Cleavage by Formamide of Intercapsomer Bonds in Adenovirus Types 4 and 7 Virions and Hemagglutinins

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When crude preparations of adenovirus types 4 and 7 were treated with solutions of formamide, a complete or nearly complete inactivation of infectivity could be achieved without affecting the complement fixation titers of the same preparations. Studies on purified virions showed that the intersubunit bonds within the viral capsid were broken and single capsomers were released as the result of treatment by formamide. When subjected to the same treatment, hemagglutinins were sequentially converted into incomplete hemagglutinins (pentons), which were in turn converted into penton bases and fibers.

Viruses have generally been studied under conditions considered to be favorable to their survival; however, the study of their behavior in less favorable environments can be equally important in achieving an understanding of the nature and significance of the noncovalent bonds which determine how the protein subunits are organized to form a viral capsid. Profound differences have been found among viruses (viral capsids) in their stability towards solvents supposed to affect their structural integrity. For example, the exposure of poliovirus (2) and turnip yellow mosaic virus (TYMV; 9) to alkaline buffers leads predominantly to the release of ribonucleic acid from the virions and to the formation of empty protein capsids, whereas further degradation into subunits occurs with tobacco mosaic virus (TMV; 1) and adenoviruses (35). Treatment with 8 M urea solutions results in degradation of the adenovirus capsid (H. S. Ginsberg, *personal communication*) and in denaturation of the TMV-protein (3), whereas the capsid of TYMV maintains its integrity under the same conditions (8). A knowledge of the behavior of viruses (viral capsids) toward solutions expected to affect their structural integrity might lead not only to an understanding of how a virus particle is built from its components, but might also lead to methods for preparation of viral antigens free from any genetic material (nucleic acid) and thus perhaps to the development of vaccines from oncogenic viruses.

It has been reported that the adenovirus capsid can be broken by exposure to alkaline solutions (35), heat (26), detergents (31), and *p*-chloro-

mercuribenzoate (17). In the present study, the effect of formamide solutions on the virions and the "soluble components" of adenovirus types 4 and 7 was studied. Formamide has been described as a mild denaturing agent for deoxyribonucleic acids (6, 14) and as capable of breaking catalase into subunits (27). It may also eliminate secondary binding forces during the ion exchange chromatography of polynucleotides on diethylaminoethyl (DEAE) cellulose (32). This reagent, like other amides, is supposed to exert its effect on complex proteins by modifying the structure of the solvent (water); thus, the interactions between the solvent and the macromolecule are altered, resulting in structural changes in the latter (24, 30). We found that, with adenoviruses, treatment of the virions by formamide leads to the cleavage of intersubunit bonds and to the release of capsomers from the viral capsid. Similarly, hemagglutinins are degraded into their structural components.

MATERIALS AND METHODS

Viruses. Adenovirus types 4 (strain CL 68578) and 7 (strain 55142) were obtained from R. M. Chanock, National Institutes of Health, Bethesda, Md. The viruses were propagated in human diploid WI-38 monolayer cell cultures in 1,000-ml Blake bottles. The inoculum consisted of $10^{6.7}$ to $10^{7.7}$ TCID₅₀ of each virus, as titrated on human embryonic kidney (HEK) cells. The cells were maintained in Eagle's medium with 2% agammaglobulin calf serum. The virus material was harvested 6 to 8 days after infection. The pool of cells and the culture medium were frozen and thawed, clarified by low-speed centrifugation, and concentrated 50 to 100 times by ultrafiltration. The

resulting material had an infectivity titer of about 10^9 TCID₅₀/ml (titrated on HEK cells), a hemagglutinin titer of 256 to 1,024, and a complement fixation (CF) titer of 128 to 1,024, as determined by use of homotypic adenovirus antisera. It was stored frozen at -20°C until used.

