Virus Research 153 (2010) 226–233

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

The first complete genome sequence of a non-chicken aviadenovirus, proposed to be turkey adenovirus 1☆

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ARTICLE INFO

Article history:
Received 10 May 2010
Received in revised form 6 August 2010
Accepted 6 August 2010
Available online 13 August 2010

Keywords:
DNA virus
Genome sequencing
Genome analysis
Turkey adenovirus B
Hungary
Fowl

ABSTRACT

The complete genome sequence of an adenovirus, isolated from turkey and proposed to be turkey adenovirus type 1 (TAdV-1), was determined to extend our knowledge about the genome organisation and phylogeny of aviadenoviruses. The longest adenovirus genome, consisting of 45,412 bp, with the highest G + C content (of 67.55%) known to date, was found. The central part of the TAdV-1 genome has the conserved gene set and arrangement that are characteristic for every other adenovirus analysed to date. This genome core is flanked by the terminal early regions 1 and 4 (E1 and E4). Aviadenovirus-specific genus-common genes were found in these regions, each containing nine such open reading frames (ORFs). Additionally a type-specific novel ORF, designated as ORF50, was found in E4. Phylogenetic analysis as well as the presence of the genus-specific genes, splice sites and protease cleavage sites confirmed the classification of TAdV-1 in the genus *Aviadenovirus*. Intrageneric analyses of two genus-specific genes demonstrated the distinctness of TAdV-1 from other aviadenoviruses, thus supporting the proposal for the establishment of a new species, Turkey adenovirus B for TAdV-1.

1. Introduction

Adenoviruses are double-stranded DNA viruses with a non-enveloped icosahedral capsid, with a size range of 60–100 nm and a genome size of 26–45 kbp. They infect a wide range of host species from every vertebrate class from fish to mammals (Harrach, 2008). Different adenovirus types of the same host are numbered consecutively, and closely related types are grouped together as adenovirus species marked with the host name and the letters of the alphabet (Benkő et al., 2005).

At present, the family *Adenoviridae* is divided into five genera. Members of the genera *Mastadenovirus* and *Aviadenovirus* infect mammals and birds, respectively. The host range of the genera *Atadenovirus* and *Siadenovirus* is much broader and each includes birds too. Atadenoviruses have been isolated from different reptiles, ruminants, and fowl. The egg drop syndrome (EDS) virus, officially named as duck adenovirus 1 and classified as *Duck adenovirus A* species is an atadenovirus (Harrach et al., 1997). Moreover, an atadenovirus has been detected also in a marsupial host (Thomson et al., 2002). Siadenoviruses have been isolated from multiple poultry species and from a frog (Davison et al., 2000; Davison and Harrach, 2002). The type species of siadenoviruses, the so-called turkey haemorrhagic enteritis virus, that is officially named as turkey adenovirus 3 (TAdV-3), has been moved to the genus *Siadenovirus* from the aviadenovirus genus recently. Additional siadenovirus types have been detected in wild birds (Kovács and Benkő, 2009; Kovács et al., 2009; Wellehan et al., 2009) and in turtles (Rivera et al., 2009). The fifth genus, *Ichtadenovirus*, contains a single member to date, an adenovirus originating from white sturgeon (*Acipenser transmontanus*), *Sturgeon adenovirus A* (ICTV Online).

The genus *Aviadenovirus* comprises adenoviruses (AdVs) of avian origin only. These include a dozen of fowl AdV serotypes grouped into five species, namely *Fowl adenovirus A* to *E* (Harrach, 2002). For the species classification of aviadenoviruses, the grouping based on the restriction endonuclease analysis of the FAdV genomes (Zsák and Kisary, 1984) has been adopted. This species designation was confirmed later by the phylogenetic analysis of partial hexon gene sequences (Meulemans et al., 2004; Raue and Hess, 1998). Besides the FAdVs, TAdV-1 and 2 also belong to the avi-

Abbreviations: aa, amino acid; AdV, adenovirus; DBP, DNA binding protein; E1, early region 1; E4, early region 4; FAdV, fowl adenovirus; ITR, inverted terminal repeat; MLP, major late promoter; nt, nucleotide; ORF, open reading frame; pTP, terminal protein precursor; TAdV, turkey adenovirus.

☆ The sequences reported in this work have been deposited in GenBank with accession numbers GU936707 (turkey adenovirus 1) and GU936708 (turkey adenovirus 2).

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doi:10.1016/j.virusres.2010.08.006

adenoviruses (Ursu et al., 2003). TAdV-1 and 2 have been described as distinct serotypes in Northern Ireland (Adair et al., 1980), but no DNA sequence has ever been published from them. TAdV-1 was isolated from birds showing conjunctivitis, nephritis and airsacculitis, but similar signs have been observed frequently in turkeys from which no viruses could be obtained (Scott and McFerran, 1972). TAdV-2 was isolated from day-old turkeys with inclusion body hepatitis. Experimental work demonstrated that TAdV-2 might be the potential cause of suboptimal hatchability (Guy and Barnes, 1997). Additional aviadenovirus isolates from turkeys showing respiratory signs have been published (Crespo et al., 1998; Simmons et al., 1976; Sutjipto et al., 1977), but unfortunately these viruses have not been compared serologically to the Irish TAdV-1 or -2. Furthermore, in the absence of sequence data, TAdVs could not be classified appropriately into virus species either (Benkő et al., 2005). Further species in the genus *Aviadenovirus* are the *Falcon adenovirus A* and the *Goose adenovirus* (ICTV Online).

