THE USE OF ANTIBIOTICS ACTINOMYCIN D AND DISTAMYCIN A FOR MAPPING OF PHAGE LAMBDA HINDIII FRAGMENTS

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1. Introduction

The antibiotics distamycin A and actinomycin D interact with double-stranded DNA forming non-covalently bound complexes. This interaction is rather selective: distamycin forms complexes preferentially with d(A/T) rich regions [1] whereas actinomycin binds d(G/C) rich regions [2] of DNA. This property has offered a possibility to use these antibiotics for effective and highly selective protection of the cleavage sites recognized by restriction endonucleases [3,4]. The recognition site of endo R. HindIII [5]

5'AAGCTT
3'TTCGAA

may be protected by both actinomycin D and distamycin A as long as it contains TT and GC sequences favourable for interaction of distamycin and actinomycin with template DNA. It has been demonstrated [4] that different EcoRI recognition sites on lambda DNA may be effectively blocked by different antibiotic concentrations due to the peculiarities of the environment of different recognition sites. In the present paper it is shown that certain endo R. HindIII sites of phage lambda DNA may be effectively protected with actinomycin whereas some other sites are protected with distamycin. This allows us to obtain a set of larger overlapping fragments of DNA (hereafter we shall term them as distamycin and actinomycin protected fragments) and to establish the location of all HindIII fragments on the physical map of phage lambda DNA. The data reported here and earlier [3,4] lead to a conclusion that the antibiotics may be useful for DNA mapping and for obtaining new vector molecules.

2. Materials and methods

2.1. *Bacteriophage DNA*

Lambda c1857s7 DNA was obtained from purified phage particles by phenol extraction [6].

2.2. *Restriction endonuclease and digestion*

Endo R. HindIII was purified according to R. J. Robert's unpublished procedure with insignificant modifications. The enzyme was free of non-specific nucleases. The standard incubation mixture contained 10 mM Tris–HCl, pH 7.5, 10 mM $MgCl_2$, 10 mM mercaptoethanol, 0.5 μg of lambda DNA, 1–2 μl of restriction endonuclease, distamycin A or actinomycin D in 10 μl volume. The reaction mixture was incubated for 1–3 h at 37°C. The reaction was terminated by adding EDTA. The digested samples were heated for 5 min at 65°C and then rapidly cooled in ice to prevent interaction of the cohesive ends.

2.3. *Agarose gel electrophoresis*

The 0.5–1.0% agarose gels (Sigma) were used for separation of the DNA fragments. The gels were prepared according to Helling et al. [7] and electrophoresis was carried out in a tube-type apparatus at 2.0–4.0 V/cm for about 14–17 h. The gels were stained in electrophoresis buffer containing 0.5 μg/ml ethidium bromide. The DNA fragments were visualized by long wave ultraviolet light (C50 Transilluminator, Ultraviolet Product, San Gabriel, Calif.). The size

of actinomycin and distamycin protected fragments was calculated by their electrophoretic mobility. The lambda DNA fragments generated by endo R. HindIII were used as inside molecular weight standards according to the procedure described earlier [3].

2.4. *Distamycin and actinomycin*

For the present experiments we have used distamycin A (Calbiochem) and actinomycin D (Reanal). The concentrations of the antibiotics in solution were calculated basing on their optical density. The molar absorption coefficient of distamycin A is 30 000 at 302 nm [1], and that of actinomycin D is 24 000 at 445 nm [2].

3. Results and discussion

Endo R. HindIII cleaves lambda phage DNA into seven fragments designated by letters A–G in the order of their decreasing size. These fragments were previously ordered within the map of lambda genome [8,9] with the aid of lambda phage deletion mutants and by means of digestion of lambda DNA with both endo R. HindIII and endo EcoRI. In this work the overlapping HindIII fragments were obtained by use of actinomycin D and distamycin A.

The addition of actinomycin produces partial protection of certain HindIII recognition sites and appearance of actinomycin protected fragments (fig.1(I)). To determine more accurately the molecular weight of larger actinomycin fragments the electrophoresis was carried out in a 0.5% agarose gel, and lambda DNA was added to the HindIII-digest as a marker (fig.1(II)). The effect of site protection depends on the antibiotic concentration. Figures 1(I) and 1(II) show that fragments 1A and 3A appear at low actinomycin concentrations (0.3×10^{-4} M) whereas higher actinomycin concentrations protect other HindIII sites giving rise to fragments 4A and 2A. Having determined the molecular weights of actinomycin protected fragments (see table 1), the order of their appearance

