Chromatography of Transforming Deoxyribonucleic Acid on Methylated Albumin and by Gel Filtration

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1. Native DNA from two strains of *Bacillus subtilis* was chromatographed by stepwise elution from MAK (methylated albumin on kieselguhr). 2. Transforming activity was confined to two out of the three main fractions, activity being distributed between the two peaks differently for DNA from the different strains. 3. Fractionation of DNA from both strains on 2% agarose gel gave two components. Approx. 75% of the material was eluted within the void volume of the column. Approx. 25% of the material consisted of degradation products of lower molecular weight. 4. Chromatography on MAK of the material of high molecular weight eluted from agarose gel gave a number of peaks differing in molecular weight, indicating that degradation of the DNA takes place during chromatography on MAK. 5. The distribution of transforming activity among the fractions from MAK suggests that degradation occurs preferentially in certain regions of the DNA.

It has been shown previously (Ayad *et al.*, 1968) that DNA from *Bacillus subtilis* can be partially fractionated by chromatography on MAK† into components carrying certain genetic markers. In experiments designed to extend this to separation of DNA fractions carrying a range of markers, it was observed that DNA prepared from different prototrophic strains used as donors behaved differently on chromatography. The relative transforming activities for different markers in different fractions varied, suggesting the possibility that separation of markers had occurred. However, the highest transforming activities were associated with different fractions according to the strain used as donor. Comparison of the behaviour of DNA from different donor strains on chromatography on MAK and by gel filtration suggest that the former process results in the scission of the polynucleotide chain in specific regions, and that this is the origin of the separation of fractions of DNA enriched in different genetic markers. Part of this work has been briefly recorded (Barker & Hodges, 1969).

Materials and Methods

Bacterial strains

B. subtilis strain 312 was used as recipient in all measurements of transforming activity. This strain was a gift from Dr. M. S. Kelly (Universitet I Bergen,

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† Abbreviation: MAK, methylated albumin on kieselguhr.

Biokjemisk Institutt, Bergen, Norway) and requires adenine, histidine, lysine, cystine and thiamin for growth. The prototrophic strains 234 (Kelly, 1967) and PR312 were used as sources of DNA. The former is the parent strain from which strain 312 was derived (M. S. Kelly, personal communication). Strain PR312 was obtained in the present work by transformation of strain 312 by using DNA from the prototrophic Marburg strain as donor. All strains were maintained by monthly transfer on to slopes of tryptose–blood–agar base (Difco Laboratories, Detroit, Mich., U.S.A.).

Preparation of DNA

DNA was prepared by the method of Marmur (1961) from the prototrophic strains, grown as described by Ayad *et al.* (1968). Solutions of DNA were sterilized as described by Krasna (1964) and stored at 0°C at a concentration of approx. 60 µg/ml, calculated from measurements of E_{260} by using the equation of Sueoka & Cheng (1962).

Fractionation and characterization of DNA

Chromatography of DNA on columns of MAK was carried out by using stepwise elution as described by Ayad *et al.* (1968).

Gel filtration of DNA was carried out in glass tubes (2.5 cm diam. × 45 cm) fitted with a nylon net, into which 2% agarose gel (Sephacrose 2B; Pharmacia Fine Chemicals, Uppsala, Sweden) was introduced as described by Öberg & Philipson (1967). DNA (1.0–1.5 mg) was applied to the

Fig. 1. Chromatography of DNA from *Bacillus subtilis* on columns of MAK

DNA was applied to columns of MAK in 0.02M-sodium phosphate buffer, pH 6.8, containing 0.6M-NaCl. The material was eluted stepwise with the same buffer containing increasing concentrations of NaCl as indicated. The percentage recovery of DNA in each fraction was calculated from measurements of E_{260} . (a) Whole DNA; (b) rechromatography of material eluted by 0.7M-NaCl; (c) rechromatography of material eluted by 0.75M-NaCl; (d) chromatography of material in peak I from Sepharose 2B (Fig. 2). For further details see the text.

column as a solution (1 mg/ml) in buffer containing 0.1M-NaCl, 0.002M- Na_2HPO_4 and 0.001M-disodium EDTA, adjusted to pH 6.0. The column was eluted with the same buffer at a flow rate of 10 ml/h, the eluate being continuously monitored by measure-

