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ADENAIN, THE ADENOVIRUS ENDOPROTEASE (A review)*

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With the possible exception of very simple viruses, most viruses appear to encode at least one virus specific endopeptidase. In addition to facilitating the orchestrated fragmentation of polyproteins of RNA viruses, these proteolytic enzymes may also be involved in the suppression of host protein synthesis, the regulation of virus assembly, the egress and subsequent uncoating in another cycle of infection of both RNA and DNA viruses. The endopeptidase encoded by adenoviruses (AVP or adenain) appears to be involved in several of these functions. Most of the literature concerns the protease of human adenovirus type 2, but there are good reasons to believe that the proteases of other adenovirus serotypes will be very similar. For a review see Weber [1, 2].

Keywords: adenain, adenovirus, antiadenoviral agents, endoprotease

The gene

The gene for the protease is located near the middle of the genome, just downstream of the gene for the major capsid protein hexon. The enzyme is translated from a small, tri-partite leader bearing mRNA expressed in the late phase of infection from the major late promoter. At 36 and 52 h p.i. approximately equal levels of enzyme were detected in the cytoplasmic and nuclear fractions [3]. No recognizable nuclear localization motif has been identified and it is not known by what means the enzyme is transported to the nucleus.

Currently 33 distinct protease genes have been sequenced from adenoviruses infecting a variety of species from man to snake (Table I). The translated amino acid sequences range from 201 to 214. Thirty residues are identical (15%) and 63 residues (46%) are conserved in identical positions among these sequences and most of it in the N-terminal half of the molecule.

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Table I

Adenovirus endoprotease sequences[1]

Virus	Databank identification
Human 2 *	gi209811/gbJ01917.1/ADRCG
Human 3	gi58476/embX13271.1/AD3PROT
Human 4	gi22796371/embAJ315930/HAD315930
Human 5 *	gi209842/gbM73260.1/ADRCOMPGEN
Human 7	gi21489859/gbAF515814.1
Human 11	gi24711762/gbAY163756.1
Human 12 *	gi313361/embX73487.1
Human 16	gi22796372/embAJ315931.1/HAD315931
Human 17	gi4416335/gbAF108105.1/AF108105
Human 40 *	gi303969/gbL19443.1/ADRGENOME
Human 41	gi209865/M21163.1/ADRDBPA2
Simian 25	gi17105037/gbAF394196.1/AF394196
Bovine 2	gi1574964/gbU44124.1/BAU44124
Bovine 3 *	gi60987/embX53990.1/PAPROHEX
Bovine 4	gi13129542/gbAF036092.2/AF036092
Bovine 7	gi60989/embX53989.1/PAPROT
Bovine 10	gi19225075/gbAF027599.2
Equine 1	gi2239279/gbL79955.1/AEEIIIA
Equine 2	gi2529366gbL80007.1/AEEHEXEND
Fowl 1 (CELO) *	gi1314432/gbU46933.1/AAU46933
Fowl 8 *	gi6466454/gbAF083975.2/AF083975
Duck 1 (EDS) *	gi2145321/embY09598.1/AAVEDSDNA
Turkey 3 (Hemorrhagic enteritis)	gi3769485/gbAF074946.1/AF074946
Canine 1 *	gi1526575/EMBy07760.1/CAVGENOM
Canine 2	gi1732265/gbU77082.1/CAU77082
Murine 1	gi209969/gbM81056.1/ADRL1A
Ovine A	gi7208208/gbAF153447.1/AF153447
Ovine isolate 287 *	gi5656619/gbU40839.2/OAU40839
Porcine 3 *	gi4630864/dbjAB026117.1
Porcine 5 *	gi13446708/gbAf289262.1/AF289262
Possum 1	gi18478696/gbAF338823.1
Frog 1 *	gi9295302/gbAF224336.1/AF224336
Snake 1	gi22266898/gbAY082603.1

[1]Obtained by BLAST search on 12 November 2002* sequence of complete viral genome available

The enzyme

The enzyme is packaged into virus particles (estimated at 10-40 molecules per virion) as the integral protein, possibly in association with the viral DNA via four large clusters of positive charge on the protease [4]. The ts1 mutant (P137L) of Ad2 prevents encapsidation of the otherwise active adenain, resulting in unprocessed virions at the nonpermissive temperature which fail to uncoat in a subsequent infection. Unlike many

other proteases, there is no evidence of proteolytic maturation in AVP. The basal activity of the enzyme is significantly boosted by an 11 residue cleavage product (pVIc) from the C-terminus of capsid protein pVI which forms a thiol bond with cys104 [5, 6]. The sequence of this peptide in Ad2 is GVQSLKRRRCF, with residues 1, 7, 8, 10 conserved in other virus serotypes. Activation by a peptide cofactor is not unique to adenain. For example, the NS3 protein of hepatitis C virus, is a serine protease whose activity is also enhanced by a peptide cofactor [7].

