FRACTIONATION OF BACILLUS SUBTILIS DNA BY USE OF POLY-L-LYSINE KIESELGUHR COLUMNS

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DNA has been fractionated by a variety of methods: methylated albumin on kieselguhr (Mandell & Hershey, 1960; Roger, Beckmann & Hotchkiss, 1966; Ayad, Barker & Weigold, 1967); hydroxyapatite columns (Tiselius, Hjerten & Levin, 1956; Miyazawa & Thomas, 1965); benzolyated-naptholyated DEAE cellulose (Sedat, Kelly & Sinsheimer, 1967) and silk fibroin columns (Huh & Helleimer, 1967). This report describes the fractionation of DNA, isolated from the Marburg strain of Bacillus subtilis on poly-L-lysine kieselguhr (PLK) columns.

MATERIALS AND METHODS

DNA from the Marburg strain of B. subtilis was prepared by Marmur's method (1961). A standard column of poly-L-lysine supported on kieselguhr (12" x ½") was prepared as follows. The column was packed in three layers under air pressure of 2lbs./sq.in. The first layer was cellulose powder (Whatman) suspended in 0.4M NaCl containing 0.02M KH_2PO_4, pH 6.7. The second layer consisted of kieselguhr which had been extensively washed in deionised water to remove U.V. absorbing material. This kieselguhr (10 gms dry weight)

was suspended in 0.4M NaCl/KH_2PO_4 buffer (50 ml), boiled and cooled to remove air bubbles. To this suspension a certain concentration of poly-L-lysine HBr (minimum molecular weight approx. 50,000) dissolved in 0.4M NaCl/KH_2PO_4 buffer at a concentration of 10 mg/ml was added (0.8 ml). The final layer was 1 ml of a suspension of kieselguhr (2gms/10ml 0.4M NaCl/KH_2PO_4 buffer). DNA was loaded to the equilibrated column (15ml) at a concentration of 100 ug/ml, under air pressure of 2lbs/sq.in. The column was eluted by a linear gradient of 0.4M NaCl/KH_2PO_4 (150ml) buffer and 4.0M NaCl/KH_2PO_4 (150ml) buffer pH 6.7, using the gradient producing apparatus described by Ayad, Bonsall & Hunt (1967). The flow rate was about 20 ml/hr., the extinction of the effluent was continually measured using L.K.B. uvicord ultra-violet absorptometer (257 mu) and 4 ml fractions were collected. The extinction at 260 mu of the fractions were measured using Unicam SP 500 spectrophotometer. DNA was heat denatured at 100°C for 10 min. at a concentration of 50 ug/ml in 0.4M NaCl/KH_2PO_4 buffer. DNA was sonicated at 4°C for 5 min. at a concentration of 100 ug/ml. The base composition of the dialysed fractions eluted from PLK columns were determined using the method of Frederiq, Oth & Fontaine (1961). It is important to note that the salt concentration must be kept to an absolute minimum, and the DNA concentration be kept low (between 10 to 15 ug/ml).

RESULTS AND DISCUSSION

Native DNA from the prototrophic Marburg strain of B. subtilis was fractionated on poly-L-lysine kieselguhr (PLK) columns, and the results are shown in Fig. 1.

It can be shown from Fig. 1 that the Marburg DNA was fractionated into three peaks, eluted at 0.6, 1.2 and 2.0 M NaCl. The fractions of each peak were combined, exhaustively dialysed against distilled water at 4°C, and adjusted to an O.D. of 0.2 at

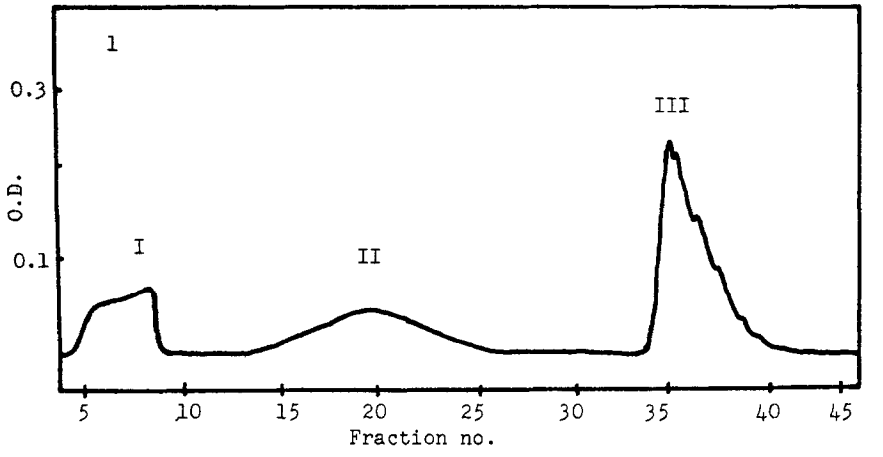

Fig. 1 The continuous elution profile (O.D. 257mu) of the effluents from a PLK column, previously loaded with native DNA (1.5mg in 15ml) and eluted with NaCl linear gradient (see text).

