CHROM. 3677

Fractionation of *Escherichia coli* DNA using poly-L-lysine Kieselguhr columns

Fractionation of DNA from complexes formed with poly-L-lysine has been reported previously[1], with increasingly concentrated salt solutions resulting in the separation of differently composed nucleic acid fractions. Recent work in our laboratory has shown that the main basis for the fractionation of the DNA–polylysine complex is that of base composition, unlike various other fractionation techniques recently reported, dependent on either molecular weight or hydrogen bonding properties or both[2]. The fractionation has been developed, using poly-L-lysine supported on Kieselguhr columns, loading with DNA, and determining the continuous elution profile of the columns with a linear gradient of sodium chloride from 0.4 *M*–4.0 *M*[3]. This communication reports the stepwise fractionation of DNA isolated from *Escherichia coli* strain B.

DNA was isolated using the method of MARMUR[4]. Poly-L-lysine Kieselguhr columns were set up as described previously[3]. DNA at a concentration of 100 μg/ml (15 ml) in 0.4 *M* NaCl/0.02 *M* KH_2PO_4 buffer was loaded onto the column under air pressure of 2 lb./sq.in. The column was eluted in a stepwise fashion with increasingly concentrated solutions of sodium chloride from 1.0 *M* to 4.5 *M*. Thirty millilitres of each concentration were found to be sufficient to complete the elution of a DNA peak. The flow rate was adjusted to about 20 ml/h, and 3 ml fractions were collected. The DNA in each fraction was determined from the extinction at 260 mμ. The elution profile is summarised in Fig. 1.

It is seen from Fig. 1 that the *E. coli* DNA was fractionated into four peaks, eluted at 1.0 *M*, 1.5 *M*, 2.5 *M* and 3.0 *M* NaCl. The fractions of each peak were combined, exhaustively dialysed against distilled water at 2°, and adjusted to an optical density of about 0.2 at 260 mμ with distilled water. They were then assayed

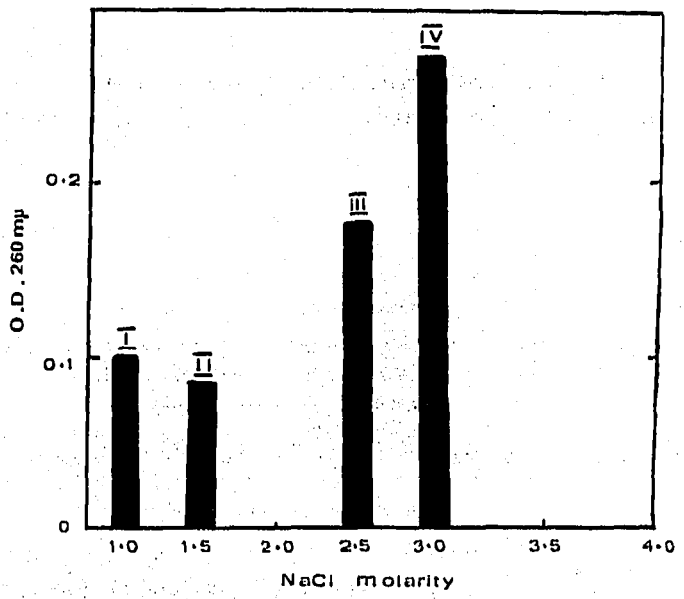

Fig. 1. Stepwise elution profile of the effluents from a PLK column, previously loaded with native *E. coli* DNA (1.5 mg in 15 ml 0.4 *M* NaCl buffer), and eluted with sodium chloride solutions of increasing concentrations from 1.0 *M* to 4.5 *M* (see text).

for base composition using the method of FREDERICQ, OTH AND FONTAINE[5]. The results are shown in Table I. The base composition of native DNA before fractionation when analysed by this method was 52 % GC.

TABLE I

BASE ANALYSIS OF DNA FRACTIONS ELUTED FROM PLK COLUMNS

Peak No.	*Salt molarity eluted*	*% GC*
I	1.0 *M*	53
II	1.5 *M*	40.5
III	2.5 *M*	45.5
IV	3.0 *M*	64

It can be seen from Table I that each of the peaks has a different average base composition. Peak I has a % GC of 53 which is very close to that of native, unfractionated DNA. Peaks II and III have a % GC substantially lower than native DNA, while that of peak IV is substantially higher than native DNA. It is also noted that the % GC of the peaks increases with salt concentration from peak II to peak IV, DNA with low GC content eluting with lower NaCl concentration.

The results obtained using the PLK column were compared with those obtained by extraction of a poly-L-lysine–DNA complex with increasingly concentrated sodium chloride solutions. 2.0 mg DNA in 15 ml 0.02 *M* KH_2PO_4/0.4 *M* NaCl buffer (pH 6.7) was mixed with 1.6 ml polylysine (10 mg/ml). A precipitate which was formed immediately was centrifuged at 12,000 g for 10 min, and the pellet washed twice with 0.4 *M* NaCl buffer. The pellet was suspended in 15 ml 1.0 *M* NaCl using a Potter homogeniser, and then centrifuged at 12,000 *g* for 10 min. This procedure was repeated twice at each concentration of sodium chloride. This technique did not give an exhaustive extraction, but gave DNA fractions suitable for qualitative evaluations. The fractions at each concentration were combined. Analysis of DNA content, and base composition of the fractions was carried out as described above. The results are summarised in Table II.

The essential similarity of this extraction pattern to that obtained on elution

TABLE II

BASE ANALYSIS OF DNA FRACTIONS EXTRACTED FROM DNA–POLYLYSINE COMPLEXES

Concentration of NaCl	*O.D. at 260 mμ*	*Base content (% GC)*
1.0 *M*	0.142 (peak I)	51
1.5 *M*	0.135 (peak II)	43
2.0 *M*	0.008	—
2.5 *M*	0.193 (peak III)	47
3.0 *M*	0.387 (peak IV)	60
3.5 *M*	0.020	—
4.0 *M*	0.030	—

from a PLK column is noted. This serves to confirm the validity of DNA fractionation on PLK columns, and also to emphasize the convenience engendered by the use of a column.

The results presented here indicate that DNA from *Escherichia coli* strain B is fractionated into peaks of different base composition on poly-L-lysine Kieselguhr (PLK) columns, DNA with low GC content eluting at low NaCl concentrations, and DNA with higher GC content eluting with increasingly concentrated NaCl solutions. These results serve as a confirmation of those obtained previously[3], using DNA from the Marburg strain of *Bacillus subtilis*.

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