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NOTES

Enhanced Infectivity of Adenovirus Type 2 DNA and a DNA-Protein Complex

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The infectivity of adenovirus type 2 DNA and a DNA-protein complex was studied in 293 cells, a human embryonic kidney cell line transformed by sheared adenovirus type 5 DNA, and in human KB cells. Adenovirus type 2 DNA was more infectious (up to about 40-fold) in 293 cells than in KB cells, whereas a DNA-protein complex (prepared by a rapid procedure) had about the same infectivity in both cell lines. These data may mean that a factor present in 293 cells (perhaps a viral-coded protein) enhances the infectivity of free viral DNA. The infectivity of DNA and the DNA-protein complex was increased up to fivefold by brief treatment of cell monolayers with 25% dimethyl sulfoxide after transfection. Under these conditions, (i) the infectivity of native adenovirus type 2 DNA ranged from 400 to 1,300 PFU/μg of DNA in 293 cells and from about 9 to 14 PFU/μg of DNA in KB cells, and (ii) the infectivity of the DNA-protein complex was 6×10^3 to 2×10^4 PFU/μg in 293 cells and 1.4×10^4 to 1.6×10^4 PFU/μg in KB cells.

The infectivity of human adenovirus DNA was first demonstrated by Nicolson and McAllister (10), who observed a typical adenovirus-type cytopathic effect when human embryo kidney cells were treated with adenovirus type 1 (Ad1) DNA in the presence of DEAE-dextran (12). No reproducible relationship was found between DNA concentration and cytopathic effect. A reproducible technique for assaying adenovirus type 5 (Ad5) DNA infectivity was developed subsequently by Graham and van der Eb (4). DNA was co-precipitated with calcium phosphate, and infectivity was quantitated by plaque assay on human KB cells. The infectivity of Ad5 DNA was low (about 20 PFU/μg) compared with other viral DNAs, such as simian virus 40 DNA (10^6 PFU/μg [8]) and herpesvirus type 1 DNA (10^3 PFU/μg [5, 15]). Recently, Sharp et al. (13) reported that the efficiency of transfection by Ad5 DNA could be increased approximately 100-fold with a viral DNA-protein complex (18); however, the infectivity of the DNA-protein complex was highly variable. It was recently reported that the infectivity of herpesvirus type 1 DNA could be increased by treatment of cell monolayers with dimethyl sulfoxide (Me_2SO) after transfection with the calcium phosphate technique (16). In this report, we have determined the infectivity of adenovirus type 2 (Ad2) DNA and a DNA-protein complex in human KB cells and 293 cells, a human embryo kidney cell line transformed by sheared Ad5 DNA. Me_2SO treatment after transfection increased the infectivity of free DNA as well as that of the DNA-protein complex. We describe a modification of the method of Robinson et al. (18) that yielded preparations of DNA-protein complex with a reproducible infectivity of about 10^4 PFU/μg.

The 293 cell line is a human embryonic kidney cell line transformed by sheared Ad5 DNA that was established by Graham et al. (3). This cell line yields plaques with Ad2 and Ad5 up to 4 days earlier than do other established human cell lines, with about the same efficiency as that observed in KB cells and in HeLa cells (F. Graham, personal communication; our unpublished data). We have been using this cell line to assay the infectivity of in vitro-constructed Ad2 mutant DNA. We have compared the infectivity of Ad2 DNA in the presence and absence of Me_2SO in 293 cells. In typical experiments, Ad2 (strain 38-2, plaque 6, free from adenovirus-associated viruses) DNA extracted from virions (6) by the standard protease-sodium dodecyl sulfate-phenol method (7) yielded from about 150 to 400 PFU/μg (different DNA preparations in different experiments) on 293 cells (Table 1). Treatment with 25% Me_2SO in *N*-2-hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid-buffered sa-

TABLE 1. *Infectivity of native Ad2 DNA in 293 cells and effect of Me_2SO*[a]

