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Biochemical Studies on Bovine Adenovirus Type 3

I. Purification and Properties

Y. NIIYAMA, K. IGARASHI, K. TSUKAMOTO, T. KUROKAWA, AND Y. SUGINO*

Biological Research Laboratories, Central Research Division, Takeda Chemical Industries, Ltd., Osaka, Japan

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Bovine adenovirus type 3 (BAV3) was purified and its properties were studied. On productive infection of CKT1 cells (a cell line derived from calf kidney) with BAV3, it was observed that viral DNA synthesis was initiated after about 24 h and its rate was maximal after about 40 h. Maturation of the virus occurred several hours after this. Purified BAV3 was separated into four discrete bands by CsCl density gradient centrifugation (complete, incomplete, empty, and degraded viruses). The complete BAV3 was similar in size and structure to human and avian adenoviruses. Polyacrylamide gel electrophoresis showed that the complete BAV3 virion contained at least 10 polypeptides. The total structural proteins of the virion had a similar amino acid composition to those of human adenoviruses. DNA of the complete virus was a linear duplex and its contour length was 12.3 ± 0.9 μm. The $S^0_{20,w}$ value of the DNA was 32.9S and its buoyant density in CsCl was 1.717 g/ml. There was about 25% homology between the DNAs of BAV3 and human adenovirus type 5 by filter hybridization. It was also noted that BAV3 produced incomplete virus. The incomplete virus was similar in morphology to the complete virus and contained almost all the structural polypeptides of the latter, but lacked infectivity. However, its DNA had a deletion(s) (13%) which seemed to locate near a terminal.

So far, nearly a hundred serotypes of adenoviruses have been isolated from human, simian, bovine, equine, porcine, canine, murine, and avian origins. The topography of the components of human adenovirus type 2 has already been reported (8). Since the discovery of type 1 bovine adenovirus (BAV) by Klein et al. (16) in 1959, nine serotypes have been reported and comparative studies on some of their properties have been reported by Rondhuis (P. R. Rondhuis, Ph.D. thesis, State Univ. of Utrecht, Utrecht, The Netherlands, 1971).

BAV3 was first isolated by Darbyshire et al. (5) in 1965 during studies on the viral etiology of a respiratory disease of cattle. This virus was later reported to have a pathogenic effect on the respiratory tract of cattle and to induce tumors in newborn hamsters (3, 4). Recently BAV3 was shown to cause abortive infection of C3H2K cells, which were derived from C3H mouse kidney (52), and to induce cellular DNA synthesis as well as morphological alterations (47). Furthermore, BAV3 was found to transform skin muscle cells from hamster embryos in vitro (26).

This paper reports the purification and biochemical characterization of BAV3, whereas the following paper is on some biological and biochemical properties of incomplete BAV3 (14).

MATERIALS AND METHODS

Virus and cells. The virus employed was BAV3, which was one of the clones purified by Y. K. Inoue (Kyoto Univ., Kyoto, Japan) by three passages at limiting dilution from the prototype (WBR1) isolated by Darbyshire (3). The seed virus is named BAV3-0 in this report. It was propagated in CKT1 cells, a clone of a cell line isolated by S. Kimura (Tokushima Univ., Tokushima, Japan) from calf kidney. BAV3-1 to BAV3-10 are plaque-purified clones derived from BAV3-0. CKT1 cells were used for propagation of BAV3. The growth medium used for cultures of cells was Eagle minimal essential medium supplemented with 10% bovine serum. Medium supplemented with 2% immobilized bovine serum (propagation medium) was used during viral growth. KB cells were used for propagation of human adenoviruses which were obtained from C. Hamada (Niigata Univ., Niigata, Japan).

Infectivity titration. Infectivity was assayed by measuring plaque formation on CKT1 monolayers after the method developed by Williams (51) for titration of human adenovirus type 5. The mean tissue culture infective dose was determined on CKT1 monolayers using the method of Reed and Muench (38). The ratio of PFU to mean tissue culture infective

dose was 5 to 10 under the conditions employed.

Purification of the virus. BAV3 was purified by a modification of the method of Green and Piña (11). The CKT1 monolayer was infected with BAV3 (10 PFU/cell), and after incubation at 37 C for 2 h to allow adsorption propagation medium was added. After 3 days, the cells were harvested by pipetting, sedimented by low-speed centrifugation, and resuspended in one-thirtieth of the original volume of 0.01 M Tris-hydrochloride (pH 8.1). The suspension was sonically treated twice in an ice bath using an ARTEK dismembrator (for 30-s periods at a dial setting of 5) and centrifuged at low speed to remove cell debris. The supernatant was then extracted three times with daiflon S-3 (Daikin Industries Co.). The latter operations were performed as described by Green and Piña except that CsCl was used in place of RbCl. Tritium thymidine ([^{3}H]Tdr) (5 Ci/mM, Daiichi Pure Chemical Co.)-labeled BAV3 was purified from CKT1 cells which were labeled for 48 h (24 to 72 h after BAV3 infection) with [^{3}H]Tdr.

Serological tests. Anti-BAV3 serum was prepared in rabbits by inoculation of purified BAV3 with an equal volume of Freund complete adjuvant intramuscularly and then three times intravenously at weekly intervals. Immunodiffusion tests were carried out by the double diffusion method of Ouchterlony (29).

Electron microscopy. CKT1 cells infected with BAV3 were fixed in glutaraldehyde and osmium tetroxide and embedded in epon. Then ultrathin sections were prepared, stained with uranyl acetate and lead citrate, and examined. Purified viruses were negatively stained with 2% phosphotungstic acid adjusted to pH 7.5 with 1 N KOH following the method of Brenner and Horne (1). DNA was examined by the protein monolayer technique of Kleinschmidt (17). The spreading solution contained 0.5 to 1.0 μg of viral DNA per ml, 0.1 mg of cytochrome *c* (horse heart, type III) per ml, 0.05 μg of fd phage replicative form DNA per ml, 0.4 M Tris-hydrochloride (pH 7.5), and 0.01 M EDTA in 40% formamide. Replicative form DNA of fd phage (a gift from H. Yamagishi, Kyoto Univ., Kyoto, Japan) was used as an internal standard. A JEM-100B (100 kV) or JEM-T7S (60 kV) electron microscope was used.

