Fractionation of Native and Denatured Transforming Deoxyribonucleic Acid from *Bacillus subtilis*

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1. Native DNA from *Bacillus subtilis* was fractionated by stepwise elution from methylated albumin, the transforming activity being confined to two out of four fractions. Partial separation of DNA active in transformation for the arginine marker from that showing activity for the histidine and tryptophan markers was achieved. 2. Partial denaturation of DNA at 90° and 93·5° resulted in the preferential destruction of transforming activity for the histidine and tryptophan markers. 3. Denaturation of DNA at 100° followed by chromatography on methylated albumin yielded five fractions, two of which exhibited residual activity. Redenaturation at 100° resulted in the interconversion of four out of the five fractions. Redenaturation of fractions labelled with ¹⁵N and ²H suggested the presence of a specific component that did not readily take part in the interconversions.

It has been shown (Ayad, Barker & Jacob, 1966) that some degree of separation of fractions of DNA from *Bacillus subtilis* carrying different genetic markers can be achieved by gradient elution with sodium chloride solutions from a methylated albumin column. Stepwise elution of DNA from methylated albumin columns has been shown to have some advantages over gradient elution (Sueoka & Cheng, 1962; Roger, 1964). The stepwise method of elution may give rise to artifacts due to trailing (Roger, Beckmann & Hotchkiss, 1966), and the possibility of separating fractions of DNA with different biological activities appeared to offer a means of assessing the significance of fractions obtained by this method. Separation of transforming DNA from inactive material in partially denatured pneumococcal DNA has been described by Roger (1964). Results initially obtained by gradient elution suggested the possibility of separating transforming DNA of *B. subtilis* from native fractions lacking transforming activity, and this is described in the present paper. By the same method of fractionation, material responsible for the residual transforming activity of heat-denatured DNA has been separated from inactive material and it has been shown that certain of these fractions are interconvertible. Experiments are described relating to the nature of the material carrying the residual transforming activity.

MATERIALS AND METHODS

Growth of bacteria. The prototrophic Marburg strain of *B. subtilis* was used as donor in transformation experiments, the recipient cells being strain 3115, which requires arginine,

histidine and tryptophan for growth. The organisms were maintained by transfer every few weeks on slopes of tryptose-blood-agar base.

The Marburg strain was grown at 37° with vigorous aeration for 14 hr. in minimal medium containing 7 g. of K₂HPO₄, 3 g. of KH₂PO₄, 1 g. of (NH₄)₂SO₄, 0·2 g. of MgSO₄·7H₂O, 0·5 g. of sodium citrate and 5 g. of D-glucose/l., to which were added 0·05% (w/v) Difco yeast extract and 0·5% (w/v) Difco bacto-peptone. Strain 3115 was grown under the same conditions in minimal medium to which was added 0·005% of each of the following amino acids: L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-cystine, L-glutamic acid, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine and L-valine. For the preparation of ²H-labelled and ¹⁵N-labelled cells, the following adaptation of the method of Marmur & Schildkraut (1961) was used. The Marburg strain was inoculated from a slope into 100 ml. of minimal medium and grown at 37° with vigorous aeration for 14 hr. A 1 ml. sample of this culture, containing approximately 10⁵ cells, was inoculated into 6 ml. of a medium containing 150 mg. of KCl, 25 mg. of NH₄Cl, 500 mg. of NaCl, 100 mg. of K₂HPO₄, 5 mg. of Na₂SO₄, 10 mg. of MgCl₂·H₂O, 0·2 mg. of CaCl₂, 0·2 mg. of FeCl₃·6H₂O, 0·2 ml. of 60% (w/v) sodium lactate and 0·15 ml. of glycerol, dissolved in ²H₂O (99·6% purity, obtained from The Radiochemical Centre, Amersham, Bucks.) up to a final volume of 100 ml. After inoculation, the medium was diluted to 10 ml. with sterile aq. 0·9% NaCl and incubated at 37° with shaking for 2 hr. A further 10 ml. of the above medium was then added and incubation was continued for 2 hr. This process was repeated a further three times, the final volume of the culture being 50 ml. After completion of the final incubation, 10 ml. of the culture were inoculated into 400 ml. of the above ²H₂O-containing medium in which the NH₄Cl used contained 99% excess ¹⁵N (Bio-Rad Laboratories, Richmond, Calif., U.S.A.). The culture was incubated

at 37° with shaking for 14 hr. In all cases, cells were harvested by centrifugation for 20 min. at 10000 rev./min. in the MSE High Speed 18 centrifuge.

