

Identification and sequence analysis of the core protein genes of bovine adenovirus 2

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Abstract

The DNA sequence of the genome of bovine adenovirus type 2 (BAdV-2) was determined between map units 42.5 and 50. By sequence analysis and homology search, the genes of five structural proteins were identified within this region: the penton base protein (III; partial sequence), the major core protein precursor (pVII), the minor core protein (V), the mu core protein precursor (pX) and the hexon associated protein precursor (pVI; partial sequence). The putative polypeptides were compared to their known counterparts from other adenoviruses. The existence of protein V and the presence and structure of certain protease cleavage recognition sites confirmed BAdV-2 as a member of the genus *Mastadenovirus*. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Bovine adenovirus; Genome DNA sequence; Mastadenovirus ; Core proteins; Sequence analysis

The ten accepted serotypes of bovine adenoviruses (BAdVs) are associated with respiratory and/or enteric diseases of cattle, as well as with subclinical infections. BAdVs originally belonged to a single genus (*Mastadenovirus*) of the family *Adenoviridae*, but were divided into two sub-

groups (Bartha, 1969). The members of subgroup 1 (BAdV serotypes 1, 2, 3, 9) share the common complement fixing antigen of the mastadenoviruses, while subgroup 2 BAdVs (types 4, 5, 6, 7, 8) do not. The subgroup 2 BAdVs are now listed as unassigned viruses in the family (Benkő et al., 2000) and have been proposed to form a new genus in the family *Adenoviridae* together with ovine adenovirus isolate 287 (OAV287) and an avian adenovirus, the egg drop syndrome (EDS) virus, officially named as duck adenovirus 1 (Benkő and Harrach, 1998). BAdV-2, a member

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of former subgroup 1 is an interesting bovine adenovirus, since it is able to bypass the species specificity and to cause natural infection both in cattle and sheep (Belák et al., 1983). Our recent serological surveys have shown that BAdV-2 is currently the most prevalent adenovirus serotype in Hungarian cattle and sheep livestock (Rusvai and Fodor, 1998). BAdV-2 sequences were earlier reported from genome regions covering map units (m.u.) 0–12.2 (Salmon and Haj-Ahmad, 1994), 13.1–24.0 (Yagubi et al., 1998), 74.8–84.4 (Esford and Haj-Ahmad, 1994) and 90.5–100 (Fitzgerald et al., 1997). The addition of the data presented in this report makes 50% of the BAdV-2 genome known.

The *EcoRI*-A fragment of the BAdV-2 prototype strain (No. 19), located between m.u. 42.5 and 90.5 (Salmon et al., 1993) was initially cloned into plasmid pBR322 (Belák et al., 1986). After cleavage with *SalI* enzyme, the *EcoRI-SalI* fragment (m.u. 42.5–65.2) was subcloned into plasmid pMOB. To create a nested set of template DNA, a transposon (TN1000) based method (Gold Biotechnology, Inc., St. Louis, USA) was used, which allows for sequencing the target DNA from the direction of the transposon. The DNA was purified using a Plasmid Mini Kit (Quiagen GmbH, Hilden, Germany), and the sequence was determined on both strands with an A.L.F. sequencer (Amersham-Pharmacia, Uppsala, Sweden). The sequences were assembled and edited using the PC/Gene sequence analysis program package (IntelliGenetics). The BLASTX algorithm (Altschul et al., 1997) was used to search for homologous proteins in the non-redundant database at the National Center for Biotechnology Information (NCBI, Bethesda, USA).

The sequenced part (approximately m.u. 42.5–50.0) of the fragment contained three complete genes, the major core protein precursor (pVII), the minor core protein (V) and the mu core protein precursor (pX), flanked by the end and beginning of the penton base protein (protein III) and the hexon associated protein precursor (pVI), respectively (Fig. 1). The order of these genes is conserved on the r-strand in all mastadenovirus genomes sequenced to date. In human adenovirus (HAdV) type 2, the genes encoding four of these

proteins belong to the major late transcription unit L2, while the gene of pVI is in L3. The 2609 nucleotide long sequence of BAdV-2 was submitted to the GenBank (accession number U44123).

