Virus Research 156 (2011) 91–97

Contents lists available at ScienceDirect

Virus Research

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

Pathogenicity and complete genome sequence of a fowl adenovirus serotype 8 isolate☆

Helena Grgić[a], Dan-Hui Yang[b], Éva Nagy[a,*]

[a] Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1, Canada
[b] Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario N1G 2W1, Canada

ARTICLE INFO

Article history:
Received 27 September 2010
Received in revised form 5 January 2011
Accepted 5 January 2011
Available online 14 January 2011

Keywords:
FAdV-8 complete nucleotide sequence
Pathogenesis
Virus shedding
Highest viral copy numbers in the cecal tonsils

ABSTRACT

In this study we determined and analyzed the complete nucleotide sequence of the genome of a fowl adenovirus serotype 8 (FAdV-8) isolate and examined its pathogenicity in chickens. The full genome of FAdV-8 was 44,055 nucleotides in length with a similar organization to that of FAdV-1 and FAdV-9 genomes. No regions homologous to early regions E1, E3 and E4 of mastadenoviruses were recognized. Along with FAdV-9, FAdV-8 has only one fiber gene and with regard to sequence composition and genome organization, FAdV-8 is closer to FAdV-9 than to FAdV-1. Moreover, our findings suggest that FAdV-1 of species *Fowl adenovirus A* as the current type species despite its historical priority is not representative of the genus *Aviadenovirus*, and that FAdV-8 or FAdV-9 in species *Fowl adenovirus E* and *Fowl adenovirus D*, respectively, would be more suitable for that designation. Additionally, pathogenicity of FAdV-8 was studied in specific pathogen free chickens following oral and intramuscular inoculations. Despite lack of clinical signs and pathological changes virus was found in tissues and cloacal swabs of all birds with the highest viral copy numbers present in the cecal tonsils. The highest virus titers in the feces for orally and intramuscularly inoculated chickens were recorded at days 10 and 3 post-infection, respectively.

1. Introduction

Adenoviruses are in the family *Adenoviridae* and grouped into five genera. While mastadenoviruses and aviadenoviruses infect mammals and birds, respectively, viruses in the genera *Atadenovirus* and *Siadenovirus* have a broader host range. The genus *Aviadenovirus* currently comprises five fowl (*Fowl adenovirus A–E*), one falcon (*Falcon adenovirus A*) and one goose adenovirus (*Goose adenovirus*) species (ICTV, Virus Taxonomy, 2009 Release). The type species is *Fowl adenovirus A* and is represented by fowl adenovirus 1 (FAdV-1) (Benkő et al., 2005).

The first molecular classification of fowl adenoviruses was based on restriction fragment length polymorphisms (RFLP) of their DNA genome (Zsák and Kisary, 1984). The genomes of fowl adenoviruses (FAdVs) are larger than that of the human adenoviruses (HAdVs), in spite of the fact that open reading frames (ORFs) for a few structural proteins such as V and IX, and early regions E1, E3 and E4 responsible mainly for host cell interaction and immune modulation in mastadenoviruses are absent in FAdVs. The complete nucleotide sequences are available for the genomes of FAdV-1 (CELO virus) and FAdV-9 (A2-A strain) from the species *Fowl adenovirus A* and *Fowl adenovirus D*, respectively. The FAdV-9 genome (45,063 bp) is 1259 bp longer than that of FAdV-1 (43,804 bp) (Ojkic and Nagy, 2000; Chiocca et al., 1996). Partial sequences are also published for the left and right ends of the genomes of FAdV-2, FAdV-4 and FAdV-10 (Corredor et al., 2006, 2008), and for the late region of FAdV-4 and FAdV-10 (Mase et al., 2010; McCoy and Sheppard, 1997; Sheppard et al., 1998). Although it is known that the highest degree of conservation among all adenovirus genomes is in the middle region that encodes the structural proteins only a few complete genome sequences are available for FAdVs.

