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Biophysical Properties of Frog Virus and Its Deoxyribonucleic Acid: Fate of Radioactive Virus in the Early Stage of Infection[1]

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Frog virus (FV-3) was banded by isopycnic centrifugation in cesium chloride, sucrose, or potassium tartrate. Two bands of infectivity were regularly found at positions in cesium chloride corresponding to densities of 1.26 and 1.30 g/cm^3, respectively. Deoxyribonucleic acid from either band had the following characteristics: double-stranded; a T_m of 76.3 C in 0.1 SSC (0.015 M NaCl plus 0.015 M sodium citrate) and a buoyant density of 1.720 g/cm^3 in cesium chloride, corresponding to a guanine plus cytosine content of 56 to 58% and a molecular weight of 130 $\times$ 10^6 daltons, determined by velocity sedimentation. These data, together with electron micrographs of sections of cells infected with material from either band suggest that two types of infectious frog virus particles exists, rather than a second virus in the frog virus stocks. The composition of frog virus was determined. It was found that highly purified preparations of frog virus were composed of 55.8% protein, 30.1% deoxyribonucleic acid, and 14.2% lipid. The kinetics of adsorption and uncoating of FV-3 was studied with radioactive virus. Uncoating is comparatively rapid and in contrast to poxvirus is unaffected by inhibitors of protein synthesis.

Although frog virus (FV-3) is herpeslike in a number of its characteristics, it replicates within the cytoplasm of mammalian cells in suspension culture (16). This raises the question of whether the molecular aspects of replication resemble the herpesviruses or the poxviruses. We can say that the kinetics of FV-3 deoxyribonucleic acid (DNA) synthesis in suspension cultures of mammalian cells resembles those of the herpesviruses in that viral DNA synthesis takes place throughout the course of viral maturation (16) rather than in a discrete prematuration step.

With regard to recent work on the early stages of poxvirus replication (14), it is pertinent to inquire whether all deoxyriboviruses that replicate within the cell cytoplasm require a two-stage uncoating mechanism and possess a structural ribonucleic acid (RNA) polymerase. Poxvirus uncoating involves a two-stage process, the second of which is dependent upon protein synthesis (12). This is in contrast to the herpesviruses for which it has been reported that uncoating does not require protein synthesis (11).

With these points in mind, we prepared purified radioactive frog virus and studied the kinetics of its intracellular uncoating as defined by the increase in accessibility of input viral DNA to exogenous deoxyribonuclease.

MATERIALS AND METHODS

Virus and cells. Frog virus was grown and titrated in fathead minnow (FHM) cells and purified as previously described (16). Suspension cultures of BHK 21/13 cells were grown as previously described (16).

Preparation of radioactively labeled frog virus. Radioactively labeled frog virus specifically labeled in viral DNA with either ^{14}C- or ^{3}H-thymidine was prepared in suspension cultures of BHK 21/13 cells. Cells (5 $\times$ 10^8) were harvested by centrifugation (600 $\times$ g, 5 min) and washed once in growth medium lacking serum. The cells were then resuspended at a concentration of 5 $\times$ 10^6 cells per ml in complete growth medium. Frog virus was added to the cells at an input multiplicity of 10 plaque-forming units (PFU) per cell and allowed to adsorb for 1 hr at 27 C. Cells were then diluted to a concentration of 5 $\times$ 10^5 cells per ml with complete growth medium and incubated at 27 C. At 4 hr postinfection, 2 to 5 mc of ^{3}H-thymidine (specific activity = 6.7 mc/mmole) or 200 μc of ^{14}C-thymidine (specific activity = 25 to

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50 mc/mmole) was added to the infected culture which was then incubated for 20 hr at 27 C. At the end of this time, the cells were harvested by centrifugation and washed once in buffer [10^{-2} M tris(hydroxymethyl)aminomethane(Tris)-hydrochloride, *p*H 7.5, containing 0.5 mg of unlabeled thymidine per ml]. The cells were then fractionated into nuclear and cytoplasmic fractions as previously described (16). The cytoplasmic fraction was centrifuged at 59,364 × *g* for 30 min to pellet the virus. The supernatant fluid from the centrifuged cytoplasmic fraction was discarded, and the virus-containing pellet was suspended in 20 ml of RSB (19). Pancreatic deoxyribonuclease (100 μg/ml) was added and the preparation was incubated at 37 C for 0.5 hr. The preparation was then layered onto 36% sucrose in phosphate-buffered saline (w/v) and centrifuged (51,505 × *g* for 60 min). The pellets containing the virus were resuspended in the same buffer.