Protein III is a subunit of the penton base located on each of the 12 vertices of the adenovirus capsid. The penton base itself is composed of five copies of this polypeptide which is the second largest structural protein of the adenovirus capsid. It plays an important role in the penetration process of the virus, since cellular integrins helping the internalisation of the virion recognise this capsomer (Wickham et al., 1993). The first 464 nucleotides of the sequenced BAdV-2 DNA fragment encode the carboxyl terminal portion of the penton base.

The major core protein (VII) is tightly bound to the viral DNA. Some observations even indicate that this close association is very likely preserved during the transport of the viral genome to the nucleus, i.e. the uncoating is incomplete (Russell and Precious, 1982). The precursor polypeptide (pVII) is cleaved by the adenoviral protease. The protease cleavage site in BAdV-2 is presumably the sequence MYGG↓A, which is conserved in other mastadenoviruses. We have examined the pVII protein alignment from several adenoviruses. Apart from the conserved amino terminal quarter containing the protease cleavage site, pVII seems to be a rather variable protein. Its length in BAdV-2 is comparable to that in HAdV-2 (196 and 155 amino acids, respectively), whereas the pVII in two members of the genus *Aviadenovirus*, in fowl adenovirus (FAdV) 1 and 10 is only 72 and 77 residues long (Chiocca et al., 1996; Shepard and Trist, 1993). The high degree of conservation among the different serotypes near to the amino terminus of the pVII shows the significance of this region, nevertheless the consensus protease cleavage recognition sites could not be identified in the candidate members (EDS and OAV287) of the proposed genus *Atadenovirus* (Fig. 2A).

The minor core protein (V) and the mu core protein (X) are also closely associated with the viral DNA, and are likely not removed in the process of uncoating like the major core protein (Chatterjee et al., 1985). Chemical cross-linking studies showed that these three core proteins (V,