Biological determinations. Viral infectivity titrations were performed on HEK cells. The final reading of cytopathic effect was performed 10 to 14 days after infection. CF tests were carried out by the microtechnique described by Sever (29); adenovirus types 4 and 7 human convalescent sera, which were supplied, respectively, by G. Micheljohn, University of Colorado, and R. M. Chanock, were used. Titrations of hemagglutinins (HA) were performed as described by others (19, 22). Incomplete hemagglutinins (IHA) of adenovirus types 4 and 7 were titrated under conditions described for types 4 (22) and 3 (20), with adenovirus type 2 rabbit antiserum (Microbiological Associates, Inc., Bethesda, Md.) and 7S-antibodies from adenovirus type 4 human convalescent serum, respectively. (In preliminary experiments, we were able to demonstrate the efficacy of human convalescent type 4 antiserum for the titration of type 7 IHA. Rabbit antisera against different adenovirus types, supplied either by the National Institutes of Health or by Microbiological Associates, Inc., were less suitable for this purpose. The possibility cannot be excluded that antibodies to other adenovirus types, which probably exist in human sera, were in part responsible for the enhancement of hemagglutination.) The 7S γ -globulin fraction was obtained by precipitation with ammonium sulfate (40% saturation), followed by precipitation by rivanol of proteins other than 7S γ -globulin (7, 28). Adenovirus type 4 fiber antigen was assayed as described by Norrby and Wadell (22), with adenovirus type 2 antiserum. Adenovirus type 7 penton bases and fiber antigen were titrated by the hemagglutination enhancement-antibody consumption (HEC) and the hemagglutination inhibition-antibody consumption (HIC) tests, respectively. These tests were described recently for the determination of the corresponding "soluble components" of adenovirus type 3 (21). A suitable dilution of the adenovirus type 4 immunoglobulin preparation described above was used for the HEC test. For the HIC test, purified HA of adenovirus type 7 (see below) and antisera against adenovirus type 7 (Microbiological Associates, Inc.) were used as the indicator system. The quantitative fluorescent precipitin test for adenovirus antigens has been described in a previous report (18).

Purification of virions and HA. The partial purification of virions and HA by high-speed centrifugation was performed under conditions previously described (17). Further purification of virions was achieved by equilibrium density gradient centrifugation in solutions of CsCl. A 0.5-ml amount of the suspension of partially purified virions was layered over 4 ml of a CsCl solution prepared by dissolving 44.5 g of CsCl in 100 ml of phosphate-buffered saline (PBS; 0.075 M phosphate and 0.072 M NaCl, pH 7.2). Centrifugation was performed for 48 hr at $109,000 \times g$ in a Beckman L-4 preparative ultracentrifuge with the

SW65 rotor. The viral band that formed during centrifugation was collected through a hole in the bottom of the tube. CsCl was removed from the purified virus preparation by dialysis against 0.01 M phosphate buffer (pH 7.0).

Further purification of HA was achieved by two cycles of rate zonal centrifugation (2 hr at $101,500 \times g$) under conditions described below. Fractions with the highest titers, corresponding to the middle of the gradient (17), were pooled and dialyzed against PBS.

Rate zonal centrifugation. Rate zonal centrifugation of virions, HA, and other "soluble" virus-specific components was performed with linear sucrose gradients (10 to 25% sucrose in PBS) having volumes of 4 ml in centrifuge tubes for the SW65 rotor. Samples of 0.2 to 0.4 ml were layered on the top of the gradients. The time and speed of centrifugation differed with different viral preparations and is given below for each case. Consecutively numbered fractions of 7 drops were collected from the bottoms of the tubes.

Approximate values of sedimentation coefficients were calculated according to the method of Martin and Ames (15), either with a standard with a known sedimentation coefficient (bovine albumin, $S_{w,20} = 4.4$; bovine γ -globulin, $S_{w,20} = 7.3$) or without such a standard. In the latter case, the time of centrifugation was corrected for the time of acceleration and deceleration of the rotor, and changes of density and viscosity (as tabulated in the *Handbook of Chemistry and Physics*, Chemical Rubber Publishing Co., Cleveland, Ohio) throughout the gradient were taken into account. When the latter method was applied to the standard bovine γ -globulin, the calculated sedimentation coefficient was about 5% higher than the theoretical value. In the case of formamide-PBS-sucrose gradients, the densities of the three-component solutions were determined pycnometrically and their viscosities were determined by use of the V2100 Falling Ball Viscosimeter (Du Pont). For the calculations, the density of the "soluble" adenovirus antigens was considered as 1.30.