FAdVs are common infectious agents of poultry although most of them are subclinical or associated with mild clinical signs (McFerran and Smyth, 2000). However, some FAdV strains are associated with a variety of diseases, such as inclusion body hepatitis (IBH) which is diagnosed in almost every part of the world (Adair and Fitzgerald, 2008). The disease affects mostly 3–7 weeks old meat-type chickens (McFerran et al., 1976; Ojkic et al., 2008a,b), although Bickford (1974) and Hoffmann et al. (1975) also reported cases in pullets and replacement layers, respectively. IBH outbreaks are characterized by sudden onset of mortality which usually ranges from 2 to 10%. (Adair and Fitzgerald, 2008). The varying degree of mortality correlates to the pathogenicity of the virus, secondary infections and susceptibility of the chickens. In Canada, the

Abbreviations: FAdV-8, fowl adenovirus 8; d.p.i., days post-infection; ORF, open reading frame; pfu, plaque forming unit; SPF, specific pathogen free.

☆ The GenBank accession number of the sequence reported in this paper is GU734104.

* Corresponding author. Tel.: +1 519 824 4120x54783; fax: +1 519 824 5930.
E-mail address: enagy@uoguelph.ca (É. Nagy).

0168-1702/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.virusres.2011.01.002

most commonly isolated FAdVs belong to serotypes 2, 7, 8, and 11 (Ojkic et al., 2008a,b). Following initial multiplication the virus spreads to virtually every organ, although the main sites of virus replication are the respiratory and alimentary tracts (Cook, 1983; Saifuddin and Wilks, 1991; Hess, 2000).

The goals of this study were to (1) determine the complete nucleotide sequence of the genome of a fowl adenovirus serotype 8 (FAdV-8) isolate from an IBH outbreak representing species *Fowl adenovirus E*, (2) investigate its genomic organization and relationship with other adenoviruses (AdVs) and (3) study its pathogenicity in specific pathogen free (SPF) chickens.

2. Materials and methods

2.1. Virus and cells

The serotype 8 FAdV was isolated by the Animal Health Laboratory (AHL), University of Guelph from an IBH outbreak that occurred in an Ontario broiler farm. The virus was plaque purified in our laboratory. Virus propagation and titration were done in a chicken hepatoma cell line (CH-SAH) as described by Alexander et al. (1998).

2.2. Nucleic acid preparation

CH-SAH cells were infected at a multiplicity of infection (m.o.i.) of 0.5. Cells and supernatants were collected when extensive cytopathic effect was seen. Virus purification and viral DNA preparation were performed as described (Ojkic and Nagy, 2001).

2.3. Cloning, sequencing and sequence analysis

The FAdV-8 DNA was digested with *Bam*HI and the fragments were separated in 0.8% agarose gel. DNA from the desired fragments was extracted and cloned into *Bam*HI digested pBluescript (SK−). Sequencing was first carried out with universal forward and reverse primers and then completed by primer walking. Multiple nucleotide sequence alignments using Vector NTI Advance™ 11 were done to identify the conserved regions. The sequence data were subjected to BLAST analysis with the GenBank database to determine the nucleotide sequence homologies and amino acid identities and similarities. ORFs encoding polypeptides over 50 amino acids were identified by the ORF finder from Vector NTI Advance™ 11.

2.4. Pathogenicity assessment of FAdV-8 in SPF chickens

A trial was conducted to assess the pathogenic potential of the virus. One hundred white Leghorn SPF chickens were hatched at the Arkell Poultry Station from eggs obtained from the Canadian Food Inspection Agency (CFIA), Ottawa. The birds were housed at the University Isolation Unit according to the Animal Care Committee of the University of the Guelph in accordance with the *Guide to the Care and Use of Experimental Animals* of the Canadian Council on Animal Care and received food (21% unmedicated starter ration) and water *ad libitum*. At 9 days of age the chickens were tagged with wing bands (Ketchum, ON) and sera were collected from all of them. Ten-day-old chicks were randomly divided into four groups: Group I (FAdV-8)-oral, Group II (FAdV-8)-intramuscular (im), Group III (control)-oral and Group IV (control)-im. Groups I and II had 30 birds each and negative control groups III and IV had 20 birds each. Chicks were each inoculated with 2×10^8 plaque forming units (pfu) of the virus. The control birds received PBS. All chickens were re-inoculated at 14 days of age with the same dose of virus.