Fig. 2. Chromatography of DNA from *Bacillus subtilis* on a column of Sepharose 2B

A column (2.5 cm $\times$ 45 cm) of Sepharose 2B was equilibrated with buffer, pH 6.0, containing 0.1M-NaCl, 0.002M- Na_2HPO_4 and 0.001M-disodium EDTA. DNA (1.0–1.5 mg; 1 mg/ml) was applied to the column in the same buffer. The column was eluted with the same buffer at a flow rate of 10 ml/h and the extinction of fractions (4 ml) was measured at 254 nm.

ment of the extinction at 254 nm. Fractions (4 ml) were collected and, when necessary, were concentrated by ultrafiltration.

'Melting' temperatures and sedimentation coefficients were measured under the conditions described by Ayad *et al.* (1968). Sedimentation coefficients were derived by using the equation of Schachman (1957); values at infinite dilution (Aten & Cohen, 1965) and molecular weights were calculated as described by Eigier & Doty (1965).

Measurement of transforming activity

Transforming activity was measured as previously described (Ayad & Barker, 1969; Akrigg *et al.*, 1967) with the following modifications. Medium I contained 50 μg each of adenine, L-cystine, L-histidine, L-lysine and thiamin/ml; in medium II, the concentration of these components was 5 μg /ml. All transformation frequencies are plateau values.

Table 1. Chromatography of transforming DNA from *Bacillus subtilis* on MAK

Values in parentheses are the numbers of transformants/ml relative to those for the lysine marker.

Donor strain	Concn. of NaCl in eluate (M)	Mol. wt. of DNA eluted	$10^{-1} \times$ Transformants/ml		
			Adenine marker	Histidine marker	Lysine marker
PR312	0.6	—*	0	0	0
	0.7	1×10^7	50 (10)	10 (2)	5 (1)
	0.75	2×10^7	885 (4.4)	355 (1.8)	200 (1)
234	0.6	—*	0	0	0
	0.7	1×10^7	4485 (4.5)	1955 (2.0)	990 (1)
	0.75	2×10^7	320 (2.2)	240 (1.7)	145 (1)

*Too low to be measured by sedimentation.

Results

Fractionation of DNA on MAK

Fig. 1(a) shows the distribution of DNA among the three fractions eluted respectively with 0.6M-, 0.7M- and 0.75M-NaCl. These results are typical of many experiments. In a few experiments, small fractions were eluted by 0.8M-, 0.85M- and 1.0M-NaCl, but these were not investigated. In some experiments referred to below, a component was eluted by 0.65M-NaCl and a small fraction was obtained under these conditions with DNA that had been stored for some time.

Of the three main fractions, that eluted by 0.6M-NaCl consisted of degraded material, which did not sediment satisfactorily in the ultracentrifuge and exhibited no transforming activity. The transforming activities for the other main fractions are shown together with chemical characteristics in Table 1.

Fractions eluted in several experiments with 0.7M-NaCl and with 0.75M-NaCl were separately pooled, dialysed against 0.6M-NaCl and rechromatographed. The results are shown in Figs. 1(b) and 1(c). They exhibited 'melting' temperatures of 88°C and 87°C respectively, which correspond to 0.46 and 0.43 mole fraction of guanine plus cytosine according to the equation of Marmur & Doty (1962).

Fractionation of DNA by gel filtration

Closely similar results were obtained by gel filtration of DNA prepared from *B. subtilis* strains 234 and PR312. A typical elution profile is shown in Fig. 2, in which approx. 75% of the material loaded is accounted for in peak I and approx. 25% in peak II. Transforming activity was confined to peak I, peak II showing no activity for any of the genetic markers used, and failing to sediment as a discrete peak in the analytical ultracentrifuge. Samples from individual fractions comprising peak I were examined in the analytical ultracentrifuge and $s_{20,w}^0$ values between 30S and 35S were found. Fractions from peak I were combined, and after being concentrated to small volume were chromatographed again on 2% agarose gel. A single peak was obtained which coincided in position with peak I in the initial chromatography.