The atomic structure of the Ad2 enzyme complexed with its activating peptide has been solved [4]. Surprisingly, the disposition of active site residues (H54, E71, C122) is identical to that of papain (H159, N175, C25) and so is the location of Q115 of AVP and Q19 of papain which is presumed to participate in the formation of the oxyanion hole of the latter. The identity of the active site triad is confirmed by mutational analysis [8, 9].

The Ad2 enzyme is a 204 amino acid monomer of 24,838 Daltons. AVP complexed with the pVIc peptide has a K_m of 5μM and its activity is optimal at pH8, and 45 °C in the presence of 1mM thiol compounds and positively charged polymers. The enzyme is specific for two consensus sites (M,I,L)XGG-X or (M,I,L)XGX-G, where X is apparently any amino acid [1]. The rate of hydrolysis is influenced by the nature of the variable residues at the X sites. Furthermore, as might be predicted by the consensus sites on capsid precursor protein pVI, GX-G sites are cleaved 3-4 times faster than GG-X sites [10]. The GX-G site which gives rise to the pVIc activating peptide is conserved on all pVI sequences known so far. A survey of the sequences of all known precursor proteins reveals a remarkable conservation of the position of cleavage and the consensus sites. Human Ad4 contains one glaring exception, the iTP site (the first of two sites digested) is QRGF-G, suggesting either that the consensus sequence includes Q in the P4 position (in addition to M,I,L), or, more likely, that the specificity of the Ad4 enzyme is changed [11].

The substrates

All of the seven viral proteins, accounting for approximately 3326 cleavage sites digested by the enzyme are either internal or in part disposed internally in the virion: pIIIa, pVI, pVIII hexon-associated capsid proteins, pVII and pX core proteins, pTP linked to the viral DNA, and L1-52K DNA packaging protein. The order of cleavages and their relationship to virus assembly and maturation is not known. Mutants, such as in capsid proteins, which prevent virus assembly fail to execute these cleavages, yet assembly occurs in the absence of active enzyme, as in the case of ts1, suggesting that virus assembly triggers the activation of the enzyme. It has been suggested that the 50

copies of protease per virion might slide along the viral DNA to the 3326 cleavage sites disposed in the semi crystalline interior of the virion [12].

In addition to viral proteins, cellular proteins may also be cleaved and indeed cytokeratin K18 and possibly K7 have been shown to be digested [13]. Cleavage results in the depolymerization of the cytoskeletal network and may accelerate cell lysis thereby promoting viral spread. Other proteins which contain cleavage sites have also been digested *in vitro* but only after denaturation to expose the site.

Several types of indirect experimental evidence suggest that the protease is also required early in infection to mediate the release of virus particles from endosomes [14, 15]. The failure of ts1 particles, which are devoid of protease, to leave the endosome, is the most compelling evidence for a role of the protease in endosomal lysis.

Recent advances

Li and Hochstrasser [16] have observed sequence similarities among the members of a large group of cysteine proteases including African swine fever virus, vaccinia virus, fowlpox virus and the Ubl-specific proteases of yeast and Chlamydia. *Saccharomyces cerevisiae* also encodes a related protease, SMT4, with a function in chromosome condensation [17]. Their catalytic residues are conserved and they cleave the corresponding protein precursors at consensus sites similar or closely related to the glycine-glycine-X cleavage sites [18, 19]. The similarities observed between their sequences, their catalytic sites and substrate cleavage sites suggest that these viral proteases are structurally related. In a recent review, Barrett and Rawlings [20], have described the evolutionary lines of cysteine peptidases and concluded that adenain, the eukaryotic Ulp1 protease and the bacterial YopJ proteases belong to the same clan CE. These enzymes show minor sequence similarity but their secondary structures especially around the catalytic residues and their substrate specificity are clearly similar.