^{14}C-leucine was used to prepare frog virus labeled in viral structural proteins. A suspension culture of 5 × 10^8 BHK21/13 cells was infected as described above, except that all procedures were carried out in leucine-free medium. At 3 hr postinfection, 300 μc of ^{14}C-L-leucine (specific activity = 15–25 mc/mmole) was added to the culture. The cells were harvested 15 hr later, and virus was prepared from the cytoplasmic fraction of these cells as described above.

Isopycnic centrifugation. The following conditions were employed for isopycnic centrifugation: 27 to 37% (w/w) potassium tartrate (10-ml gradient, SW41 rotor, 4 hr at 100,000 × *g*); 20 to 40% (w/w) sucrose, or 25 to 35% (w/w) CsCl gradients prepared in 0.05 M Tris, *p*H 7.5 (5-ml gradients, SW50 rotor, 100,000 × *g* for 4 hr). Fractions were collected, and the acid-precipitable radioactivity and the optical density at 260 nm of each fraction were determined.

Electron microscopy. Frog virus-infected cells were prepared for electron microscopy as described by Darlington et al. (6). Purified suspensions of virus were treated with an equal volume of 2.0% aqueous phosphotungstic acid adjusted to *p*H 7. A drop of this mixture was placed onto a carbon-coated grid for about 1 min and then washed off by dipping the grid into distilled water. All preparations were examined in a Siemens Elmiskop I electron microscope.

Buoyant density of viral DNA. Radioactive DNA was extracted from viral particles by a modification of the procedure reported by Davern (7). A 4-ml amount of 60% (w/w) cesium chloride dissolved in 0.05 M phosphate buffer (*p*H 6.4) was placed into a centrifuge tube. On the surface of this was layered 0.5 ml of 0.01 M Tris-hydrochloride (*p*H 8.5) containing 0.01 M ethylenediaminetetraacetic acid (EDTA), 0.5% sarcosyl, and 100 μg of Pronase. An amount of radioactive virus equivalent to about 1 μg of viral DNA was added to the upper layer, and the tube was then filled with mineral oil and allowed to stand at room temperature for at least 20 hr. The gradients were then centrifuged (SW50 rotor, 70 hr, 204,035 × *g*, at 25 C). After centrifugation, two-drop fractions were collected from the bottom of the tubes, and the acid-precipitable radioactivity of each fraction was determined. Refractive indices readings were obtained for every fifth fraction. Under these conditions, virtually all of the viral DNA is released in a form degradable by exogenous deoxyribonuclease.

Molecular weight determination of viral DNA. An estimate of the molecular weight of frog virus DNA was obtained by a modification of the technique of Burgi and Hershey (3), with radioactively labeled T2 phage DNA as a molecular weight marker. Since the viral DNA is subject to shear damage, radioactive viral DNA was released as described above. An amount of radioactively labeled virus equivalent to 0.2 to 0.5 μg of DNA in the sarcosyl-Pronase solution was layered onto the surface of a 4-ml 20 to 40% (w/w) sucrose gradient in 0.01 M EDTA and 1.0 M NaCl. The gradients were then overlayed with mineral oil and allowed to stand overnight at room temperature. The gradients were then centrifuged (60 min, 73,449 × *g*, SW50 rotor). Three-drop fractions were collected from each tube.

Melting profile of viral DNA. The melting profile was measured in a Gilford spectrophotometer fitted with a water-jacketed cell holder and equipped to automatically record the absorbancy at 260 nm and temperature of the samples. Viral DNA was obtained from sucrose gradients. The fractions containing viral DNA were pooled and dialyzed against 0.1 × SSC (0.015 M sodium chloride plus 0.015 M sodium citrate, *p*H 7.4). The concentration of viral DNA was adjusted to 10 μg/ml, and the preparation was briefly evacuated to remove air bubbles.

Binding of DNA to cellulose nitrate membrane filters. The binding of DNA to cellulose nitrate membrane filters was determined by the method of Wohlhieter et al. (22). Viral DNA was denatured by raising the *p*H of the sample to 12.3 with 3 N NaOH for 1 min at 5 C, and then neutralizing with 3 N HCl.

Chemical determinations. The DNA and protein content of purified virus preparations were determined by using micromodifications of the procedures of Burton (4) and Lowry et al. (15). Calf thymus DNA (Sigma Chemical Co., St. Louis, Mo.) and bovine plasma albumin (fraction V, Armour Pharmaceuticals, Chicago, Ill.) were used as standards. Viral lipid was extracted by the method of Kates et al. (13), evaporated to dryness, and measured gravimetrically.

Measurement of adsorption. Measurements of adsorption of radioactive frog virus were carried out under the following conditions. BHK cells in suspension were washed once in fresh growth medium and suspended at 6 × 10^6 cells per ml. Radioactive frog virus was added to the cells at an input multiplicity of 10 PFU per cell. Samples of the culture were withdrawn at times and washed twice with cold growth medium; the cell-associated radioactivity was determined. The supernatant fluids from the washes were combined with the supernatant fluid from the cells and precipitated with trichloroacetic acid to determine the unadsorbed radioactivity.

