HETEROGENEITY OF *STREPTOMYCES* DNA

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Received 26 April 1977

1 . Introduction

Reassociation kinetic analysis of DNA led to the discovery of some important differences between prokaryotic and eukaryotic DNA. Ten years ago it was found that some of the nucleotide sequences of eukaryotic DNAs were repeated several tens to a million times [1]. Repetitive sequences in prokaryotic DNAs have not been found by the conventional methods. Bacause of the second-order kinetics of reassociation of the prokaryotic DNAs they were considered as consisting of non-repeated sequences only.

Until recently it was thought that the ribosomal RNA genes are the only repeated DNA sequences in prokaryotes (see [2]). Chiscon and Kohne [3] revealed a small fraction of repeated DNA in *Escherichia coli* cells and presented evidence for its extrachromosomal (plasmid) origin. Recently, a fast renaturing fraction in *E. coli* DNA representing 2% the whole genome was described [4,5]. Basing on the properties of this fraction the authors exclude its plasmid origin. These data give reason to assume that repetitive sequences may also exist in the genome of some other prokaryotes. In this respect, studies on DNA of the highest prokaryotes are of greatest interest.

This paper describes the results of studying the heterogeneity of DNA of *Streptomyces coelicolor* A3(2) which belongs to the highest prokaryotic organisms with a genome 2–3-times larger than that of *E. coli* [6]. The data obtained led to the conclusion that about 2% of the genome of *Streptomyces coelicolor* consist of 'foldback' DNA and about 5% are repetitive sequences presented in about 4 copies per haploid genome on the average.

Abbreviations: NaPB, sodium phosphate buffer (an equimolar mixture of NaH_2PO_4 and Na_2HPO_4, pH 6.8); HAP, hydroxyapatite; Cot, the product of the DNA concentration (mol nucleotides/liter) and time (s)

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2. Materials and methods

2.1. *Isolation of DNA*

Streptomyces coelicolor A3(2), strains 39 NF and 165 IF, *E.coli* K12 and *Shigella boydii* 13 were used for the isolation of DNA by the MUP method [7]. Prior to lysis the lyophilized mycelium cells of *Streptomyces* were ground with quartz sand to disrupt the cell wall. DNA was sonicated in an MSE ultrasonic power unit for 30 s at 1 A to obtain fragments of 200 base pairs average length. Before every step of reassociation the DNA samples were purified from bivalent cations by passing through a column of SE-Sephadex C-25 (Na^+-form).

2.2. *Kinetics of reassociation*

Precipitated fragmented DNA was dissolved in distilled water to concentration of 5–6 mg/ml. The

solution was heated in a boiling water bath for 10 min, adjusted to 0.14 M NaPB with 0.7 M NaPB and divided into samples of 5 $A_{260\ nm}$-units DNA in sealed glass tubes. The samples were then heated at 160°C for 10 min and incubated to appropriate Cot at 73°C. This temperature is optimal for reassociation in this buffer of the *Streptomyces* DNA which contains 71% GC [8]. After incubation, the DNA preparations were loaded onto HAP columns pre-equilibrated with 0.14 M NaPB at 73°C, the single-stranded DNA was eluted with 0.14 M NaPB and the double-stranded DNA with 0.48 M NaPB at the same temperature.

2.3. *Thermal chromatography on HAP*

Melting of the native fragmented DNA and of the duplexes of reassociated DNA was carried out on HAP by raising the temperature in 3–5°C steps and eluting the single-stranded DNA with 0.14 M NaPB.

To separate preparatively the DNA duplexes according to their thermal stability fragmented *Streptomyces* DNA was allowed to reassociate to Cot 10 in 0.14 M NaPB at 60°C instead of 73°C. The thermal elution was performed at 75°C in two steps: the material melted between 60–75°C was eluted with 0.14 M NaPB and the duplexes which remained stable were eluted with 0.48 M NaPB.

2.4. *Sedimentation analysis of DNA*

$s_{20,w}$-Values were determined by analytical ultracentrifugation in alkaline solution according to Studier [9].

2.5. *Base composition of DNA*

Base composition analysis was carried out by paper chromatography after hydrolysis of DNA with 85% HCOOH at 175°C for 30 min.

