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Cell-Binding Domain of Adenovirus Serotype 2 Fiber

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The adenovirus fiber appears as a long, thin projection terminated by a knob (head). The fiber consists of a trimeric protein whose head domain is thought to interact with cell receptors. The head part (amino acids 388 to 582) of adenovirus type 2 fiber was produced in a baculovirus expression system. The purified protein was shown to cross-link into trimers. It was very resistant to proteolytic attack and seemed to attain a high degree of compactness. The head domain efficiently inhibited attachment of adenovirus to receptors on the surface of HeLa cells, thereby confirming the hypothesis that the head domain interacts with viral receptors.

One of the major constituents of the adenovirus outer capsid is an elongated structure, the fiber, protruding from each of the twelve fivefold vertices of the icosahedral virion (for a review, see reference 10). These fibers play a crucial role in adenovirus infection by attaching the virus to specific receptors on the cell surface (2, 13, 18).

The adenovirus type 2 (Ad2) fiber appears as a long, thin projection terminated by a knob (head) (15, 16) and is a trimer of three identical subunits (17). Ad2 fiber polypeptide (582 amino acids) can be divided into three regions (4): a short amino-terminal tail region, a shaft consisting of repeating units each of approximately 15 amino acids whose relative hydrophobicities rather than strict identities tend to be conserved, and a carboxy-terminal part of about 180 residues.

The polarity of the polypeptide chain in the fiber structure has been determined; the fiber is attached to the virion through its N terminus located in the tail region, and the distal head contains the C terminus (3). The globular head of the fiber is thought to interact with the cellular adenovirus receptor, but this was never confirmed by experimental data. This paper describes the structural and functional properties of the recombinant head domain of Ad2 fiber.

Cloning, expression, and purification. Total DNA was extracted from Ad2 virions (12) and digested with *Taq*I. Fragment *Taq*I-B (nucleotides 29860 to 33055, numbering after Roberts et al. [14]), which includes the fiber gene (nucleotides 31054 to 32883), was cloned into the unique *Acc*I site of pUC19, resulting in the plasmid pTaq. A fragment coding for the head of the fiber protein (nucleotides 32215 to 32883) was obtained by PCR, with priming oligonucleotides containing *Bam*HI restriction sites and with pTaq as a template. Amplified DNA was digested with *Bam*HI and cloned into the *Bam*HI-cleaved and dephosphorylated vector pAcCL29 (8). The recombinant plasmid containing the insert in the correct orientation relative to the polyhedrin promoter was cotransfected with linearized baculovirus DNA in the presence of lipofectin (6), into Sf9 cells. Expression of the head protein in Sf9 cells infected with recombinant virus was demonstrated by Western immunoblot analysis with rabbit serum prepared against a peptide consisting of the last 16 C-terminal amino acids of fiber polypeptide. Recombinant baculovirus isolates were subjected to three rounds of plaque purification. Three days after infection with recombinant baculovirus, cells were collected, incubated in 10 mM Tris buffer (pH 8) containing protease inhibitors for 20 min at 4°C, and broken by homogenization. The supernatant obtained after lysis of expressing cells was applied to a column of Q-Sepharose Fast Flow equilibrated with 20 mM Tris buffer (pH 8) containing protease inhibitors. The recombinant protein was eluted with the same buffer containing 0.05 M NaCl. The head protein obtained had a purity of better than 95%.

Biochemical and electron microscopic analysis. The C-terminal fragment starts at Ala-388 and ends at Glu-582. Its monomer has an apparent molecular weight of about 21,000. Upon cross-linking with glutaraldehyde, monomers, dimers, and trimers of head protein could be observed on a denaturing

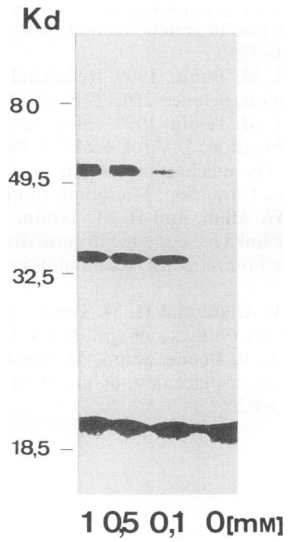

FIG. 1. Cross-linking studies of the recombinant head domain. A freshly prepared solution of glutaraldehyde was added to each portion (10 μg) of protein to the required final concentration. After 30 min at room temperature, samples were boiled for 5 min in Laemmli (7) sample buffer, subjected to 0.1% sodium dodecyl sulfate–15% polyacrylamide gel electrophoresis, and analyzed by Western blot with a serum prepared against the last 16 C-terminal amino acids of fiber polypeptide.

