ISOLATION OF DOUBLE-HELICAL REGIONS RICH IN GUANINE-CYTOSINE BASE PAIRING FROM BACTERIOPHAGE f1 DNA

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Summary: Bacteriophage f1 DNA was digested with single-strand specific nuclease S1 at 30°for 5 hours. About 1.9% of the treated DNA was resistant to S1 under the conditions and the fraction was termed core fraction. The core fraction was characterized by high(G+C)content and exhibited reversible thermal denaturation. It was considered on these bases that the f1 DNA might be looped out at the GC-rich regions.

We reported in a previous paper(1) that a double-helical fraction rich in adenine-thymine base pairing(AT-rich core) could be obtained from bacteriophage f1 DNA by successive digestion with DNase K2 and nuclease S1. In contrast to this, Schaller, Voss, and Gucker(2) succeeded recently in the isolation of GC-rich core fraction from bacteriophage fd DNA by successive digestion with endonuclease of Neurospora crassa and exonuclease I of Escherichia coli. Since fd is a bacteriophage related closely to f1, we thought that a fraction rich in guanine and cytosine base pairing might also be obtained from f1 DNA by digestion with nuclease S1. S1 is an endonuclease which specifically attacks single-stranded portion of a DNA molecule(3).

MATERIALS AND METHODS

Bacteriophage f1 DNA was prepared as described previ-

ously(1). The final DNA preparation formed a single sharp peak in a CsCl density of 1.725 g/cm^3, a value approximately that reported by Rossomando & Zinder(4). Nuclease S1 was kindly supplied by Dr. T. Ando of this laboratory, and its incapability of attacking double-stranded DNA was confirmed by use of E. coli DNA as the substrate. The digestion of f1 DNA with nuclease S1 was performed in a mixture consisting of 5.0 ml of f1 DNA solution(2 mg/ml), 0.5 ml of 0.2 M acetate buffer(pH 5.0), 1.0 ml of 1 M NaCl, 1.0 ml of nuclease S1 solution(1.2x 10^5 units/ml), and 2.5 ml of distilled water. During incubation at 30°, the S1 activity was enforced by addition of fresh S1 solution at indicated times. When 5 hours passed, the reaction was terminated by addition of an equal volume of phenol saturated in advance with standard saline citrate solution(SSC) and the mixture was shaken for 5 min. Phenol in aquous layer was removed with ether. The digest thus prepared was passed through a Sephadex G-50 column (1.2 x 50 cm) equilibrated with SSC.

Molecular size of the filterable fraction was estimated from its sedimentation profile in a sucrose density gradient and melting profile of the same fraction was recorded by use of a Hitachi Perkin-Elmer 139 type spectrophotometer equipped with a Komatsu thermostat.

RESULTS

Fig. 1-a shows a result of the Sephadex G-50 filtration stated above. Among the two fractions obtained, the first fraction (peak I) contained materials resistant to nuclease S1. Fig. 1-b shows yields of the nuclease S1-resistant fraction as a function of digestion period.

