Analysis of the genome of type 7 simian adenovirus using restrictases

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ABSTRACT

the effect of the restricting endonucleases R.EcoRI, R.BamI and R.SalI on the genome of type 7 simian adenovirus (SA-7) has been studied. Since the DNA has only one site of R.EcoRI recognition the enzyme cleaves SA-7 DNA into two fragments with the molecular weights 12.0 and $10.0 \cdot 10^6$. The restrictase R.BamI cleaves the SA-7 DNA at six sites producing 7 fragments with the molecular weights 6.6, 5.9, 3.8, 2.7, 1.3, 0.7 and $0.6 \cdot 10^6$. R.SalI cleavage yields 6 fragments with the molecular weights 8.1, 5.5, 4.3, 2.45, 1.2 and $0.6 \cdot 10^6$. The R.BamI and R.SalI fragments are arranged in the orders E-A-D-F-C-G-B and A-B-D-F-E-C, respectively. The only R.EcoRI recognition site is localized in the C fragment produced by R.BamI and in the B fragment produced by R.SalI.

INTRODUCTION

Up till now there has been practically no studies of the DNAs of the simian adenoviruses (SA) using restricting endonucleases. In the available literature we know only of the paper¹ which reports the preliminary results on the effects of some enzymes (R.HindIII, R.HpaI, II, R.EcoRI, R.EcoRII and R.Hga) on the SA-7 DNA. However, this paper does not report any characteristics of the fragments apart from their approximate amounts. But for the R.EcoRI fragments the authors have shown that this enzyme recognizes one site in SA-7 DNA and they have determined the molecular weight and the buoyant density of the R.EcoRI fragments.

The capacity of the virus to produce tumours in the newborn hamsters is associated with the smaller of the two DNA fragments. It is assumed also that the "oncogenic" part of the genome is in the middle region of the DNA. Therefore, it would be of interest to compile the physical map of the SA-7 DNA using several restrictases which produce a not too large

number of the fragments and to test subsequently the capacity of the resulting fragments to produce tumours in the hamsters.

MATERIALS AND METHODS

The cell culture and the virus. The SA-7 virus was grown on the renal cells of green monkeys². The virus was extracted from the infected cells by freezing and thawing; the extracted virus was treated with Freon-113, concentrated by centrifugation on cushion of CsCl and purified by centrifugating twice in CsCl gradients³.

Extraction of DNA and agarose electrophoresis. The viral DNA was extracted according to the modified procedure⁴. The DNA and its fragments were analyzed in agarose gel slabs⁵ or tubes using 0.7-0.9% agarose gel (Bio-Red) with the field up to 1.5 V/cm at 4°C. The time of electrophoresis varied from 16 to 24 hours. The gels were stained with ethidium bromide and the DNA fragments in the gel were detected by their fluorescence under ultraviolet illumination. The molecular weights of the fragments were calculated using as the standard the R.EcoRI fragments of the λ phage DNA. Elution of the DNA from the agarose gel was carried out according to the procedure⁶.

Isolation of enzymes and the conditions of cleavage. The endonuclease R.EcoRI was isolated from E.coli of RY-13 strain according to the modified procedure of Yoshimori⁷. The enzymes R.BamI and R.SalI were isolated according to the authors' original procedure (Naroditzky, B.S. and Karamov, E.V., unpublished results).

The enzyme reactions were carried out in the following mediums: for R.EcoRI - 70 mM tris-HCl (pH 7.5), 50 mM NaCl, 10 mM MgCl₂ and 7 mM 2-mercaptoethanol⁷; for R.BamI - 6 mM tris-HCl (pH 7.4), 6 mM MgCl₂ and 6 mM 2-mercaptoethanol⁸; and for R.SalI - 8 mM tris-HCl (pH 7.6), 150 mM NaCl, 6 mM MgCl₂, 0.2 mM EDTA, 50 μ g/ml bovine serum albumin⁹. The temperature was 37 °C, the incubation time was 180 min, the incubation mixture volume varied from 20 to 50 μ l and the DNA content varied from 0.5 to 2 μ g. The amount of enzyme added to the mixture varied from 0.5 to 5 μ l. To produce the partial cleavage the viral DNA was incubated either with the

diluted enzyme or in a high ionic strength medium at 32 °C. The reaction was discontinued by adding EDTA or by incubation for 5 min at 65 °C.