Gel filtration. For these experiments, K 25/45 columns filled with Sephadex G-25 (Pharmacia, Uppsala, Sweden) were used, and 1-ml fractions were collected.

Chromatography on triethylaminoethyl (TEAE) cellulose. Portions (1.25 ml) of a crude adenovirus type 7 preparation from which the bulk of virions had been removed by centrifugation (17) were dialyzed overnight against 0.01 M Tris (hydroxymethyl) aminomethane (Tris)-acetate buffer (pH 8.0; TB) and were applied to a column (1.6 by 24 cm) of TEAE cellulose (Serva, Heidelberg, Germany; lot # B2253) equilibrated with TB. For elution, a continuous gradient of increasing NaCl concentration was used (180 ml of TB to 180 ml of 0.4 M NaCl in TB). The effluent was collected in 5-ml fractions, which were titrated for HA, IHA, and CF antigen. Protein in the fractions was determined according to the method of Lowry et al. (13), and the molarity of NaCl was determined by measurements of electrical conductivity.

Treatment of virions and "soluble" virus-specific

components with formamide. If not otherwise stated, purified virions and HA were treated with formamide (stabilized; Fisher Scientific Co., Fair Lawn, N.J.) by mixing two volumes of the viral preparation with one volume of the reagent and incubating the mixture at 37 C for 1 hr. To follow the kinetics of inactivation of HA (IHA) at different formamide levels, appropriate volumes of the viral preparation and of the reagent were mixed and incubated at a chosen temperature. At time intervals, 0.1-ml samples were withdrawn into 0.9 ml of PBS and were titrated for residual HA (IHA). For similar experiments with the CF antigens, crude viral preparations from which the bulk of virions and HA had been removed by centrifugation were used. Formamide was removed from the sample (2 ml) by gel filtration on Sephadex G-25. The fractions with the highest protein content were titrated for CF antigen with homotypic antisera.

To follow the effect of treatment with formamide on the infectivity of the viruses, crude infectious tissue culture fluids were used. Portions (0.25 ml) of the fluid were mixed with 0.01 to 0.12 ml of formamide (in increments of 0.01 ml), and the mixtures were incubated for 1 hr at 37 C. Formamide was removed from the samples by dialysis (at 4 C) against PBS.

RESULTS

Preliminary survey of the effect of formamide on the virions and the "soluble" components of adenovirus types 4 and 7. To determine whether formamide might be a suitable reagent for disrupting the adenovirus capsid, the effect of different dosage levels on the biological activities of virions and "soluble" viral components was investigated. In preliminary experiments with adenovirus type 7, treatment with formamide (1 hr at 37 C) at a 7.4% (v/v) concentration resulted in a one log loss of infectivity. Treatment at higher levels (10.7 and 13.8%) resulted in three and five log decreases of infectious titer, respectively. The type 4 virions appeared to be more resistant to the action of formamide; treatment with 19.3% formamide resulted in only a three-log reduction of infectious titer. In numerous other experiments with both virus types and with levels of formamide reaching 33%, infectivity either was lost completely or was reduced to very low levels (about 10^2 TCID₅₀/ml from an initial titer of about 10^9 TCID₅₀/ml).