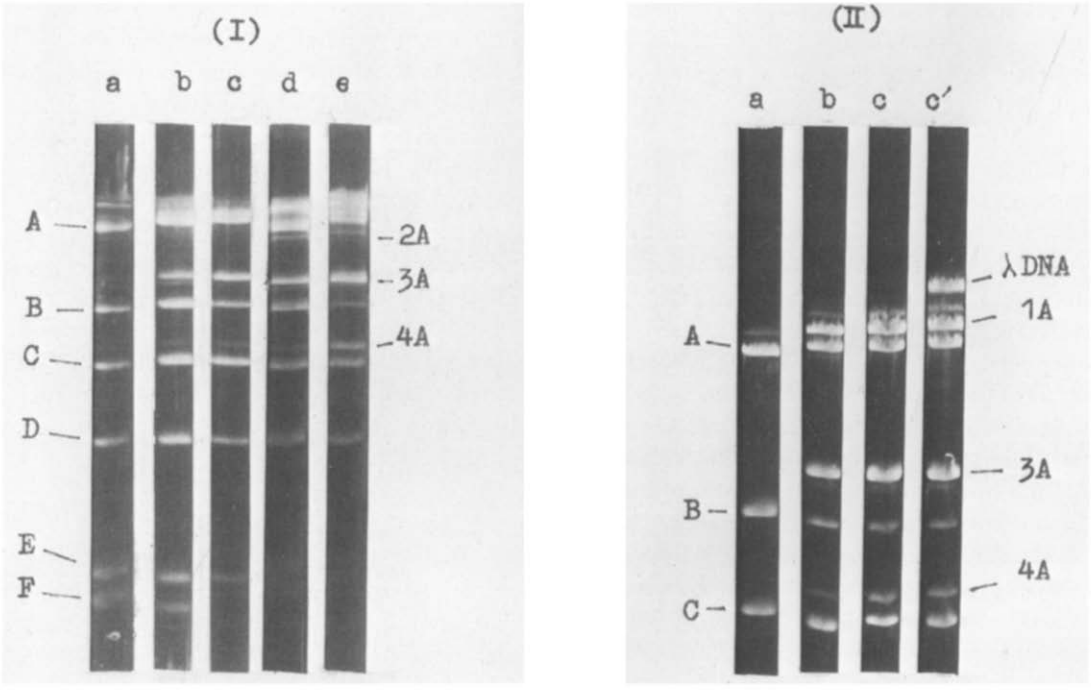

Fig.1. Electrophoretic pattern of lambda phage DNA digested with endo R. HindIII in the presence of actinomycin. (I) Electrophoresis carried out at 2.0 V/cm for 16 h; 1% agarose gel. Concentrations of actinomycin D in reaction mixture (10^{-4} M): (a) none; (b) 0.3; (c) 0.5; (d) 0.7; (e) 1.0. (II). Electrophoresis carried out at 3V/cm for 14 hrs; 0.5% agarose gel. (a)–(c) the same as in I; (c') the same as (c) plus lambda phage DNA added as a marker.

Table 1
Molecular weight and composition of distamycin and actinomycin protected fragments of DNA (λc1857s7 DNA cleaved by endo R. HindIII)

DNA fragments	Mol. wt. × 10^{-6} experimental	Composition	Mol. wt. × 10^{-6} calculated
Actinomycin fragments (see fig.1)			
1A	15.70 ± 0.55	A,F	15.88
2A	12.14 ± 0.35	B,C,E,G	12.04
3A	7.66 ± 0.20	B,E	7.56
4A	4.50 ± 0.12	C,G	4.48
Distamycin fragments (see fig.2)			
1D	16.35 ± 0.50	B,C,D,E,F,G	16.15
2D	9.35 ± 0.30	B,E,F,G	9.17
3D	9.00 ± 0.28	B,E,F	8.82
4D	7.15 ± 0.20	C,D	7.33
5D	6.34 ± 0.10	B,G	6.36

and the order of disappearance of the original fragments we identified fragments 1A and 3A as AF and EB, and fragments 4A and 2A as GC and EBGC, respectively.

The electrophoretic patterns of endo R. HindIII fragments of lambda DNA obtained in the presence of distamycin A are given in fig.2. It is obvious that the increase of distamycin concentration has induced the disappearance of the original fragments as well as the formation of larger distamycin protected fragments of DNA. At relatively high concentrations of distamycin ($> 1.5 \times 10^{-4}$ M) the enzyme splits the phage lambda DNA into two fragments (see fig.2c,d). One of them is fragment A, and the other one is fragment 1D which can be identified by its molecular weight as a block of fragments FEBGCD (see table 1), i.e. at this concentration distamycin protects all the endo