RESULTS AND DISCUSSION

The R.BamI and R.SalI enzymes following extraction and purification were tested using as standards the λ , SV-40 and colEI DNAs (the results are not given); the results agree with the data reported in literature^{8,9,10}.

SA-7 DNA cleavage by R.EcoRI. The SA-7 DNA has one recognition site for R.EcoRI (Fig. 1h); the cleavage gives rise to two DNA fragments with the molecular weights 12.0 and $10.0 \cdot 10^6$ (Table 1). These results are in a good agreement with the data reported by Burnet et al.¹.

SA-7 DNA cleavage by R.BamI and the sequence order of the fragments. The complete cleavage of SA-7 DNA with R.BamI gives rise to seven fragments (Fig. 1d). The molecular weights of these fragments vary from 6.6 to $0.6 \cdot 10^6$ (Table 1). The sums of the molecular weights of the R.EcoRI fragments ($22 \cdot 10^6$); and the R.BamI fragments ($22.15 \cdot 10^6$) are in a good agreement.

The ³H-labelled SA-7 DNA was cleaved with R.BamI and fractionated in the agarose gel; six radioactivity peaks were found which correlated well with the mobility of the R.BamI fragments of the unlabelled SA-7 DNA detected by fluorescence under ultraviolet illumination. The label was not found in the gel slices corresponding to the mobility of the DNA fragments with the lower molecular weight than that of the G fragment. The seven R.BamI fragments of the SA-7 DNA give rise to six radioactivity peaks owing to incomplete separation of the two high-molecular fragments A and B (which have close molecular weights) in 0.9% agarose gel. These data and the results of partial cleavage of SA-7 DNA (see below) suggest that the the fragment G is the smallest R.BamI fragment of SA-7 DNA. (Fig. 2)

To determine the order of the R.BamI fragments in the SA-7 DNA the genome was simultaneously cleaved by R.BamI and R.EcoRI (Fig. 1e), the isolated R.EcoRI fragments A and B were cleaved by R.BamI (Fig. 1c and f) and both the intact SA-7 DNA and the isolated R.EcoRI fragments were partially cleaved by R.BamI

Fig. 1. Agarose gel fractionation of the R.BamI fragments of SA-7 DNA and the R.EcoRI fragments of SA-7 DNA. The capital letters denote the complete cleavage products, the figures denote the partial cleavage products. DNA was analyzed in slabs of 0.7% agarose gel, 1.5 V/cm, 18 - 22 hours at 4°C. a - partial R.BamI cleavage of the DNA (0.5 µl enzyme, 32°C, 10 min incubation in 0.3 M NaCl). b - isolated R.EcoRI fragment A cleaved by R.BamI under the conditions of partial cleavage. c - the R.EcoRI fragment A cleaved by R.BamI under the conditions of complete cleavage. d - the intact DNA cleaved by R.BamI under the conditions of complete cleavage. e - simultaneous cleavage of SA-7 by R.EcoRI and R.BamI. f - complete cleavage of the R.EcoRI fragment B by R.BamI. g - cleavage of the R.EcoRI fragment B by R.BamI under the conditions of partial cleavage. h - cleavage of SA-7 DNA by R.EcoRI.

