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Characterization of Adenovirus Particles Made by Deletion Mutants Lacking the Fiber Gene

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H2*dl*802, H2*dl*807, and H5*dl*1021 are defective deletion mutants of human adenovirus which do not make the capsid protein fiber yet which can make substantial amounts of virus particles. Virions made by the mutants contain very little fiber (which comes from helper virus contaminants in the deletion virus stocks): less than 6% as much as that contained by wild-type virions. This demonstrates that fiber is not an essential structural component of the adenovirus virion and suggests that fiber is nonessential for virion assembly. These fiber-deficient particles are poorly adsorbed to cells, consistent with the proposed role of fiber in virus attachment. Further, virion protein precursors, including that of the virion protease, are poorly processed in these particles, suggesting a relationship between the presence of fiber and the maturation of the virus particle.

Recently, we described three defective deletion mutants of human adenovirus, H2*dl*802, H2*dl*807, and H5*dl*1021 (Fig. 1), none of which can make the capsid protein fiber yet all of which are capable of producing virus particles at nearly wild-type levels (10). These mutants also lack large segments of early region 3, and in addition, H2*dl*807 lacks the promoter distal half of early region 4 (E4). Because of the loss of E4, H2*dl*807 produces substantial numbers of virus particles only in the W162 cell line, a Vero cell derivative which contains E4 DNA and which supports the growth of E4 mutants (22). H2*dl*802 and H5*dl*1021 produce particles at nearly wild-type levels in all cells tested. It came as a surprise that these mutants could make particles in the absence of fiber, since previous results obtained with temperature-sensitive (*ts*) mutants (4, 6, 9) had suggested that fiber was essential for the assembly of virions. There is some fiber synthesized in cells infected by these mutants because of residual helper virus in the deletion virus stocks, but the amount of fiber synthesized is small, 0.03 to 0.3% of the wild-type level. This trace amount of fiber in deletion mutant-infected cells could be enough to allow the assembly of wild-type levels of virus particles with a wild-type protein composition. Alternatively, fiber may not be an essential structural component of adenovirions. This report describes experiments which investigated the protein composition of the virus particles made by H2*dl*802, H2*dl*807, and H5*dl*1021.

The amount of fiber on the virions made in deletion mutant infections was compared with that on adenovirus type 2 (Ad2) and adenovirus type 5 (Ad5) virions. W162 cells growing in a 9-cm-diameter dish were labeled with 100 μCi of [^{35}S]methionine from 24 to 30 h postinfection by H2*dl*802, H2*dl*807, Ad2, or Ad5 and then were chased with nonradioactive medium until 48 h postinfection. H5*dl*1021 and Ad5 were grown similarly on Vero cells. The cells were then scraped from the dishes, and virus particles were purified as described previously (2). The virions were exhaustively dialyzed against 0.05 M Tris hydrochloride (pH 6.8)–10% glycerol and then were adjusted to 1% sodium dodecyl sulfate (SDS)–1% 2-mercaptoethanol. The samples were boiled and briefly centrifuged, and the supernatants were adjusted to 10% Nonidet P-40–0.15 M NaCl. Portions were then immune precipitated with a rabbit antiserum raised against SDS-denatured purified Ad2 fiber, and the precipitates were analyzed by SDS-polyacrylamide gel electrophoresis and fluorography. The wild-type samples were diluted to facilitate comparison of the wild-type and mutant fiber signals. The fluorograph in Fig. 2 demonstrates that there was some fiber on the virions produced in deletion mutant-infected cells but that the amount of fiber was much less than that on wild-type virions. The fiber on the H2*dl*807 and H5*dl*1021 particles was the Ad5 type, whereas the fiber on the H2*dl*802 particles was the Ad2 type; the differences arise because the mutant stocks are contaminated with different helper viruses, as previously described (10). The extra bands visible in the fluorograph are caused by nonspecific precipitation of other virion proteins; these bands appear darker in the mutant lanes because larger fractions of the immune precipitates were loaded in those lanes than in the wild-type lanes.

The fiber signals were quantitated by densitometry, and the fiber signal of each deletion mutant was compared with the appropriate standard curve of the wild-type fiber signals. The particles produced in H2*dl*802-infected cells had less than 6% as much fiber as Ad2 virions, H5*dl*1021-infected particles had 3.5% as much fiber as Ad5 virions, and H2*dl*807-infected cells produced virions which had only 2% of the wild-type level of fiber. This demonstrates that the level of contaminating fiber in deletion mutant infections is not sufficient to produce particles with the wild-type fiber stoichiometry. Fiber is not required as a structural component at the level found in wild-type virions; H2*dl*807-infected cells made virus particles containing 50-fold less fiber than Ad5 virions.