Uncoating of frog virus. Uncoating was determined by the susceptibility of viral DNA to degradation by exogenous deoxyribonuclease. The method used was as described by Joklik (12).

RESULTS

Isopycnic centrifugation of frog virus. Samples of tritium-labeled or unlabeled frog virus were centrifuged to equilibrium in either cesium chloride, sucrose, or potassium tartrate gradients. The gradients were fractionated by collecting drops from the bottom of the tubes. Radioactivity, optical density, and infectivity profiles were determined (Fig. 1 and 2). Radioactivity, optical density at 260 nm, and infectivity profiles coincide, and there are two bands of infectivity in each gradient corresponding to densities of 1.26 g/cm³ and 1.30 g/cm³ in cesium chloride or potassium tartrate. Routinely, in most of our stock preparations the ratio of band I (1.26 g/cm³) to band II (1.30 g/cm³) material is about 3:1 or more.

Electron microscopy of infective bands. Electron microscopic examination was made of negatively stained preparations of material from band I and band II. The particles banding at a density of 1.26 g/cm³ appear (Fig. 3) to be typical frog virus particles (6) measuring 135 nm along the long axis. The material banding at 1.30 g/cm³ appears to be disrupted or collapsed frog virus particles (Fig. 4); however, this was difficult to ascertain because the preparation did not respond well to any of the usual negative staining procedures.

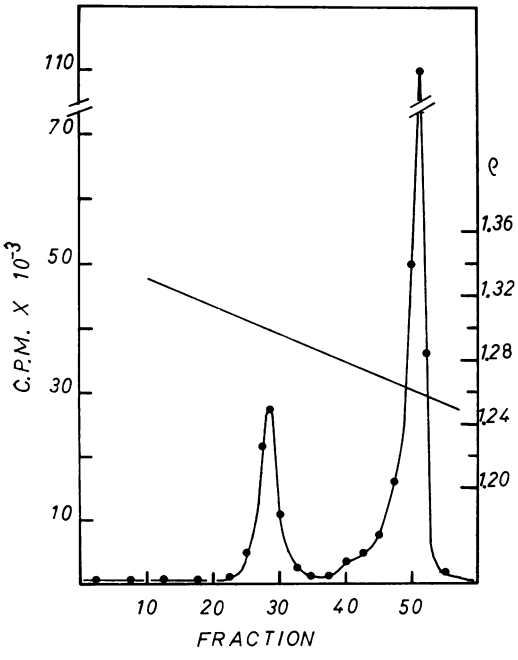

FIG. 1. *Centrifugation of FV-3 in 25 to 35% (w/w) CsCl (SW50 rotor, 4 hr, 100,000 × g). Fractions (5 drops) were collected, and the acid-precipitable radioactivity of each fraction was determined. Refractive indices were measured before precipitation.*

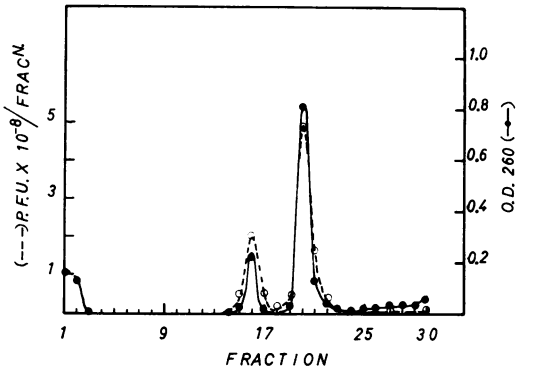

FIG. 2. *Isopycnic centrifugation of FV-3 in 27 to 37% (w/w) potassium tartrate (4 hr, 100,000 × g). Ten-drop fractions were collected. The peaks correspond to densities of 1.26 and 1.30 g/cm³ as determined by refractive indices when compared to standards. Centrifugation of FV-3 in 20 to 40% (w/w) sucrose, under the same conditions, gave results similar to those seen in Fig. 1 and 2.* (○) *Infectivity;* (●) *optical density at 260 nm.*

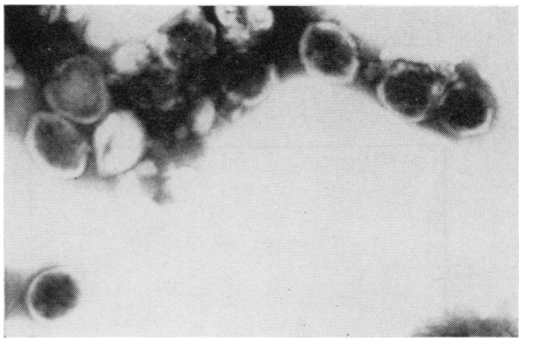

FIG. 3. *Electron micrograph of material from band I. The preparation was negatively stained with phosphotungstic acid and contains only typical frog virus particles.*