Preparation of DNA. DNA was prepared from the Marburg strain of *B. subtilis* by the method of Marmur (1961), and stored at 0° at a concentration of approx. 60 µg./ml. in 1.0 M-NaCl containing 20 mM-KH₂PO₄ adjusted to pH 6.8 with 2 N-NaOH.

DNA was denatured by heating for 10 or 20 min. at 100° (Schildkraut, Marmur & Doty, 1961; Subirana, 1966) at a concentration of 20 µg./ml. in 0.15 M-NaCl containing 20 mM-KH₂PO₄ adjusted to pH 6.8 with 2 N-NaOH, and cooling rapidly in an ice bath. Variation in the time of heating did not affect the results obtained. The volume of solutions treated in this way did not exceed 5 ml. Partial denaturation was carried out similarly at 90° and 93.5°. Concentrations of solutions of DNA were calculated from measurements of extinction at 260 mµ by using the equations of Sueoka & Cheng (1962).

Fractionation and characterization of DNA. Methylated albumin was prepared by the method of Hayashi, Hayashi & Spiegelman (1965). Methylated albumin adsorbed on kieselguhr was prepared by the following modification of the method described by Mandell & Hershey (1960) for the second layer of their column, chromatography being carried out at 20–25°. A suspension of 20 g. of kieselguhr in 100 ml. of 0.1 M-NaCl buffered with 20 mM-KH₂PO₄ adjusted to pH 6.8 with 2 N-NaOH was boiled and cooled to remove air bubbles. An aq. 1% (w/v) methylated albumin solution (5 ml.) was added and the mixture stirred for 10 min. The suspension was then poured into a column and washed with 300 ml. of 0.4 M-NaCl containing 20 mM-KH₂PO₄ adjusted to pH 6.8 with 2 N-NaOH. The solid was removed from the column and made up to 100 ml. with the same solution. Of this suspension, 10 ml. was poured into a column of 1 cm. diameter. Pressure of 2 lb./in.² was applied to the top of the column until the level of liquid reached that of the solid. The column was then washed with 20 ml. of 0.6 M-NaCl containing 20 mM-KH₂PO₄ adjusted to pH 6.8 with 2 N-NaOH. DNA (0.5–1.0 mg.) was dissolved in the same solution at a concentration of 25 µg./ml. and applied to the column. The DNA was eluted by applying successive 20 ml. portions of 20 mM-KH₂PO₄ adjusted to pH 6.8 with 2 N-NaOH containing increasing concentrations of NaCl. In all cases the eluting solution was forced down to the level of the solid in the column by applying pressure as above. The rate of flow was 20 ml./hr., the extinction of the effluent was continuously measured by an LKB Uvicord ultraviolet spectrophotometer, and 4 ml. fractions were collected. The extinctions at 260 mµ of some of the fractions were measured with a Unicam SP.500 spectrometer. In most cases the extinction of the effluent had dropped to not greater than 0.03 by the time each 20 ml. portion of eluting solution had been forced through. When this was not so, a further 5 ml. of buffer of the same composition was added and forced through to ensure the decrease of extinction to the base-line value.

Unfractionated DNA and fractions eluted from the columns were examined for temperature dependence of extinction. DNA samples were dialysed against 0.15 M-NaCl containing 15 mM-sodium citrate adjusted to pH 7.0 with N-NaOH, and the extinctions of the resulting solutions were measured at 260 mµ with a Unicam SP.500 spectro-

meter having an electrically heated cell holder. Temperatures were measured with a calibrated thermistor fitted into the top of the cuvette. Solutions of DNA were placed in 1.0 cm. cuvettes, degassed under reduced pressure and covered with a layer of liquid paraffin to prevent evaporation. Some loss by evaporation was observed unless cuvettes with circular necks were used. Measurements of extinction were made every 10° up to 75° and every 2° in the melting region. Measurements were corrected for thermal expansion of the solution.