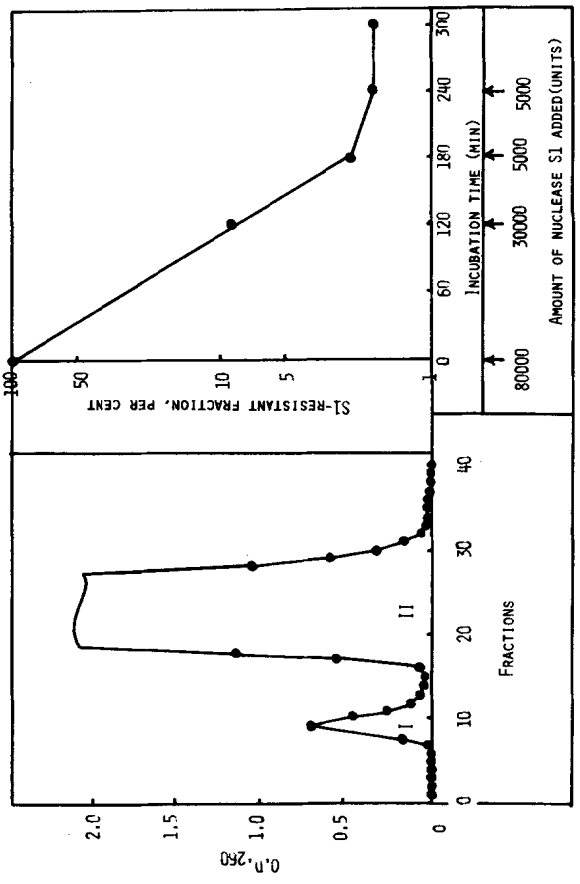

Fig. 1 Nuclease S1-resistant fraction in f1 DNA.
a): Elution profile from a Sephadex column. 10 mg of f1 DNA was digested with nuclease S1 (1.2×10^5 units) at 30° for 5 hours and then loaded on a Sephadex G-50 column. Elution was made with standard saline citrate solution.
b): Per cent of fraction I in Fig.1-a as a function of digestion period. Indicated amount of nuclease S1 was added newly to the reaction mixture at the arrowed time.

This figure indicates that about 1.9 % of the f1 DNA are not degraded extensively even when exposed to an excess amount of nuclease S1. Moreover, the 1.9 % fraction was resistant to nuclease S1 even after it was heated at 100° for 10 min or treated with 0.1 M KOH. Hereafter, the peak I fraction will be referred to as core fraction.

Fig. 2 shows a sedimentation profile of the core fraction in a sucrose density gradient. By comparison with the profiles of E. coli transfer RNA (purchased from

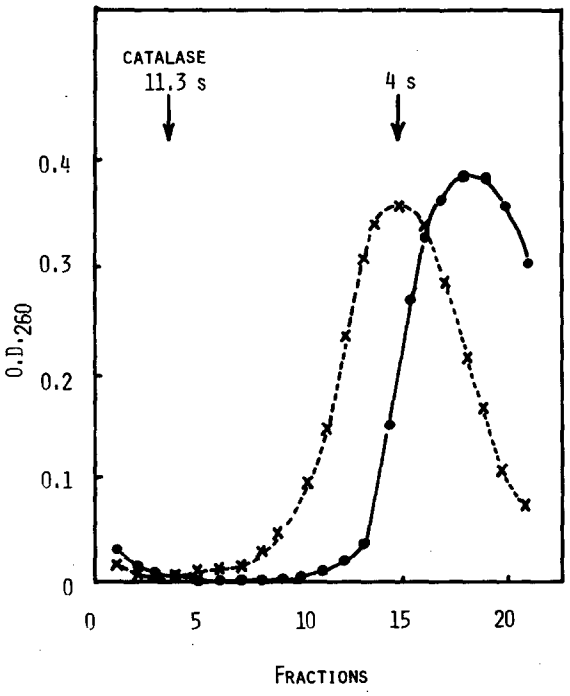

Fig. 2 Sedimentation profiles of core fraction and E. coli transfer RNA in sucrose density gradient.
A sample which had been dissolved in 0.1 ml of distilled water was layered on 4.5 ml of sucrose density gradient (3 to 15 % sucrose supplemented with 10 mM Tris-HCl buffer, pH 7.0, and 10 mM $MgCl_2$) and subjected to centrifugation in a Hitachi model 55 PA ultracentrifuge using RPS 40 rotor at 38000 rpm for 14.5 hours at 10°. 0.22 ml fractions were collected from the bottom of the tube and $O.D._{260}$ was measured in a Hitachi Perkin-Elmer 139 type spectrophotometer.
——●——, core fraction; ------×------, E. coli transfer RNA.

Miles Lab.) and catalase preparation generously supplied by Dr. K. Horikoshi, sedimentation constant of the core fraction was estimated to be about 1.8 s, corresponding to the size of 35-40 nucleotides. Since yield of the core fraction is 1.9 % of the total f1 DNA (105 out 5,500 nucleotide residues(5)), the core fraction is assumed to consist of three S1-resistant fragments.