260 mu with distilled water. They were then assayed for base composition using the method of Frederiq, Oth & Fontaine (1961). The results are shown in table 1.

TABLE 1

Base analysis of DNA fractions eluted from PLK columns

Fraction No.	Salt Molarity eluted	%GC
4 - 8 (peak I)	0. 6 - 0.78	44%
16 - 24 (peak II)	1.16 - 1.54	34%
35	2.07	49%
36 - 37 (peak III)	2.12 - 2.15	57%
38 - 41	2.21 - 2.36	68%

It can be seen from Table 1 that each peak has a different average base composition. Peak I (fractions 4 - 8) has a %GC of 44% which is very close to that of native DNA (Native DNA has a %GC of 45% before fractionating, when analysed by this method). Peak II

is a small broad peak with an average %GC of 34% and peak III varies from 49% (2.07M NaCl fraction No.35) to 68% (2.21 - 2.36M NaCl fractions 38 - 41). All the peaks gave a positive result with the diphenylamine assay (Burton, 1956).

To investigate whether base composition was the only basis of fractionation, a sample of DNA was sonicated for 5 min, and another sample heat denatured (see text), and then fractionated on a PLK column. The results are shown in Fig. 2a and 2b.

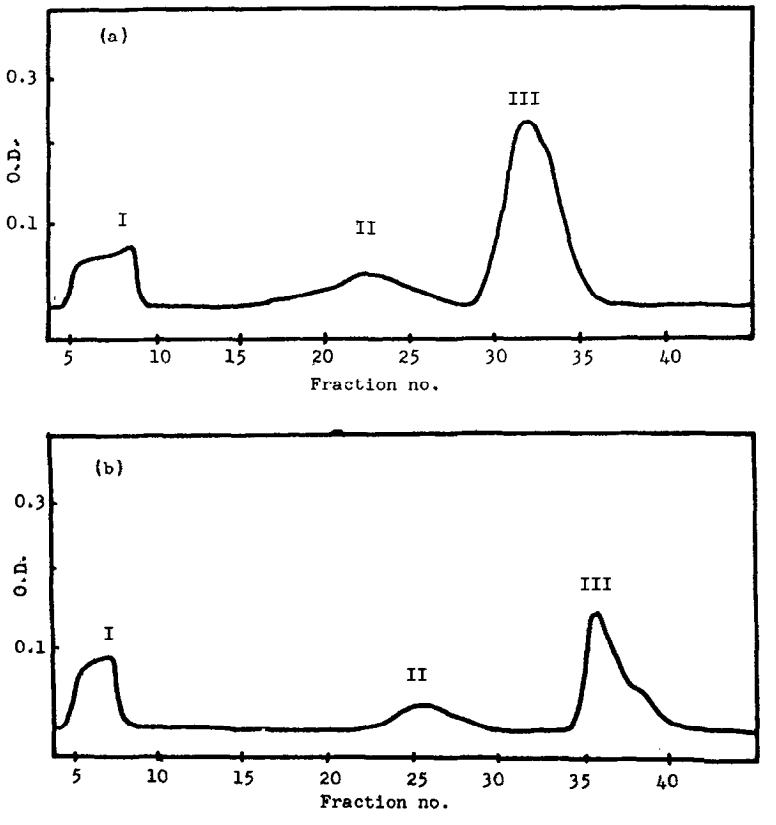

Fig.2 **The continuous elution profiles (O.D. 257mu) of the effluents from PLK columns, previously loaded with (a) sonicated DNA (see text) and (b) heat denatured DNA (see text), and eluted with NaCl linear gradient.**

In Fig. 2a, the DNA is resolved into 3 main components, eluted at 0.6, 1.25 and 1.84M NaCl, and these three peaks correspond in shape, size and salt molarity very closely with those of native DNA. Fig. 2b shows the elution profile of heat denatured DNA, from a PLK column. Three peaks are obtained, eluted at 0.6, 1.35 and 2.07M NaCl, and the similarity of the profile (O.D. at 257 mu) to that of native DNA is noted.

Several controls were also tried; a PLK column was eluted with a NaCl buffer gradient (see text), but without prior loading of DNA. No peaks were observed. A sample of DNA, incubated with DNase in 0.005M MgCl, (5 ug/ml) for 20 mins at 37°C and dialysed against 0.4M NaCl, was also loaded onto a PLK column. On elution it was seen that all three peaks previously noted had disappeared.

In order to determine whether or not the three peaks represent distinct components, the material eluted at 2.0M NaCl was dialysed against 0.4M NaCl/KH_2PO_4 buffer, concentrated by rotory evaporation at 30°C, redialysed against 0.4M NaCl/KH_2PO_4 buffer and rechromatographed on a PLK column (see text). A single fraction was eluted at a concentration of 2.0M NaCl.

It has been concluded that the main basis for fractionation on PLK columns is base composition, since sonication, and the subsequent reduction in molecular weight, did not affect the elution profile. Also heat denaturation, which disrupts the secondary structure of the DNA, gave the same elution profile as that of native DNA.

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