It has been reported that there are only 36 fiber monomers per wild-type adenovirion (19). This implies that the particles produced in H2*dl*807-infected cells have an average of only 0.72 monomer each. The distribution of this fiber among the population of these virions could not be ascertained directly from our data. However, fiber capsomers consist of three fiber monomers (7, 16, 19), and newly synthesized fiber is

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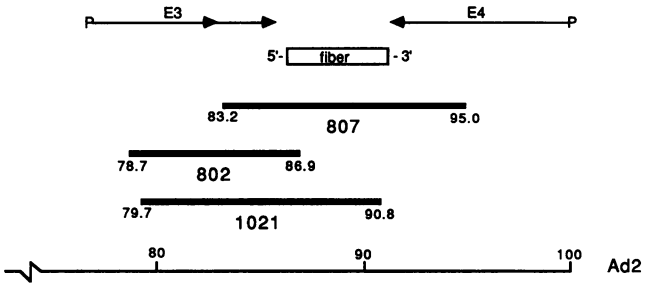

FIG. 1. Deletions in H2*dl*802, H2*dl*807, and H5*dl*1021. The line at the bottom represents the right-hand end of Ad2 marked off in map units (one map unit is 1% of the genome length). Symbols: ▬, DNA sequences removed by the deletions in H2*dl*802 (802), H2*dl*807 (807), and H5*dl*1021 (1021), with endpoints indicated (in map units); ▭, protein-coding region for fiber, with transcriptional polarity indicated; →, transcriptional early regions 3 (E3) and 4 (E4); ▶, polyadenylation sites. P, Promoter.

rapidly assembled into fiber capsomers (20). This suggests that the fiber present on particles made in these infections should be present as trimers. Even if a random distribution of fiber trimers on particles is assumed, the majority (78%) of the virions produced by H2*dl*807-infected cells must lack fiber completely. This suggests that fiber is not required as a structural element in the assembly of the adenovirus particle.

Previous observations with *ts* mutants had suggested that fiber was essential for virion assembly. One possible explanation for the conflicting findings is that fiber could have some essential, nonstructural role in assembly, in which case the trace amount of fiber in H2*dl*807-infected cells (0.1% of the wild-type level; 10) must be sufficient. Alternatively, the fiber protein might be dispensable for particle assembly. The failure of *ts* fiber mutants to produce particles at the nonpermissive temperature would then be caused by interference by denatured fiber in the assembly process, perhaps through the sequestering of an essential virion component in a

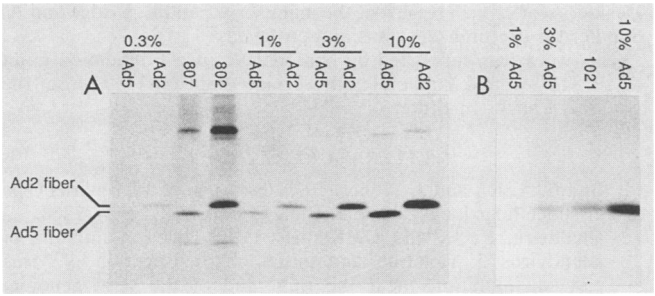

FIG. 2. Quantitation of fiber on virions made in H2*dl*802-, H2*dl* 807-, and H5*dl*1021-infected cells. (A) W162 cells were infected at a multiplicity of infection of 40 PFU per cell with H2*dl*802 (802), H2*dl*807 (807), Ad2, or Ad5, and ^{35}S-labeled virions were prepared as described in the text. (B) Vero cells were infected at a multiplicity of infection of 15 PFU per cell with H5*dl*1021 (1021) or Ad5, and radioactive virions were prepared. Virion proteins were immune precipitated with a fiber-specific antiserum, and the precipitates were examined on an SDS–17.5% polyacrylamide gel (A) or on a 10% gel (B). Various fractions of the wild-type samples were compared with the deletion mutant samples. The percentages above the Ad2 and Ad5 lanes indicate the fractions of the samples loaded in those lanes. The positions of Ad2 and Ad5 fibers are indicated. Fiber signals were quantitated by scanning the gel fluorograph with a densitometer.

