Adenovirus core protein VII displays a linear epitope conserved in a range of human adenoviruses

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A monoclonal antibody (MAb) which recognized a linear epitope on polypeptide VII of human adenovirus (Ad) serotype 4 also interacted with polypeptides VII of Ad serotypes 2, 5, 7 and 10, but not with 12 and 40, in Western blotting. Utilizing a hexapeptide phage display library, the MAb was found to recognize the consensus sequence RXYXPX. A peptide based on a similar sequence from Ad2, viz. VEEARNYPPTPPV, was synthesized and shown to inhibit binding of the MAb to polypeptide VII. Direct sequencing of the Ad4 polypeptide VII gene validated these observations, the sequence RNYTPA being detected. Comparison with gene sequences from other Ads indicates that this sequence is preserved in polypeptide VII of types 2 and 5 but in types 12 and 40 insertion of another residue disrupts this motif.

Adenoviruses (Ads) have a complex architecture with at least 12 structural proteins. The major protein, the hexon, is the main component of the capsid and there are a number of other capsomeres which complete the capsid and link with the core of the virus (Stewart et al., 1993). The latter consists of the viral double-stranded DNA genome in association with a number of other proteins – the principal ones being proteins VII and V as well as the small peptide µ. The spatial organization of the core has been the subject of a number of studies, sometimes with conflicting conclusions (for reviews see Nermut, 1984; Vayda et al., 1983). Protein VII is a highly basic protein very tightly attached to the viral DNA and most likely plays a role analogous to histones in cellular chromatin. However, very little is known of its structure and function although some studies have suggested that it is responsible for holding the genome in a supercoiled configuration (Wong & Hsu, 1989). It is clear, however, that along with some other structural proteins, VII results from processing of a precursor pVII by the virion-associated protease (Weber, 1976). Protein V appears to be associated with the protein VII–DNA complex since it is a component of the core structures isolated following disruption of the virus by a variety of procedures (Russell et al., 1971; Prage et al., 1968). However, it can be removed from the nucleoprotein core if the virus is disrupted in denaturing conditions with urea (Maizel et al., 1968). This suggests that protein V is not tightly attached to the protein VII–DNA complex and other results suggest that it may function as a linker protein to the capsid, since it has been shown to interact with both protein VII and protein VI – a capsid protein associated with hexons at the virus vertices (Matthews & Russell, 1998).

Table 1. Sequences of hexapeptide inserts in selected phage colonies

A 1/100 dilution of the MAb ascitic fluid was bound to biotinylated anti-mouse globulin linked to streptavidin-coated microwells and then incubated with the primary hexapeptide phage library. Three rounds of biopanning and amplification of phage in K91 E. coli cells were carried out as described by Scott & Smith (1990) and after cloning 14 phage colonies were selected, amplified and purified. The phage DNA was extracted and sequenced using the appropriate primer and 11 of these demonstrated a consensus sequence as shown.

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We have been studying the properties of human Ad type 4 (strain RI-6) and as a tool to dissect the antigenic structure of the virus we prepared monoclonal antibodies (MAbs) against purified virus which had been disrupted by incubation in 6 M urea using protocols described previously (Watson et al., 1988). We were particularly interested in detecting epitopes which were group specific and therefore we screened for hybridomas which bound to both Ad4 and Ad2 using immunofluorescence and immunoblotting (Tarassishin & Russell, 1994). One of these was found to detect polypeptide VII under denaturing conditions. By utilizing a phage hexapeptide expression library, generously supplied by G. P. Smith, we were able, using established protocols (Scott & Smith, 1990), to determine a consensus sequence, RXYXPX (Table 1),
for the epitope detected by this MAb. By sequencing the Ad4 gene for polypeptide VII by standard methodologies (accession no. U70921) we confirmed that a sequence corresponding to this consensus was present, viz. RNYTPA (Fig. 1). The validity of this finding was tested by preparing a peptide, VEEARNYTPPTPPPV, based on the Ad2 sequence. This peptide blocked the activity of the MAb in competitive immunoblotting (Fig. 2a). The MAb could also detect processed forms of pVII by immunoblotting (Fig. 2b) extracts of cells after different times of infection but did not immunoprecipitate the polypeptide from labelled infected cell extracts, implying an inability to interact with the native protein (data not shown). An interaction with extracts of Ad2, Ad4, Ad7 and Ad10 but not with Ad12 or Ad40 could be demonstrated by Western blotting (Fig. 2c). A parallel blot (not shown) using a group-specific MAb against Ad fibre (Watson et al., 1988) confirmed that the antigenicities of all the extracts were similar. These viruses are representative of the six subgroups of human Ads and the results therefore suggest that the epitope is widely conserved. A MAb against Ad2 polypeptide VII has also been obtained by Lunt et al. (1988) using purified protein as an antigen and could detect the polypeptide in a Western blot. However, no further characterization of the epitope was made.

The comparative sequence analysis of polypeptide VII shows (Fig. 1) that the consensus sequence is present in serotypes 2, 4, 5 but not in 12 and 40 where insertion of another residue disrupts the motif. These findings are in agreement with the blotting tests (Fig. 2c). It is significant that the sequence found in type 4 (at residues 69–74) is proximal to a well-preserved relatively acidic stretch (residues 55–82) of the very basic polypeptide. Similar considerations also apply to serotypes 2 and 5. It is also notable that, although the exact consensus sequence is not apparent in the pVII gene of serotypes 12 and 40, a similar relatively acidic stretch of residues is present (see Fig. 1). This assertion is also valid for the smaller polypeptide pVII from canine adenovirus (a mastadenovirus). In this case, the consensus sequence is deleted but acidic residues are still prominent at residues 61–73. The polypeptide pVII from another mastadenovirus — ovine adenovirus — is much shorter at 111 residues and also has the consensus sequence deleted but in this case there is no similar cluster of acidic sequences. These findings are, in general, compatible with the supposition that this acidic portion of the VII polypeptide is not interacting with the virus DNA and is therefore available for association with other virus polypeptides such as polypeptides V or VI (see Matthews & Russell, 1998). Indeed, earlier work had suggested that the DNA binding region was towards the C terminus of VII (Chatterjee et al., 1986). The sequence data also confirm the presence of a protease consensus sequence (MYGGA) (Webster et al., 1989) at residues 21–25 consistent with the evidence for pVII processing in Ad4 infection (Fig. 1b). All polypeptide pVII sequences of mastadenoviruses have a protease cleavage site in approximately this position which
reflects the importance of this protein in the final folding of the virus DNA coincident with maturation of the virion. It should be noted, however, that the ovine adenovirus gene does not contain a standard protease consensus site, although there seems to be a cleavage at a similar position in the gene, viz. at residue 23 (Vrati et al., 1996).

Fig. 1 also provides the sequence for polypeptide pVII from CELO virus, a member of the *Aviadenoviridae*. It is evident that this polypeptide is very much smaller with 72 residues and will presumably be fragmented further on processing, since it has two putative protease cleavage sites (at residues 27 and 40). Furthermore, there is no acidic region in this peptide and this may be related to the apparent absence of polypeptide V from this virus (Chiocca et al., 1996). A similar situation occurs with ovine adenovirus (Vrati et al., 1996), suggesting that the virus core is packaged in a different fashion in these viruses. Such a conclusion had been reached earlier after comparison of the results of micrococcal nuclease digestion of virus chromatin from human Ad5 and CELO virus (Li et al., 1984). It is also interesting that the 14 amino acids at the N termini of all these Ads demonstrate significant conservation, implying that this relatively hydrophobic portion of the polypeptide is required for some important function yet to be determined.

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References


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