To further resolve the question of whether the two peaks of infectivity represent two different viruses or two forms of the same virus, two experimental approaches were tried. (i) If the two bands of infectivity represent two different viruses, then this might be reflected in the base composition and molecular weights of the DNA obtained from these bands. (ii) If the two peaks of infectivity represent two different forms of the same virus, then infection of cells with either form of the virus might yield progeny of both forms, and electron microscopy of thin sections of these

infected cells should show typical frog virus particles.

Buoyant density of DNA. It has been demonstrated that the buoyant density of double-stranded DNA in cesium chloride is a reflection of the guanine plus cytosine (GC) content of that DNA (21). If the two viral bands described above represent different forms of the same virus, then the GC content of their DNA should be identical.

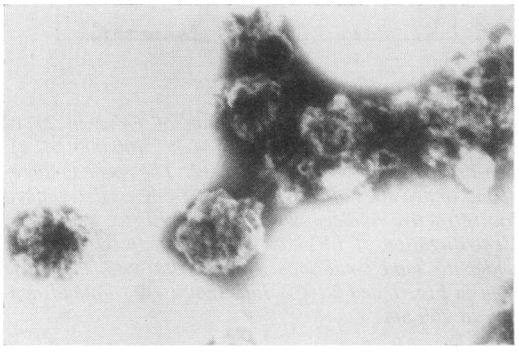

FIG. 4. *Electron micrograph of material from band II. The preparation was treated as in Fig. 3 and appears to contain disrupted or collapsed frog virus particles.*

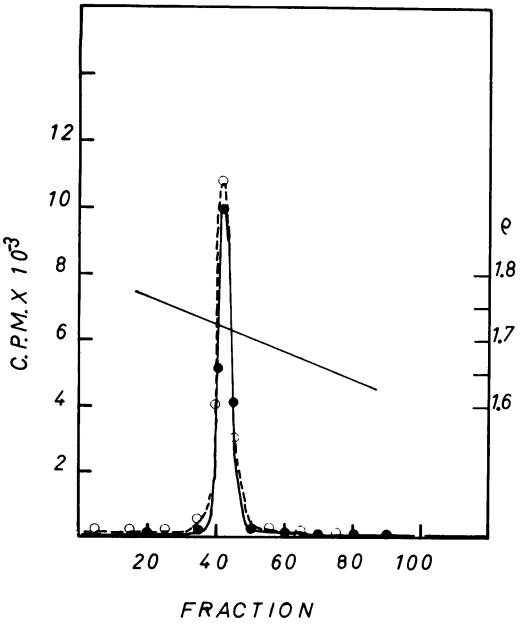

FIG. 5. *Buoyant density determination of DNA from band I and band II particles.* ^{3}*H-DNA from band I particles was centrifuged with* ^{14}*C-DNA from band II particles as described.* (○) *Band I DNA;* (●) *band II DNA.*

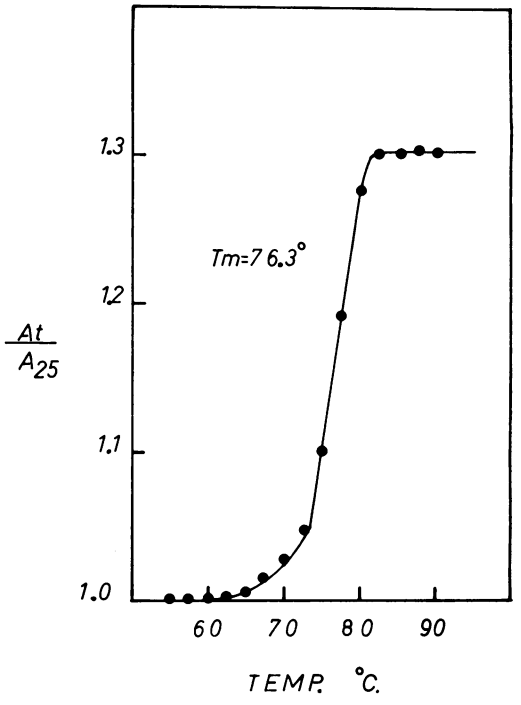

FIG. 6. *Melting profile of band I particle DNA (10 μg/ml) in 0.1 × SSC. Only the high-molecular weight DNA peak was collected for the melting profile. Identical results were obtained with band II particle DNA.*

To test this, ^{14}C-thymidine-labeled DNA from the particles having a density of 1.30 g/cm^3 was centrifuged to equilibrium in a cesium chloride gradient with ^{3}H-thymidine-labeled DNA from the less dense particles. At the end of the centrifugation, two-drop fractions were obtained and the acid-precipitable radioactivity of each fraction was determined. The DNA of particles from each band formed a single peak at a density of 1.720 g/cm^3, corresponding to a GC content of 56 to 58% (Fig. 5) assuming that the viral DNA is double-stranded.

