A Comparison of the Terminal Protein and Hexon Polypeptides of Avian and Human Adenoviruses

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SUMMARY

It was found that the virion terminal protein of chick embryo lethal orphan (CELO) virus had a molecular weight of 46000, and the hexon a molecular weight of 100000. 125I-labelled tryptic and chymotryptic peptide maps of the hexons and terminal proteins from CELO virus and human adenovirus type 5 (Ad5) differed. However, limited proteolysis of CELO virus and Ad5 terminal proteins by protease V8 showed similarities which were not detected in the case of the two hexons.

The adenovirus terminal proteins (Robinson et al., 1973; Robinson & Bellett, 1974) from members of each of five different subgroups of human adenovirus were all found to have a mol. wt. of 52000 to 55000 and to have closely related primary sequences as judged by 125I-labelled peptide analysis (Rekosh et al., 1977; Green et al., 1979). The terminal protein found in the virion is derived by cleavage from an 80000 to 87000 mol. wt. precursor which primes replication of the DNA molecule. This may involve binding to a DNA sequence near the origin of replication (Stillman et al., 1981; Challberg et al., 1982; Ikeda et al., 1982; Tamao & Stillman, 1982). Chick embryo lethal orphan (CELO) virus DNA and adenovirus type 2 (Ad2) DNA have two regions of homology. One is close to the coding sequence for Ad2 terminal protein, and the other is within the coding sequence for Ad2 hexon (Alestrom et al., 1982). In this paper we compare the molecular weights and peptide compositions of terminal proteins and hexons from CELO virus and Ad5.

Ad5 and CELO virus were grown and purified as described by Laver et al. (1971) and DNA-protein complexes were prepared by twice centrifuging to equilibrium in CsCl containing 4 M-guanidine·HCl at an initial density of 1.45 g/ml. Complexes were dialysed against phosphate-buffered saline (PBS) containing 0.2 mM-phenylmethylsulphonyl fluoride (PMSF), boiled in 0.1% SDS for 2 min, and then labelled with 125I at 4 °C using the method of Fraker & Speck (1978), an iodination procedure which was shown not to alter the mobility of virion proteins (data not shown). After extensive dialysis, 125I-labelled proteins were cleaved from the DNA by incubation in 0.5 M-piperidine at 37 °C for 2 h.

When 125I-labelled terminal proteins were compared by polycrylamide gel electrophoresis, the CELO virus protein migrated slightly faster (Fig. 1 a), with a mol. wt. of 46000 compared with 52000 for the terminal protein of Ad5 (Stillman et al., 1981). When [35S]methionine-labelled virion proteins were compared, the CELO virus hexon also migrated faster than the Ad5 hexon (Fig. 1 b). Taking the mol. wt. of Ad5 hexon as 103000 (Bahr-Lindstrom et al., 1982), we calculated a mol. wt. of 100000 for CELO virus hexon. Yasue & Ishibashi (1977) reported a mol. wt. of 115000 for the CELO virus hexon. However, using more recent values for marker proteins with mol. wt. over 100000 (Jornvall et al., 1981), a value of 101000 mol. wt. was calculated for the CELO virus hexon from the data of Yasue & Ishibashi.

For peptide analysis, 125I-labelled CELO virus and Ad5 hexon polypeptides were visualized by brief Coomassie Brilliant Blue staining and cut from the wet gel. The slices were soaked in 50% methanol for 16 h at 4 °C, dried under a lamp, and digested with 0.7 ml L-(1-tosylamide-2-phenyl)ethyl chloromethyl ketone (TPCK)-treated trypsin at a concentration of 50 μg/ml in 50
Fig. 1. The molecular weights of CELO virus terminal protein and hexon polypeptides. (a) $^{125}$I-labelled Ad5 and CELO terminal proteins were cleaved from DNA–protein complexes as described in the text and analysed by 10 to 20% linear gradient SDS–polyacrylamide gel electrophoresis followed by autoradiography. Lanes 1, Ad5 terminal protein, 52000 mol. wt.; lanes 2, CELO virus terminal protein, 46000 mol. wt. Ad5 virion polypeptides were used as markers. (b) Polypeptides of purified virions of $[^{35}S]$methionine-labelled Ad5 and CELO virus were analysed on a 10% SDS–polyacrylamide gel and detected by fluorography. Lane 1, CELO virion polypeptides, hexon polypeptide 100000 mol. wt. (arrow); lane 2, Ad5 virion polypeptides used as markers. The mol. wt. of hexon was taken as 103000 (Bahr-Lindstrom et al., 1982).