Sedimentation coefficients were determined in a Spinco model E ultracentrifuge fitted with ultraviolet optics. DNA was dissolved at a concentration of 50 µg./ml. in 0.15 M-NaCl containing 10 mM-sodium citrate adjusted to pH 7.0 with N-NaOH. Photographs were taken at intervals of 4 min. after the speed of 35000 rev./min. had been reached. Densitometer tracings obtained with a Beckman model R Analytrol showed sharp boundaries with native DNA but more diffuse boundaries with denatured DNA. The position of the boundary was taken as the mid-point between the solvent and plateau regions. In each case, experiments were carried out with three dilutions of the above solution, and sedimentation coefficients were corrected to infinite dilution and 20°.

Equilibrium sedimentation in caesium chloride solution. Solid CsCl was added to solutions containing approximately 0.1 mg. of DNA in 5 ml. of 20 mM-KH₂PO₄ buffer adjusted to pH 6.8 with 2 N-NaOH containing 1.0 M-NaCl to give a refractive index of 1.421. The solution was centrifuged at 35000 rev./min. for 66 hr. in the SW 39 rotor of the Spinco model L centrifuge, the solution being covered by 0.5 ml. of mineral oil. The base of the centrifuge tube was pierced and fractions (60 µl.) were collected. One drop was used for measurement of refractive index, and the rest was diluted to 0.3 ml. with 1.0 M-NaCl containing 20 mM-KH₂PO₄ adjusted to pH 6.8 with 2 N-NaOH and the extinction at 260 mµ measured. Densities of solutions were calculated from refractive indices by the method of Weigle, Meselson & Paigen (1959).

Measurement of transforming activity. Competent cells were prepared from *B. subtilis* strain 3115 by the following modification of the methods of Saito & Miura (1963) and K. S. Kirby (personal communication). Strict adherence to the conditions was found to be necessary. *B. subtilis* strain 3115 was inoculated from a slope into glucose-minimal medium (Saito & Miura, 1963) supplemented with 22.3 µg. of MnSO₄/ml. and 50 µg. of each of the 20 amino acids listed above/ml. and grown for 17 hr. at 37° with slow aeration. A sample of the culture (approx. 3 ml.) was added to 40 ml. of the same medium in a flask with a side arm, such that the extinction at 450 mµ was 0.2. The culture was aerated vigorously at 37° and the extinction at 450 mµ was measured every 15 min. When the extinction reached 1.7 (about 3–4 hr.), a 10 ml. sample of the culture was transferred to 30 ml. of the above medium modified by the omission of MnSO₄ and the decrease of the concentrations of L-arginine, L-histidine and L-tryptophan to 5 µg./ml. The culture was incubated at 37° with very slow aeration for 105 min. and then diluted to 300 ml. with minimal medium. Samples (3 ml.) of the diluted culture were incubated for 90 min. with DNA dissolved at a final concentration of 5.0 µg./ml. in saline-phosphate buffer. Incubation was carried out in sterile tubes (19 mm. × 125 mm.) at 37° with vigorous shaking for 90 min. One sample (culture

control) was incubated under the same conditions without the addition of DNA. After incubation, the samples were inoculated in duplicate on to plates of minimal medium solidified with 1.8% agar and supplemented with 50 μ g. of each of L-alanine, ammonium L-aspartate, L-cysteine, glycine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-proline, L-serine and L-threonine/ml. together with 50 μ g. of each of L-histidine and L-tryptophan/ml. for measurement of transforming activity for the arginine marker, 50 μ g. of each of L-tryptophan and L-arginine/ml. similarly for the histidine marker and 50 μ g. of each of L-histidine and L-arginine/ml. for the tryptophan marker. Samples were also similarly inoculated after dilution with cold minimal medium to 10, 100 and 1000 times the original volume. Plates were incubated at 37° for 48 hr. and colonies were counted.

Preliminary experiments in which competent cells were incubated with DNA in the range 3–7 μ g./ml. gave identical results within experimental error, indicating that the transformation activities measured were plateau values.

RESULTS

Fractionation of native DNA by stepwise elution from methylated albumin. Fig. 1(a) shows the course of the elution from methylated albumin of native DNA prepared from the Marburg strain of *B. subtilis* with increasing concentrations of sodium chloride. These results are typical of numerous experiments with different preparations of DNA in which the relative proportions of each fraction varied by not more than 10%. In one experiment with an old specimen of DNA, a small peak was eluted by 0.9M-sodium chloride, but this was not observed with freshly prepared material. In order to determine whether or not the least widely separated fractions, eluted by 0.7M- and 0.75M-sodium chloride, properly represent distinct components, material eluted by 0.7M-sodium chloride was dialysed against 0.6M-sodium chloride containing 20mM-potassium dihydrogen phosphate adjusted to pH 6.8 with 2N-sodium hydroxide and rechromatographed. A single fraction was eluted at a concentration of 0.7M-sodium chloride.