3. Results and discussion

For studying the heterogeneity of *Streptomyces* DNA two experimental approaches were applied:

(i) *Streptomyces* DNA was allowed to reassociate under low experimental criterion (0.14 M NaPB and 60°C – a temperature about 40°C lower than T_m of the native *Streptomyces* DNA). Under these conditions a larger amount of imperfect duplexes was formed. As shown [10] the analysis of their thermal stability may be a useful approach to detect the existence of similar nucleotide sequences in the DNA studied, i.e., to reveal the presence of DNA heterogeneous in respect to similarity.

(ii) Separation of a DNA fraction enriched in repetitive sequences by reanealing to a relatively low Cot (Cot 0.5), an approach similar to that applied by Kato et al. [5] to *E. coli* DNA.

As fig.1 shows, the melting of the duplexes of *Streptomyces* DNA follows a biphasic curve with a well-expressed transition between 75–80°C. However, this was not observed in the melting curves of *E. coli* and *Shigella* DNA duplexes, formed also upon reassociation under low experimental criterion (fig.2). On the other side such a transition is typical for duplexes of eukaryotic DNAs containing repetitive [11] or similar sequences [10].

The biphasic melting curve was taken as a basis for separating *Streptomyces* DNA into two fractions: fraction I (DNA I), containing the duplexes melted to 75°C and fraction II (DNA II), containing the duplexes which remained stable to this temperature.

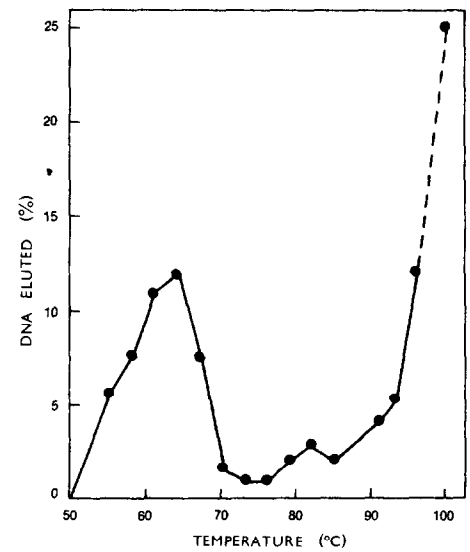

Fig.1. Derivate melting curve of duplexes of *Streptomyces* DNA after reassociation in 0.14 M NaPB at 60°C to Cot 10. The dashed line indicates non-melted duplexes eluted with 0.48 M NaPB.

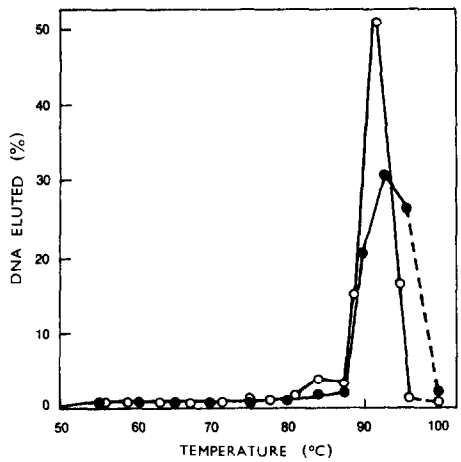

Fig.2. Derivative melting curve of duplexes of *E. coli* K12 DNA (○———○) and *Shigella boydii* DNA (●———●) after reassociation in 0.14 M NaPB at 50°C to Cot 5.

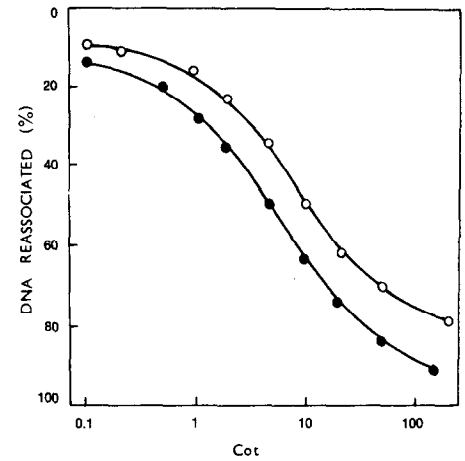

Fig.4. Kinetics of reassociation of the two fractions of *Streptomyces* DNA. Symbols as in fig.3.

The sedimentation analysis gave equal $s_{20,w}$- values (4.8 S) for the DNA of the two fractions, thus excluding the possibility that the differences in the thermal stability of the duplexes were due to differences in the molecular weight of the DNA fragments.

The sequences of the two fractions were compaired in respect to thermal stability of the duplexes formed upon reannealing and to the rate of reassociation. It was found that the sequences of DNA II gave more stable duplexes (fig.3) and ressociated about two times faster than those of DNA I (fig.4).

