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The capsid protein of infectious bursal disease virus contains a functional α4β1 integrin ligand motif

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ABSTRACT

Infectious bursal disease virus (IBDV), a member of the dsRNA *Birnaviridae* family, is an important immunosuppressive avian pathogen. We have identified a strictly conserved amino acid triplet matching the consensus sequence used by fibronectin to bind the α4β1 integrin within the protruding domain of the IBDV capsid polypeptide. We show that a single point mutation on this triplet abolishes the cell-binding activity of IBDV-derived subviral particles (SVP), and abrogates the recovering of infectious IBDV by reverse genetics without affecting the overall SVP architecture. Additionally, we demonstrate that the presence of the α4β1 heterodimer is a critical determinant for the susceptibility of murine BALB/c 3T3 cells to IBDV binding and infectivity. Our data suggests that the IBDV might also use the α4β1 integrin as a specific binding receptor in avian cells.

Introduction

Infectious bursal disease virus (IBDV) is the best characterized member of the *Birnaviridae* family that groups naked viruses with a bipartite double-stranded (dsRNA) RNA genome (Delmas et al., 2004). IBDV is responsible for an immunosuppressive disease that affects young domestic chickens (*Gallus gallus*), and causes major economic losses to the poultry industry world-wide (van den Berg et al., 2000).

The knowledge about IBDV structural and molecular biology has experienced a great progress over the last few years, thus the crystal structure of all structural polypeptides has been completely or partially solved (Casañas et al., 2008; Coulibaly et al., 2005; Feldman et al., 2006; Garriga et al., 2007, 2006; Lee et al., 2006; Pan et al., 2007), and the virus assembly pathway has been extensively characterized (Chevalier et al., 2005; Luque, 2007; Oña et al., 2004; Saugar et al., 2005). However, our understanding about fundamental aspects of the virus replication cycle, e.g. the entry and egress mechanisms is, as yet, scarce.

Viruses are obligate intracellular parasites, thus the first requirement to initiate a successful infection consists on the recognition of a suitable receptor(s) on the surface of target cells. This recognition sets off a series of events, generally involving an elaborated interplay between different cellular and viral components, allowing the virus to reach the appropriate cellular compartment for virus replication (Smith and Helenius, 2004). Indeed, the presence and distribution of specific virus receptor(s) molecules are in many cases critical determinants for virus host-range, tropism, and pathogenesis.

The IBDV capsid (65–70 nm in diameter) is a single shell built by 260 trimers of the VP2 polypeptide arranged in an icosahedral lattice with T = 13l symmetry (Bottcher et al., 1997; Coulibaly et al., 2005; Saugar et al., 2005). VP2 is produced by proteolytic maturation of a precursor polypeptide, known as pVP2 (Birghan et al., 2000; Feldman et al., 2006). VP2 is the only component of the virus capsid, and thus responsible to the interaction with host cell receptors. Although the tropism of IBDV for lymphocytic cell populations found at the Fabricius bursa has been extensively characterized (Becht, 1980; Kibenge et al., 1988), the identification of specific cell binding receptor (s) has remained elusive.

The VP2 crystal structure, independently solved by three different groups (Coulibaly et al., 2005; Garriga et al., 2006; Lee et al., 2006), revealed the presence of three distinct domains designated base (B), shell (S), and projection (P) (Fig. 1A). Domain P, formed by a β-barrel with a jelly roll topology, is well exposed to the solvent, thus being a likely candidate to interacting with the host cell binding receptor(s). This hypothesis is supported by the presence within this domain of several neutralizing epitopes (Fahey et al., 1989), and amino-acid residues directly involved both in virulence and tissue-culture adaptation (Brandt et al., 2001; van Loon et al., 2002). Additionally, the P domain shows a high degree of structural homology with projection domains of reovirus capsid polypeptides directly involved

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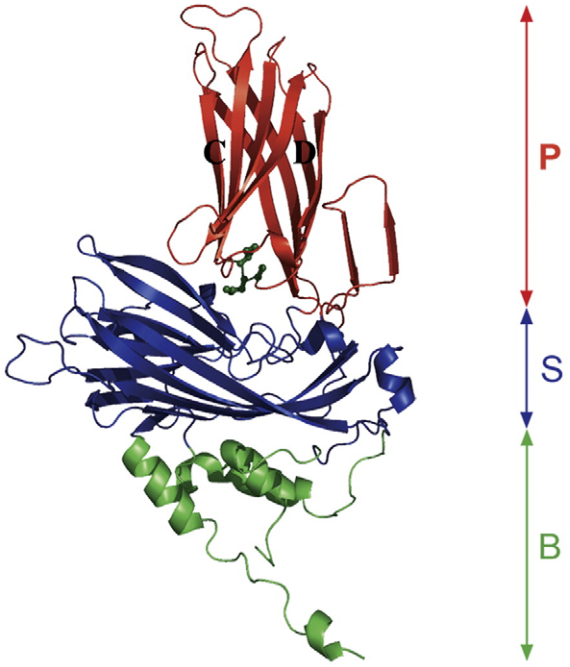

B

Soroa	TITLFSANIDAITSLSVGGE	(226–245)	AAD30136
D78	TITLFSANIDAITSLSVGGE	(226–245)	AAO15768
52/70	TITLFSANIDAITSLSIGGE	(219–238)	BAA00745
UK661	TITLFSANIDAITSLSIGGE	(226–245)	NP690838
Variant_E	TITLFSANIDAITSLSVGGE	(226–245)	AAD32617
OH	KTTLFTANIDALTSLSVGGE	(226–245)	AAC55351