The results of inactivation studies on the HA of both adenovirus types at different concentrations of formamide are presented in Fig. 1 and 2; the type 4 HA were more resistant to formamide than the type 7 HA. The hemagglutination titrations performed in the presence of suitable heterotypic antisera gave higher results than those performed in PBS only, which suggests the possibility that formamide treatment disrupts complete HA, which consist of 12 pentons (19, 22, 33), forming IHA; i.e., single pentons (5, 20,

FIG. 1. Inactivation of adenovirus type 7 HA by formamide. The titrations were performed in the presence (○) or absence (●) of adenovirus type 4 antibodies. Crude tissue culture material from which virions had been removed was mixed with suitable volumes of formamide and PBS to achieve the desired final concentrations of formamide. The mixtures were incubated 1 hr at 37 C.

FIG. 2. Inactivation of adenovirus type 4 HA by formamide. Symbols and experimental conditions as in Fig. 1, except that IHA was titrated in the presence of adenovirus type 2 antibodies.

22). This problem will be considered later in this paper and in a subsequent publication.

Formamide failed to affect the CF titer at concentrations that caused the destruction of more than 99.9% of both infectivity and HA. Figure 3 shows that decreases in CF could only be achieved with much higher levels of formamide and that the effect of formamide was greater on type 7 than on type 4.

Disruption of virions of adenovirus types 4 and 7 by formamide. Purified virions were treated with

formamide and were submitted to rate zonal centrifugation (30 min at 18,000 rev/min) after sufficient formamide had been removed by dialysis (for 1 hr against distilled water) to permit flotation on a sucrose gradient. Simultaneously, untreated virions were also centrifuged. No visible virus band was observed, as compared with the band in untreated material, and no CF antigen (and fluorescent precipitating antigen as tested with the type 4 material only) was recovered from the gradient fractions in which intact virus particles, if present, would have occurred (Fig. 4 and 5). Instead, increased levels of CF antigen (and precipitating antigen) were found in the top fractions from the density gradients with treated virions, indicating a degradation of the latter. The occurrence of low levels of CF antigen (and precipitating antigen) in the top fractions from the untreated virus material may be ascribed to a partial damage of the virions in the course of the experimental procedures.

When the formamide-treated purified virus was submitted to rate zonal centrifugation at higher speeds ($300,000 \times g$ for 2 hr), the CF antigen (and precipitating antigen) was recovered in a single peak from the gradient. This peak was at a position similar to the CF antigen peak obtained after (similar) gradient centrifugation of crude tissue culture material from which the virions and hemagglutinins had been removed. The position of this CF peak was the same in such culture supernatant fluids whether they had or had not been treated with formamide (Fig. 6 and 7). The position of the CF antigen peaks in all of these gradients corresponded to a sedimentation coefficient of about 12S, in reasonable agreement with the value suggested for the hexon antigen of several adenovirus types (4, 10, 19, 34). It may be

FIG. 3. Inactivation of adenovirus types 4 (●) and 7 (○) CF antigens by formamide. Experimental conditions as in Fig. 1. Formamide was removed prior to titration by gel filtration on Sephadex G-25.

FIG. 4. Distribution of CF and precipitating antigens after rate zonal centrifugation ($24,000 \times g$ for 30 min) of formamide-treated (top) and untreated (bottom) virions of adenovirus type 4. CF antigen is indicated by columns with diagonal lines slanting down to the left; precipitating antigen is indicated by columns with diagonal lines slanting down to the right. For formamide treatment, 0.25 ml of the virus suspension in PBS was mixed with 0.125 ml of the reagent and the mixture was incubated at 37 C for 1 hr.

concluded from this finding that the main products resulting from the treatment of virions by formamide are single hexon antigens. It will be further shown that penton antigens are degraded under the same conditions of treatment with formamide.