All fractions obtained from native DNA showed increases in extinction at 260m μ on being heated, the transition temperatures for the fractions eluted by 0.6M-, 0.7M-, 0.75M- and 1.0M-sodium chloride being 86°, 87°, 86° and 83° respectively. Under the same conditions, unfractionated native DNA showed a transition at 85°. The above fractions of native DNA had sedimentation coefficients of 6.2s, 26.0s, 27.9s and 29.7s. Unfractionated native DNA had a sedimentation coefficient of 26.0s. Photographs taken early in the centrifugation of unfractionated DNA showed a diffuse boundary, but a sharp boundary was observed in later photographs. The transforming activities for the arginine, histidine and tryptophan markers obtained with 0.6M-, 0.7M-, 0.75M- and 1.0M-sodium

Fig. 1. DNA from *B. subtilis* was fractionated by elution from methylated albumin with increasing concentrations of NaCl. E_{260} of the eluate was measured continuously, as described in the text. (a) Native DNA; (b) DNA denatured at 90°; (c) DNA denatured at 93.5°; (d) DNA denatured at 100°; (e) DNA eluted with 0.7M-NaCl, Fig. 1(a), denatured at 100° in the presence of formaldehyde; (f) DNA eluted with 0.7M-NaCl, Fig. 1(a), denatured at 100°; (g) DNA eluted with 0.8M- and 0.85M-NaCl, Fig. 1(d), denatured at 100°; (h) DNA eluted with 0.9M- and 0.95M-NaCl, Fig. 1(d), denatured at 100°.

chloride fractions of native DNA eluted from methylated albumin are shown in Table 1. The results are expressed as percentages of the activities obtained with native unfractionated DNA. With unfractionated material, the numbers of colonies of transformants corresponding to a 0.2ml. sample, after making allowance for dilution, were 223×10^3 (0.84%), 198×10^3 (0.75%) and 210×10^3 (0.79%) for the arginine, histidine and tryptophan markers respectively, the figures in parentheses being the number of transformants expressed as a percentage of the viable cells.

Fractionation of denatured DNA by stepwise elution from methylated albumin. Figs. 1(b), 1(c) and 1(d) show the course of the elution from methylated albumin of DNA from the Marburg strain of *B. subtilis* after denaturation for 10 min. at 90°, 93.5° and 100° respectively. No fraction obtained from DNA denatured at 100° showed hyperchromicity. Of the fractions obtained from

Table 1. *Transforming activities of samples of DNA prepared from the Marburg strain of B. subtilis*

DNA sample	Elution diagram	Concn. of NaCl in eluted fractions (M)	Plateau transforming activities, expressed as percentages of the value with an unfractionated native DNA control		
			Arginine marker	Histidine marker	Tryptophan marker
Native	Fig. 1(a)	0.60	0	0	0
		0.70	161	82	71
		0.75	63	157	141
		1.0	0	0	0
Denatured at 90°	Fig. 1(b)	Unfractionated	89	71	66
		0.70	121	15	7
		0.75	23	85	80
		0.85	0	7	7
		0.90	0	0	0
		0.95	0	0	0
Denatured at 93.5°	Fig. 1(c)	1.0	0	0	0
		Unfractionated	57	8	11
		0.70	60	0.5	0.3
		0.75	1	8	7
		0.80	9	0	0
		0.85	0	4	4
		0.90	0	0	0
		0.95	0	0	0
Denatured at 100°	Fig. 1(d)	1.0	0	0	0
		Unfractionated	0.6	0.7	0.8
		0.80	0.7	0	0
		0.85	0	0.7	0.6
		0.90	0	0	0
		0.95	0	0	0
0.8M- and 0.85M-NaCl fractions (Fig. 1d) heated at 100°	Fig. 1(g)	1.0	0	0	0
		0.80	0.5	0	0
		0.85	0	0.7	0.7
		0.90	0	0	0
0.9M- and 0.95M-NaCl fractions (Fig. 1d) heated at 100°	Fig. 1(h)	0.95	0	0	0
		0.80	0.4	0	0
		0.85	0	0.8	0.8
		0.90	0	0	0