Heteroduplex formation. Formation of heteroduplex DNA between complete and incomplete viruses was performed by the method of Davis et al. (6). After denaturation of both DNAs in 0.2 N NaOH for 10 min at room temperature, renaturation was performed in the presence of 0.15 M Tris-hydrochloride, 0.001 M EDTA, and 40% formamide (pH 8.6 to 8.8) for 1 h. The spreading solution contained 1 μg of DNA per ml, 0.1 mg of cytochrome *c* per ml, 0.02 μg of fd phage replicative form DNA per ml, 0.1 M Tris-hydrochloride (pH 7.5), and 0.01 M EDTA in 40% formamide. The hypophase contained 0.01 M Tris-hydrochloride (pH 7.5) and 0.001 M EDTA in 10% formamide.

Rate of DNA synthesis. Confluent CKT1 monolayers in glass petri dishes of 30-mm diameter were infected with BAV3 (10 PFU/cell) or subjected to mock infection. At various times the medium was replaced by minimal essential medium containing 1 μCi of [^{3}H]Tdr. After pulse labeling for 1 h at 37 C, the cells were scraped off the dish, centrifuged, and washed with 0.85% NaCl. Then 1 mg of bovine serum albumin was added as carrier followed by cold perchloric acid. The resulting precipitate was washed twice with cold 0.4 N perchloric acid and dissolved in 2 N NH_4OH and a 0.1-ml aliquot was counted in 10 ml of Brays solution in an Aloka liquid scintillation spectrometer.

Preparation of DNA. The viral DNA was extracted as described by Green and Piña (12) with the following modifications: after dialysis against 0.01 M Tris-hydrochloride (pH 8.1), the virus was treated overnight with 2 mg of self-digested Pronase per ml and 0.01 M EDTA. The digested virus solution was then mixed with one-twentieth volume of 10% sodium dodecyl sulfate and the mixture was incubated at room temperature for 30 min and then extracted three times with phenol. After removal of the phenol with ether, the aqueous phase was dialyzed against 0.1× SSC (0.15 M NaCl plus 0.015 M sodium citrate) plus 0.01 M EDTA and was used as the viral DNA preparation. Cellular DNA was extracted as described previously (47).

DNA-DNA hybridization. The procedure described by Warnaar and Cohen (50) with modifications (37) was used for DNA-DNA annealing (47).

Sedimentation coefficient of DNA. Sedimentation coefficients were measured in a Spinco model E ultracentrifuge using ultraviolet optics. The method of Vinograd et al. (48) was employed. Sedimentation was performed at 35,800 rpm in an An-F rotor equipped with cells containing band type III centerpieces (charcoal-filled epon) at 20 C. The solvent used was 1 M NaCl in 0.01 M Tris-hydrochloride (pH 8.1). Values of $S^0_{20,w}$ were calculated as described by Studier (43).

Buoyant density of DNA in CsCl. The buoyant density of [^{3}H]Tdr-labeled viral DNA was determined by equilibrium density gradient centrifugation in CsCl with a Spinco L2-65B preparative ultracentrifuge (SW65 Ti rotor, 34,000 rpm for 72 h at 25 C). The initial density of CsCl (Merck Chemical Co. Spurapur grade) was 1.700 g/ml. The density was calculated from the refractive index using the equation of Thomas and Berns (46).

Amino acid analysis of viral protein. Highly purified samples of viruses obtained by equilibrium density gradient centrifugation three times in CsCl were extensively dialyzed against distilled water. They were then hydrolyzed with 6 N HCl at 110 C for 24 h, and the hydrolysates were analyzed by the method of Moore et al. (25) using a Hitachi amino acid analyzer (KLA-3B).

Acrylamide gel electrophoresis. Acrylamide gel electrophoresis of viral protein was performed by the method of Maizel (21). The purified virus sample, corresponding to bands I, II, III, or IV, was dialyzed against the solution of 0.01 M Tris-hydrochloride (pH 8.1) and dissociated in the solution of 1% sodium dodecyl sulfate, 0.1% 2-mercaptoethanol, 10% glycerol, and 0.002% bromophenol blue at 100 C for 1 min. Electrophoresis was done in 13% polyacrylamide gel (0.5 by 12 cm) for 6 h at 10 V/cm. Gels were stained with Coomassie brilliant blue (R250). The densitomet-

ric scanning of the gel was performed with a Joyce-Loebl instrument. The relative ratio was calculated by measuring the weight of the areas of the polypeptides in the trace. Human gamma globulin fraction II, bovine albumin, ovalbumin, and beef pancreas chymotrypsinogen A (Schwarz/Mann Co.) were used as molecular weight standards.

RESULTS

Growth of BAV3 on CKT1 cells. One-step growth of BAV3 was followed by determining the rate of viral DNA synthesis and by infectivity assay of mature virus on permissive CKT1 cells. On infection, incorporation of [^{3}H]Tdr into the acid-insoluble fraction began to increase after about 24 h and was maximal after about 42 h (Fig. 1). When minimal essential medium supplemented with 2% calf serum was used instead of virus suspension (mock infection), [^{3}H]Tdr incorporation into cellular DNA was maximal after 18 h and then decreased (Fig. 1). Similar results on cellular DNA synthesis were observed with human adenovirus type 2 and 12 (20). A DNA-DNA hybridization experiment was performed to determine whether the DNA synthesized after virus infection was viral or cellular. As shown in Table 1, the DNA synthesized after virus infection was viral. Maturation of infectious virus was observed several hours after this viral DNA synthesis (Fig. 1). When CKT1 cells were harvested 48 h after BAV3 infection and observed by electron microscopy, viral crystalline arrays were found in the nucleus (Fig. 2). Viral particles in these crystalline arrays displayed three distinct types of nucleoids: (i) a uniformly dense type, (ii) a weakly staining vesicular type, and (iii) a dense type which with its surrounding capsid had a doughnut-like configuration.