Hexon antigens are thought to consist of six subunits (H. S. Ginsberg, *personal communication*). During the process of treatment with formamide, the hexons might be degraded into such subunits, and, during subsequent removal of the reagent by dialysis or gel filtration, reaggregation might occur. To exclude this possibility, tissue culture fluids from which virions and HA had been removed were treated with formamide (33% for 1 hr at 37 C) and were submitted to density gradient centrifugation ($300,000 \times g$ for 4 hr) with a 10 to 25% sucrose gradient in 33% formamide (two volumes of PBS plus one

volume of the reagent). Bovine γ -globulin was centrifuged simultaneously as a standard. Each fraction from the gradient was then dialyzed against PBS and titrated for CF antigens. The CF antigens sedimented more rapidly than the 7.3S γ -globulin and had a position in the gradient corresponding approximately to a 12S particle, though the presence of larger aggregates (especially with type 7 material) cannot be excluded (Fig. 8). These data suggested that hexon antigens are not degraded into smaller units during treatment with 33% formamide. Gel filtration experiments with the same formamide-treated tissue culture fluids, using Sephadex G-200 with 33% formamide as the eluent, led to a similar conclusion (*unpublished data*).

Identification of IHA of adenovirus type 7, and the sequential conversion of complete HA into IHA and of the latter into penton bases and fibers. Study of the kinetics of inactivation of adenovirus type 7 HA by formamide (7.4% at 37 C), using virion-free tissue culture supernatant material, revealed different speeds of inactivation of HA activity, depending on whether heterotypic antibodies had been incorporated into the

FIG. 5. Distribution of CF antigen after rate zonal centrifugation of formamide-treated (top) and untreated (bottom) adenovirus type 7 virions. Symbols and experimental conditions as in Fig. 4.

FIG. 6. Distribution of CF and precipitating antigens after rate zonal centrifugation ($300,000 \times g$ for 2 hr) of virion- and HA-free adenovirus type 4 crude tissue culture fluid (top), the same fluid treated with formamide (middle), and purified virions treated with formamide (bottom). Symbols and experimental conditions as in Fig. 4.

diluent used for titration (Fig. 9). Taking into consideration that IHA of type 7 may be like IHA (pentons) of type 3, which are detectable only in the presence of heterotypic antibodies (20), the data presented in Fig. 9 suggested that such IHA either may have been present originally in the adenovirus type 7 tissue culture material and was more resistant to the inactivating effect of formamide, or was formed as a result of a possible degradation of complete HA. To determine whether either or both of these explanations were valid, the original adenovirus type 7 tissue

culture material was submitted to rate zonal centrifugation (2 hr at $133,000 \times g$) and the fractions were titrated for HA in the presence of heterotypic antibodies. Two peaks of HA activity could be distinguished; the one nearer the bottom of the tube was also detectable in the absence of heterotypic antibodies and represented complete HA with a sedimentation coefficient of about 55S to 65S (17), and the other, slower sedimenting peak represented IHA (Fig. 10). The latter peak seemed to sediment more rapidly than hexon antigen; this was confirmed by additional rate zonal centrifugation experiments at higher speeds ($228,000 \times g$ for 2 hr), which clearly separated IHA and hexon antigen (*unpublished data*). In addition, by chromatography on TEAE-cellulose, two different entities with hemagglutinating activity, representing complete HA and IHA,

FIG. 7. Distribution of CF antigen after rate zonal centrifugation ($300,000 \times g$ for 2 hr) of virion- and HA-free adenovirus type 7 crude tissue culture fluid (top), the same fluid treated with formamide (middle), and purified virions treated with formamide (bottom). Symbols and experimental conditions as in Fig. 4.

FIG. 8. Distribution of protein (black columns) and CF antigen (columns with diagonal lines) after rate zonal centrifugation ($300,000 \times g$ for 4 hr) of bovine γ -globulin (top) and of adenovirus types 4 (middle) and 7 (bottom) tissue culture materials in sucrose gradients (10 to 25% sucrose in 33% formamide). Formamide treatment as in Fig. 4. Distribution of CF antigen throughout the gradients was the same whether titrated with homotypic or heterotypic antisera.

could be distinguished (Fig. 11) in a way analogous to the chromatography of the "soluble" components of adenovirus type 3 on DEAE-Sephadex (20). Thus, the occurrence of IHA of adenovirus type 7 in crude tissue culture material could be established.