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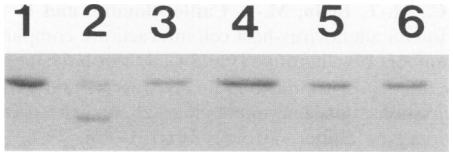

FIG. 2. Chymotrypsin digestion of the head domain. Portions (5 μg) of the head domain were treated with chymotrypsin in 50 mM Tris buffer (pH 8) containing 5 mM EDTA–10 mM $CaCl_2$ at room temperature at different enzyme/protein (e/p) ratios. Samples were analyzed by sodium dodecyl sulfate–15% polyacrylamide gel electrophoresis and stained with Coomassie brilliant blue. Lane 1, untreated sample; lane 2, molecular weight markers; lane 3, 30-min treatment, e/p = 1:100; lane 4, 30-min treatment, e/p = 1:10; lanes 5 and 6, 10- and 30-min treatments, respectively, e/p = 1:1. The molecular weight markers were soybean trypsin inhibitor, M_r = 21,500, and lysozyme, M_r = 14,400 (Bio-Rad).

gel (Fig. 1). The amount of trimer increased with increasing concentration of the cross-linking agent.

Native fiber can be cleaved with chymotrypsin after Tyr-17 (in the tail domain) and then at Met-448 (in the head domain), at rather high enzyme/protein ratios (3). The head protein expressed in baculovirus could be partially cleaved with chymotrypsin, giving rise to two fragments with approximate M_rs of 15,000 and 6,000, but only when the ratio of enzyme to protein was 1:1 (Fig. 2, lanes 5 and 6). These results suggest that, as for native protein, the head domain can be cleaved at Met-448.

Native Ad2 fiber contains O-linked GlcNac (1, 5, 9), and it seems that the site of glycosylation is in the shaft (11). Accordingly, mass spectroscopy performed on the sample of head protein expressed in the baculovirus system showed that this protein does not contain any carbohydrate residue.

In the electron microscopic images of the purified head protein expressed in the baculovirus system, we observed globular particles that often appeared triangular. Some of them are shown in Fig. 3 by arrowheads. The mean (± standard distribution) dimension of the side of these triangles is 58 ± 4 Å (5.8 ± 0.4 nm) (n = 105), which is similar to the side view of the heads of the native Ad2 fibers, 56 ± 4 Å (5.6

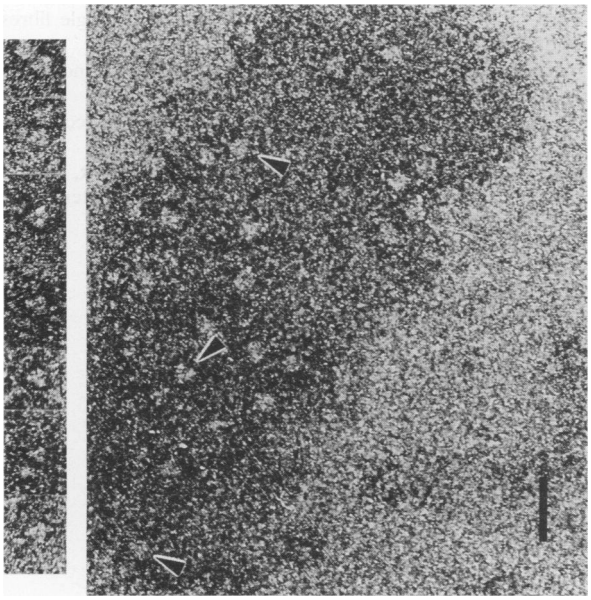

FIG. 3. Electron microscopy of the Ad2 fiber head expressed in the baculovirus system. The protein was negatively stained with 1% sodium silicotungstate. Low-dose electron microscopy and measurements were as described elsewhere (15). Arrowheads in the main image indicate triangular shapes. The particles in the left panel show triangles made up of three subunits. The bar represents 20 nm.