Purification of BAV3. When BAV3-0 was propagated on CKT1 cells using roller bottles (NBS, RC-94) and purified by the method of Green and Piña (11), the final purified preparation separated into four bands (I, II, III, and IV) on equilibrium density gradient centrifugation in CsCl (Fig. 3a and c).

(i) Band I: d = 1.340 g/ml. Only this virus had infectivity (Fig. 3c). It showed an icosahedron structure (about 75 nm in diameter) similar to that of human adenoviruses by electron microscopy (Fig. 4a). These results indicate that this is the complete virus of BAV3. Structures which seemed to be hexon (Fig. 4d) and fiber subunits were observed in weakly sonicated preparations of purified complete BAV3 virion. The former were 7.5 to 9 nm in diameter and the latter were 12 to 18 nm in length. These subunits were similar in size to those of adenoviruses (31) (see Table 5).

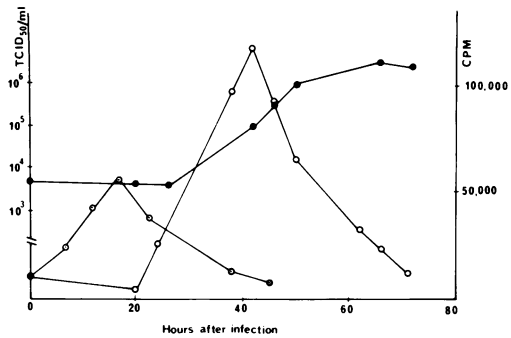

FIG. 1. *One-step growth of virus on CKT1 monolayers. Confluent CKT1 monolayers (glass petri dish of 45-mm diameter) were infected with BAV3 (10 PFU/cell), and then the virus propagation medium (5 ml) was added. Cultures were harvested with a policeman at various times after infection and freeze-thawed three times. Then the BAV3 in these samples was titrated (●). Duplicate samples were titrated at each time. The rate of DNA synthesis was measured from 1-h pulse incorporation of [^{3}H]Tdr into the acid-precipitable fraction after virus infection (○) or mock infection (⊙).*

TABLE 1. *Hybridization of DNA in BAV3-infected CKT1 cells with cellular and viral DNA*

DNA on filter (μg)	Input DNA (μg)	Input counts/min	Net counts/min bound[a]	% of input
BAV3(3)	^{3}H-labeled (BAV3-infected) CKT1[b] (0.19)	38,803	27,912	71.9
BAV3(3)	^{3}H-labeled (BAV3-infected) CKT1[b] (0.38)	77,606	55,075	71.0
CKT1(10)	^{3}H-labeled (BAV3-infected) CKT1[b] (0.19)	38,803	152	0.4
CKT1(10)	^{3}H-labeled (BAV3-infected) CKT1[b] (0.38)	77,606	212	0.3
BAV3(3)	^{3}H-labeled BAV3 (0.46)	2,273	1,540	67.8
CKT1(10)	^{3}H-labeled CKT1[c] (2.1)	9,325	1,698	18.2

[a] Average of duplicate measurements. The background (counts/min) was subtracted from the value bound to the filter.

[b] DNA extracted (see Materials and Methods) from CKT1 cells pulse-labeled with [^{3}H]Tdr for 5 h (26 to 31 h after BAV3 infection).

[c] DNA extracted (see Materials and Methods) from growing CKT1 cells labeled with [^{3}H]Tdr for 24 h.

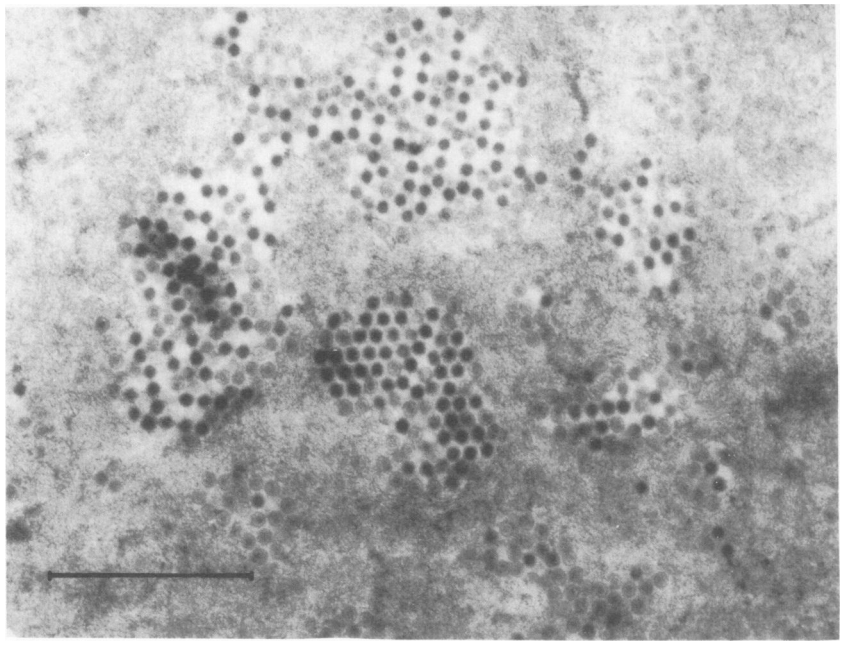

FIG. 2. *Electron micrograph of a BAV3-infected CKT1 cell. BAV3-infected CKT1 cells were harvested 48 h after infection, fixed, and examined by electron microscopy. The electron micrograph shows intranuclear cystalline arrays. The bar represents 1 μm.*

(ii) Band II: d = 1.338 g/ml. This virus was similar to the complete virus in morphology (Fig. 4b), in the composition of its structural proteins, in the homology of its DNA, and in some immunological characteristics, as described later. However, it lacked infectivity as shown in Fig. 3c and so was designated as an incomplete virus.