To demonstrate that the complete HA of adenovirus type 7 was degraded into smaller fragments having IHA activity, the original and the formamide-treated tissue culture fluids were submitted to rate zonal centrifugation (2 hr at $145,000 \times g$) and the fractions were titrated in duplicate for HA in the presence of heterotypic

antibodies. The tissue culture fluids used in this experiment had been treated for 1 hr at 37 C with formamide at concentrations of 7.7 and 9.1%. Because of spontaneous breakdown, the use of purified HA offered no advantage. The ratio of the HA titer for each fraction before and after treatment was calculated and plotted (Fig. 12). The data showed that treatment with formamide resulted in a decrease of concentration of the more

FIG. 9. Kinetics of inactivation of adenovirus type 7 HA in 7.4% formamide at 37 C. The titrations were performed in PBS (●) and in a preparation (diluted 100-fold) of adenovirus type 4 antibodies (○).

FIG. 10. Rate zonal centrifugation ($133,000 \times g$ for 2 hr) of adenovirus type 7 HA. Fraction 1, bottom; fraction 18, top. Titrations were performed in the presence of adenovirus type 4 antibodies. Arrows show positions in the gradient corresponding to the indicated sedimentation coefficients. A sedimentation coefficient between 55 and 65S has been ascribed to the complete HA of adenovirus type 7 (17).

FIG. 11. Distribution of biological activities after fractionation of adenovirus type 7 tissue culture material by chromatography on TEAE-cellulose. CF antigen, columns with diagonal lines slanting down to the left; HA detected in the presence of heterotypic adenovirus type 4 antibodies, dotted columns; HA detected in the absence of heterotypic antibodies, columns with diagonal lines slanting down to the right; molarity of NaCl, dotted line; protein, dashed line. The CF antigens were titrated in convalescent human serum which (as shown here) does not fix complement with HA or IHA.

FIG. 12. Conversion of adenovirus type 7 HA into smaller degradation products with IHA activity by treatment with formamide. Virion-free tissue culture fluid was treated with formamide at concentrations of 7.7 (●) or 9.1% (○) for 1 hr at 37 C. Treated and untreated materials were subjected to rate zonal centrifugation at $145,000 \times g$ for 2 hr. Fractions were titrated for HA in the presence of adenovirus type 4 heterotypic antibodies. The ratio of after-treatment to before-treatment titers was calculated for each numbered fraction and plotted as shown.

rapidly sedimenting HA and their conversion into slower sedimenting particles. Similar results were obtained with purified HA of adenovirus type 4 (Fig. 13).

When a higher concentration of formamide (33%) was used to treat the purified HA (30 min

at 37 C), no IHA of adenovirus type 7 could be recovered (see Fig. 1). When HA treated in this way were submitted to rate zonal centrifugation ($300,000 \times g$ for 3 hr), fiber antigen and penton bases could be detected in some of the fractions (Fig. 14) by use of appropriate serological techniques (21). The fiber antigen sedimented at about the same or slightly higher speed than the bovine albumin standard and the penton bases sedimented about twice as rapidly. From the gradient with adenovirus type 4 HA treated in the same way, only slowly sedimenting IHA (i.e., fiber antigen; 22) could be recovered. The latter sedimented slightly more rapidly than bovine albumin. These data suggested that, at higher levels of formamide (33%), penton antigens are largely degraded into penton bases and fibers.

DISCUSSION

Virions of adenovirus types 4 and 7 are degraded by treatment with 33% formamide into CF antigens sedimenting during centrifugation at about the same rate as hexon antigens and are morphologically indistinguishable from hexons

FIG. 13. Distribution of adenovirus type 4 HA after (top) and before (bottom) treatment with formamide in sucrose gradients after centrifugation at $145,000 \times g$ for 2 hr. The purified HA was treated with 9.1% formamide for 1 hr at 37 C. Titrations were performed in the presence of heterotypic adenovirus type 2 antibodies.