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GAATTCCAATGCCACGCTGTAGTGACCCCTGACGTAACCTGCGGCATGGAGCAAGTATACTGGAGCATCC 72
>> I R N N A T L L V T P D V T C G M E Q V Y W S I
protein III >
CAGATATGATGTTGGAGCCAGTGACGTTTAAAGCTAGTCAAAAACGTGTCTAACATCCAGTGTAGTGTAG 144
P D M Y V E P V T F K A S Q N V S N Y P V I G V
AGTTAATGCCTCAGCGGCCCTCGTAGTTTTTACAATGCTCAAGCGGTGTATCTCAATGATTCAAGAAAACA 216
E L M P Q R P R S F Y N A Q A V Y S Q M I Q E N
CTAATCAGACAAATGGTCTTAAACCGCTTCCCTGACAACCGATTTCCTCGCGCCGCCAGAGTCTACTATCA 288
T N Q T M V F N R F P D N Q I F L R P P E S T I
CCAGCATCAGTGAAAACGTGCCAACCGACAGACAGACCAGGACCGCTTCTCTATTAGAAAACAGTGTCTGGTG 360
T S I S E N V P T Q T D H G T L P I R N S V S G
TTACCGCGCTCAGCTTAAACAGACCGAGACGTAGAGCGAGTCTTACGTGTATAAAAAGCATAGCTGTGAGCC 432
V A Q R V T L T D A R R R A S P Y V Y K S I A V A
AACCTAAAGTCTTGTCTAGTCGGACTTTTAAATGGCCATTTTAGTTTCCACGTAGCAATAACACAGGGTGG 504
Q P K V L S S R T F - M A I L V S P S N N T G W
pVII >
GCCTTGGTTGCAAGTCCATGTACGGCGGCCCGCCGATTGACCGAGCACCCAGTCTTGTGCGCCGCC 576
G L G C K S M Y G ↓ A R R R L T E H H P V L V R R
ATTTACGGCTTCTGGGGCAGCAAGCGGGACGCACTACCGTCCACAGTCCCATCAGTATCTGATCTCGT 648
H F R A S W G S K R G R T T V P T V P I T D D P
TTGCTGACGTAGTCAATGCCATTGCCAAGGCACAACCCGCGCCGACGACAGCAGAGCGCCGCGCAGGC 720
V A D V V N A I A E G T T R R R R R A E R R R R
GTCGTCAAGCAAGCGCCAAATGCCAGCCGCCAGAGCTTAGTCAGGTCACTAGGCGCGCTTAGCCAGGC 792
R R Q A T S A M R A A R A L V R S A R R L L A R
GAGGCGGTGACGTCGAACCGGCAACCCAGTGGCTGACGTAGTGGCGCGCGTCCGAAGAAGCAACTCGAGCTA 864
R G R V R R T R N P V A D V V R A V E E A T P S L
ACCCCTCCGCGCAAGCGCGGTATCAGGGCTAGAACCTGTGGTACTGTTAACCCATTGGGCGCCGAAACA 936
N P P R R S A R I R A R T V A T V N P L G P R N
TTTACTGGGTGCGCAGCAGAGCGGCAACGCATTCCTGTAACATCACGCCCTTCACGCGCTCTGGTTACT 938
I Y W V R D Q S G K R I P V T S R F S R A L G Y
TGGTTTAAATAAACCTCATTGTCGCATTATACAGTGTCCCGCCCGTGTGTTTTCCGTACAGATGATTTCCGG 1080
L V -
protein V > M S S R
AAAAATAAAGAAGAAATGCTTGAATCATAGCGCCGAACCTTACGCCCCACGGCACCCCGCCGATAAA 1152
K I K E E M L E I I A P E L Y A P R H R R S V K
AGCTGAAACCAATCAAGAGTTAAAAAAGAGGAAATAAAGTCCAAAAGAAAGTGGAAACGCTCTCAATAGA 1224
A E T K S R V K K E E I K S K R K W K R P Q Q I D
CGACTGTAAACAGAGGATGTTGAAGTCGTAGGGCCACGGCGCTAGACGCCCTTATCAGTGGCGGTGAG 1296
D L L T E D V E V V G A T A P R R P Y Q W R G R
AAAAGTAAAGCCGCTGCTTCGACAGGCACTGTCATCACGTTACGCTTCCGCTCCGCGCCGCTGAAAGGGC 1368
K V K R V L R P G T V I T F T P G V R S R E R A
AAGCAAGCGCTTTCAGATGAAATCTTTGCAGATGAAGACATTTTAGACAGTATGAGAGAGCGGAGGGCGA 1440
S K R S S D E I F A D E D I L E Q Y E R G E G E
GTTCCGTTACGGGAAACCGCAGCAAGCTGAGGCTGCTGTTGCTAGACACTTCTAACCCCACTCCACGTT 1512
F R Y G K R S K A E A A V V L D T S N P T P S L
GCACCAGTACTCCTCAGATGCCGATGTTCACTACTAGTCTGCCAAAAGAACGCTGTGGCTACTGTGA 1584
H P V T P Q M P I V H T S A A K R S A V P T V E
AGTCTTAGCCCTAAAAGCGCGCTTCACAGAGTCTTCTGATCAGTTCAGTGGACATGGTACTGAAAC 1656
V L A P K K R R F T E S S D Q L A V D M V T E T
AAGCACTGTTCTCCAGAACGGCTGTGCTTCTCCAGTAGGGCTGTAAAACAAGCCAGCGCGGCTTCCC 1728
S T V P P G T A V L L P A R A V K Q A R R R F P
AGTGGCAGTAGAGTCAAAAAGCCAGACATGGTAGTTGAAGAAGTAAAAGTGCAGCGCATTAAGCCAGT 1800
V A V E S K K P E H M V V E E V K V R D V K P V
GGCTCCAGCATAGGGCTGCAGACAATAGATTTTAAAGTGCCTGTAGATGCCCTAAGCCCTGTATCCGAT 1872
A P G I G V Q T I D F K V P V D A P K P P V S I
CACTGAGCAGATGGATATCAGCTCTACCCCGCCAAAGAAAGTACGCTACGGCCAGTAAATAAATTTATCC 1944
T E Q M D I S S T P A K K V A Y G P A N K I I P
AGTAGGCTGGCAGCATCTTAGTCAGATGGGATTTCCAAAATACGTGCGCCCAACGAAGAAGCGCGTGGC 2016
V A W Q H P S Q M G F P K Y V R P K R R R V A
TCGACGACGACAGCACTGGGCGGTTTGTCCGACGCCACGCAAGCCACTCCAAGACGCAAAATTTGTGCT 2088
R R S K S T G R F V A A P R K R T P R R K I V L
TCCGGCTGTGGCTACCACTCCAGCTTGGACACTGTTCTCGCTCACAAAGTGGCCATTTGGCGTTAACTGTT 2160
P A V R Y H P S L D T V P R S Q V A I W R -
AATAAACATATTGCTGCTACTGCGTTTAGGGTCTCCTTTTTCGCGATGACTGGAGTCCAAGAGTAACATA 2232
pX > M T G V P R V T Y
CCGCGTCCGAGTTCAGTGCCTACTCGGCTTCTACGACTAGACGCCACGGAAGACTGGTTCGAGCGGTAGC 2304
R V R V P V R T R V L R P R R H G R L V R V A
GC CGC AAAAGCATGCGTGGCGGATTTCTGCGGTTCTTGTTCCTTTGATTGCTGGCGCCATAGCGCGGC 2376
R R K S M R G G ↓ F L P F L V P L I A A A I G A A
GCCTGGAAATCGCTTCACTAGCCTCGAGGCTTCGCGACGCTAGTACCTCGTTCATGACTGTGTAGACTGAT 2448
P G I A S V A L Q A S R R -
TTTACTCTTTATTTCGCCACGCTGCCCTCCATGGAAGGAATTAATTTCTCCGCTTGGCTCCAGATACG 2520
pVI > M E G I N F S A L A P R Y
GGTCAAGGCCATGCTTAGCAGTGGTCTGACATCGGCACCGACTCCATGAATGGGGCGCCCTTAACTGGG 2592
G S R P M L S S W S D I G T S S M N G G ↓ A F N W
GCAGCTTGGAGCGGG 2609
G S L W S G >>