The birds were observed daily (3×) for clinical signs. Four chicks from treatment groups I and II and three chicks from the control groups were drawn randomly for necropsy at each of 0, 3, 5, 7, 14, 21, and 28 days post-infection (d.p.i.) (except day 0 when only two chicks from the controls were euthanized). The chickens were euthanized, necropsied, examined for the presence of gross lesions and tissues from thymus, lung, heart, liver, kidney, bursa of Fabricius and cecal tonsils were collected. Each organ was sectioned into three portions: one was placed into 10% formalin for histology, the second was stored at −70 °C for virology and the third was stored at −70 °C for qPCR. Cloacal swabs were taken from all birds at 0, 3, 5, 10, 14, 21, 28 d.p.i. and their virus titers were determined in CH-SAH cells. In addition, chickens were bled at weekly intervals, 0, 7, 14, 21, 28 d.p.i. and serum samples were tested for FAdV-specific antibodies (Abs) by enzyme-linked immunosorbent assay (ELISA) as described (Ojkic and Nagy, 2003).

2.5. Quantitative real time PCR (qPCR) analysis

To determine the viral load in tissues, real time PCR using Syber Green as intercalating dye was developed. The FAdV-8 ORF-1 A/B gene (Corredor et al., 2006) was used as an indicator for the presence of viral DNA with primers; forward primer: 5′-AAATGGTAAACGCGTGGGATC-3′ and reverse primer: 5′-TTCTCCGTCTCCGATCTGG-3′. The 20 μl qPCR reaction containing 1 μl (10 pmol) of each primer, 10 μl of QuantiTec SYBR Green I PCR Master Mix (Qiagen), 2 μl DNA and 6 μl nuclease free water, was prepared using the computerized automated liquid handling robotics CAS-1200 (Corbett Research). The qPCR program was first run at 15 min at 95 °C to activate the *Taq* polymerase, followed by 40 cycles of 95 °C for 20 s, 57 °C for 15 s, 72 °C for 20 s, with an acquiring step at 75 °C for 15 s, using the Corbett Research Rotor-Gene 6000 System. Quantitative measurement of the PCR product was carried out by incorporation of the SYBR Green I fluorescent dye. To establish the standard curves, 10-fold serial dilutions (10^{-1} to 10^{-7}) of FAdV-8 genomic DNA starting at 12.2 ng/μl (2.4×10^8 copies/μl) were made and used as qPCR templates. Melting curve analysis was performed after each run to monitor the specificity of the PCR products. Negative control using DNA extracted from uninfected chicken and no template control (NTC) were routinely included in each experiment.

2.6. Statistical analysis

Kruskal–Wallis test was performed to evaluate whether viral copy numbers differed among tissues. Following statistically significant *P*-value, pair-wise comparison was performed between different tissues. *P*-values of 0.05 were considered to be statistically significant.

3. Results

3.1. Genome size and organization of FAdV-8

The full genome of FAdV-8 was found to be 44,055 base pairs (bp) with a G+C content of 58%. The position and size of 46 ORFs with potential to encode polypeptides of at least 50 amino acid (aa) residues are listed in Supplementary Table 1. A schematic of the genome map is presented in Fig. 1. The GenBank accession number of the sequence reported in this paper is GU734104.

The sequences of the left and right ends of this FAdV-8 isolate were determined earlier in our laboratory (Corredor et al., 2006, 2008). Briefly, the first 6 kb at the left terminal region of the genome have no significant sequence homology to known mastadenoviral sequences either at the DNA or protein level. Homologues to the first 10 ORFs (ORF0 to IVa2) in this region described previously for FAdV-1 and FAdV-9 were present in the genome of FAdV-8 (Corredor et al., 2006). None of the leftward oriented ORFs at the right end of FAdV-8 showed homology to identified E4