(iii) Bands III and IV. The buoyant densities of bands III and IV were 1.302 and 1.300 g/ml, respectively, and these bands were broader than bands I and II. Electron microscopy showed that bands III and IV contained many empty particles (Fig. 4c) and aggregated proteins. Moreover, the protein components of both bands III and IV consisted of viral polypeptides lacking core proteins, as described later. These bands contained little or no DNA (at most 10% of that of the complete virus). These results indicate that band III mainly consisted of empty virus and band IV of degraded virus. So they were designated as empty and degraded viruses, respectively.

In contrast to BAV3-0, when any one of the plaque isolates (BAV3-1 to BAV3-10) was propagated under standard culture conditions and then purified, no incomplete virus was observed on CsCl equilibrium density gradient centrifugation (Fig. 3b). When BAV3-0 was passaged at 10 PFU/cell, the incomplete virus was found in about 30% of the amount of complete virus of BAV3, whereas when it was passaged at high multiplicity of infection (20 to 50 PFU/cell), the relative amount of the incomplete virus increased. In contrast, BAV3-1 has never yielded incomplete virus during over 50 passages, even at a high multiplicity of infection (20 to 50 PFU/cell).

Properties of structural components of BAV3. The properties of the structural components (nucleic acid and protein) of complete BAV3 virion were studied, using complete BAV3-1 virion to avoid contamination with incomplete virus. Complete BAV3-1 virion is also called "BAV3" in the following sections. The properties of the structural components of incomplete BAV3 virion were studied using purified incomplete virus of BAV3-0. To minimize contamination with the complete virus, only the upper half of the single opalescent band of the incomplete virus obtained after centrifugation three times in CsCl density gradients was used. The extent of contamination of incomplete virus sample with complete virus was estimated by infectivity assay as less than 1%. No differences were observed between complete viruses of BAV3-0 and BAV3-1 in their buoyant density in CsCl, their morphology, the

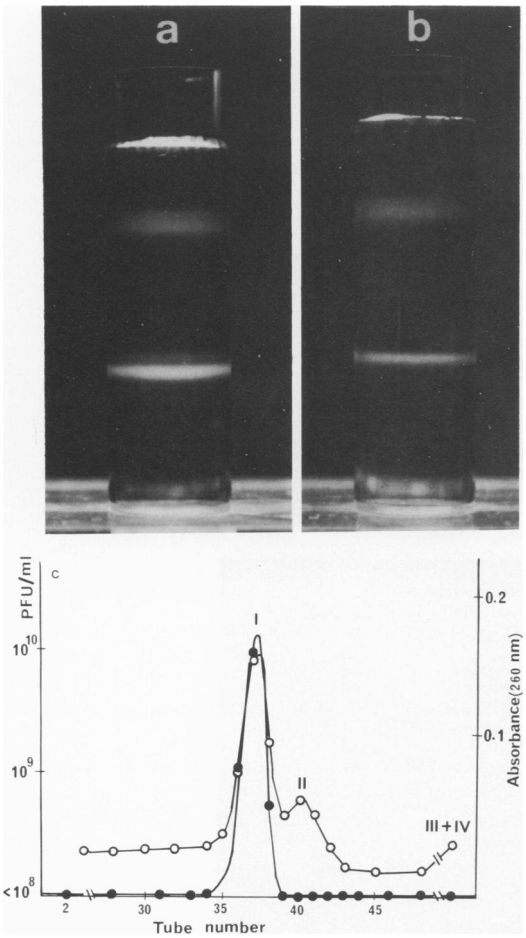

FIG. 3. *Equilibrium sedimentation profiles of BAV3-0 and BAV3-5 in CsCl density gradients. (a) Profiles of BAV3-0. (b) Profiles of BAV3-5 which is one of the plaque-purified clones from BAV3-0. (c) The infectivity (●) and absorbance at 260 nm (○) (×40 diluted sample) of BAV3-0 were determined. Bands III and IV were collected in the same tube in this experiment. The hemagglutinating activity of mouse erythrocytes and absorbance at 280 nm showed similar patterns to that of absorbance at 260 nm (data not shown).*

properties of their structural proteins, and the homology of their DNA.

Properties of structural components of BAV3: properties of viral nucleic acid. The *S* value of the complete virus DNA, determined in a Spinco E analytical ultracentrifuge, was 32.9*S*. The buoyant density of DNA in CsCl was 1.717 g/ml, corresponding to a content of guanine plus cytosine of 58% as calculated by the equation of Schildkraut et al. (41). Electron microscopy showed that the DNAs of both complete and incomplete viruses were linear duplexes with contour lengths of 12.3 ± 0.9 μm and 10.9 ± 1.0 μm, respectively, under the conditions employed (Fig. 5a and b).

The method of heteroduplex formation was used to observe the deletion in incomplete virus DNA. Many DNA molecules with a single-stranded loop (about 13% of the total length) near one terminal were observed in the heteroduplex DNA samples (Fig. 6). Such molecules were not observed in the homoduplex DNA samples. Most of incomplete virus DNA, therefore, seems to have about 13% deletion near one terminal. Single-stranded circular DNA molecules were also observed in the heteroduplex and the homoduplex DNA samples. Both complete and incomplete virus DNAs seemed to form single-stranded circular DNA molecules under the conditions employed. Moreover, the complete virus DNA was cleaved into five fragments (I-V) by a restricting endonuclease, EcoRI (32), whereas upon the same treatment the incomplete virus DNA gave only four fragments, lacking fragment IV of the complete virus DNA. The fragment IV had a size equivalent to about 13% of the total molecular weight of the complete virus DNA (unpublished observation). These results suggest that most of incomplete virus consisted of virions containing DNA with a deletion near one terminal.

The homology between the DNAs of BAV3 and human adenovirus type 5 was about 25%. BAV3 DNA showed practically no homology with CKT1 cellular DNA. Incomplete virus DNA showed homology only with viral DNA (Table 2).

Properties of structural components of BAV3: properties of viral protein. (i) Amino acid analysis of viral structural protein. The amino acid composition of the total structural protein of BAV3 was similar to that of human adenovirus type 2 (Table 3). The result of human adenovirus type 2 virion showed a good coincidence to that of Polasa and Green (35).