FIG. 14. Conversion of adenovirus type 4 (bottom) and 7 (middle) HA into fiber antigen and penton bases by treatment with formamide. Fiber antigen, dotted columns; penton bases, columns with diagonal lines; protein, black columns. Purified HA was treated with 33% formamide for 1 hr at 37 C and was submitted to rate zonal centrifugation at $300,000 \times g$ for 3 hr. As a standard, bovine albumin (top) was centrifuged simultaneously. The HEC and HIC tests (see text) were used to titrate adenovirus type 7 penton bases and fiber antigen, respectively. Adenovirus type 4 fiber antigen was detected by titration of HA in the presence of heterotypic adenovirus type 2 antibodies.

(in preparation). It is therefore suggested that formamide causes a cleavage of intercapsomer bonds in the virions, resulting in the release of single capsomers. Studies on HA suggest that at the same time penton antigens are further degraded into penton bases and fibers.

When sedimented in sucrose gradients, the IHA of adenovirus type 7 behaved differently from the IHA of adenovirus type 3 (20), which have a sedimentation coefficient of about 13S (19) and are thus not separable from the hexon antigen. The IHA of adenovirus type 7 sedimented more rapidly, with a speed corresponding

to particles with a sedimentation coefficient of 20S to 30S. Since slight differences in the size of fibers and penton bases between the adenovirus types 3 and 7 could hardly explain the observed large differences in rate of sedimentation of the corresponding IHA, we assume that IHA of adenovirus type 7 might exist in the form of aggregates of penton antigens (probably two to three per aggregate). Attempts to detect such aggregates by electron microscopy of the preparations of IHA have thus far failed, possibly as a result of degradation during the staining procedures.

Complete HA of adenovirus types 4 and 7 are degraded by formamide, the extent of degradation depending on the concentration of the reagent and the time of treatment. The experimental results suggest that, in formamide solutions, the bonds between the individual pentons within the complete HA (4, 19, 20, 22, 33) are more labile than are the bonds between the penton bases and fibers. A similar breakdown of the penton antigen of adenovirus type 3 into the corresponding two components has also been achieved by treatment with 1.6 to 2.0 M solutions of guanidine hydrochloride (22).

The described sedimentation behavior of the fiber antigens is in reasonable agreement with the value of 5.5S found for the fiber antigen of adenovirus type 2 by analytical ultracentrifugation (10). Methods other than rate zonal centrifugation would probably be more suitable for detecting possible differences in the size of the fiber antigens of the different adenovirus types (21).

The rate of HA inactivation by formamide increases with increasing pH values of the medium and with increasing ionic strength (*unpublished data*). The latter observation, together with the fact that the presence of formamide leads to an increase of the dielectric constant of the solvent (12), suggests that formamide affects salt links and hydrogen bonds rather than hydrophobic bonds within the HA (3).

In the process of degradation of TMV by urea, alkali, or sodium dodecyl sulfate, protein subunits are stripped from one end of the virus rod (3, 16). It would be of interest to determine whether the degradation by formamide of adenoviruses, i.e., virions with icosahedral symmetry, proceeds randomly or whether it is initiated at "weak points" of the viral capsid. Electron microscopic observations (*in preparation*) indicate that the latter is true. Studies on the kinetics of HA inactivation and electron microscopic studies on the breakdown of virions by solutions containing different amounts of formamide show that the degradation is not immediate and that it consists of an ordered sequence of events.

The rate of HA inactivation is strongly temperature-dependent; although no difference in the inactivation at 0 and at 22°C could be detected, an increase to 37°C enhanced the rate of inactivation considerably (*unpublished data*), indicating that an activation energy threshold must be reached to initiate the degradation induced by formamide.

Recent studies (11, 23, 25, 26) suggest the existence of an internal adenovirus protein associated with the viral deoxyribonucleic acid. The fate of these two viral components during the degradation induced by formamide and the physical nature of the occasionally observed residual infectivity will be the subject of a separate report.

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