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Fig. 1. The nucleotide and deduced amino acid sequence of the BAdV-2 genome region between map units 42.5 and 50. The cleavage sites of restriction endonucleases *Eco*RI, *Not*I, *Xho*I, *Cla*I and *Pst*I (in this order) are printed in bold. With the exception of the *Xho*I site their location corresponded to the results of Salmon et al. (1993). The protease recognition sites are underlined, the presumed actual cleavage sites are marked by arrow (↓). The putative NLS sequence (³¹⁷RR–SKSTGRFVAA–PRKRT³³³) in the gene of protein V is printed in bold.

VII, X) make contact with each other. In different HAdVs, the presence of a nuclear localisation signal (NLS) was demonstrated close to the carboxyl terminus of protein V and this signal is considered to be connected with the putative function of binding the DNA (Russell and Kemp, 1995). A bipartite NLS motif, consisting of two basic residues separated by approximately 10 amino acids from a stretch of five amino acids, three of which are basic (Dingwall and Laskey, 1991) was found in the protein V sequence of BAdV-2 (Fig. 1). The localisation of this putative NLS is comparable to that in HAdVs. It should be mentioned that the protein V gene is missing

from the aviadenoviruses (Chiocca et al., 1996) and also from the members of the proposed genus *Atadenovirus* (Benkő and Harrach, 1998) i.e. from OAV287 (Vrati et al., 1996), from the EDS virus (Hess et al., 1997) and from every former subgroup 2 BAdVs investigated so far (Szathmáry et al., 1997; Benkő and Harrach, 1998).