DNA denatured at 90° and 93.5°, only those eluted by 0.7M- and 0.75M-sodium chloride showed hyperchromicity, the melting temperatures being 87° and 85° respectively for material denatured at the lower temperature and 65° and 69° for material denatured at the higher temperature. The sedimentation coefficient of unfractionated DNA after denaturation at 100° was 18.5s. The corresponding values for fractions eluted by 0.8M-, 0.85M-, 0.9M-, 0.95M- and 1.0M-sodium chloride (Fig. 1d) obtained from this material were respectively 16.0s, 16.3s, 18.9s, 19.8s and 21.4s. The transforming activities for the arginine, histidine and tryptophan markers, expressed as percentages of the corresponding activity for native unfractionated DNA, are shown in Table 1.

Fractions obtained from native DNA eluted by 0.7M-sodium chloride (Fig. 1a) were combined, dialysed against 0.6M-sodium chloride containing 20mM-potassium dihydrogen phosphate adjusted to pH 6.8 with 2N-sodium hydroxide, made 1.0M with respect to formaldehyde, denatured at 100° and chromatographed as described above. The course of the elution is shown in Fig. 1(e). A similar experiment in which formaldehyde was omitted gave the elution profile shown in Fig. 1(f).

Interconversion of fractions from denatured DNA. Solutions corresponding to fractions eluted by 0.8M- and 0.85M-sodium chloride (Fig. 1d) were combined to give fraction A, concentrated to 20ml. under reduced pressure at 10° and dialysed against 0.6M-sodium chloride containing 20mM-potassium

dihydrogen phosphate adjusted to pH 6.8 with 2N-sodium hydroxide. The resulting solution was heated at 100° and chromatographed on methylated albumin as described above. The course of the elution is shown in Fig. 1(g). In one experiment, the DNA was denatured as described in the Materials and Methods section and dialysed against 0.6M-sodium chloride before chromatography. The same elution profile was obtained. The transforming activities for the arginine, histidine and tryptophan markers for fractions eluted by 0.8M-, 0.85M-, 0.9M- and 0.95M-sodium chloride (Fig. 1g) are shown in Table 1.

Solutions corresponding to fractions eluted at 0.9M- and 0.95M-sodium chloride were combined to give fraction *B* and treated exactly as above. The material eluted by 0.8M-, 0.85M-, 0.9M- and 0.95M-sodium chloride (Fig. 1h) had transforming activities shown in Table 1. A similar experiment in which the solution was made 1.0M with respect to formaldehyde before denaturation gave an elution profile identical with that shown in Fig 1(e).

Interconversion of fractions from isotopically labelled DNA. DNA was prepared from cells grown

in ^2H - and ^{15}N -labelled medium and, after denaturation at 100°, was fractionated by elution from methylated albumin as described above. Fractions eluted by 0.8M- and 0.85M-sodium chloride respectively were combined (fraction *AH*) and subjected to equilibrium sedimentation in caesium chloride solution. A single component having a density of 1.746g.cm. $^{-3}$ was obtained. The combined fractions eluted with 0.9M- and 0.95M-sodium chloride (fraction *BH*) gave a single component having a density of 1.763g.cm. $^{-3}$ under the same conditions. Corresponding fractions (*AL* and *BL*) obtained from cells grown in normal medium gave single components having densities of 1.704g.cm. $^{-3}$ and 1.722g.cm. $^{-3}$ respectively.

A sample (2ml.) of fraction *AH* was mixed with 2ml. of fraction *BL*, each sample containing approx. 90 μg . of DNA, and the solution was heated at 100° for 20min. and cooled to 0° within 20sec. The resulting solution was subjected to equilibrium sedimentation in caesium chloride solution and gave four components having densities of 1.762g.cm. $^{-3}$, 1.747g.cm. $^{-3}$, 1.723g.cm. $^{-3}$ and 1.706g.cm. $^{-3}$ (Fig. 2 *AH/BL*). A similar experiment was carried out by mixing fractions *AL* and *BH*; equilibrium sedimentation gave components having densities of 1.762g.cm. $^{-3}$, 1.746g.cm. $^{-3}$, 1.720g.cm. $^{-3}$ and 1.705g.cm. $^{-3}$ (Fig. 2 *AL/BH*).