Expt	DNA concn μg/dish	No. of plaques/dish		PFU/μg	
		Without Me_2SO	With Me_2SO	Without Me_2SO	With Me_2SO
1	0.1	40, 32, 41	117, 85, 139	400, 320, 410	1,170, 850, 1,390
	0.2	84, 73, 91	233, 227, 209	420, 365, 455	1,165, 1,825, 1,045
	0.5	158, 180, 195	—[b]	316, 360, 390	—
2	0.2	26, 29	84, 83	130, 145	420, 415
	0.5	75, 72	—	150, 144	—

[a] Each experiment was carried out with a different DNA preparation on different days. The transfection assays were carried out by the calcium phosphate technique of Graham and van der Eb (4) with minor modifications. Briefly, 293 cells growing in Eagle minimal essential medium containing 10% horse serum were removed from monolayers with a mixture of 0.05% trypsin and 0.025% EDTA in phosphate-buffered saline, diluted with growth medium, centrifuged, resuspended in growth medium, and seeded (2×10^6 cells) in 60-mm Falcon petri dishes. Cell sheets were used on the following day for transfection. Calcium phosphate precipitation of viral DNA was performed at room temperature for 15 to 20 min in *N*-2-hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid-buffered saline (4) containing 20 μg of phenol-chloroform-extracted salmon sperm DNA per ml (Sigma Chemical Co.) and 125 mM $CaCl_2$. The calcium phosphate precipitate (0.5-ml suspension) was added to each cell monolayer after removal of growth medium. After 20 min at room temperature, 5 ml of fresh growth medium was added, and cultures were incubated at 37°C for 4 h. The medium was removed, and plates were overlaid with 10 ml of agar overlay medium or treated with Me_2SO followed by the addition of agar overlay. The overlay medium consisted of minimal essential medium in 0.9% agar (Difco) supplemented with 5% horse serum and 0.1% yeast extract for 293 cells or with 5% horse serum and 5% chicken serum for KB cells. A second overlay (5 ml) containing 0.0022% neutral red was added on day 7 for 293 cells or on day 10 for KB cells. Plaques were counted 2 days later. Treatment with Me_2SO was carried out essentially as described by Stow and Wilkie (16). Cell monolayers at 4 h after transfection were washed with minimal essential medium (2 or 5% serum) and treated with 1 ml of sterile 25% Me_2SO in *N*-2-hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid-buffered saline at room temperature for 4 min. Me_2SO was removed, and cell sheets were washed with 3 ml of minimal essential medium containing serum, followed immediately by the addition of agar overlay as described above.

[b] —, Not tested.

line for 4 min at 4 h after the addition of the calcium phosphate DNA precipitate yielded from 400 to 1,300 PFU/μg. In other experiments, plates treated with Me_2SO always yielded more plaques than parallel plates receiving no Me_2SO.

Recently, Sharp and co-workers (13) have reported that the DNA-protein complex prepared by disruption of Ad2 virions with 4 M guanidinium chloride followed by sedimentation in a sucrose density gradient containing 4 M guanidinium chloride was about 100-fold more infectious than free DNA when assayed in human embryonic kidney cells. As noted by these authors, a wide variability in the number of plaques (47 to 7,820 PFU/μg) was observed. We prepared the DNA-protein complex by a modification of the method of Robinson et al. (18). Virions were disrupted with 4 M guanidinium chloride in the presence of mercaptoethanol, and the DNA-protein complex was purified on Sepharose 4B. The entire operation was carried out in 2 to 3 h at 0 to 4°C to avoid possible denaturation of the complex. The DNA-protein complex prepared from ^{32}P-labeled Ad2 by this procedure yielded a single homogeneous peak (Fig. 1) with a ratio of absorbancy at 260 nm to that at 280 nm greater than 1.9 (indistinguishable from that of free DNA).

The DNA-protein complex prepared as described above was further characterized, inasmuch as it was prepared in a somewhat different manner than previously described. When the DNA-protein complex was cleaved with restriction endonuclease *Eco*RI and subjected to electrophoresis through agarose gels, the terminal A and C fragments (9) were retained on the gel surface (Fig. 2, lane 3). When digested with Pronase, the fragments readily entered the gel (Fig. 2, lane 4). Digestion of the DNA-protein complex with other restriction endonucleases, including *Bam*HI, *Sal* I, and *Xba*, followed by electrophoresis on agarose gels, gave identical results (data not shown). Similar observations have been made with Ad2 and Ad5 DNA-protein complexes prepared by different methods (2, 11, 13). Therefore, the DNA-protein complex used in the present study contained the protein attached to both terminal fragments of Ad2 DNA.