Melting temperature of viral DNA. As an alternative method for the estimation of the GC content of the viral DNA, the melting temperatures of DNA obtained from band I and band II particles were compared. The melting temperature of DNA from both bands was 76.3 C (Fig. 6), corresponding to a GC content of 57% (16), which is in agreement with the above results.

The sharp thermal transition seen in the melting profiles of DNA from bands I and II (Fig. 6) indicate that the viral DNA is double-stranded (17). To confirm this, the method of Wohlhieter et al. (22) was used. Single-stranded DNA is re-

tained by cellulose nitrate filters, whereas double-stranded DNA is not. Table 1 shows the results of such an experiment. Native DNA from either viral band mixed with single-stranded fd phage DNA passes through the filter, whereas the phage DNA is retained. When the viral DNA is alkali-denatured and mixed with native fd DNA, all of the DNA is retained, demonstrating that the DNA in both band I and band II particles is double-stranded.

Molecular weight of viral DNA. The molecular weight of the DNA from each band was estimated by measurement of velocity sedimentation as described. ^{3}H-thymidine-labeled T2 DNA was used as a marker. The DNA of particles from each viral band sedimented at a position corresponding to a molecular weight of 130 $\times$ 10^{6} daltons (Fig. 7).

TABLE 1. *Binding of viral DNA to cellulose nitrate filters*

Prepn	Percentage of radioactivity bound to filter	
	^{3}H	^{14}C
Native ^{3}H-fd DNA + native ^{14}C-frog virus DNA	100	0
Native ^{3}H-fd DNA + native ^{14}C-SVP DNA	100	0
Denatured ^{3}H-fd DNA + denatured ^{14}C-frog virus DNA	100	100
Denatured ^{3}H-fd DNA + denatured ^{14}C-SVP DNA	100	100
Native ^{3}H-*Escherichia coli* DNA	2.6	
Denatured ^{3}H-*E. coli* DNA	100	

Electron microscopy of cells infected with particles from band I and band II. BHK monolayers were infected with particles from either band after further purification by velocity sedimentation in Ficoll gradients (details to be published in a subsequent communication). At 24 hr after infection, the cells were harvested and processed for electron microscopy. In this sections of these cells (Fig. 8), only typical frog virus particles can be seen, regardless of whether the cells were infected with particles from band I or band II.

Characterization of progeny from cells infected with particles from each band. BHK monolayers were infected with particles from each band as described above, and the viral progeny of these cells were harvested. Samples from each of these viral preparations were then centrifuged to equilibrium in cesium chloride gradients, fractions were collected, and the optical density at 260 nm of each fraction was determined. Cells infected with particles from either band yielded populations of each type of particle in about the same proportion of dense to less dense particles. Similar results were obtained from infected FHM cells.

In view of these results, it seems likely that our virus stocks are composed of two forms of the same virus—a typical frog virus particle, with a

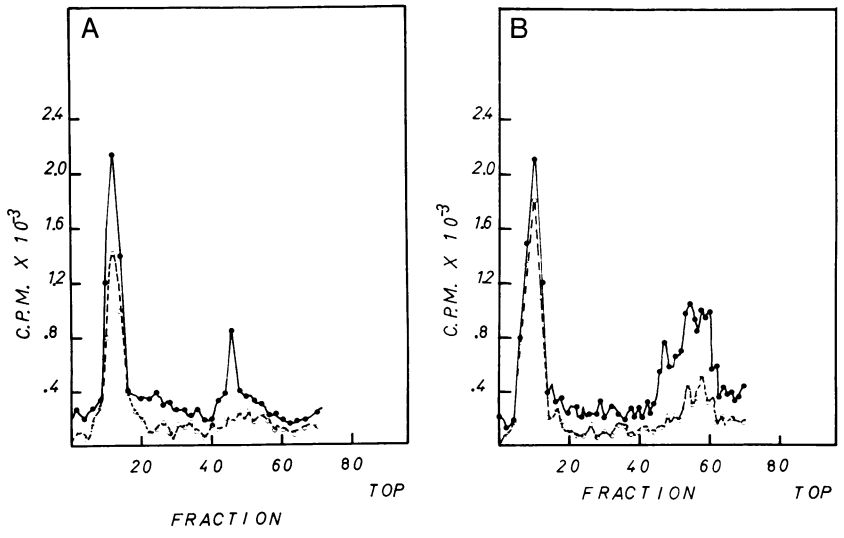

FIG. 7. *(A) Sedimentation velocity of ^{14}C-DNA from band I particles. (B) Sedimentation velocity of ^{14}C-DNA from band II particles. ^{3}H-DNA from T2 phage was used as a molecular weight marker (molecular weight = 130 $\times$ 10^{6} daltons).* (●) *Band I or II DNA;* (○) *T2 DNA.*