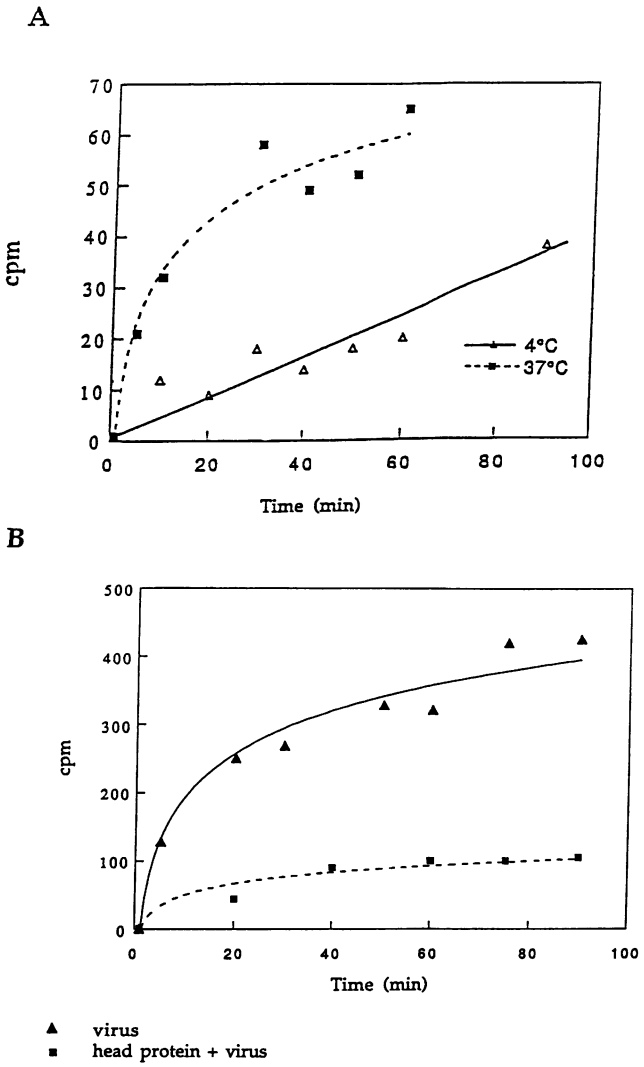

FIG. 4. Interaction of the head domain with HeLa cells. (A) Binding of head domain to HeLa cells. Cells were washed in phosphate-buffered saline (PBS) and resuspended in PBS containing 3% bovine serum albumin at a concentration of 5 × 10^7 cells/ml. [^{35}S]methionine-labeled recombinant protein (1 μg; specific activity, 8,500 cpm/μg) was incubated at 37 and 4°C with 1 ml of cells. At the times indicated, 100-μl aliquots were withdrawn, the cells were collected by centrifugation and washed twice in PBS, and the radioactivity was measured in a scintillation counter. Nonspecific binding was measured by addition of a 100-fold excess of unlabeled protein. (B) Inhibition of Ad2 binding by the recombinant head protein. Two PBS-washed cell samples (1 ml each) were prepared. To one sample, 1.2 μg of recombinant protein was added. After 15 min of incubation at 37°C, ^{14}C-labeled Ad2 was added (2.5 × 10^{12} virions; specific activity, 1 cpm/6.25 × 10^7 virions) to both samples, and the incubation at 37°C was continued. Samples of 100 μl were withdrawn at different times and diluted in 900 μl of PBS, the cells were recovered by centrifugation, and their radioactivity was determined in the scintillation counter.

± 0.4 nm) (15). Sometimes, the triangles were resolved into three subunits, which clearly indicates the trimeric nature of this recombinant head domain. Some of them are presented in the left panel of Fig. 3.

In conclusion, proteolytic analysis, cross-linking, and electron microscopy of the C-terminal fragment expressed in the baculovirus system show that it is a compact, trimeric protein with morphology similar to that of the head domain in the native Ad2 fiber.

Functional analysis of baculovirus-expressed head domain. Radioactive recombinant protein was allowed to interact with HeLa cells alone or in the presence of excess nonlabeled protein. The results obtained after subtraction of nonspecifically bound proteins (approximately 35%) showed that each HeLa cell fixed about 10^5 molecules of head domain at 37°C (Fig. 4A). This result agrees well with the amount of native Ad2 fiber which can be attached to cells (13). In order to determine if the recombinant protein recognizes Ad2 cellular receptors, we used a competition binding assay between Ad2 and the recombinant head domain. HeLa cells could bind about 4,500 virus particles in the absence of the competitor. When the cells were incubated with the recombinant protein 15 min prior to the addition of virus, binding of virus was inhibited by over 70% (Fig. 4B). This value is close to the 90% inhibition of Ad2 attachment observed for native fiber protein (13, 18).

In conclusion, the recombinant head domain produced in the baculovirus system is trimeric, structurally similar to the appropriate part of native protein, and is competent in binding to adenovirus receptors. When the fiber polarity was resolved, revealing that the N-terminal part is embedded in the penton base in the virion structure (3), it was hypothesized that the fiber head interacts with the cell during infection. This work supplies an experimental confirmation that this is indeed so. The biochemical, morphological, and functional analysis of the Ad2 fiber head domain allows us to define it as a cell-binding domain.

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