Table 1. The molecular weights of the R.EcoRI, R.BamI and R.SalI fragments of the SA-7 DNA

Fragment	Molecular weights*		
	R.EcoRI	R.BamI	R.SalI
A	12.0 ± 0.3	6.6 ± 0.3	8.1 ± 0.3
B	10.0 ± 0.3	5.9 ± 0.2	5.5 ± 0.2
C		3.8 ± 0.2	4.3 ± 0.2
D		2.7 ± 0.2	2.45 ± 0.2
E		1.3 ± 0.2	1.2 ± 0.2
F		0.7 ± 0.1	0.6 ± 0.1
G		0.6 ± 0.1	
Total	22.0 ± 0.3	22.16 ± 0.4	21.6 ± 0.4

*

The weights are means of 8 independent experiments. Molecular weights are given in 10^6 D.

Fig. 2. Label distribution in the 0.9% agarose gel following fractionation of the R.BamI cleavage products of SA-7 ^3H -DNA. Arrows point the R.BamI fragments of the SA-7 DNA detected by fluorescence under ultraviolet illumination following staining with ethidium bromide.

(Fig. 1a, b, g; Table 2). The results show that the recognition site for R.EcoRI is in the R.BamI fragment C. The fragment C is cleaved into the fragment α with the molecular weight $0.1 \cdot 10^6$ and the fragment C' whose molecular weight is less by $0.1 \cdot 10^6$ than that of the R.BamI fragment C (see Fig. 1 e and f; Table 2). The R.EcoRI fragment A is cleaved by R.BamI into 5 fragments - A, D, E, F and α ; the fragment α can be found only under the conditions of partial cleavage since its molecular weight is $0.1 \cdot 10^6$. The results of the partial R.BamI cleavage of the R.EcoRI fragment A (see Fig. 1 b and Table 2) make it possible to determine the order of the R.BamI fragments in this part of the SA-7 DNA which is E - A - D - F (see Fig. 5). The R.EcoRI fragment B of the SA-7 DNA is cleaved by R.BamI into three fragments - B, G and C'; its partial cleavage by R.BamI yields fragments whose molecular weights correspond to the pairs C'G and BG (Fig. 1g and Table 2). These results suggest the following order of the R.BamI fragments in this DNA region: C' - G - B. The above results on the orders of the R.BamI fragments in the R.EcoRI fragments A and B can be used to determine the order of all the R.BamI fragments in the SA-7 DNA which proves to be E - A - D - F - C (α + C') - G - B (see Fig. 5).

SA-7 DNA cleavage by R.SalI and the sequence order of the fragments. Complete cleavage of the SA-7 DNA by R.SalI yields six fragments (Fig. 3c) with the molecular weights varying from 8.1 to $0.6 \cdot 10^6$ (Table 1). To demonstrate the absence of the fragments with the molecular weight lower than $0.6 \cdot 10^6$ the products of the complete R.SalI cleavage of DNA were fractionated in the agarose - polyacrylamide gel copolymer. Fig. 4 shows that no fragments with the molecular weight lower than $0.6 \cdot 10^6$ were found in the agarose - polyacrylamide gel. There is a good correlation between the total molecular weights of the R.SalI fragments ($21.6 \cdot 10^6$), the R.BamI fragments ($22.15 \cdot 10^6$) and the R.EcoRI fragments ($22.0 \cdot 10^6$).

A comparison of the measured and predicted molecular weights of the partial R.SalI cleavage products of SA-7 DNA (Fig. 3b; Table 3) yielded a tentative order of the R.SalI fragments in the viral genome: A - B - D - F - E - C. To substantiate the

Table 2. Molecular weights* of the products of R.BamI cleavage of the isolated R.EcoRI fragments of the SA-7 DNA

R.EcoRI fragments	R.BamI fragments			Fragment composition	Overlapping order
	Complete cleavage	Partial cleavage	fragments		
		Measured mol. wt.	Predicted mol. wt.		
A	A	I - 10.2 ± 0.4	10.6	A,E,D	E-A-D
	D	II - 9.2 ± 0.3	9.3	A,D	A-D
	E	III - 7.8 ± 0.3	7.9	A,E	E-A
	F	IV - 3.5 ± 0.1	3.4	D,F	D-F
	**	V - 0.78 ± 0.1	0.8	F,L	F-L
B	B	VI - 6.5 ± 0.3	6.45	B,G	G-B
	C'	VII - 4.3 ± 0.2	4.3	C',G	C'-G
	G				

* Molecular weights are given in 10^6 D; the values in the table are the means of 5 independent experiments.

