Transformation by Heat-denatured Deoxyribonucleic Acid

Lerman & Tolmach (1960) showed that the transforming biological activity of DNA decreases, upon heating to temperatures higher than the "melting point", to a few per cent of its initial value. This residual activity could arise in three ways (Rownd, Lanyi & Doty, 1961): (1) a small fraction of the DNA molecules is resistant to thermal denaturation; (2) a small degree of renaturation occurs on cooling; (3) denatured DNA can be taken up by the recipient cells to a small extent and participate in bacterial transformation.

The presence of a heat-resistant fraction has been considered unlikely, since transforming DNA which has been heated to 120°C still possesses residual activity (Marmur & Lane, 1960). The occurrence of a small degree of renaturation is ruled out by the finding of Ginoza & Zimm (1961) that the level of the residual activity is independent of the DNA concentration at denaturation over a hundredfold range. The results of Guild (1961) with *Diplococcus pneumoniae* DNA and Rownd *et al.* (1961) with *Haemophilus influenzae* DNA indicate that the residual activity forms bands in the denatured DNA region, in the CsCl density-gradient. Barnhart & Herriott (1962) showed that a very extensive digestion of denatured *H. influenzae* DNA by *Escherichia coli* exonuclease I (Lehman, 1960) did not affect the residual transforming activity, thus providing evidence that the remaining biologically active fragments have a native-like structure. Work by Rownd, Green & Doty (1963, *Abst. Biophys. Soc.* TB7) with hybrid *Bacillus subtilis* DNA indicated that the residual transforming activity only formed bands, in the CsCl density-gradient, in the density range expected for native and partially denatured hybrid molecules. The conclusions to be drawn from the work of Rownd *et al.* (1963) as well as from the results of Barnhart & Herriott (1962) are therefore at a variance with those suggested by Guild (1961) and by Rownd, Green & Doty (1961, *Abst., Biophys. Soc.*, TB7).

TABLE 1

Fractionation of heat-denatured H. influenzae *DNA on hydroxyapatite*

Experiment number	O.D. 260 mμ			Biological activity			Specific biological activity			
	Total recovery	Fractions I	Fractions II	Total recovery	Fractions I	Fractions II	Native DNA	Heat-denat. DNA	Fractions† I	Fractions† II
42‡	100	82·3	17·7	91	8	92	22,200	486	64	5673
45	98	69	31	79	10	90	30,600	154	15	1750
49	73	84	16	92	4·2	95·8	41,200	240	26	2208

† The specific activities are those of the peak fractions. The specific activities of fractions II are likely to be underestimated for the following reasons: (a) the base line was not subtracted from the optical density of the fractions; (b) the phosphate concentration of the fractions was high enough to cause a light inhibition in the transformation. Specific activity is given in transformed cells/mμg DNA.

‡ Experiment no. 42 was performed in the cold room; experiments 45 and 49 were carried out at room temperature.

The development in this laboratory of chromatography of nucleic acids on hydroxyapatite, a technique capable of fractionating DNA molecules according to their secondary structure (Bernardi, 1961,1962, and manuscript in preparation), offered a new approach to the problem. Chromatographic experiments were performed as described elsewhere (Bernardi, manuscript in preparation). Solutions containing about 1 mg *H. influenzae* DNA, prepared according to Goodgal & Herriott (1961) and purified from residual ribonucleic acid by chromatography on hydroxyapatite, were loaded on 1 cm × 10 cm columns; elution was performed with a linear molarity gradient of phosphate buffer pH 6·8 (0·01 to 0·5 M; 100 + 100 ml.). No ultraviolet monitoring system was used.

Preliminary experiments showed that native *H. influenzae* DNA was eluted as a single peak at about 0·23 M-phosphate, the recovery of both optical density and transforming activity for three markers (erythromycin, viomycin and streptomycin) being higher than 90%.