TABLE 1. Adsorption of virus particles

Virus	N[a]	Adsorption rate constant (SEM)[b]	Ratio[c]
H5*dl*1021	5	5.43×10^{-3} (3.73×10^{-4})	0.112
Ad5	10	4.84×10^{-2} (1.58×10^{-3})	

[a] N, Number of experiments.
[b] Rate constants expressed per minute and determined from the initial slope of the plot of the natural logarithm of the fraction of unadsorbed virus versus time.
[c] Adsorption rate constant of H5*dl*1021 divided by that of Ad5.

nonproductive interaction. Fiber deletion mutants, which produce no fiber protein, would be expected to assemble particles normally. A similar explanation has been proposed to explain discrepancies in the behavior of *ts* and deletion mutants of early region 2a (15). Abnormal proteins have been shown to be capable of disrupting the structure of complex assemblies in nonviral systems. For example, in *Caenorhabditis elegans*, the products of missense alleles of a presumed actin gene can interfere with the assembly of structurally normal muscle, even in the presence of a full complement of normal components (13, 21). Mutations conferring such phenotypes are frequently dominant or semidominant; it would be of interest to determine whether *ts* alleles of the fiber gene exhibit dominance in their effect on particle assembly. Finally, it should be noted that at least one other adenovirus capsid protein, IX, is nonessential for the assembly of virions (5).

It has been proposed that the attachment of adenovirus particles to receptors on susceptible cells occurs via fiber (14). This proposal is based on the ability of purified fiber protein to block the attachment of virions to cells. The fiber-deficient particles made in H2*dl*1021 infections were used to test this hypothesis. Purified ^{35}S-labeled or ^{32}P-labeled (2) virions were allowed to adsorb to HeLa cells as described previously (14). The cell density in these experiments was 3×10^7 cells/ml, and the incubations were carried out at 37°C in a CO_2 incubator. At various times, samples were removed and centrifuged briefly to pellet the cells, and the radioactive signals in the supernatant and pellet were determined. The natural logarithm of the fraction of the radioactivity in the supernatant was plotted versus time, and the initial slopes of the wild-type and H5*dl*1021 adsorption curves are reported as adsorption rate constants (Table 1). The adsorption rate constant of H5*dl*1021 was about 11% that of the wild type, confirming that fiber-deficient particles are defective for adsorption. This rate constant is higher than would be expected if the adsorption rate constant was simply proportional to particle fiber content and may reflect fiber-independent adsorption via penton base (17).

In addition to measurements of the fiber content of mutant particles, comparisons were made of the protein compositions of deletion mutant and wild-type virions by electrophoresis directly on SDS-polyacrylamide gels. Figure 3 shows two representative gels. In addition to the lack of fiber, the pattern of viral proteins in particles produced by deletion mutant-infected cells was quite different from that of wild-type virions. Most strikingly, the mutant virions still had large amounts of the precursor proteins pVI, pVII, and pVIII and had correspondingly reduced levels of the processed forms VI, VII, and VIII (and, in the cases in which these species were visualized, X to XII), whereas wild-type particles had only the processed proteins. Also, the deletion mutant virions contained a slightly reduced level of protein III. Finally, in Fig. 3A, the band which ran just below

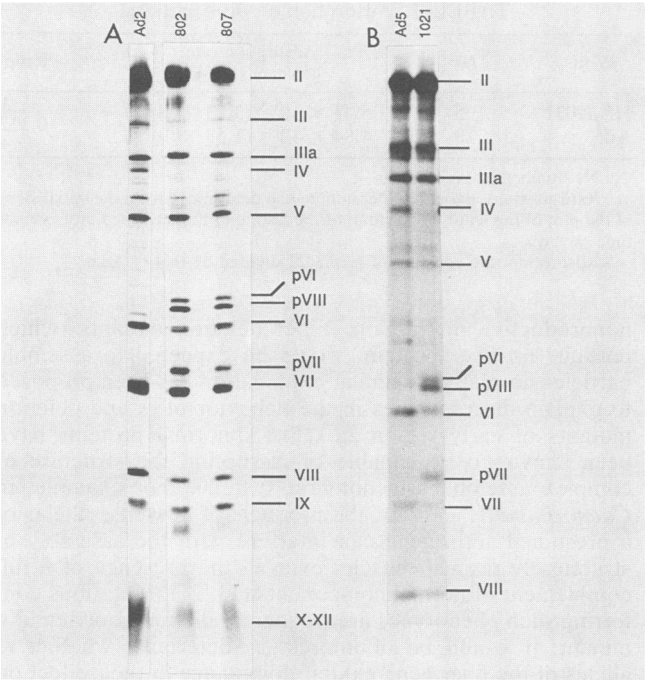

FIG. 3. Protein composition of particles made in H2*dl*802-, H2*dl*807-, and H5*dl*1021-infected cells. ^{35}S-labeled wild-type and deletion mutant virions were electrophoresed on an SDS–17.5% polyacrylamide gel and autoradiographed (A) or were electrophoresed on a 10% gel and fluorographed (B). The positions of the viral proteins are indicated.