The pathogenicity of aviadenoviruses, especially of FAdVs in chickens, is not well understood. Typical disease entities include inclusion body hepatitis (IBH), gizzard erosion, and hydropericardium syndrome. While the causative agent of this latter disease is most likely FAdV-4, the involvement of different types has been linked to IBH and gizzard erosion cases. Gizzard erosion of broilers is characterised by patchy erosions within the koilin layer of the gizzard, and it is most likely to be caused by FAdV-1 (FAdV-A). Hydropericardium syndrome is associated with FAdV-4 (FAdV-C) in most cases. Other FAdV types mainly from species FAdV-E may cause IBH characterised by large, round eosinophilic intranuclear inclusion bodies in the hepatocytes but most probably only in immunocompromised animals (Adair and Fitzgerald, 2008; Smyth and McNulty, 2007).

Birds, as hosts for adenoviruses, represent an interesting and special group as they can harbour very different AdVs from as many as three genera. The significant differences in the genome size and organisation between the poultry AdVs of different genera have been recognized early.

Previously, the complete genome sequence has been determined only from two serotypes of the genus *Aviadenovirus*: FAdV-1 (Chiocca et al., 1996) and FAdV-9 (Ojkic and Nagy, 2000), representing species FAdV-A and -D, respectively. The other two bird adenoviruses with complete genome sequences, duck adenovirus 1 (*Duck adenovirus A*, egg drop syndrome virus) (Hess et al., 1997) and turkey adenovirus 3 (*Turkey adenovirus A*) (Pitcovski et al., 1998), do not belong to the genus *Aviadenovirus*.

The main goal of this study was to uncover the differences among aviadenoviruses originating from different hosts to deepen our knowledge about the genome organisation and phylogenetic relationships of these viruses. Aviadenoviruses possess the longest genomes among adenoviruses, and a number of putative ORFs of yet unknown function have been detected in them. With the analysis of a new full genome, the supposedly genus-common ORFs and predicted splice sites can be confirmed. Finally, we also aimed at testing the theory on the co-evolution of adenoviruses with their host animals in the case of birds.

2. Materials and methods

2.1. Virus propagation, DNA purification and molecular cloning

In Hungary, a virus strain (designated as D90/2) was isolated from the trachea of 10-week-old turkeys showing respiratory signs. It was propagated on chicken embryo liver cells. When the cytopathic effect was maximal, the tissue cultures were frozen to detach the cells from the surface. The cells and the supernatant were combined and subjected to two additional freezing and thawing cycles. After low-speed clarification, the virions were concentrated by ultracentrifugation.

The viral DNA was purified by phenol–chloroform extraction. Random cloning of the genome was carried out with different restriction endonucleases (*Bam*HI, *Eco*RI, *Pst*I, *Sac*I). The resulting fragments were ligated into the pBluescript II KS phagemid. Larger DNA fragments were subcloned by *Pst*I enzyme. For cloning the genome ends, the terminal protein was removed by the alkaline treatment (Zakharchuk et al., 1993), and the *Bam*HI end fragments were cloned into pBluescript II KS double-cut by *Eco*RV and *Bam*HI.

For comparative purposes, samples of formerly published TAdV-1 and -2 were also examined. These samples, kindly provided by Brian M. Adair, originated from the reference strain collection established by J. B. McFerran, Belfast, Northern Ireland.

2.2. DNA sequencing and PCR

The nucleotide sequence of the cloned viral genome fragments was determined with the use of the BigDye Terminator v3.1 Cycle Sequencing Kit and an ABI PRISM 3100 machine. Subcloning and primer walking approaches were also applied.

The identity of TAdV-1 and -2 was tested by a nested PCR system published by Meulemans et al. (2004). This PCR can amplify a 553–559-bp-long fragment from the hexon gene of aviadenoviruses.

2.3. Bioinformatical and phylogenetic analysis

The newly determined sequences were identified using the NCBI database and the blastx homology search program (NCBI Blast; Altschul et al., 1997). The *Bam*HI physical map was thus gradually assembled, the sequences were handled and merged using the Staden Package (Staden et al., 2000).

Virtual restriction endonuclease analysis of the viral genome was achieved using the program pDRAW32 (pDRAW32 DNA analysis software). The resulting picture was compared to the restriction endonuclease assays of the Irish TAdV-1 and -2 types (Guy and Barnes, 1997; Scott and McFerran, 1972).

The full genome sequence was annotated with the help of the Artemis program (Rutherford et al., 2000). Every ORF longer than 40 amino acids (aa) was marked first, then subjected to blastp homology search (Altschul et al., 1997) in BioEdit (Hall, 1999) against a custom database containing every aviadenovirus ORF. Homologues were accepted and named after their counterparts according to the nomenclature as defined by Davison et al. (2003). ORFs showing no homology to any protein sequences in the NCBI GenBank were numbered continuing the numbering of Corredor et al. (2008). The derived amino acid sequences were searched for the presence of putative domains by InterProScan Sequence Search (Zdobnov and Apweiler, 2001) or Pfam (Finn et al., 2010).

Multiple protein alignments were made using the Tcoffee program (Poirot et al., 2003), or in the case of the partial hexon sequences derived from PCR products (Meulemans et al., 2004) using the ClustalW program (Thompson et al., 1994). The alignments were edited with BioEdit (Hall, 1999). Phylogenetic calculations were performed using the Phylip package (Felsenstein, 1989) online (Mobyle@pasteur) by Protdist with Categories model, or in the case of the partial hexon sequences by Dnadist with Kimura two parameters model (Kimura, 1980). The Fitch program was used by the Fitch–Margoliash method with global rearrangements for phylogenetic tree reconstruction. Bayesian method was also applied using MrBayes (Ronquist and Huelsenbeck, 2003) (number of runs: 2, number of generations: 1,000,000, sample frequency: 10, burn: 40%) online by Topali (Milne et al., 2009), for which model selection was also performed using Topali. The trees were visualised using Mega (Tamura et al., 2007). The tail, shaft and knob