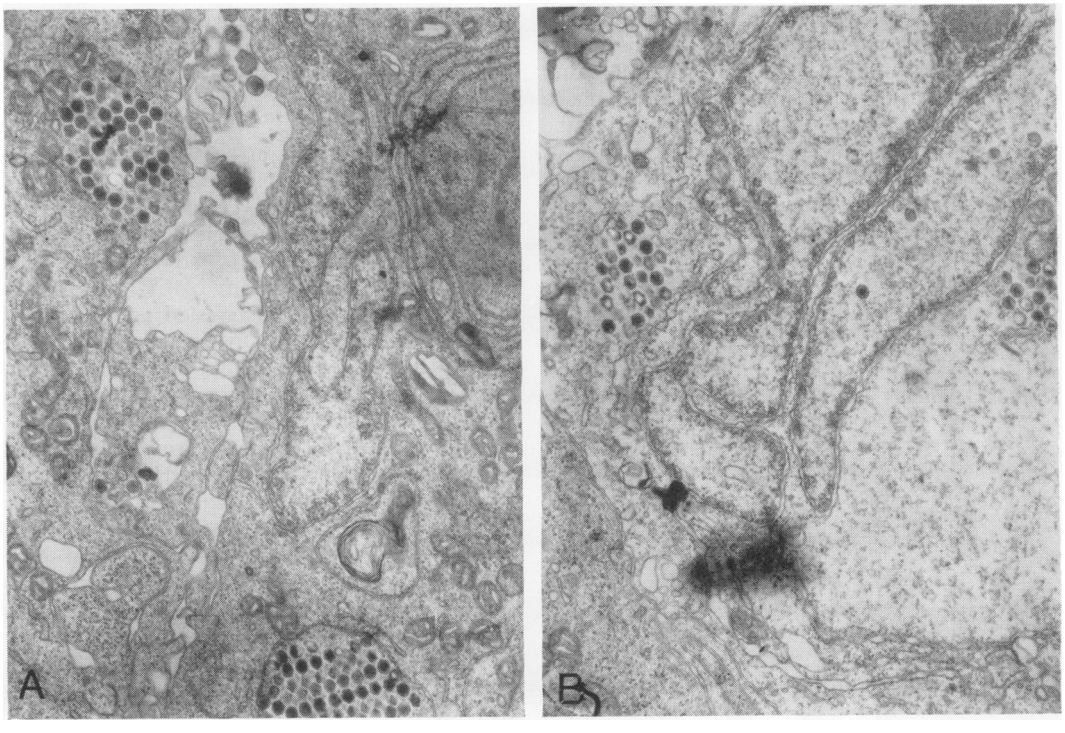

FIG. 8. *Electron micrograph of thin sections of BHK cells infected with material from band I (A) and from band II (B). Regardless of the source of the infecting virus, only typical frog virus is seen in these cells.*

TABLE 2. *Chemical composition of frog virus*

	Protein	DNA	Lipid
	(μg/ml)	(μg/ml)	(μg/ml)
Prepn I			
Assay 1	505.5	283.4	124.4
Assay 2	534.3	279.9	129.4
Assay 3	521.2	266.7	137.2
Average	520.3	276.7	130.3
Per cent	56.1	29.8	14.1
Prepn II			
Assay 1	473.1	254.0	120.1
Assay 2	461.9	259.5	125.3
Assay 3	476.1	257.3	121.3
Average	470.6	256.9	121.3
Per cent	55.4	30.3	14.3

density of 1.26 g/cm^3, and an infective subviral particle (SVP) with a density of 1.30 g/cm^3.

Chemical composition of frog virus. The chemical composition of typical frog virus particles banding at a density position of 1.26 g/cm^3 was determined. The virus used in this study was first purified by velocity sedimentation in Ficoll gradients and then banded in a cesium chloride gradient. Table 2 shows the results of protein, DNA, and lipid determinations for such preparations of frog virus. Virus prepared in this manner was found to consist of 55.8% protein, 30.1% DNA, and 14.2% lipid, assuming that no other constituents were present. An infectivity of 1.11×10^9 PFU/optical density unit at 260 nm or 6×10^9 PFU/mg of protein was determined for the preparations used for chemical analysis.