(ii) Polypeptides of viral structural protein. The electrophoresis in sodium dodecyl sulfate polyacrylamide gel revealed that the complete BAV3 virion contained at least 10 polypeptides. Molecular weight and relative quantity of each polypeptide were determined (Fig. 7 and 8; Table 4). The estimation of relative quantity was rather arbitrary for two reasons. Different polypeptides may be stained differently with the dye, Coomassie brilliant blue (R250). In addition, some polypeptides were applied on the gel in excess over the proportional staining range with the dye. BAV3

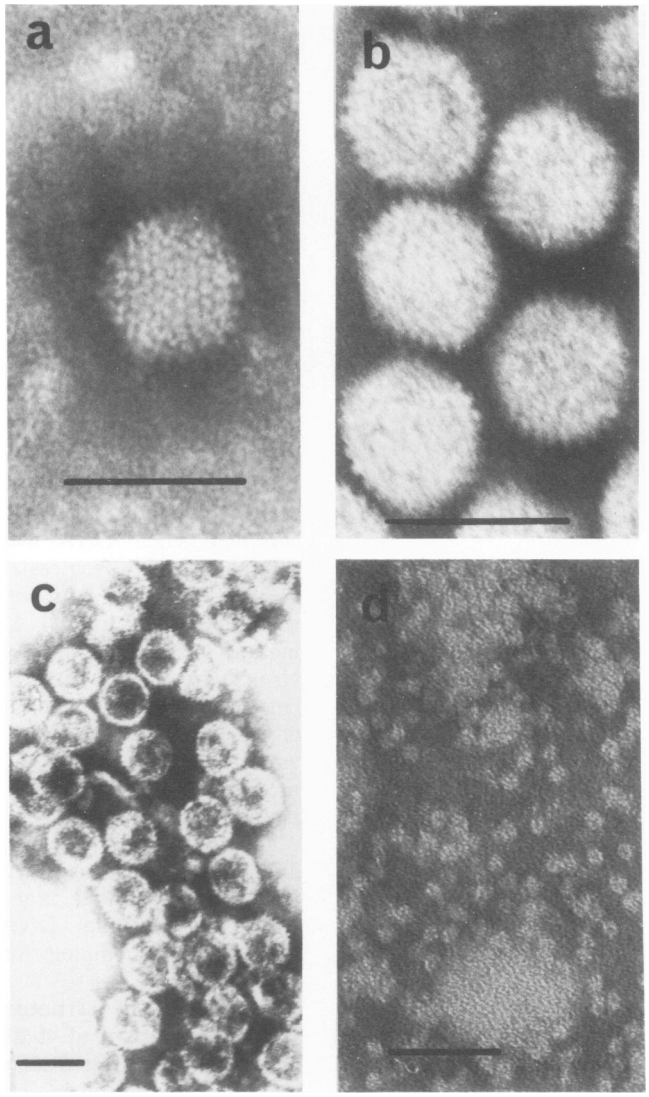

FIG. 4. *Electron micrographs of BAV3 particles and subunits. BAV3 viruses purified by equilibrium density gradient centrifugation in CsCl were stained with phosphotungstic acid and examined. (a) Band I (complete virus); (b) band II (incomplete virus); (c) band III (empty virus); (d) subunits of the complete virus (from a weakly sonicated preparation of complete virus). The bars represent 100 nm.*

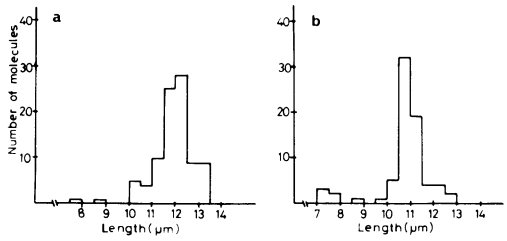

FIG. 5. *Length distribution of complete and incomplete virus DNA. The contour length of DNA was determined by measuring the length of enlarged DNA filaments on a Nikon shadow-graph (final magnification, ×50,000). Length distribution of complete virus DNA (a) and incomplete virus DNA (b).*

structural polypeptides were similar to those of human adenovirus type 2 especially in bands 1 (II) and 6 (VII). The location of BAV3 structural polypeptides in the virion has not yet been determined. However, band 1 seems to be hexon. The analysis of core proteins obtained from the virion by the urea degradation method of Maizel et al. (23) suggested that band 6 was the main component of core proteins (further experiments are needed to clearly identify the