The infectivity of the DNA-protein complex in 293 cells and the effect of Me_2SO treatment are given in Table 2. The DNA-protein complex yielded an average of from 3×10^3 to 1.6×10^4 PFU/μg in three different experiments. Me_2SO treatment increased the specific infectivity from two- to fivefold. The infectivity of the DNA-protein complex was completely abolished by treatment with DNase; moreover, no plaques resulted in the absence of precipitation with

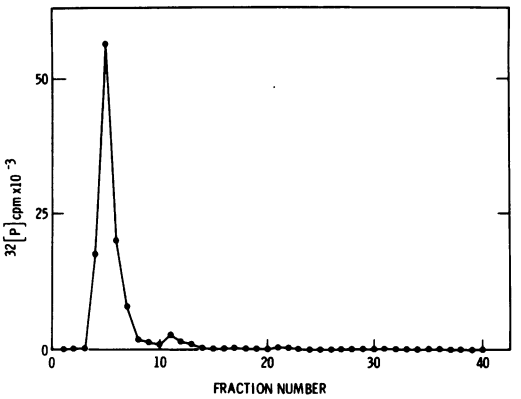

FIG. 1. *Isolation of an Ad2 DNA-protein complex by chromatography on Sepharose 4B. CsCl-purified Ad2 (6) was diluted to an absorbancy at 260 nm of 8 to 10 (^{32}P-labeled virus mixed with unlabeled virus) and dialyzed against two changes of 500 volumes of 10 mM Tris-hydrochloride (pH 8.1) containing 1 mM EDTA and 2 mM β-mercaptoethanol at 0°C for 6 h. An equal volume of 8 M guanidinium chloride (Schwarz/Mann) in Tris-hydrochloride-EDTA-mercaptoethanol was added, and the suspension was incubated at 0°C for 10 min and loaded onto a Sepharose 4B (Pharmacia) column (2 by 26 cm) equilibrated with 4 M guanidinium chloride in Tris-hydrochloride-EDTA-mercaptoethanol at 4°C. Fractions were collected at a flow rate of about 18 ml/h. Fractions containing the DNA-protein complex were detected by measurement of radioactivity or absorbancy at 260 nm, pooled, and dialyzed against 10 mM Tris-hydrochloride (pH 7.6) containing 1 mM EDTA and 2 mM mercaptoethanol. The DNA-protein complex (30 to 50 μg/ml) was stored at 0°C up to 2 months for transfection studies without appreciable loss in infectivity. The concentration of DNA in preparations of the DNA-protein complex was determined from the specific radioactivity or from the absorbancy at 260 nm (the absorption spectrum of the DNA-protein complex is indistinguishable from that of DNA purified by treatment with pronase-sodium dodecyl sulfate-phenol).*

calcium phosphate (data not shown). Thus, Me_2SO had a stimulatory effect on the infectivity of both Ad2 DNA and the DNA-protein complex in 293 cells.

KB cells are used extensively as a host cell for the study of Ad2 replication. We therefore studied the effect of Me_2SO on the infectivity of Ad2 DNA and the DNA-protein complex in these cells. Ad2 DNA yielded an average of 5 and 8 PFU/μg in KB cells in two experiments (Table 3). Similar values have been obtained by Graham and van der Eb (4) with Ad5 DNA. When Me_2SO treatment was used, the infectivity increased to an average of 9 and 14 PFU/μg of Ad2 DNA (Table 3). Thus, the infectivity of native DNA in KB cells is about 40-fold less than that in 293 cells (Table 1).