Thermal denaturation was carried out by heating DNA (20 μg/ml. in 0·15 M-NaCl–0·01 M-phosphate buffer, pH 6·8) for 10 minutes at 100°C. Solutions were then rapidly cooled by pouring them into flasks cooled in an ice bath and used for the chromatographic experiments. The fractions were assayed by the method of Luzzati (1962) for biological activity at a non-saturating DNA concentration (0·05 or 0·1 μg/ml.), using as a genetic marker the resistance to 1 μg/ml. of novobiocin. Heat-denatured DNA, in contrast to native DNA, was eluted in two fractions, a major one at about 0·12 M-phosphate and a minor one at about 0·22 M-phosphate (Fig. 1). This is the usual chromatographic behaviour of heat-denatured DNA samples from several different sources (Bernardi, 1962, and manuscript in preparation). It has already been shown that the first fraction reacts completely at 25°C with 1% formaldehyde in 0·13 M-NaCl–0·01 M-phosphate (pH 6·8), whereas the second one reacts only partially, and a

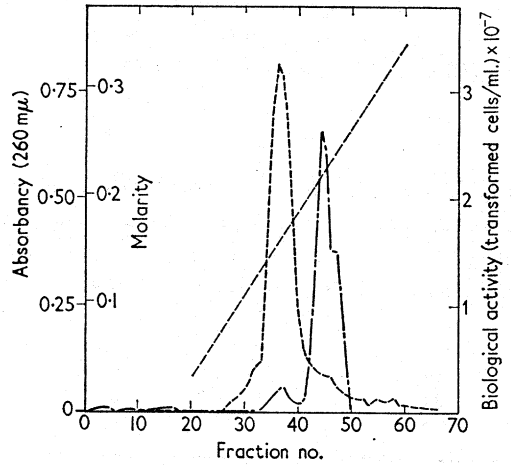

FIG. 1. Chromatography of heat-denatured *H. influenzae* DNA (experiment no. 42) on hydroxyapatite. – – – – – – – –, absorbancy of the fractions at 260 mμ; — · — · — · —, biological activity of the fractions. The linear molarity gradient of phosphate buffer is also indicated. In this experiment tubes 30 to 40 were taken as fraction I and tubes 41 to 50 as fraction II. The specific biological activities given in Table 1 were those of tubes 37 (peak I) and 44 (peak II), respectively. See text and Table 1 for further details.

further increase (of about 15%) in its optical density at 260 mμ may be obtained after heating the fraction in the presence of formaldehyde. Furthermore, this second fraction, in contrast to the first one, melts sharply, and is therefore supposed to contain a substantial portion of DNA in a double-stranded, native-like structure (Bernardi, 1962, and manuscript in preparation).

The residual biological activity was found to be almost completely associated with the minor fraction eluted at the higher molarity, the specific transforming activity being about 10 times higher than in unfractionated, heat-denatured DNA (Table 1). The activity detected in the major fractions was extremely low, the specific activity being about 10 times less than in the starting DNA; it is possible that this low activity is due to contamination from the active fraction.

These results lead to the conclusion, also supported by the data of Barnhart & Herriott (1962), of Rownd, Green & Doty (1963, *Abst.*, *Biophys. Soc.* TB7) and also of Roger & Hotchkiss (1961), that the residual activity is bound to molecules which still have, to a significant extent, a native-like structure. One would expect that these molecules would form bands, in a CsCl density-gradient, in a density range intermediate between native and denatured DNA, possibly closer to the latter than to the former. Guild's results (1961) are therefore not considered by us to contradict our conclusion, since they only show that activity is found at a density close to that expected for denatured molecules. So far as the nature of the partially denatured molecules is concerned, we are inclined to think that they are formed by strands which did not come apart completely upon heating, an event which is now known to occur to some extent even in bacterial DNA's (Marmur, Rownd & Schildkraut, 1963).

A more detailed presentation of this work will be given elsewhere.

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