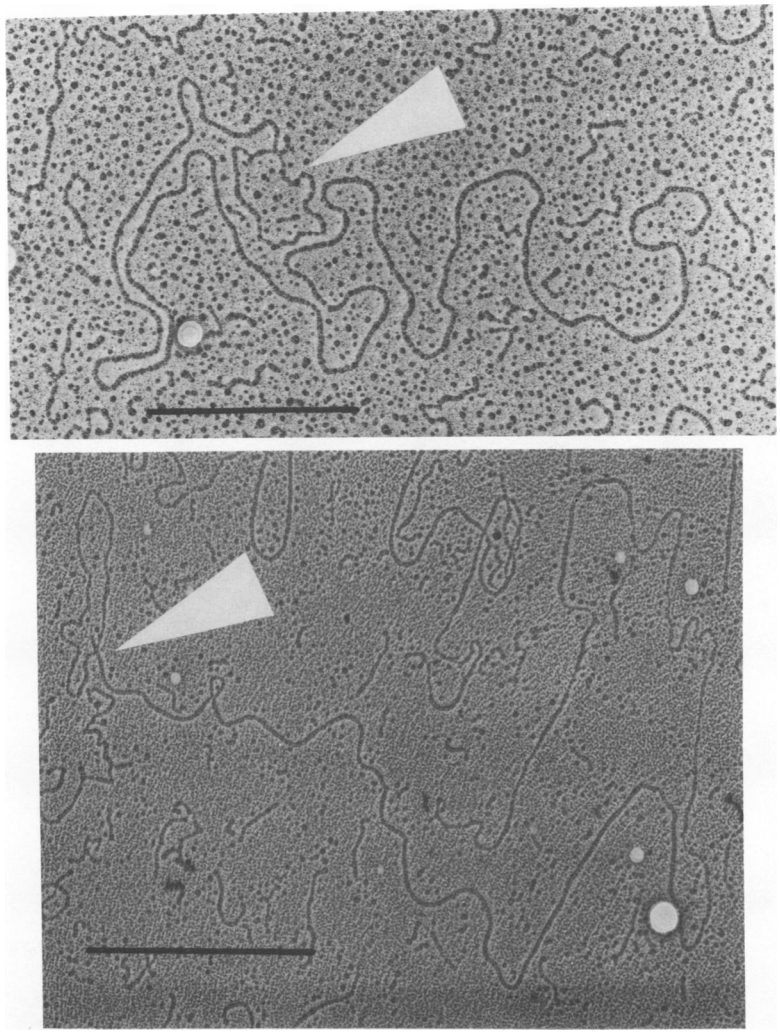

FIG. 6. *Heteroduplex between complete and incomplete virus DNAs. Heteroduplex formation and electron microscopic analysis were described in Materials and Methods. The bar represents 1 μm. Arrows indicate deletion loops.*

TABLE 2. *Hybridization of complete BAV3 DNA with incomplete BAV3, human adenovirus type 5, and cellular DNA*

Experiment	DNA on filter[a]	Input DNA (μg)	Input counts/min	Net counts/min bound[b]	% of input	Homology (%)
A	BAV3 complete	BAV3 complete (0.07)	14,588	12,579	86.6	100
	HAV5[c]	BAV3 complete (0.07)	14,588	3,108	21.4	25
	BAV3 complete	CKT1 cell (0.8)	18,168	58	0.3	0.4
B	BAV3 incomplete	BAV3 complete (0.3)	2,198	1,786	81.3	
	BAV3 incomplete	CKT1 cell (0.8)	18,120	48	0.3	
C	BAV3 complete	BAV3 incomplete (1.0)	19,300	14,286	74.0	
	BAV3 complete	BAV3 complete (1.5)	60,100	40,557	67.5	

[a] 3 μg of DNA on the filter.

[b] Average of duplicate measurements. The background (counts/min) was subtracted from the value bound to the filter.

[c] Human adenovirus type 5.

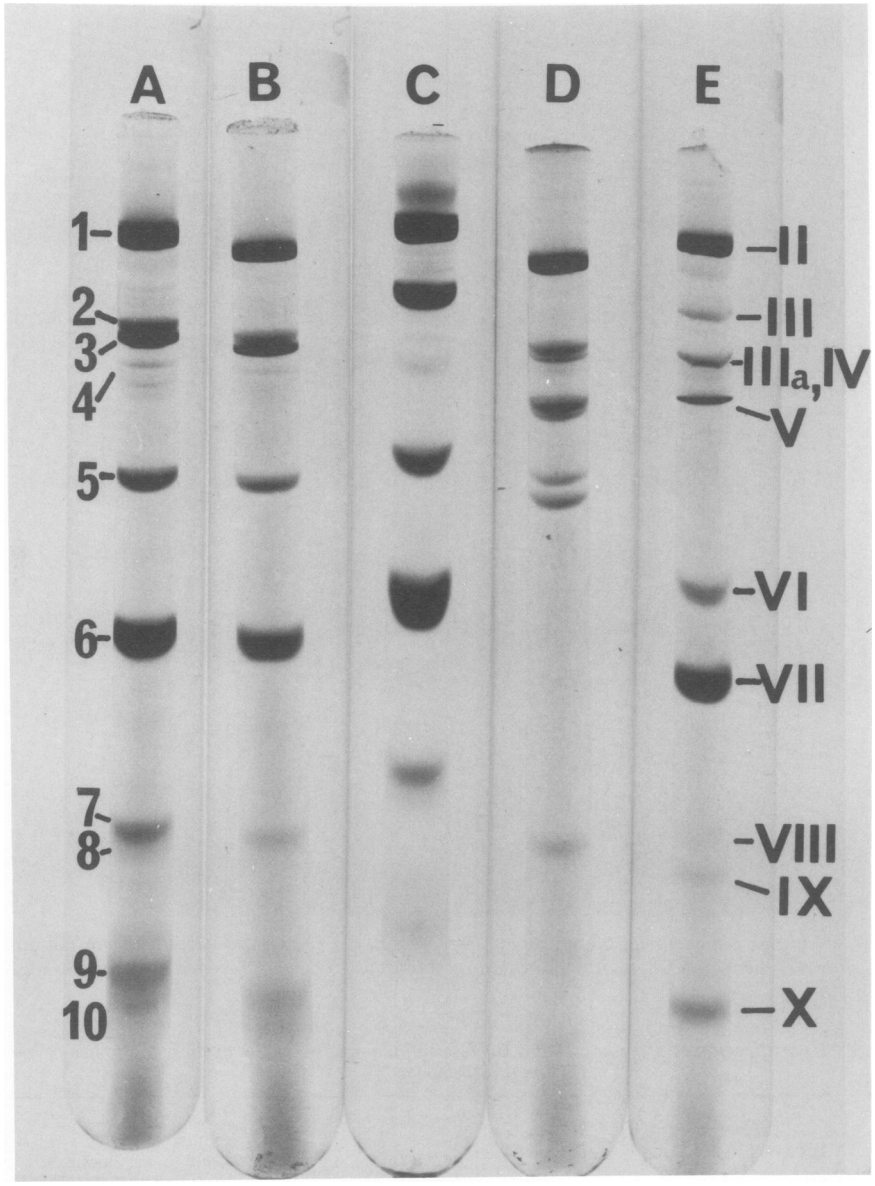

FIG. 7. *Electrophoresis of structural proteins of complete, incomplete, and empty viruses of BAV3 and human adenovirus type 2 in polyacrylamide gels. Purified viruses were analyzed in 13% sodium dodecyl sulfate-polyacrylamide gels. (A) Complete virus of BAV3 (50 μg); (B and C) incomplete virus of BAV3 (25 and 50 μg); (D) empty virus of BAV3 (40 μg); (E) human adenovirus type 2 (50 μg). The polypeptides of human adenovirus type 2 was referred to by Roman numerals.*

polypeptides corresponding to human adenoviruses).