Adsorption of frog virus to BHK cells. Figure 9 shows the adsorption of ^{3}H-thymidine-labeled frog virus. Under these conditions, maximal adsorption of the input radioactivity occurred within about 40 min. At the time of the initial observation, a relatively large fraction (20% of the adsorbed radioactivity) was already adsorbed to the cells. Since this fraction was so large, it could have represented nonspecific attachment of radioactive, nonviral impurities. To test this possibility, an adsorption experiment was carried out at 5 C, under which condition nonspecific adsorption would be expected to occur, but specific viral adsorption might not occur. It has been shown that the adsorption of frog virus 3 is temperature-dependent (10). Very little radioactivity was adsorbed at this temperature and no significant in-

crease in the amount adsorbed occurred over the observation period. Thus, a large fraction of frog virus is very rapidly adsorbed to BHK cells, accounting for the large initial amount of cell associated radioactivity.

Effect of increased Mg^{2+} concentration on the adsorption of frog virus. The rate of adsorption of viruses can be markedly affected by divalent cation concentration (1, 12). There is a significant increase in the rate of frog virus adsorption to BHK cells if the concentration of Mg^{2+} in the adsorption medium is increased to 2×10^{-2} M (Fig. 9A). Within 60 min, over 50% of the input radioactivity had become cell-associated in the presence of increased Mg^{2+}. In view of this result, all subsequent studies were performed with adsorption medium containing 2×10^{-2} M Mg^{2+}.

Adsorption of SVP to BHK cells. The adsorption of labeled SVP to BHK cells was compared with the adsorption of frog virus to these cells. ^{3}H-thymidine-labeled virus was separated on cesium chloride gradients into frog virus and SVP fractions. BHK cells were then exposed to either SVP or frog virus as described, and the cell-associated radioactivity was determined at various times. Little difference exists in the rate of adsorption of SVP or frog virus to BHK cells, and maximal adsorption of both particles occurred within 40 min (Fig. 9B).

Uncoating of frog virus. The uncoating of frog virus in BHK cells was examined by determining the fraction of input viral DNA accessible to exogenous deoxyribonuclease. About 35 to 40% of the adsorbed radioactivity becomes degradable by deoxyribonuclease during the observation period of 4 hr. Uncoating is apparently a very rapid process, since about 20% of the virus destined to become uncoated has already become so by the time of the initial observation. Similar results were obtained when BHK cells were infected with radioactively labeled SVP. No deoxyribonuclease-susceptible material was present in the viral preparation prior to adsorption.

Effect of inhibition of protein synthesis upon the uncoating of frog virus. The uncoating of frog virus was measured after pretreatment of cells with streptovitacin A, a potent inhibitor of protein synthesis in this system (16). BHK cells were pretreated with 200 μg of streptovitacin A per ml for 1 hr before infection. The cells were then infected with frog virus in the presence of the inhibitor, which was left in for the duration of the experiment, and uncoating was measured at intervals. Parallel control cultures without the inhibitor were also maintained (Table 3). In cells infected with frog virus, no effect of protein inhibition on the rate of uncoating was apparent.

Fate of viral coat protein. The fate of ^{14}C-leucine-labeled viral coat protein in infected cells was determined by measuring the acid-soluble and acid-precipitable radioactivity associated with these cells at various times after infection with labeled frog virus. At least 90% of the viral protein remained acid-precipitable (Table 4), even at a time when viral uncoating is maximal. However, we detected a small fraction of the label which is acid-soluble. This acid-soluble fraction was apparent soon after infection but was not detectable in the virus preparation used.

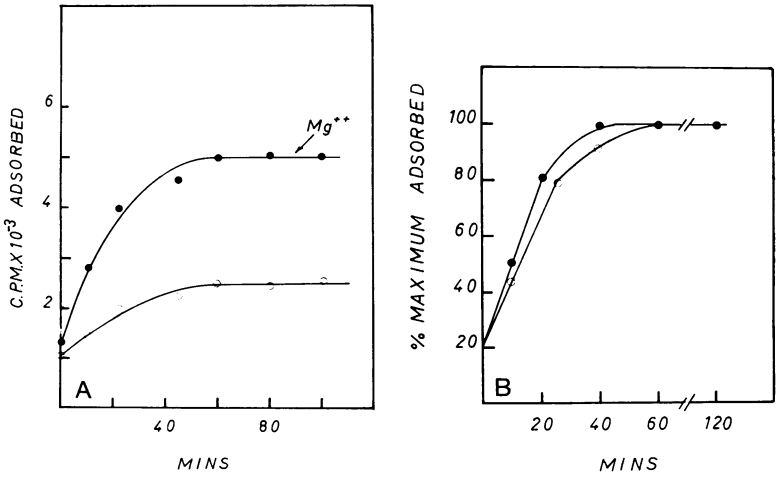

FIG. 9. *(A) Adsorption of frog virus to BHK cells with (●) and without (○) added Mg^{2+}. (B) Adsorption of frog virus (●) and SVP (○) in the presence of added Mg^{2+}. The maximal amount of radioactivity adsorbed in each case was approximately 5,000 counts/min and represents 79% of the input virus.*