An amino acid sequence alignment of the adenoviral precursor protein X (pX) is presented in Fig. 2B. The mu protein (protein X) is cleaved from the precursor by the viral protease. There are two protease cleavage sites in HAdV-12, 40 and 41, while HAdV-2 even has a third one adjacent to the first site. On the other hand,

A

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H2  MSILISPSNNTGWGLRF-PSKMFGGAKKRSDQHPVVRVGRHYRAPWGAHKRGRTGR-TTVDDAIDAVVEEARNYT-PTPPPV
H5  MSILISPSNNTGWGLRF-PSKMFGGAKKRSDQHPVVRVGRHYRAPWGAHKRGRTGR-TTVDDAIDAVVEEARNYT-PTPPPV
H12 MSILVSPSNNTGWGL--GAARMYGGAKTRSSQHPVVRVGRHYRAPWGAHTRGRTGR-TTVDDVIDSVVADARKYRAPAETAG
H4  MSIFISPSNNTGWGLRA-PSKMYGGAXQRSTQHPVVRVGRHFRAPWGALKGRVRSR-TTVDDVIDQVADARNYT-PAAAPV
H40 MSILISPDNNTGWGL--CSAGMYGGAKKRSSQHPVVRVGRHYRAPWGAYTRGVISRRTTVDVVIDSVVADAQRYTRPVAT--
B3  MAILISPSNNTGWGL--GCNKMYGGARIRSDLHPVKVRSYHRAWGSRTGRVGRRATAALADAVATGDPVADTIEAVVAD
B2  MAILVSPSNNTGWGL--GCKSMYGGARRLTEHHFVLVRRHFRASWGSKRGR---TTVPTVPIITDDPVADVNAIAEGTTR
E1  MAILISPTNNTGWGLGLGNNGSFSAVLTRSDHEPVYVKAHYRASWGSSRTRSRTRRVRRAVPRVVPMDPVTAQAQVAETTD
C1  MAILISPSNNTGWGL--GTHKLFGGAKQKSDQHPVYVQAHYRAPWGGKGRRRP-GRARGVPLDPKTEAEVVATIDEVA---
C2  MAILISPTNNTGWGL--GTHKLFGGAKQKSDQHPVYVQAHYRASWGSSKGRRRRQGRARGAPLDPKTEAEVVATIDEVA---
M1  MSILISPSDNNTGWGL--GTGKMYGGARRRSAEHPVHVRSYWRASWGSSRNRVVATVAEDAEPQLEDVAQAPATVPIVRR
F1  MSILISPSDNRTGWGA-----NMRVRRRASMRGVCRRR-----LTLRQLLGLGSRRRRRSRP-TTVSNRLVVST-----
F10 MSILISPNNTGWG-----MRRRSRSSMRGVCMRRRARP-LTLRSLGLGTRRRRGRSRSRPRTTSSLVVRT-----
EDS MSILMSPADNTGWGL--GTRMLRATGLRFSEREPVVRVRSYRAQWGQLNGRKSARQLRRGKYRYQAIHKGRQLAKQRRAVL
O287 MSILVSPSDNTGWGI--GTSSMRATGLKFSKKQPVVRVRYRAQWGQLNARTSLEKLTKLKYYEKLVRDLRKRKTVVPPK