The effects of Me_2SO on the infectivity of the DNA-protein complex in KB cells are given in Table 4. The DNA-protein complex gave an average of 1.2×10^4 PFU/μg. Me_2SO treatment increased infectivity to about 1.6×10^4 PFU/μg. Although KB cells were not as sensitive as 293 cells to Me_2SO treatment, a definite enhancement of plaque formation in Me_2SO-treated monolayers was always observed. It is noteworthy that the infectivity of the DNA-protein complex is similar in 293 cells (Table 2) and KB cells (Table 4), whereas the infectivity of native DNA was much higher in 293 cells than in KB cells.

In this study we have determined the infectivity of Ad2 DNA and a DNA-protein complex in both 293 cells and KB cells and shown that infectivity is enhanced by treatment with Me_2SO. The infectivity of herpesvirus type 1 DNA was increased about 100-fold by Me_2SO treatment (16). Although we did not observe as dramatic an increase in the infectivity of Ad2 DNA and DNA-protein complex, a consistent enhancement of infectivity compared with that in untreated monolayers (up to about fivefold) was observed. The mechanism by which Me_2SO

TABLE 2. *Infectivity of DNA-protein complex in 293 cells and effect of Me_2SO*[a]

Expt	DNA concn μg/dish	No. of plaques/dish		PFU/μg	
		Without Me_2SO	With Me_2SO	Without Me_2SO	With Me_2SO
1	0.01	—[b]	111, 134, 118	—	11,100, 13,400, 11,800
	0.02	47, 44, 53	142, 109, 116	2,350, 2,200, 2,650	7,100, 5,420, 5,800
	0.05	165, 173, 167	—	3,300, 3,460, 3,340	—
2	0.01	—	175, 177, 195	—	17,500, 17,700, 15,950
	0.02	165, 138, 160	339, 338, 319	8,250, 6,900, 8,000	16,950, 16,900, 15,950
	0.05	266, 211, 250	—	5,320, 4,220, 5,000	—
3	0.01	164, 81, 100	213, 183, 220	16,400, 8,100, 11,000	21,300, 18,300, 22,000

[a] Experiments 2 and 3 were carried out with the same DNA-protein complex preparation; experiment 1 was conducted with a different preparation.

[b] —, Not tested.