TABLE 3. *Uncoating of frog virus in the presence and absence of streptovitacin A*

Time (min)	Fraction of adsorbed virus uncoated	
	Streptovitacin-treated	Untreated
0	100.0[a]	100.0
15	81.4	82.5
30	69.3	70.1
60	54.5	53.2
120	42.8	41.0
180	42.5	40.9
240	41.9	41.2
300	41.8	39.3

[a] One-hundred per cent represents 2,400 counts/min.

TABLE 4. *Fate of viral protein*

Time (mins)	Frog virus		SVP	
	Acid-soluble counts	Acid-precipitable counts	Acid-soluble counts	Acid-precipitable counts
0	169	1,090	206	1,265
15	170	1,066	266	1,335
30	200	1,076	200	1,100
60	127	1,188	240	1,225
120	143	1,033	178	1,266
180	150	1,214	233	1,230

DISCUSSION

Radioactive frog virus can be prepared easily and purified. Two species of infective particles are usually found in stock viral preparations. The bulk of the particles and infective virus prepared from BHK cells or FHM cells has a density in cesium chloride of 1.26 g/cm^3. The minor species of particles bands in cesium chloride at a position corresponding to a density of 1.30 g/cm^3. The particles banding at a position corresponding to a density of 1.26 g/cm^3 are typical frog virus particles when observed by electron microscopy. The more dense particles are ill-defined, as they do not respond easily to standard negative staining procedures. We suggest that they are either derived from frog virus particles during the preparation procedure or represent immature forms of frog virus. The DNA from both types of particles is double-stranded and has the same buoyant density and T_m (corresponding to a GC content of 57%); and the same molecular weight of 130×10^6 daltons (determined by sedimentation velocity). Infection of cells with either particle gives rise to both species of particles; examination of thin sections of cells infected with either species of particle shows the presence of only typical frog virus particles. It is difficult to eliminate the possibility of contaminating band II with band I during collection, but the analysis of the DNA reinforces our argument that we are dealing with two forms of the same virus. The experimental conversion of frog virus to the denser particle has been achieved, but the method is not consistently successful, and we are attempting to improve the technique to determine the specific infectivity of the denser particle and the components lost during the conversion.

In preparations of frog virus grown in chick fibroblasts, Morris et al. (18) found that the majority of their virus banded in cesium chloride at a density of 1.305 g/cm^3, and a minor fraction was found at a density of 1.24 g/cm^3. However, virus infectivity banded at a position corresponding to 1.287 g/cm^3. In that study, the authors found that the DNA from either component had a buoyant density of 1.720 g/cm^3 in cesium chloride, which is in agreement with our determination of the buoyant density of band I and band II particles (1.720 g/cm^3). Since our preparations of frog virus banding at a density of 1.26 g/cm^3 are typical frog virus particles when observed in an electron microscope, we suggest that either the growth of frog virus in chick fibroblasts or, more likely, the method of preparation used by Morris et al. (18) favored the generation of a large proportion of denser particles of low infectivity.

The adsorption of both types of particles to cells is rapid. Maximal amounts of virus are adsorbed within about 30 to 40 min. About 40% of the adsorbed virus becomes uncoated within 60 min after adsorption.

In marked contrast to poxvirus, frog virus uncoating takes place even if protein synthesis is blocked. In this respect, frog virus resembles the herpesviruses (11), and the complex two-stage uncoating mechanism observed for poxvirus (12) is not characteristic of all deoxyriboviruses that replicate within the cytoplasm of host cells.

To date, the classification of frog virus has been difficult primarily because of the lack of information concerning the nature of the viral DNA. In many respects frog virus resembles the herpesviruses. Like the herpesviruses, frog virus which has been released from the cell possesses an envelope, a characteristic which is infrequently found among the vertebrate deoxyriboviruses (8). Both frog virus and herpesviruses are ether-sensitive, and the GC content of frog virus (57%) resembles that of several of the herpesviruses (5, 20). However, in marked contrast to the herpesviruses, frog virus replicates within the cytoplasm

of infected cells (10, 16). If we accept the classification scheme proposed by Gibbs et al. (9), frog virus could be described by the following cryptogram:

D/2:130/30; S/S; V/*

This cryptogram is distinct from that describing any group of vertebrate viruses. The only other virus group which has a similar cryptogram are the cytoplasmic iridescent viruses of insects, but these viruses are ether-resistant and have a GC content of 28 to 31% (2).

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