Because of the absence of currently identifiable protein motifs on AVP, functions other than proteolysis are unlikely. A recent report implicated adenain in the deubiquitination of cellular proteins during infection [21]. It was suggested that this function could protect viral proteins from ubiquitin-dependent proteasomal degradation as well as leading to inhibition of antigen presentation by major histocompatibility complex class I molecules.

Antivirals targeting adenain

Currently there are no anti-adenoviral agents in use in clinical practice. *In vitro* experiments and *in silico* approaches have identified a number of potentially useful agents which target the viral protease, adenain (Table II). Of these agents the acetonitriles and glycilnitriles were efficient inhibitors of adenain *in vitro*, they are however, insufficiently specific and are consequently toxic to cells [22]. A tetranitrofluorenone inhibitor was identified by means of a computer docking program and its properties have yet to be tested *in vitro* [23]. Surprisingly, the peptide cofactor inhibited adenovirus growth by activating adenain prior to encapsidation [24, 25]. With appropriate modifications to improve stability and cell penetrance, the cofactor appears to be a promising lead compound. Selective serpins and cystatins had activity *in vitro* but because of their size they are less promising than the peptide cofactor [26]. In addition to these agents, the chinese green tea polyphenol, epigallocatechin-3-gallate, inhibited adenain at an IC50 of 25 μM [27].

Table II

Antiadenoviral agents acting through adenain

Agent	Mechanism/target	Reference
Benzamidoacetonitrile	substrate analogue	Sircar et al., 1998 [22]
Acetamidoacetonitrile	substrate analogue	Sircar et al., 1998 [22]
n-Methoxyphenylalanine Glycylnitrile	substrate analogue	Sircar et al., 1998 [22]
2,4,5,7-Tetranitro-9-fluorenone	active site	Pang et al., 2001 [23]
Carboxybenzyl-LAGG-acetonitrile	substrate analogue	Cornish et al., 1995 [28]
Serpins	active site	Ruzindana-Umunyana et al., 2000 [24]
Cystatins	active site	Ruzindana-Umunyana & Weber, 2001 [26]
Activating cofactor peptide pvic	activation mechanism	Ruzindana-Umunyana et al., 2000; Baniecki et al., 2001 [24, 25]
Epigallocatechin-3-gallate	unknown	Weber et al., 2003 [27]

Outlook

Major unanswered questions concerning adenain include the following: What prevents the protease from digesting its substrates prior to virus assembly? How is the protease transported and encapsidated? Do the cleavage fragments have any function? Are other cellular proteins cleaved? Are there any endogenous inhibitors of the protease? The unique nature of AVP and the prospect that all adenovirus proteases function similarly offers an ideal target for the development of specific inhibitors effective for the control of all adenovirus infections [22, 24, 26, 28].

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References

1. Weber, J.M. The adenovirus endopeptidase and its role in virus infection. In "Molecular Repertoire of Adenoviruses" Vol. 199/I, Curr. Top. Micro. Imm. (W. Doerfler and P. Bohm, Eds), pp. 227-235, Springer-Verlag, Berlin (1995).
2. Weber, J.M. Role of endopeptidase in adenovirus infection. In "Adenoviruses: From Basic Research to Gene Therapy Application" (P. Seth, Ed.), Humana Press, Clifton, NJ (1999).
3. Webster, A., Leith, I.R., Hay, R.T. Activation of adenovirus-coded protease and processing of preterminal protein. J. Virol. **68**:7292-7300 (1994).
4. Ding, J., McGrath, W.J., Sweet, R.M., Mangel, W.F. Crystal structure of the human adenovirus proteinase with its 11 amino acid cofactor. EMBO J. **15**: 1778-1783 (1996).
5. Mangel, W.F., McGrath, W.J., Toledo, D.L. et al. Viral DNA and a viral peptide can act as cofactors of adenovirus virion proteinase activity. Nature **361**:274-275 (1993).
6. Webster, A., Hay, R.T., Kemp, G. The adenovirus protease is activated by a virus-coded disulphide-linked peptide. Cell **72**:97-104 (1993).
7. Butkiewicz, N.J. et al. Enhancement of hepatitis C virus NS3 proteinase activity by association with NS4A-specific synthetic peptides: identification of sequence and critical residues of NS4A for the cofactor activity. Virology **225**: 328-338 (1996).
8. Rancourt, C., Keyvani-Amineh, H., Diouri, M., Weber, J.M. Mutagenesis of conserved residues of the adenovirus protease. Virology **224**:561-563 (1996).
9. Grierson, A.W., Nicholson, R., Talbot, P. et al. The protease of adenovirus serotype 2 requires cysteine residues for both activation and catalysis. J. Gen. Virol. **75**:2761-2764 (1994).
10. Diouri, M., Keyvani-Amineh, H., Geoghegan, K.F., Weber, J.M. Cleavage efficiency by adenovirus protease is site-dependent. J. Biol. Chem. **271**:32511-32514 (1996).
11. Webster, A., Leith, I.R., Nicholson, J., Hounsell, J., Hay, R.T. Role of preterminal protein processing in adenovirus replication. J. Virol. **71**:6381-6389 (1997).
12. Mangel, W.F., Toledo, D.L., Jianzhong, D. et al. Temporal and spatial control of the adenovirus proteinase by both a peptide and the viral DNA. TIBS **22(10)**:393-398 (1997).
13. Chen, P.H., Ornelles, D.A., Shenk, T. The adenovirus L3 23-kilodalton protease cleaves the amino-terminal head domain from cytokeratin 18 and disrupts the cytokeratin network of HeLa cells. J. Virol. **67**:3507-3514 (1993).
14. Cotten, M., Weber, J.M. The adenovirus protease is required for virus entry into host cells. Virology **213**:494-502 (1995).
15. Greber, U.F., Webster, P., Weber, J.M., Helenius, A. The role of the adenovirus protease in virus entry into cells. EMBO J. **15**:1766-1777 (1996).
16. Li, S., Hochstrasser, M. A new protease required for cell-cycle progression in yeast. Nature **398**:246-251 (1999).