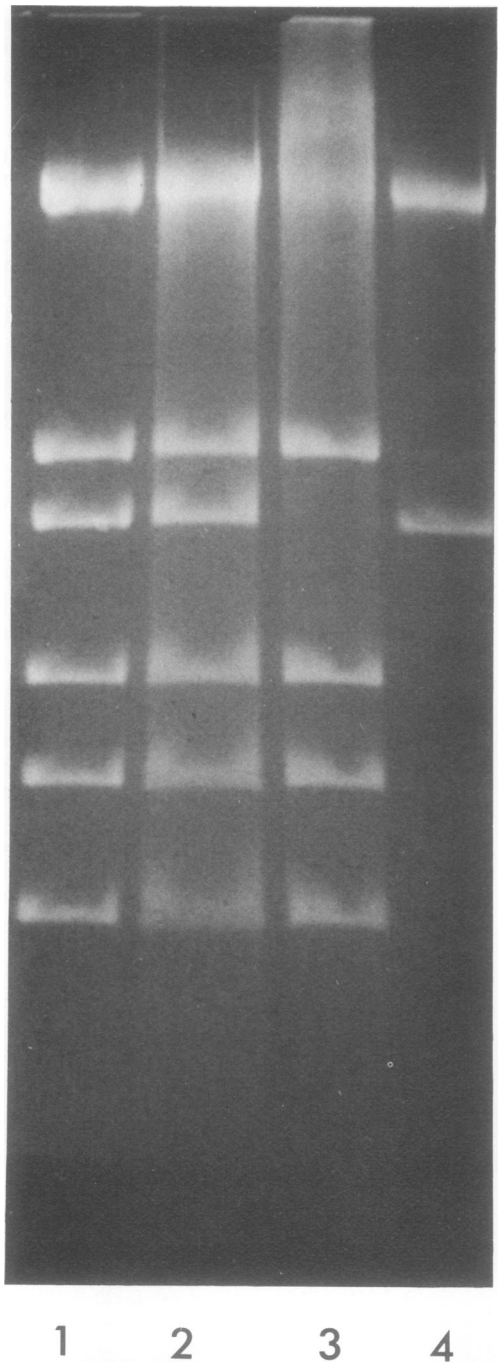

FIG. 2. *Gel electrophoresis of Ad2 DNA and the DNA-protein complex after cleavage with restriction endonuclease EcoRI. Electrophoresis was carried out in 1.4% agarose slab gel at 40 V for 14 h as described previously (14). (1) EcoRI-digested Ad2 DNA. (2) EcoRI-digested DNA-protein complex after incubation with 500 μg of Pronase per ml at 37°C for 1 h. (3) EcoRI-digested DNA-protein complex. (4) Fragments retained on the surface of the gel after electrophoresis of EcoRI-digested DNA-protein complex were eluted, incubated with 500 μg of Pronase per ml at 37°C for 1 h, and subjected to electrophoresis as described above.*

TABLE 3. *Infectivity of Ad2 DNA on KB cells and effect of Me_2SO*[a]

Expt	No. of plaques/dish	
	Without Me_2SO	With Me_2SO
1	5, 6, 12 (8)	17, 13, 11 (14)
2	5, 7, 4 (5)	8, 12, 6 (9)

[a] These experiments were carried out at a DNA concentration of 1 μg/dish. A single DNA preparation was used in both experiments. Numbers in parentheses are averages.

increases the infectivity of viral DNA is not known.

Transfection of KB cells with Ad2 DNA gave about 5 to 8 PFU/μg. Similar results were reported for Ad5 DNA (3). On the other hand, about 150 to 400 PFU/μg of DNA were observed in 293 cells. Graham, who developed the 293 cell line, has also observed in 10- to 50-fold stimulation of infectivity of Ad5 DNA in 293 cells compared with that in other established human cell lines (quoted in reference 3).

It has been shown that the Ad5 DNA-protein complex (13) and the Ad2 DNA-protein complex (present study) are substantially more infectious than DNA purified by the standard protease-sodium dodecyl sulfate-phenol method (7). The 293 cell line retains about 14% of the left end and about 5% of the right end of the Ad5 genome (R. Weinman, personal communication) and expresses only the left end as mRNA (3). Graham et al. (3) reported evidence that some viral-induced polypeptides are synthesized by 293 cells. These polypeptides may play some role in enhancing the infectivity of viral DNA. The protein bound covalently to adenovirus DNA (11, 17) appears necessary for the increased infectivity of viral DNA (13; present study). It is therefore possible that this protein may be synthesized by 293 cells and thereby may account for the enhanced infectivity of free Ad2 DNA. Other possible explanations include (i) lower levels of nucleases in 293 cells, by analogy with *Escherichia coli* strains deficient in *recBC* nucleases that are responsible for the enhanced infectivity of bacteriophage DNAs (1), and (ii) more efficient uptake of viral DNA by 293 cells than by KB cells.

In the present study, we have shown that the infectivity of the Ad2 DNA-protein complex ranged from about 6×10^3 to 2×10^4 PFU/μg in 293 and KB cells. DNA-protein complex prepa-

TABLE 4. *Effect of Me_2SO on infectivity of the Ad2 DNA-protein complex in KB cells*[a]

Expt	No. of plaques/dish		PFU/μg	
	Without Me_2SO	With Me_2SO	Without Me_2SO	With Me_2SO
1	218, 268, 261	271, 312, 342	10,900, 13,400, 13,050	13,550, 15,600, 17,100
2	215, 262, 250	322, 315, 316	10,750, 13,100, 12,500	16,100, 15,750, 15,800

[a] These experiments were carried out at a DNA concentration of 0.02 μg/dish.

rations used over a period of 4 months exhibited only about a fivefold variation in infectivity. Others (13) have reported a wider variation (47 to 7,820 PFU/μg) in the infectivity of an Ad5 DNA-protein complex. Our method for the preparation of the DNA-protein complex is somewhat different and may be responsible for the increased reproducibility of transfection. The improved transfection technique described here should be very useful for manipulation of the adenovirus genome in vitro.

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