The structural polypeptides of incomplete BAV3 virion were almost identical to those of complete BAV3 virion (Fig. 8).

The structural proteins of empty virus of BAV3 showed at least eight polypeptides. These polypeptides did not include band 6 at all but some additional polypeptides (40,000 to 50,000 daltons) not included in the complete virion (Fig. 7).

The structural polypeptides of degraded virus

of BAV3 were similar to those of empty virus except that they contained one additional polypeptide (about 40,000 daltons).

DISCUSSION

There have been many studies on adenoviruses from various sources (30, 42). However, the physicochemical properties of bovine adenoviruses have not been studied. This paper reports detailed studies on the physicochemical properties of the protein and nucleic acid components of BAV3, which is oncogenic to hamsters.

TABLE 3. *Amino acid composition of viral protein*

Amino acid	BAV3	HAV2[a]
Aspartic acid	9.8[b]	9.6[b]
Threonine	6.1	7.2
Serine	6.1	7.2
Glutamic acid	9.8	8.4
Proline	6.1	7.2
Glycine	8.5	8.4
Alanine	11.0	10.8
Half cystine	<1.0	<1.0
Valine	7.3	6.0
Methionine	1.2	2.4
Isoleucine	3.6	3.6
Leucine	9.3	7.2
Tyrosine	3.6	3.6
Phenylalanine	3.6	2.4
Lysine	2.4	3.6
Histidine	1.2	1.2
Arginine	11.0	10.8
Tryptophan	ND[c]	ND[c]

[a] Human adenovirus type 2.
[b] Molar percentage.
[c] ND, Not determined.

Our preparation of BAV3-0, purified by the method of Green and Piña, formed four virus bands (I, II, III, and IV) on equilibrium density gradient centrifugation in CsCl. It was found that these bands consisted of complete, incomplete, empty, and degraded viruses. On the other hand, plaque-purified virus clones so far obtained in our laboratory did not produce incomplete virus under standard culture conditions even after repeated undiluted passages.

There are several reports on incomplete viruses of human adenoviruses: types 2 and 3 (2, 36, 39, 44), type 12 (2, 24, 40), and type 16 (49). However, all these incomplete viruses were empty viruses which appeared as particles de-

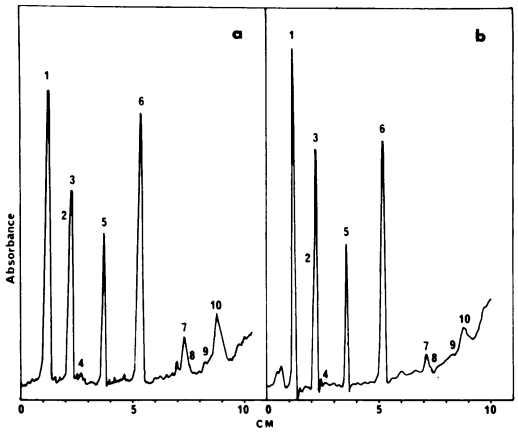

FIG. 8. *Comparison of structural polypeptides of complete and incomplete viruses of BAV3. The Coomasie brilliant blue-stained gels were traced densitometrically by a Joyce-Loebl instrument. (a) Complete virus; (b) incomplete virus.*

TABLE 4. *Polypeptide composition of viral protein*

Band	BAV3		Band[b]	HAV2[a]	
	Mol wt	Ratio(%)		Mol wt	Location
1	113,000	30.2	II	120,000[c]	Hexon[c]
2	68,000	4.8	III	70,000[c]	Penton base[c]
3	64,000	12.6	IIIa	62,000[c]	Peripentonal area[d]
4	52,000	1.0	IV		Fiber[c]
5	35,000	9.9	V	44,000[c]	Core[c]
6	20,000	25.5	VI	24,000[d]	Ninemer[d]
7	10,500	5.2	VII	18,500[d]	Core[c]
8	9,500	1.2	VIII	13,000[c]	Ninemer[c, d]
9	7,200	2.3	IX	12,000[d]	Cementing polypeptide[c, d]
10	6,500	8.0	X	6,500[d]	

[a] Human adenovirus type 2.
[b] Ishibashi and Maizel (15) reported that there are five more polypeptides characteristic of young virions.
[c] Maizel et al. (22).
[d] Everitt et al. (8).

void of cores by electron microscopy, except incomplete virus of human adenovirus type 12 described by Mak (24) and Burlingham et al. (2). Mak reported that the density of the incomplete virus was 0.003 g/ml lower than that of complete virus and that it lacked infectivity. Burlingham et al. designated the incomplete virus as band IIIa. Although it has not been completely separated from the complete virus, it seems similar to our incomplete virus of BAV3.

BAV3-0 produced incomplete virus in a large amount. This incomplete virus appeared rather homogeneous in both structure and functions. No remarkable differences were observed in the morphological or antigenic properties between the complete and the incomplete viruses. The structural polypeptides of the incomplete virus were almost identical to those found in the complete virus both in their molecular weights and relative quantities. However, the incomplete virus seemed to contain DNA with 11 to 13% deletion near one terminal. The extent of this deletion may account for the observed difference between the buoyant densities of the two viruses in CsCl (0.002 g/ml) assuming that complete virus contained 13% DNA in the virion (11) and its density is 1.717 g/ml in CsCl and that the amounts of viral structural protein did not significantly differ between two viruses. The mechanism on the production of incomplete virus of BAV3 remains to be solved. The function deficient in the virus is to be described in the following paper (14).

Purified complete BAV3 virion had almost all characteristic properties of known adenoviruses. The properties of BAV3 particles and DNA are summarized in Tables 5 and 6, respectively.

Adenoviruses have been known to show the icosahedron structures with an average diameter of 75 to 80 nm by electron microscopy. Both the morphology and size of the hexon subunits of BAV3 resembled those of human adenovirus type 2 and the fiber length of BAV3 was similar to those of a subgroup of human adenoviruses with shorter fibers (28).