17. Strunnikov, A.V., Aravind, L., Koonin, E.V. *Saccharomyces cerevisiae* SMT4 encodes an evolutionarily conserved protease with a role in chromosome condensation regulation. Genetics **158**:95-107 (2001).
18. Lopez-Otin, C., Simon-Mateo, C., Martinez, L., Vinuela, E. Gly-Gly-X, a novel consensus sequence for the proteolytic processing of viral and cellular proteins. J. Biol. Chem. **264**:9107-9110 (1989).
19. Lee, P., Hruby, D.E. Proteolytic cleavage of vaccinia virus virion proteins. Mutational analysis of specificity determinants. J. Biol. Chem. **269**:8616-8622 (1994).
20. Barrett, A.J., Rawlings, N.D. (2001) Evolutionary lines of cysteine peptidases. Biol. Chem. 382:727-733.
21. Balakirev, M.Y., Jaquinod, M., Haas, A.L., Chroboczek, J. Deubiquitinating function of adenovirus proteinase. J. Virol. **76**:6323-6331 (2002).
22. Sircar, S., Ruzindana-Umunyana, A., Neugehauer, V., Weber, J.M. Adenovirus endopeptidase and papain are inhibited by the same agents. Antiviral Res. **40**:45-51 (1998).
23. Pang, Y.P., Xu, K., Kollmeyer, T.M., Perola, E., McGrath, W.J., Green, D.T., Mangel, W.F., Discovery of a new inhibitor lead of adenovirus proteinase: steps toward selective, irreversible inhibitors of cysteine proteinases. FEBS Letters **502**:93-97 (2001).
24. Ruzindana-Umunyana, A., Sircar, S., Schick, C., Silverman, G.A., Weber, J.M. Adenovirus endopeptidase hydrolyses human squamous cell carcinoma antigens in vitro but not ex vivo. Virology **268**:141-146 (2000).
25. Baniecki, M.L., McGrath, W.J., McWhirter, S.M., Li, C., Toledo, D.L., Pellicena, P., Barnard, D.L., Thorn, K.S., Manger, W.F. Interaction of the human adenovirus proteinase with its 11-amino acid cofactor pVIc. Biochem. **40**:12349-12356 (2001).
26. Ruzindana-Umunyana, A., Weber, J.M. Interactions of human lacrimal and salivary cystatins with adenovirus endopeptidase. Antiviral Res. **51**:203-214 (2001).
27. Weber, J.M., Ruzindana-Umunyana, A., Imbeault, L., Sircar, S. Inhibition of adenovirus infection and adenain by green tea catechins. Antiviral Res. In press. (2003).
28. Cornish, J.A., Murray, H., Kemp, G.D. et al. Specific inhibitors of the adenovirus type 2 proteinase based on substrate-like tetrapeptide nitriles. Bioorganic & Medicinal Chem. Lett. **5**:25-30 (1995).