, they should show a comparable increment in plateau transforming activity. It was found that they do. The plateau yield of transformants is obtained at a concentration of DNA sufficiently high to saturate the competent cells, and it is of particular value because it is a criterion independent of precise DNA concentration. The high concentration of NaCl in the column fractions interferes with this biological assay, and to obtain plateau transformation, it was necessary to dialyze the solutions to lower the salt content. Complete concentration response curves for transformation to aminopterin resistance, obtained with native and unfractionated 91.3°C heat-denatured DNA, as well as with a dialyzed 0.80 M NaCl column fraction from the denatured DNA, are given in Figure 4. This is a

![Figure 4](image-url)

**Fig. 4.—Enrichment of specific and plateau transforming activities of aminopterin marker.** The concentration response curve for unheated native pneumococcal DNA is compared with that for the same DNA heated at 91.3°C and a dialyzed fraction obtained from the 91.3°C heat-denatured DNA by 0.80 M NaCl elution from a MAK column. Experimental details are as described for Fig. 2. The doubly circled point is the average of several assays obtained for the 0.80 M fraction before dialysis.

duplicate of the previous experiment at 91.3°C, in which similar results were obtained although different preparations of DNA and of MAK were used. Heat treatment reduced the specific activity of the aminopterin marker to 50 per cent in this instance, although again the plateau yield is equal to that of unheated DNA.
In this experiment, the 0.80 M NaCl column fraction (native DNA is eluted at 0.80 M NaCl with this MAK) shows a 3-fold increase in both specific and plateau activity when compared with native DNA. With respect to the heated DNA applied to the MAK column, the enrichment of aminopterin specific activity is again 6-fold. Similar complete concentration curves have been obtained in the two experiments described in Figures 2 and 3. In each case, the specific and plateau activities were increased to the same extent. Thus, the native fraction recovered from the MAK column is significantly richer in those molecules which carry the more heat-resistant markers.

Renaturation of heated DNA and column fractions: The column fractions obtained in the two 91.3°C denaturation experiments were tested for their ability to become renatured. The conditions for annealing the different samples were maintained as uniform as possible. The results are shown in Figure 5. Annealing resulted in no recovery of the 50 per cent loss in aminopterin activity, although in the same solution streptomycin activity increased from 3 per cent to 27 per cent. This behavior of the aminopterin marker has been consistently observed. The 0.80 M salt fraction, which is 6-fold enriched in aminopterin activity, shows no further increase for either aminopterin or streptomycin activity after annealing, as would be expected if it is truly a native fraction and contains no denatured DNA. The renaturable streptomycin activity appears, as expected, where denatured DNA is normally eluted. It is interesting that the two denatured DNA fractions which
were obtained are renatured to different extents. The 0.90 M salt fraction is renatured to 10 per cent of native streptomycin activity, while the 1.0 M salt fraction attains a value of 22 per cent. Only a small amount of aminopterin activity is recovered in these fractions.

Summary.—The mixtures of native and denatured DNA produced by partial heat denaturation at temperatures which inactivate some of the genetic markers of pneumococcal DNA, while leaving others intact, have been separated on MAK columns. In the DNA fractions which are eluted from MAK where native DNA is expected, the specific as well as plateau transforming activities for the preserved markers are substantially increased over those for untreated, native DNA.

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STUDIES ON THE MECHANISM OF THE EFFECT OF FLUORODEOXYURIDINE ON CHROMOSOMES

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5-Fluorodeoxyuridine (FUrD) has been found in experiments with Escherichia coli to inactivate irreversibly thymidylate synthetase, the enzyme involved in the conversion of deoxyuridylic to thymidylic acid. In the presence of FUrD and consequent absence of thymidylate both RNA synthesis and protein synthesis continue for a time, whereas DNA synthesis is stopped.

Volkin and Ruffili, however, found that the yield of T2 phage following infection of E. coli with T2 is so low in the presence of FUrD as to indicate a lack of utilization of host DNA as well as an absence of new DNA synthesis. This result could not be explained solely on the basis of the inhibition of thymidyl acid synthesis by FUrD.

Taylor reported that FUrD induced chromosome gaps and fragments in lateral roots of the broadbean, Vicia faba. At the concentration used (10⁻⁶ M) cells had to be treated for 4 hr before broken chromosomes could be detected. Because 4 hr is approximately the average time required for an untreated Vicia root tip cell to proceed from the end of the DNA synthetic period (S) of the cell cycle through the postsynthetic interphase (G2) to metaphase (M) where chromosomes could be seen, and because treatment with exogenous thymidine seemed to "cure" the chromosome lesions, Taylor postulated that the chromosome breaks arose in the latter part of S