DISCUSSION

Fractionation of native DNA. It has been shown by Sueoka & Cheng (1962) that fractionation of DNA on columns of methylated albumin depends on the molecular size, the degree of hydrogen bonding and the base composition of the component polynucleotides. Although no attempt has been made to determine the molecular weights of DNA fractions, and no comparison can be made between fractions obtained from native and denatured DNA, observed sedimentation coefficients suggest that, in a given fractionation, components of low molecular weight are eluted earlier than those of higher molecular weight. It is considered that the lack of transforming activity in the fraction of native DNA eluted by 0.6M-sodium chloride (Fig. 1a) is due to its low molecular weight, in agreement with the findings of Fluke, Drew & Pollard (1952) and Goodgal & Herriott (1957). No explanation can be put forward at present for the inactivity of the fraction of native DNA eluted by 1.0M-sodium chloride (Fig. 1a) since it appears to have neither a lower sedimentation coefficient nor less secondary structure than the active fractions, as judged by its melting behaviour (Marmur & Doty, 1961; Schildkraut *et al.* 1961).

The results reported in Table 1 indicate that the

Fig. 2. Equilibrium sedimentation in CsCl solution of fractions of denatured DNA labelled with ^2H and ^{15}N . (*AH/BL*) Fractions of heavy denatured DNA eluted by 0.8M- and 0.85M-NaCl (fraction *AH*) were mixed with fractions of unlabelled DNA eluted by 0.9M- and 0.95M-NaCl (fraction *BL*) and redenatured at 100°. The resulting solution, after centrifugation in CsCl solution, gave fractions *BH*, *AH*, *BL* and *AL* as shown. (*AL/BH*) A mixture of fraction *A* from unlabelled DNA (fraction *AL*) and fraction *B* from heavy DNA (fraction *BH*), after similar treatment, gave fractions *BH*, *AH*, *BL* and *AL* as shown. Each of these fractions, when centrifuged separately in CsCl solution, gave single peaks banding in the same positions as the corresponding peaks obtained from redenatured mixtures.

transforming activity of the native DNA appears only in fractions eluted by 0.7M- and 0.75M-sodium chloride and that partial separation of genetically marked segments of the molecule occurs. The different transforming activities associated with the two fractions indicate that they are not artifacts of the method of elution. It can be seen from Table 1 that in the case of fractions eluted by 0.7M- and 0.75M-sodium chloride, transforming activities for the histidine and tryptophan markers are similar, and that they differ considerably from the activities for the arginine marker. The fact that no separation occurs of segments of DNA carrying histidine and tryptophan markers is in agreement with the high co-transfer index recorded for this pair of markers (Nester & Lederberg, 1961; Saito & Masamune, 1964) and confirms the close linkage between them. In contrast, DNA carrying the arginine marker is partially separated from that carrying the histidine and tryptophan markers, indicating no close linkage between the arginine marker and the histidine or tryptophan markers. This is in accordance with the lower co-transfer indices for the arginine marker in relation to the histidine and tryptophan markers.

Fractionation of heat-denatured DNA. It can be seen from Fig. 1(b) that heating native DNA at 90° for 10min. completely destroys the 0.6M-sodium chloride fraction and gives rise to new fractions eluted by 0.85M-, 0.9M- and 0.95M-sodium chloride. Treatment at 93.5° has a similar effect except that a fourth new peak is eluted by 0.8M-sodium chloride. In the above experiments, the proportion of material eluted by 0.7M and 0.75M-sodium chloride falls progressively and there is a simultaneous increase in the proportion of material eluted by 0.9M-sodium chloride; that eluted by 0.95M-sodium chloride shows a slower increase. Heating at 100° for 10min. completely destroys the peaks eluted by 0.7M- and 0.75M-sodium chloride, fractions being obtained only with 0.8M-, 0.85M-, 0.9M-, 0.95M- and 1.0M-sodium chloride. The fractions eluted by 0.7M- and 0.75M-sodium chloride from DNA after denaturation at 90° and 93.5° appear to possess some double-helical structure, since they show melting behaviour. However, transition occurs at considerably lower temperatures with material denatured at 93.5°; no explanation for this can be given. No fraction obtained from DNA after being heated at 100° exhibits melting behaviour characteristic of double-stranded DNA, and it is concluded that the material eluted by 1.0M-sodium chloride is not identical with the corresponding fraction obtained from native DNA.