mm-ammonium bicarbonate at 37 °C for 16 h. The supernatants were freeze-dried and mapped on 20 × 20 cm cellulose-coated thin-layer plates by electrophoresis at pH 3·5 followed by ascending chromatography (for details, see Fig. 2). Terminal proteins were cut from gels according to their autoradiograms and mapped in the same way, except that chymotrypsin was used, since several of the tryptic peptides remained at the origin during electrophoresis. The results (Fig. 2) showed that the hexons and terminal proteins of human and avian adenoviruses differed, with few detectable common peptides.

Another approach to the analysis of protein structure is limited proteolysis, which has been widely used to analyse adenovirus terminal proteins (Challberg et al., 1982; Binger et al., 1982; Rekosh, 1981). Hexon or terminal protein bands were cut from gels as described above, and partial proteolysis by Staphylococcus aureus protease V8 was carried out using the method of Cleveland et al. (1977), except that 10 mM-EDTA was used to soak the gel slices to prevent non-specific proteolysis, and in the case of the terminal proteins, 1 μg casein (also cut from a gel) was used as a carrier protein. The proteolytic cleavage patterns showed that while the two hexons exhibited no detectable similarity, the terminal proteins showed similarities in the number and sizes of the peptides generated by partial proteolysis (Fig. 3). It should be noted that iodination does not seem to affect the enzymic digestion patterns.
Avian adenoviruses are only distantly related to their human counterparts (for review, see Flint, 1980). Although one of the two regions of homology between CELO virus DNA and Ad2 DNA is within the Ad2 hexon gene, the basically different peptide maps of hexons might still be expected, since the homology covers less than 15% of the two genes. The chymotryptic maps of the two terminal proteins indicate that their primary sequences are different. This agrees well with the DNA homology study (Alestrom et al., 1982) and the sequencing data (Smart & Stillman, 1982), which together suggest that the homologous region is just to the left of the coding sequence for the terminal protein precursor. The similarities in partial proteolysis patterns of the terminal proteins of avian and human adenoviruses could be coincidental. Alternatively, an overall conformational structure may have been conserved in evolution to preserve function in priming DNA replication, although homology is no longer detectable in the primary sequence.

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Fig. 3. Comparison by partial proteolysis of terminal proteins and hexons from CELO virus and Ad5. 
(a) Coomassie Brilliant Blue-stained partial proteolysis maps of CELO virus and Ad5 hexons. Lanes 1, 
3, 5 and 7, approx. 5 μg Ad5 hexon polypeptide digested with 0, 10, 50 and 500 ng *S. aureus* protease V8 
respectively; lanes 2, 4, 6 and 8, approx. 5 μg CELO virus hexon polypeptide digested with 0, 10, 50 and 
500 ng *S. aureus* protease V8 respectively; lane 9, 500 ng protease alone. (b) 125I-labelled partial pro-
teolysis maps of CELO virus and Ad5 hexons. Approx. 0.5 μg Ad5 hexon or CELO virus hexon 
polypeptide was used in each track. Digestion conditions were the same as in (a). Note that a com-
parable pattern was obtained by 125I-autoradiography to that obtained by staining. (c) 125I-labelled 
partial proteolysis maps of CELO virus and Ad5 terminal proteins. Lanes 1, 3, 5 and 7, approx. 50 ng 
Ad5 terminal protein together with 1 μg casein in a separate gel slice digested with 0, 500, 50 and 10 ng 
*S. aureus* protease V8 respectively; lanes 2, 4, 6 and 8, approx. 50 ng CELO terminal protein together 
with 1 μg casein digested with 0, 500, 50 and 10 ng *S. aureus* protease V8. (The top of the separation gel 
is indicated by a bar in each panel.)
REFERENCES


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