Purified BAV3 and human adenovirus type 5 formed spurs with anti-BAV3 serum on Ouchterlony plates (Fig. 9). This indicates that they have partially cross-reactive antigenic components (probably hexons) (42).

It was found that BAV3 DNA was a linear duplex with a contour length of 12.3 ± 0.9 μm and $S^0_{20,w}$ value of 32.9S. The values for the molecular weight of the DNA calculated from the contour length and S value were 24.1×10^6 and 27.4×10^6, respectively (43, 45). Both

TABLE 5. *Comparison of properties of particles of BAV3 with those of human adenovirus type 2*

Determinants	BAV3 complete	BAV3 incomplete	HAV2[a]
Diameter (nm)	75–80	75–80	85–95 (18)
Buoyant density in CsCl (g/ml)	1.340	1.338	1.334 (7)
Diameter of hexon (nm)	7.5–9	ND[b]	8–11 (31)
Fiber length (nm)	12–18	12–16	28–31 (28)
Number of polypeptides	10	10	10 (8)–15 (15)
Immunological cross-reaction with human adenoviruses	+	+	

[a] Human adenovirus type 2. Parentheses indicate reference.
[b] ND, Not determined.

TABLE 6. *Comparison of properties of DNA of BAV3 with those of human adenovirus type 2*

Determined	BAV3 complete	BAV3 incomplete	HAV2[a]
(1) Length (μm)	12.3	10.9	12.6 (13)
(2) Molecular weight from (1) (45)[b]	24.1×10^6	21.4×10^6	24.4×10^6 (13)
(3) Sedimentation coefficient	32.9	ND[c]	31.1 (13)
(4) Molecular weight from (3) (43)[b]	27.4×10^6	ND	23.0×10^6 (13)
(5) Buoyant density in CsCl (g/ml)	1.717	ND	1.716 (33)
(6) % G + C[d] content from (5) (41)[b]	58	ND	57 (33)

[a] Human adenovirus type 2.
[b] Reference
[c] ND, Not determined.
[d] G + C, Guanine plus cytosine.

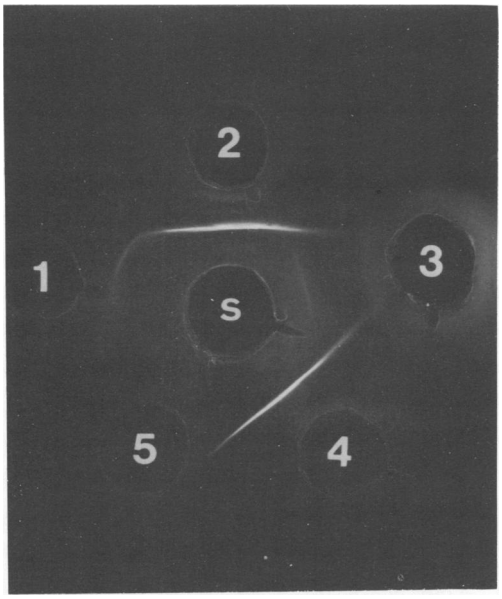

FIG. 9. *Immunodiffusion test of BAV3 and human adenovirus type 5. Rabbit antiserum to purified complete BAV3-1 virion was placed in the central well (S). Wells 1 and 4, Complete BAV3-0 virion; well 2, incomplete BAV3 virion; well 3, human adenovirus type 5; well 5, CKT1 cell homogenate. These purified virions were used after freezing and thawing. Precipitation lines showed that complete and incomplete BAV3-0 virions and complete BAV3-1 virions are antigenically identical. A reaction of partially cross-reactive antigenic sites was observed between BAV3 and human adenovirus type 5.*

complete and incomplete virus DNA of BAV3 seemed to have terminal redundancy as previously reported by Garon et al. (9) in human adenoviruses. The buoyant density of BAV3 DNA in CsCl was 1.717 g/ml and the guanine plus cytosine content was calculated as 58% from the equation of Schildkraut et al. (41). DNA-DNA homology measurements by filter hybridization showed that BAV3 DNA shared 25% of its base sequences with human adenovirus type 5 DNA (Table 2). Heteroduplex analysis (though it has not been done) would be more convincing. It has been reported that the homologies between the DNAs of human adenoviruses of different subgroups are 10 to 26% (10, 19), whereas those between human and simian adenoviruses are 10 to 24% (34). These findings suggest that the sequence of about 20% in their DNA chain of viruses of the adenovirus group may be identical or very similar.

No remarkable difference was observed between BAV3 and human adenovirus type 2 in the amino acid composition of total structural proteins. (The amino acid composition of each subunit has not been determined separately. It cannot be denied that one or more subunits could differ considerably in the amino acid composition between two viruses.) Both molecular weights and relative quantities of the structural polypeptides of BAV3 also resembled those of human adenovirus type 2. However, the polypeptides which constituted the penton subunit seemed to be considerably different from those of the human adenovirus.

It has been reported that two different pathological lesions were observed in BAV3 (WBR1)-infected hamsters (4) and that BAV3 represented a heterogeneous population of virus particles with different tumorigenic potentialities (27). We observed that purified BAV3-0 complete virus induced two types of tumors in newborn hamsters: an undifferentiated, hemorrhagic, transplantable type and a differentiated, nonhemorrhagic, nontransplantable type. The incomplete virus induced tumors to the same extent as the complete virus (14). As described in Results, no difference was found between the physicochemical properties of complete viruses of BAV3-0 and BAV3-1. Moreover, both complete viruses showed about the same extent of early functions (synthesis of viral mRNA, induction of cellular DNA synthesis, and focus formation of the cells) on abortive infection of C3H2K clones (K. Tsukamoto, K. Igarashi, Y. Niiyama, M. Suno, and Y. Sugino, submitted for publication). Plaque isolates other than BAV3-1 have not yet been investigated in detail.

Finally, it should be mentioned that the incomplete virus of BAV3 can be obtained in a relatively large amount by adjusting culture conditions (14), and that the incomplete virus DNA(s) has about 13% deletion(s) not randomly distributed on the molecule but mainly located near one terminal.

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