Heat stability of different markers. It can be seen from Table 1 that transforming activity for the arginine marker is retained to a greater extent than

is that for the histidine and tryptophan markers in unfractionated DNA after partial denaturation at 90° or 93.5°. In agreement with this, the fraction carrying residual activity for the histidine and tryptophan markers (eluted by 0.85M-sodium chloride) is observed after denaturation at 90° (Fig. 1b), whereas that carrying residual activity for the arginine marker is only produced after denaturation at a higher temperature (Fig. 1c). The same general tendency is seen in the results obtained with fractions eluted with 0.7M- or 0.75M-sodium chloride after partial denaturation, but the situation is more complex in that the relative stabilities of the markers to heat is different in the different chromatographic fractions.

Nature of material carrying residual transforming activity. It can be seen from Figs. 1(g) and 1(h) that redensaturation of either of the combined fractions *A* and *B* results in the redistribution of the material between the two fractions. Moreover, treatment of inactive denatured fractions in this way regenerates material having transforming activity. This, together with the fact that it is eluted at a different position from native DNA, indicates that residual transforming activity is not associated with material that has escaped denaturation.

Rownd (1965; see also references cited therein) and B. M. Alberts & P. Doty (personal communication) have obtained evidence that the residual activity of heat-denatured DNA from *B. subtilis* is associated with a specific fraction possessing covalent cross-linkages between strands. This is inconsistent with a random interconversion of fractions *A* and *B* and the interchange of such fractions was therefore studied with isotopically labelled material. Preliminary experiments showed that fractions *A* and *B* eluted from methylated albumin, when separately subjected to equilibrium sedimentation in caesium chloride solution, gave single bands at the same positions as components *AL* and *BL* (Fig. 2) respectively. Similarly, corresponding fractions from DNA labelled with ¹⁵N and ²H gave single peaks corresponding with components *AH* and *BH* (Fig. 2). When fraction *A* from heavy DNA was mixed with fraction *B* from light DNA, redensaturated and subjected to equilibrium centrifugation in caesium chloride solution, the heavy component of fraction *A* (component *AH*) predominated over the light component (component *AL*), whereas in fraction *B*, the light component (component *BL*) was present in greater proportion than the heavy component (component *BH*) (Fig. 2, *AH/BL*). Corresponding results were obtained by redensaturing a mixture of light fraction *A* and heavy fraction *B* (Fig. 2, *AL/BH*). These results are not due to failure to attain equilibrium between fractions *A* and *B* because, under the conditions used, the proportions of total fraction

A and total fraction *B* are approximately equal (Figs. 1*g* and 1*h*). It is therefore concluded that the interconversion of fractions *A* and *B* is not entirely a random process. The small proportion of component *AL* appearing in Fig. 2 (*AH/BL*) and of component *AH* in Fig. 2 (*AL/BH*) are believed to have been formed in a random process. The predominance of heavy material in fraction *A* in the first of these experiments and the high proportion of light material in fraction *A* in the second is believed to be due to a specific component in fraction *A* of denatured DNA that preferentially reverts to its fraction of origin on being heated and cooled. The nature of this component cannot as yet be clearly defined. It probably possesses some hydrogen-bonded structure, since it is not observed after denaturation in the presence of formaldehyde (Figs. 1*e* and 1*f*). Under these conditions, no hydrogen-bonded structure would be expected to be formed (Stollar & Grossman, 1962). Since the presence of formaldehyde does not affect the formation of fraction *B*, this latter material is believed to be devoid of double-stranded structure. It cannot be ascertained whether fraction *A* contains any material composed of one heavy and one light strand, because this would be likely to band with component *BL*. Fraction *A* appears to have some native-like properties and this is in agreement with the fact that the buoyant density of the material is in the same range as that found for native DNA (Schildkraut *et al.* 1961). These results are in general agreement with those of Rownd (1965; see also references cited therein), but it cannot be concluded unequivocally that the component in fraction *A* that preferentially appears in the same fraction after redensaturation is responsible for the residual activity. If this is, in fact, the case, then it is suggested that the molecules

carrying the residual activity can adopt a configuration closely similar to that of fully denatured material but are able preferentially to revert to a partly native-like structure.

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