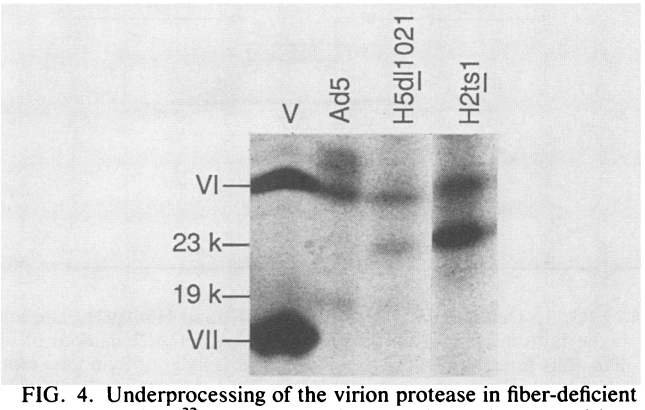

FIG. 4. Underprocessing of the virion protease in fiber-deficient particles. Purified ^{32}P-labeled H5*dl*1021, Ad5, and H2*ts*1 virions were electrophoresed on an SDS–12.5% polyacrylamide gel which was treated with 1 M NaOH at 50°C for 2 h and then was dried and autoradiographed. Lane V contains ^{35}S-labeled Ad5 virion proteins. Pertinent bands are identified by viral protein designation or molecular mass. Lanes containing commercial molecular weight markers (New England Nuclear Corp.) and ^{35}S-labeled infected cell proteins are not shown. k, Kilodalton.

protein VI might be an intermediate in a proposed two-step processing pathway for the conversion of protein pVIII to VIII (19). It is evident that mutant particles, which are made with subnormal amounts of fiber, are not properly proteolytically processed as they age; they maintain a protein pattern reminiscent of that of young virions (8, 12).

The precursor proteins are processed by a protease which is a virion component (1, 18). Our results show that the action of the protease is correlated with the presence of fiber on the particle, since processing did not occur efficiently in the absence of fiber. The gene for this protease maps to late region 3 (11, 23), so fiber is not itself the protease. Chatterjee and Flint have recently demonstrated that the protease is a 19-kilodalton (kDa) phosphoprotein that is synthesized as a 23-kDa precursor which, they suggested, is self-cleaving (3). By using H2*ts*1, they also correlated the processing of virion protein precursors with the presence of the 19-kDa form of the protease.

We have investigated the degree of processing of the protease in H5*dl*1021 virions. ^{32}P-labeled virions were purified from H5*dl*1021- or Ad5-infected Vero cells and also from H2*ts*1-infected cells grown at 39.5°C. Virion proteins were electrophoresed on an SDS–12.5% polyacrylamide gel, and the gel was base treated as described previously (3). An autoradiograph of such a gel is shown (Fig. 4). It is apparent that the 19-kDa protease species is present in Ad5 but not in H2*ts*1 virions, whereas the 23-kDa protease precursor is present in H2*ts*1 but not in the wild type. H5*dl*1021 particles have an obvious signal at the position of the 23-kDa precursor and a faint signal at the position of the 19-kDa protease. Thus, the protease itself is underprocessed in these mutant virions. This finding is consistent with the proposal (3) that the 23-kDa protease precursor is inactive in the processing of virion proteins and suggests that in the absence of fiber, the virion protease is not cleaved and remains inactive.

The addition of fiber to the immature virion might thus act as the stimulus that precipitates maturation. How this would occur is unknown. Perhaps fiber interacts directly with the protease, inducing an allosteric change that enables the protease to cleave (and activate) itself. Alternatively, the addition of fiber to the virion precursor might induce a structural change in the particle that leads to protease self-cleavage and activation. Further study of these and other fiber mutants may lead to a better understanding of the events involved in the activation of the virion protease.

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