Fig. 1. Identification of a conserved putative integrin binding motif on the VP2 capsid polypeptide. (A) Ribbon diagram of the VP2 polypeptide. The three VP2 domains, namely P, S, and B, colored in red, blue, and green, respectively, are delimited by arrows. The lateral chains of the IDA motif amino acid residues are shown in dark green. The position of the C and D β-strands is indicated. (B) Multiple alignment comparing the predicted amino acid sequences of the IBDV VP2 from attenuated (D78), classical virulent (52/70), very virulent (UK661), and variant (Variant E) serotype 1, and classical serotype 2 (OH) strains to that of the virulent serotype 1 Soroa isolate. The image shows 20 residues-long protein regions containing the putative IDA integrin binding motif (shown in bold). Non-strictly conserved residues are shown in red. The amino acid positions and the GenBank accession numbers of the different proteins are indicated on the right.

in cell adhesion and/or penetration processes (Graham et al., 2005, 2006; López and Arias, 2004; Maginnis et al., 2006; Tan et al., 2001).

Early studies on the nature of the IBDV cell receptor(s) showed that the treatment of chicken B lymphoblastoid cells with proteases and/or N-glycosylation inhibitors cause a major reduction on IBDV infectivity, thus strongly suggesting the involvement of a N-glycosylated membrane polypeptide(s) during the first stages of virus infection (Ogawa et al., 1998). Initial attempts to identifying IBDV specific receptor polypeptides were based on the use of virus overlay protein binding assays. This approach led to the detection of several membrane polypeptides specifically interacting with immobilized IBDV particles (Nieper and Muller, 1996; Setiyono et al., 2001). However, their identity and the actual implication on IBDV cell binding and entry remain uncharacterized. More recently, the use of IBDV-derived subviral particles (SVP), generated by VP2 recombinant expression, has led to the identification of the chicken heat shock protein 90 (cHsp90) as a major component of a putative IBDV receptor complex including other cellular polypeptides (Lin et al., 2007).

Similarly to many other icosahedral viruses (Boulanger, 1999), some members of the dsRNA *Reoviridae* family use integrins as binding receptors and/or essential entry molecules (Graham et al., 2005, 2006; López and Arias, 2004; Maginnis et al., 2006; Tan et al., 2001). Integrins form a superfamily of adhesion receptors involved in a wide variety of matrix–cell and cell–cell interactions. Members of this superfamily are heterodimers formed by two glycosylated transmembrane proteins termed α and β, respectively. Both the structural similarity of the IBDV VP2 P domain to the corresponding projection domains of reovirus capsid polypeptides (Coulibaly et al., 2005), and the glycosylated nature of the integrins suggested that IBDV might also employ an integrin(s) during its cell entry process.

Integrins mediate cell adhesion to extracellular matrix, cell membrane, and virus capsid polypeptides through the recognition of short linear ligand sequence motifs. Many integrin-binding motifs have been extensively characterized (Hynes, 1999; Komoriya et al., 1991; Plow et al., 2000; Staatz et al., 1991), facilitating the search for putative integrin-binding motifs within the VP2 sequence. This search resulted in the identification of the Ile-Asp-Ala (IDA) sequence within the VP2 P domain. That motif matches the XDY triplet (X = G, L, I, E; Y = V, A) used by the α4β1 integrin in binding to fibronectin (Fn) (Mould and Humphries, 1991). This finding prompted us to perform a series of experiments aimed to characterizing the roles of both the VP2 IDA motif and the α4β1 integrin on the binding of IBDV to susceptible cells.

The results described in this report show that, with the virus strain and cell lines used for the present study, the VP2 IDA motif plays a critical role in the binding of IBDV-derived SVP and virus particles to IBDV-susceptible cells, and that a single point mutation on this motif completely abrogates SVP cell binding and virus infectivity. Additionally, we demonstrate that transformation with a recombinant expression plasmid harboring the α4 subunit gene is sufficient to turn naturally α4-lacking murine BALB/c 3T3 fibroblasts from resistant to IBDV attachment and infection to permissive for both phenomena.

Results

Identification of a putative integrin-ligand motif within the IBDV capsid polypeptide

The amino acid sequence of the VP2 polypeptide from the IBDV Soroa strain was scanned for integrin-binding sequences. This led to the identification of the Ile-Asp-Ala sequence (IDA, residues 234–236) (Fig. 1B) matching the XDY (X = G, L, I, E; Y = V, A) amino acid triplets used by the α4β1 integrin in binding to Fn, and in particular to the IDA motif found in the Fn H1 fragment (Mould and Humphries, 1991). The identified VP2 IDA sequence is located within a flexible loop, connecting the C and D β strands, at the base of the P domain (Fig. 1A).

A multiple alignment performed with over 100 VP2 sequences from antigenic variant, classical virulent, very virulent, as well as tissue culture-adapted IBDV serotype 1 and serotype 2 isolates, indicated that the IDA triplet is strictly conserved in all members of the *Avibirnavirus* genus. Fig. 1B, showing a multiple alignment of VP2 sequences from representative IBDV strains, summarizes this finding.

Binding of IBDV-derived SVP to DF-1 cells

In order to assess the feasibility of using IBDV-derived SVP as a model to analyze the role of the IDA motif on cell attachment, it was critical to determine whether they were able to bind to IBDV-susceptible cells. IBDV and SVP were produced, purified, and analyzed by EM as previously described (Castón et al., 2001a). DF-1 monolayers, grown on coverslips, were incubated for 1 h at 4 °C with a suspension of either purified virus or SVP. Thereafter, cells were processed for CLSM using an anti-VP2 specific antiserum. As shown in