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B

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H2  MA----LTCRLRFVPGFRGRMHRRCMAGHGLTGG-----MRAHRRRRRASHRRMRGGILPLLIPLIAAAIGAVPGIASVALQAQR--H- 80
H5  M-----LTCRLRFVPGFRGRMHRRCMAGHGLTGG-----MRAHRRRRRASHRRMRGGILPLLIPLIAAAIGAVPGIASVALQAQR--H- 48
H12 MA----LTCRMRIPIPGYRGRPRRKGLTNG-----RFRRRSMRRMKGGVLPFLIPLIAAAIGAVPGIASVALQAQR--KN- 72
H40 MA----LTCRFRIPVPSYRGRSRRRRCMAGSG-----R--RRLRRIKGGFLPALIPIIAAAIGAI PGVASVALQAAR--KQ- 70
H41 MA----LTCRFRIPVSSYRGRSRRRRCMLGSG-----P-A-SLPGHMKGGFLPALIPIIAAAIGAI PGIASVALQA--R-KQ- 68
C1  MA-----G-RNVTLRLRVVVRTKTGAC-----RRRGRRTR-I-RCGHMKGGFLPALIPLIAAAIGAVPGIASVALQAAR--H- 68
C2  MA-----G-RNVTLRLRVVVRTKTGAC-----RRRGRRPGI-RCGHMRGGFLPALIPLIAAAIGAVPGIASVALQAAR--H- 69
B3  MSPRGNLTYRLRIPVA-LSGRRRRRTCLRCG-----SAYLLGRRRRRAGGGLRGGFLPLLAPIIAAAIGAI PGIASVAIQAAR--NK- 80
B2  MT-----GVPRTVYRVVVRTLVLR-----P-RRHGRLVRRVARRKSMRGGFLPFLVPLIAAAIGAAPGIASVALQASR--R- 70
O287 MKV-----VHVLKSPHRRRHTRRYKL-----KKINLSPIYLPKLQGGFLPALIPIIAAAISAAPAIAGTVIAAKNANRS 71
EDS  MRR-----SRSYGGLRYGHSVVRYR-----SSQVRRRRRRLKGGFLPAIPIIAAAISAAPAIAGTVIAAKNAR-- 67

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Fig. 2. Conservative cleavage sites of the viral protease. (A) Alignment of the amino terminus of the pVII protein sequences. The recognition sites of the protease are boxed. The presumed cleavage occurs after the fourth residue. The conserved amino acids are printed in bold, and the consensus sequence is underlined. (B) Alignment of the pX amino acid sequences. HAdV-41 sequence is shown presuming a frame shift error at the most probable location of the published sequence. Conserved residues of the protease recognition sites are boxed. The pX sequence of FAdVs are not shown because of their exceptional length and low similarity to mastadenoviruses and atadenoviruses.

HAdV-5 lacks the common first site because of a large deletion. The pX in BAdV-2 also seems to have only one putative cleavage site (MRGG↓F) closer to its carboxyl terminus (Figure 2B), and thus the predicted size of the mu core protein should be 41 amino acid residues. The first protease cleavage site is missing also from the two viruses (EDS and OAV287) representing the proposed atadenovirus genus. In BAdV-3, however, which is a typical mastadenovirus and a deemed close relative of BAdV-2, there are two putative protease cleavage sites, and the one situated closer to the amino terminus (LRGG↓) is unique. Sheppard and Trist (1993), comparing sequences of FAdV-10 and HAdV-2 found little homology between the avian and human mu proteins, but the M/L/IXGXG↓ and the MRGG↓ cleavage sites were found in both precursor proteins. The putative mu protein precursors of HAdV-2 and FAdV-10 consist of 80 and 179 amino acids respectively, while the size of the presumed mature mu protein (the peptide between the two cleavage sites) is comparable (19 and 15 residues, respectively).

The hexon associated protein (VI) is thought to have a role in linking the core complex to the capsid (Russell and Precious, 1982). The sequenced portion of the putative pVI gene of BAdV-2 contains a protease cleavage site close to its amino terminus (MNGG↓A) resembling most the one found in canine adenoviruses (Fig. 1).

Our data supported BAdV-2 as a member of the genus *Mastadenovirus*: its genome contains the genes missing from every studied candidate member of the other (proposed) genus (*Atadenovirus*) comprising BAdVs (Benkő and Harrach, 1998). Namely, the minor core protein gene (V), the hexon associated protein IX gene, and the E1A region (Salmon and Haj-Ahmad, 1994), as well as the E3 region between the pVII and the fiber genes (Esford and Haj-Ahmad, 1994) are all present in BAdV-2.

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