Note

Analysis of Base Composition of Sequenced DNA's by High Performance Liquid Chromatography of Their Nuclease P₁ Hydrolysate

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The base composition of DNA (G + C content) is one of the most important criteria for chemical taxonomy of microorganisms. Recently a direct method has been developed for measuring the base composition of DNA. The method consists of complete hydrolysis DNA with nuclease P₁ and high performance liquid chromatography (HPLC) of the hydrolysate. In this note, this method will be proved to be highly accurate by comparing observed and calculated data on the base composition of several sequenced DNA's.

A *penicillium* enzyme, nuclease P₁,¹⁾ degrades not only single strand RNA into four ribonucleotides but also heat-denatured single strand DNA into four deoxyribonucleotides. Therefore, the base composition of DNA can be measured directly by HPLC of its nuclease P₁ hydrolysate.^{2~4)} This direct method can be applied even if the DNA sample contains RNA or the amount of DNA is very small.⁵⁾ Furthermore, this method requires neither expensive instruments nor expert technique.

The purpose of this study is to prove the absolute accuracy of the method by analyzing several sequenced DNA's; a coliphage lambda DNA (48502 bp), a plasmid vector pUC19 (2686 bp), and a cloned yeast DNA (2171 bp).

The DNA sequence of lambda was reported by Sanger *et al.*,⁶⁾ and pUC19 was well characterized by Yanisch-Perron *et al.*⁷⁾ Both lambda DNA and pUC19 DNA prepared by Takara Shuzo Co., Ltd. are commercially available.

The cloned yeast DNA we analyzed is a *Hind*III-fragment of *Saccharomyces cerevisiae* chromosome DNA, and contains the glyceraldehyde-3-phosphate dehydrogenase (GAP-DH) gene. It was first cloned and sequenced by Holland and Holland.⁸⁾ We have independently cloned this fragment and inserted it into the *Hind*III site of pUC19. The resultant plasmid was named pUC-GAP. Plasmid pUC-GAP DNA was prepared as described by

Birnboim and Doly⁹⁾ using *E. coli* JM109 as a host cell. Finally, the ccc (covalently closed circular) from DNA of plasmid was isolated by CsCl-EtBr centrifugation. To isolate the 2171 bp-DNA fragment, pUC-GAP DNA was digested with *Hind*III and fractionated by agarose gel electrophoresis. After electrophoresis, the 2171 bp-DNA fragment was electroeluted from agarose gel by the method of Mcdonell *et al.*¹⁰⁾

The DNA samples were dissolved in distilled water (20 µg/ml). These DNA solutions were heated at 100°C for 5 min, and then rapidly chilled in an ice bath. One-tenth ml each of the denatured DNA solutions was mixed with 0.01 ml of nuclease P₁ (2 units/ml of 40 mM sodium acetate buffer containing 0.2 mM ZnCl₂, pH 5.3), and was incubated at 50°C for 1 hr. Both the nuclease P₁ hydrolysate and the standard solution consisting of equimolar amounts of sodium salt of dCMP, dAMP, dGMP, and dTMP were separately applied to HPLC.

The G + C content (mol%) was calculated as follows:

$$G + C(\text{mol}\%) = \frac{Cx/Cs + Gx/Gs}{Cx/Cs + Ax/As + Gx/Gs + Tx/Ts} \times 100$$

where Cx (Ax, Gx, or Tx) was the peak area of dCMP (dAMP, dGMP, or dTMP) in the nuclease P₁ hydrolysate, and Cs (As, Gs, or Ts) was the peak area of dCMP (dAMP, dGMP, or dTMP) in the standard solution.

Results are shown in Table I. The analyzed value were essentially the same as those calculated from the DNA

TABLE I. MEASUREMENT OF THE BASE COMPOSITION OF SEQUENCED DNA'S

HPLC was done under the following conditions: column CHEMCOSORB 7-ODS-H, 4.0 × 250 mm; temp., 30°C; mobile phase, 10 mM potassium phosphate buffer (pH 7.0).

DNA (size)	Base com- ponent	Base number	Content (mol%)	
			Calculated	Analyzed
Lambda (48502 bp)	A	12336 + 11988	25.1	24.9
	G	12818 + 11360	24.9	24.5
	C	11360 + 12818	24.9	25.3
	T	11988 + 12336	25.1	25.3
	G + C		49.8	49.8
pUC19 (2686 bp)	A	666 + 659	24.7	24.8
	G	686 + 675	25.3	25.2
	C	675 + 686	25.3	26.2
	T	659 + 666	24.7	23.8
	G + C		50.7	51.4
GAP-DH (2171 bp)	A	647 + 645	29.8	30.9
	G	417 + 462	20.2	20.3
	C	462 + 417	20.2	19.4
	T	645 + 647	29.8	29.5
	G + C		40.4	39.7

sequence. The result indicates that this method is quite accurate for measuring the base composition of DNA.

In this study, we were able to analyze a slight amount of a DNA fragment electroeluted from agarose gel. Some microorganisms have plasmids with large size or high copy number, whose G+C content is different from that of chromosome DNA. For measuring the accurate base composition of chromosome DNA (or plasmid DNA), separation and isolation of chromosome DNA (or plasmid DNA) are essential. This study apparently indicates that we can accurately measure the base composition of microbial chromosome DNA and/or plasmid DNA, using agarose gel electrophoresis.

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