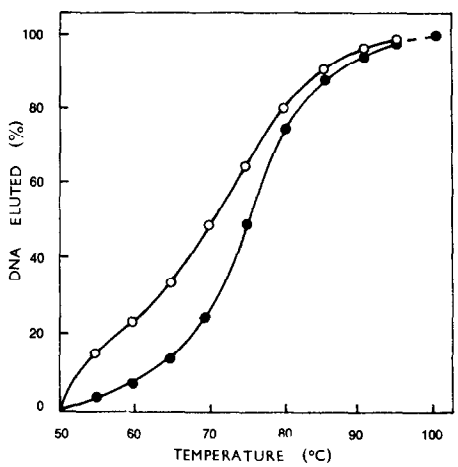

Fig.3. Integral melting curves of the duplexes of the two fractions of *Streptomyces* DNA: DNA I (○———○) and DNA II (●———●).

It should be mentioned that the fractionation of DNA by reassociation and thermal chromatography may be accompanied by additional fragmentation of the DNA (data not shown). This finding is in good agreement with the data of Kato et al. [5], who have studied in detail the changes in the molecular weight during fractionation of *E. coli* DNA. On the other hand, it is known that the thermal stability and the reassociation rate depend inversely proportional on the fragment size of the DNA [12,13]. This may explain the decrease in the thermal stability and in the rate of reassociation of the two DNA fractions as compared with those of total fragmented DNA (compare fig.1 with fig.3 and fig.4 with fig.5).

The high GC content of the *Streptomyces* DNA gave reason to assume that the different thermal stability of the DNA duplexes might be due to differences in the base composition, DNA II being enriched in GC. However, this assumption was ruled out by the base composition analysis which gave almost the same GC content (69–71%) for both DNA fractions and for the total DNA as well.
The most probable explanation of all these results is that DNA II is enriched in repeated, non-diverged nucleotide sequences.

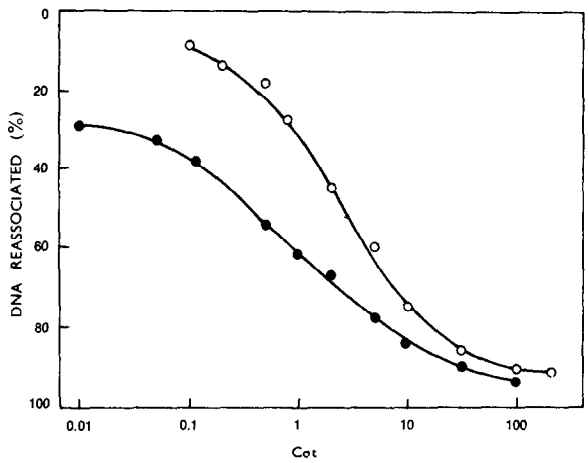

Fig.5. Kinetics of reassociation of the total *Streptomyces* DNA (o——o) and of fraction Cot 0.5 (•——•).

The presence of repetitive DNA sequences in the *Streptomyces* genome was checked directly by studying a DNA fraction which represented about 7% of the total DNA obtained after two cycles of reassociation to Cot 0.5. The reassociation analysis (fig.5) showed that even at Cot 0.01 30% of the material in this fraction was retained on HAP as double-stranded DNA. It seems very likely that it represents 'foldback' DNA. The rest of the material reassociated about 4-times faster than the total *Streptomyces* DNA. Since the GC content of this fraction was found to be the same as that of the total DNA (69–71%) it was concluded that fraction Cot 0.5 consisted of sequences repeated 4 times on the avarage. Probably the actual number of copies for different sequences varies in a wide range.

The general conclusion from the results reported is that *Streptomyces* DNA is considerably heterogeneous. Unlike typical prokaryotic DNAs, it contains a significant amount of similar nucleotide sequences giving a high percentage of mismatched duplexes upon reassociation under low criterion. In this respect *Streptomyces* DNA resembles eukaryotic DNAs. It is also concluded that the *Streptomyces* genome contains about 2% 'foldback' DNA and up to 5% repetitive sequences.

Acknowledgements

The authors are thankful to Professor Angelo Carere from the Istituto Superiore di Sanità, Roma, for supplying us with *Streptomyces coelicolor* lyophilized mycelia and to Professor E. Dore from the same Institute for the accurate sedimentation analysis.

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