The solid curves in Fig. 3 show melting profiles of the core fraction and AT-rich core fraction reported previously(1). Tm is 70° and 45°, respectively. The dotted curve shows renaturation of the core fraction. The $O.D._{260}$ returns to its original level. This reversible thermal

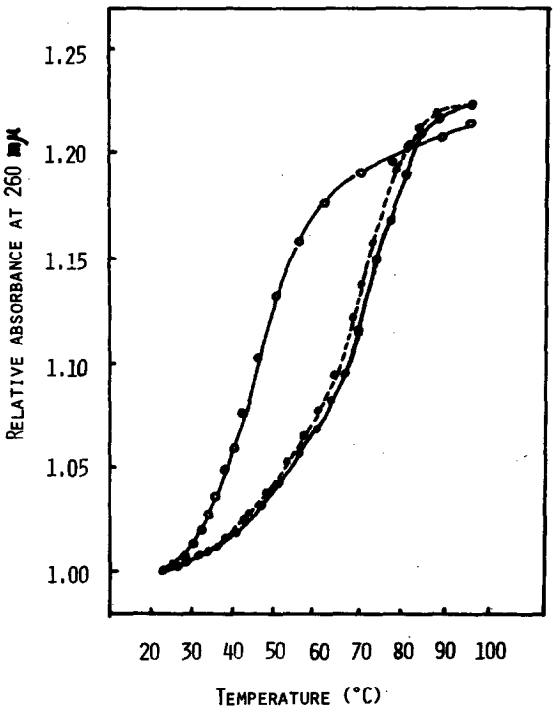

Fig. 3 Melting profiles of AT-rich core and core fraction. About 60 μg sample was dissolved in 3 ml of SSC, and changes in $O.D._{260}$ were measured upon heating or upon cooling at a rate of 1°/min.——o——,denaturation curve of AT-rich core;——●——, denaturation curve of core fraction;----●----,renaturation curve of core fraction.

denaturation was observed even at a lower concentration of core DNA(4 μg/ml).

Table 1 shows base compositions of the core fraction, AT-rich core fraction and native f1 DNA. The core fraction is characterized by high(G+C)content. Moreover, molar per cent of guanine is approximately that of cytosine and molar per cent of adenine is approximately that of thymine.

BASE COMPOSITION

	A	T	G	C	(A+T)/(G+C)	Pu./Py.
NATIVE f1 DNA*	24.7	34.2	20.3	20.8	1.43	0.82
AT-RICH CORE*	29.3	32.0	19.8	18.9	1.58	0.96
CORE FRACTION**	19.1	22.2	31.5	27.2	0.70	1.02

Table 1 Base composition of native f1 DNA, AT-rich core and core fraction.
The analysis was made by the method of Wyatt(6).
*Cited from previous paper(1).
**Average of four independent preparations.

DISCUSSION

The above stated result suggests that the core fraction may have a double-helical structure. This double-helical core may not be in the form of ordinary double-stranded DNA but be in the form of hairpin; the observation that the core fraction exhibits reversible thermal denaturation supports the latter alternative. We think that the core fraction is not contaminated with any significant amount of AT-rich core reported previously(1).

When 100 μg AT-rich core was digested with nuclease S1 (total 1.2 x 10^3 units) at 30° for 5 hours, there remained no detectable amount of core.

The double-helical core reported previously(1) was rich in adenine-thymine base pairing and the core fraction reported in this paper is rich in guanine-cytosine base pairing. Thus, bacteriophage f1 DNA appears to involve AT-rich and GC-rich paired regions. The base paired structures may be formed, by intrastrand force, between two complementary base sequences running in opposite directions. The presence of base paired structure in bacteriophage R17 and Qβ RNA's was reported recently by several investigators(7-10).

The experimental results obtained with the plus strand suggest that the replicative form itself may have both AT-rich and GC-rich regions in the structure.It is supposed also that a part of the double-helical structure at the AT-rich region may open or unwind at relatively low temperature(Fig.3). Validity of this supposition as well as the binding of RNA polymerase to the AT-rich and GC-rich cores is under investigation.

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