Fractionation on methylated albumin of DNA obtained by gel filtration

Fractions of DNA from peaks I and II, obtained by gel filtration, were separately dialysed against buffer containing 0.6M-NaCl and chromatographed on MAK. Material from peak II was not retained by MAK. Material eluted from agarose gel as

peak I gave four main components and two minor components which were not examined further. The distribution of material among the six fractions is shown in Fig. 1(d). The fraction eluted by 0.6M-NaCl did not sediment satisfactorily in the ultracentrifuge and, on rechromatography on agarose gel, was eluted at the same position as the original peak II. Material eluted from MAK by 0.7M-, 0.75M- and 0.8M-NaCl had $s_{20,w}^0$ values of 24S, 30S and 33S respectively.

Discussion

Fractionation of DNA on columns of MAK may depend on the heterogeneity in molecular weight, base composition, secondary structure, or all three. Material eluted by 0.6M-NaCl is of low molecular weight, and this is sufficient to account for the lack of transforming activity (Fluke *et al.*, 1952; Goodgal & Herriott, 1957; Ayad *et al.*, 1968). Components of DNA from both strains of *B. subtilis* eluted by 0.7M- and 0.75M-NaCl all show 'melting' behaviour typical of native DNA. The fractions eluted at the different salt concentrations show a difference in base composition, in agreement with the results of Cheng & Sueoka (1963), but this is small. It is believed that elution of DNA in these two fractions depends largely, if not exclusively, on the approximate twofold difference in molecular weight. On this basis, the fact that rechromatography on MAK of material previously eluted from MAK by 0.7M- or 0.75M-NaCl yields major fractions eluted by lower concentrations of NaCl is taken to indicate that chromatography on MAK causes degradation of DNA.

Since the components of high molecular weight (eluted in peak I from Sepharose 2B) appear as a single peak on rechromatography, it is concluded that degradation of the DNA does not occur during chromatography on Sepharose 2B and that peak II represents approx. 25% of material of low molecular weight present in DNA prepared as described above from either strain of *B. subtilis*. The material of high molecular weight in the preparations of DNA appears to be degraded progressively during chromatography on MAK, possibly through shearing during passage through the pores of the kieselguhr. The results of Burgi & Hershey (1961) suggest that degradation of DNA by shearing may consist of successive fission into half-molecules, and it would be possible on this basis to account for the molecular weights shown above for fractions eluted by 0.7M- and 0.75M-NaCl. However, in view of the distribution of transforming activities shown in Table 1 such a simple explanation is insufficient and it appears that scission occurs in a non-random manner for the reasons outlined below.

Table 1 shows that transforming activity of DNA eluted from MAK is associated largely with the fraction of higher molecular weight from *B. subtilis* strain PR312, but with that of lower molecular weight from strain 234. The former situation is in agreement with the results of Fluke *et al.* (1952) and Goodgal & Herriott (1957). The results with DNA from the latter strain indicate that the genetic markers used in measuring transforming activity are specifically associated with fractions of lower molecular weight. Regions of high lability to shearing have been recognized in DNA from various sources (Creager & Spatz, 1969, and references therein) and it has been shown that such lability may arise in different ways. The present results are consistent with breakage of chains in the middle if the assumption is made that the genetic markers used are distributed differently within the DNA molecules from the two strains, and that in strain 234 they are associated with strands of DNA that are particularly susceptible to shearing. The possibility that scission occurs other than in the middle of the DNA chains, as suggested by Burgi *et al.* (1966), Roger *et al.* (1966) and Hotchkiss *et al.* (1967), cannot be excluded, but in either case the results obtained are most readily explained by assuming first that the adenine, histidine and lysine markers are disposed differently within the chromosome in strains PR312 and 234, and secondly that scission of DNA chains occurs in a non-random manner during passage through MAK. It is suggested that the chromatographic separation of DNA fractions with different transforming activities results, at least in part, from non-random degradation of the DNA during chromatography such that fragments carrying different markers have different molecular weights. The extent to which fractionation

is affected by differences in base composition between segments of the polynucleotide chain carrying different markers cannot be assessed.

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