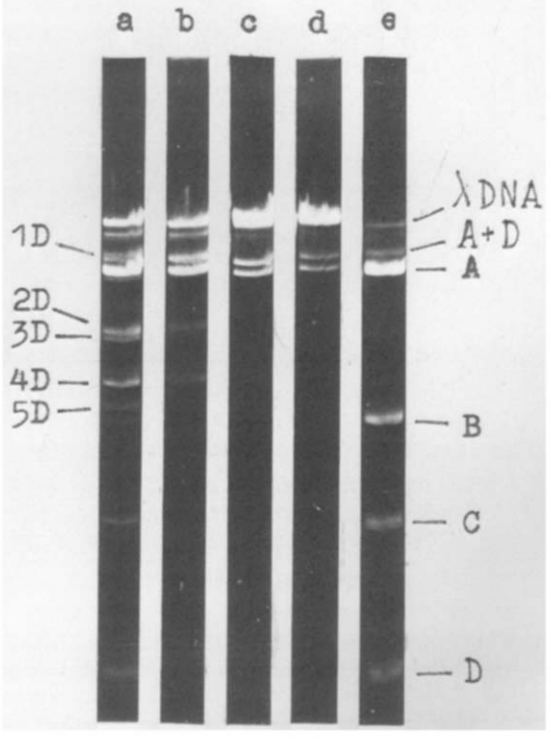

Fig.2. Electrophoretic patterns of lambda DNA digested with endo R. HindIII in the presence of different concentrations of distamycin (10^{-4} M): (a) 0.5; (b) 1.0; (c) 1.5; (d) 2.0; (e) no distamycin. Electrophoresis carried out at 2.5 V/cm for 17 h; 0.7% agarose gel.

R. HindIII sites except site 1 which remains partially available for the enzyme. A considerable part of lambda DNA remains uncleaved at these distamycin concentrations. The decrease of distamycin concentration ($0.75-1.0 \times 10^{-4}$ M) produces two additional fragments, 2D and 4D (fig.2b) that may be identified as FEBG and CD (see table 1). At moderate concentration of the antibiotic (0.5×10^{-4} M) endo R. HindIII produces a set of distamycin protected fragments (see fig.2a) which were identified by their molecular weights (see table 1).

Thus, having selective affinity for particular sequences of double-stranded DNA, distamycin A and actinomycin D protect different HindIII recognition sites in lambda DNA. This property of the antibiotics permits to obtain overlapping fragments of DNA and establish the order of HindIII fragments in lambda DNA:

AF	Actinomycin
FEB	Distamycin
EB	Actinomycin
BG	Distamycin
FEBG	Distamycin
EBGC	Actinomycin
GC	Actinomycin
CD	Distamycin
FEBGCD	Distamycin
AFEBGCD	

This result is in good agreement with the map built with the use of the deletion mutants [8,9] (see fig.3).

The proposed method for DNA mapping is based on selective protection of the recognition sites of the restriction endonuclease with an antibiotic. Different affinity of the antibiotic for the sites of one and the same restriction endonuclease is, presumably, explained by a different environment of the sites. Since distamycin interacts preferentially with d(A/T)-rich regions and actinomycin with d(G/C)-rich regions of DNA we may expect that the sequences with strong affinity for distamycin have weaker affinity for mycin, and vice versa. Indeed, at moderate concentrations, actinomycin protects completely site 1 and partially sites 3 and 5, while distamycin protects sites 2,4,6, and 3. These data are schematically shown on fig.3. At relatively high concentration of distamycin ($> 1.5 \times 10^{-4}$ M) only site 1 is available for endo R. HindIII. This allows to split lambda DNA into two large fragments.

Our results suggest that distamycin A and actinomycin D may be useful to obtain overlapping DNA fragments and to map DNA.

References

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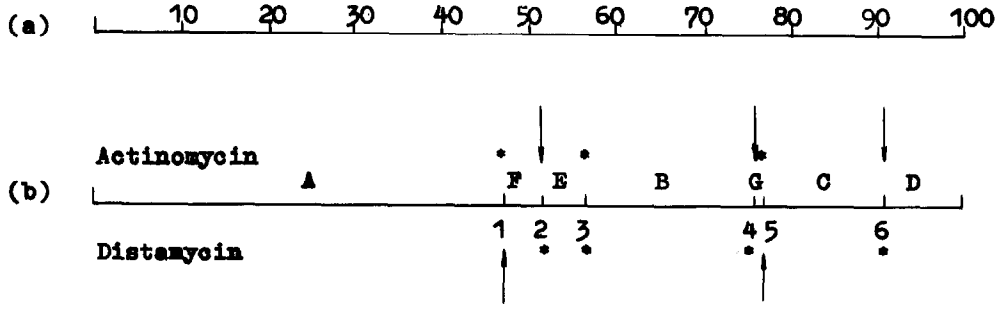

Fig.3. HindIII sites protected with actinomycin and distamycin on lambda phage DNA. (a) Physical map of lambda phage DNA; (b) HindIII cleavage map of lambda DNA [8,9]. The sites protected with moderate concentrations of the antibiotics are indicated with asterisks; the sites cleaved with the enzyme in the conditions are indicated with arrows.

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