** The R.EcoRI restriction site is localized in the DNA region corresponding to the R.BamI fragment C; when cleaved by R.EcoRI the R.BamI fragment C yields a fragment with the molecular weight $0.1 \cdot 10^6$ which is found following partial cleavage of the R.EcoRI fragment A by R.BamI (see Fig. 1 b, band V) and the fragment C' (Fig. 1e and f) whose molecular weight is lower by $0.1 \cdot 10^6$ than that of the R.BamI fragment C.

partial cleavage results the SA-7 DNA was cleaved simultaneously by R.EcoRI and R.SalI and the isolated R.EcoRI fragments A and B were cleaved by R.SalI (Fig. 3d, e and f). The R.EcoRI fragment A has been shown to be cleaved by R.SalI into the R.SalI fragment A and the fragment B'. When the SA-7 DNA is cleaved simultaneously by R.SalI and R.EcoRI there are produced, instead of the R.SalI fragment B (Fig. 3 d), two new fragments, B' and β the sum of whose molecular weights is equal to the molecular weight of the R.SalI fragment B, that is, the recognition site for R.EcoRI is localized in the R.SalI fragment B. The results show that the fragments A and B produced by R.SalI cleavage are neighbours and the fragment A is the terminal one and is localized in the region of the

Fig. 3. Agarose gel fractionation of the R.SalI fragments produced after complete and partial cleavage of the SA-7 DNA and the R.EcoRI fragments of the SA-7 DNA. The capitals denote the complete cleavage products of the DNA, the figures denote the partial cleavage products. The conditions of fractionation are described in the legend to Fig. 1. a — simultaneous cleavage of the SA-7 DNA by R.SalI and R.BamI. b — partial R.SalI cleavage of the SA-7 DNA (1 μ l enzyme, 60 min, 37°C. c — complete cleavage of the SA-7 DNA. d — simultaneous cleavage of the SA-7 DNA by R.SalI and R.EcoRI. e — cleavage of the isolated R.EcoRI fragment A by R.SalI. f — partial cleavage of the isolated R.EcoRI fragment B by R.SalI.

Fig. 4. Fractionation of the R.SalI fragments of the SA-7 DNA in 0.5% agarose - 3.5% acrylamide gel copolymer. Electrophoresis at 35 V/cm for 20 hours at 4 °C. a - R.SalI fragments of the SA-7 DNA. b - the S_D phage DNA cleaved by R.EcoRI as the marker; the arrows denote the fragments with the molecular weights $0.48 \cdot 10^6$ and $0.35 \cdot 10^6$ (B.S. Naroditzky, unpublished data).

SA-7 DNA which corresponds to the R.EcoRI fragment A.

The partial cleavage of the R.EcoRI fragment B by R.SalI (Fig. 3c) gave rise to partial cleavage products III and IV whose molecular weights correspond to the sums of the fragments C+E+F and D+F as well as the total cleavage products C, D, E, F and the fragment β . Thus, the results of partial cleavage of the R.EcoRI fragment B by R.SalI agree with the data obtained in the partial cleavage of SA-7 DNA by R.SalI indicating definitely the following sequence of the fragments: D - F - E - C.

Since the partial cleavage of the intact SA-7 DNA by R.SalI gives rise to the fragment whose molecular weight corresponds to the sum of the fragments A + B + D it is clear that the order of the R.SalI fragments of the SA-7 DNA suggested above (A - B - D - F - E - C) is correct.

Table 3. Molecular weights of the products of the partial R.SalI cleavage of the SA-7 DNA and their fragment composition

Partial cleavage fragments	Predicted mol. wt.(10 ⁶ D)	Measured mol. wt. (10 ⁶ D)	Fragment composition	Overlapping order
I	16.8	16.5	A,B,D,F	A-B-D-F
II	16.1	15.8	A,B,D	A-B-D
III	6.1	6.0	C,E,F	F-E-C
IV	5.5	5.5*	C,E	E-C
V	4.3	4.3*	D,E,F	D-F-E
VI	3.1	3.1	D,F	D-F
VII	1.3	1.8	E,F	F-E

* The molecular weights of the partial cleavage fragments IV and V are the same as the molecular weights of the fragments B and C produced in complete cleavage of SA-7 DNA by SalI so that they are not separated by agarose gel electrophoresis.

Fig. 5. The physical map of the SA-7 genome derived using R.BamI and R.SalI. Figures represent percentages of the genome length.

Our suggestions about the orders of R.BamI and R.SalI fragments in the SA-7 genome (Fig. 5) are supported also by the good agreement between the predicted and measured molecular weights of the DNA fragments produced by the simultaneous cleavage of the DNA with R.BamI and R.SalI (Fig. 3a: Table 4).

Our data concerning the order of R.BamHI and R.SalI-generated fragments in the genome of SA-7 (Fig. 5) were also corroborated by the results of determination of terminal frag-

Table 4. The molecular weights of the fragments produced by simultaneous cleavage of the SA-7 DNA by R.BamI and R.SalI

Fragments	Predicted mol. wt. (10^6 D)	Measured mol. wt. (10^6 D)
R.BamI A	6.6	6.7
R.SalI C	4.3	4.4
R.BamI + R.SalI A	2.7	2.6
R.BamI + R.SalI B	2.05	2.0
R.BamI + R.SalI C	2.0	1.85
R.BamI E	1.30	1.38
R.SalI E	1.20	1.25
R.BamI F	0.7	0.7
R.SalI F and R.BamI G	0.6	0.65
R.BamI + R.SalI D	0.45	0.50

ments by exposing SA-7 DNA associated with the molecules of terminal protein to these restriction enzymes (Fig. 6). It is known that the terminal fragments of such a complex do not enter the agarose gel¹¹. As shown in Fig. 6, fragments E and B produced by R.BamHI and fragments A and C produced by R.SalI are actually terminal. They are covalently linked with the terminal protein and under conditions used for the electrophoresis do not enter the gel. Thus the order of all the fragments except for R.SalI - D, F and E is determined by two independent methods. The position of fragments D, F and E produced by R.SalI is deduced only from the comparison of the results of partial cleavage, fact which is alone sufficient for the construction of the restriction map (see 13, 14, 15).

Fig. 6. Cleavage of SA-7 DNA - terminal protein complex by restriction enzymes R.BamHI and R.SalI and fractionation of the cleavage products in 1% agarose gel (2 v/cm, 18h). DNA-protein complex of SA-7 was extracted and characterized (B.S. Naroditsky, unpublished data) by the method¹². a. - 1 ug of DNA-protein complex incubated for 60 min. at 37°C with 1 ul of R.BamHI and then with 10 ul of pronase for 10 min. b - DNA-protein complex (1 ug) treated with R.BamHI (1 ul) alone for 60 min at 37°C. E fragment is not observed and only 8-10% of B fragment are detected. c - DNA-protein complex incubated with R.SalI and then treated with pronase. d - DNA-protein complex treated with R.SalI alone. The fragments A and C are not observed in the gel.

It is noteworthy that all the recognition sites for R.BamI and R.SalI in the SA-7 DNA (except one for R.BamI) are localized in the middle part of the genome which ac-

count for 40% of the genome length. Since the recognition sites for both these enzymes have a higher content of the GC pairs (Roberts, R.; unpublished data) it is natural to assume that it is this part of the SA-7 DNA that is rich in the GC pairs. According to Burnett et al.¹, this part of the SA-7 genome contains the DNA region which is responsible for the oncogenic capacity of the virus.

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