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Molecular characterization of Lelystad virus

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Abstract

Lelystad virus (LV), the prototype of porcine reproductive respiratory syndrome virus, is a small enveloped virus, containing a positive strand RNA genome of 15 kb. LV is tentatively classified in the family *Arteriviridae*, which consists of lactate dehydrogenase-elevating virus (LDV), equine arteritis virus (EAV) and simian hemorrhagic fever virus (SHFV). These viruses have a similar genome organization and replication strategy as coronaviruses, but the size of the genome is much smaller (12–15 kb) and they have different morphological and physicochemical properties. The genome of LV contains eight open reading frames (ORFs) that encode the replicase genes (ORFs 1a and 1b), envelope proteins (ORFs 2 to 6) and the nucleocapsid protein (ORF7). Genomic comparison of European and North American isolates has shown that the structural proteins encoded by ORFs 2 to 7 vary widely. The amino acid sequences of ORFs 2 to 7 of North American strains share only 55 to 79% identical amino acids with those of European strains. Using polyvalent porcine anti-LV serum, gene-specific anti-peptide sera and monoclonal antibodies, we have identified six structural proteins of LV and their corresponding genes. These are: the 15 kDa unglycosylated nucleocapsid protein (N) encoded by ORF7, an 18 kDa unglycosylated integral membrane protein M encoded by ORF6, a 25 kDa *N*-glycosylated protein encoded by ORF5, a 31–35 kDa *N*-glycosylated protein encoded by ORF4, a 45–50 kDa *N*-glycosylated protein encoded by ORF3 and a 29–30 kDa *N*-glycosylated protein encoded by ORF2. A nomenclature for these structural proteins is proposed. © 1997 Elsevier Science B.V.

Keywords: Lelystad virus; Molecular characterization; Genome organization; Sequence comparisons; Nomenclature

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1. Introduction

The porcine reproductive respiratory syndrome (PRRS) was first described a few years ago and is characterized by reproductive failure of sows and respiratory distress of piglets. In 1991 the Lelystad virus (LV; Wensvoort et al., 1991) also known as PRRS virus (Ohlinger et al., 1991; Collins et al., 1992) was identified as the causal agent. LV is a small, enveloped positive strand RNA virus that replicates preferentially in porcine alveolar lung macrophages, but only to a limited extent in other cells. LV is tentatively classified in the family *Arteriviridae*, which consists of LDV, EAV and SHFV (Meulenberg et al., 1993a; Plagemann and Moennig, 1991). These viruses share many characteristics such as genome organization, strategy of gene expression, the propensity to grow preferentially in macrophages and a tendency to induce persistent infections. In this review the molecular properties of LV are briefly summarized and a nomenclature for the structural proteins is proposed.

2. Genome organization

LV contains a polyadenylated positive strand RNA genome of 15.1 kb (Fig. 1). Eight open reading frames (ORFs) that encode virus-specific proteins were identified. ORFs 1a and 1b comprise about 80% of the viral genome and are predicted to encode the viral RNA dependent RNA polymerase. Their amino acid sequences contain elements conserved in RNA polymerases of the torovirus Berne virus, EAV, LDV and coronaviruses (Conzelmann et al., 1993; Meulenberg et al., 1993a). The ORFs 2 to 7 encode structural proteins as will be discussed further below. Analogous to RNA synthesis during replication of EAV and LDV, multiple subgenomic RNAs are synthesized in LV-infected alveolar macrophages. These subgenomic RNAs have been identified in Northern blot hybridization analysis using LV-specific oligonucleotides located in the unique part of the various ORFs and at the extreme 5′ and 3′ end (Meulenberg et al., 1993a). They form a 3′ coterminal nested set and all contain a leader sequence derived from the 5′ end

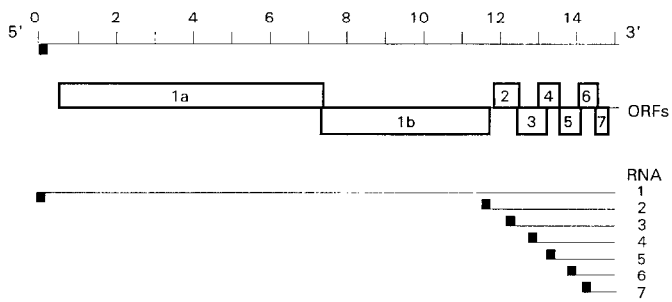

Fig. 1. Organization of the genome of LV. In the upper part the ORFs identified in the nucleotide sequence are shown, in the lower part the 3′ nested set of six subgenomic RNAs are shown. The leader sequence is indicated by a solid box.

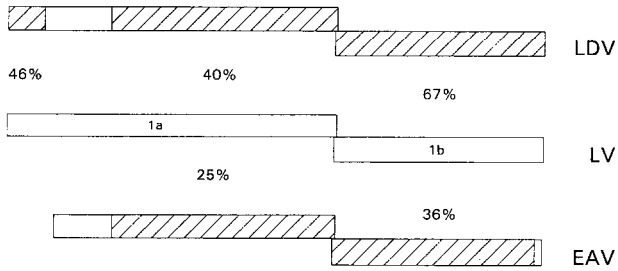

Fig. 2. Comparison of amino acid sequences of ORFs 1a and 1b of LV, EAV (den Boon et al., 1991) and LDV (Godeny et al., 1993). The percentage of identical amino acids is shown. The regions of the ORFs of EAV and LDV, which have identity with LV are shaded.

of the viral genome (Fig. 1). The junction site i.e. the site where the leader is fused to the body of the subgenomic RNA was identified for all six subgenomic RNAs (Meulenberg et al., 1993b). These junction sites contain a conserved six nucleotide motif, UCAACC, or a very similar sequence.

3. Sequence comparisons

Comparison of the amino acid sequences encoded by the ORFs identified in the genome of LV with those of other viruses indicates that LV is distantly related to coronaviruses and toroviruses and closely related to LDV and EAV. As is shown in Fig. 2, the amino acid sequences of ORF1a (apart from a gap at the N-terminal half) and ORF1b of LV are highly identical with the corresponding ORFs of LDV (Chen et al.,

Table 1
Amino acid sequence identity of ORFs 2 to 7 of LV with those of other isolates of PRRSV and of other arteriviruses

	PRRSV-10 [a]	VR2332 [b]	VR2385 [c]	IAFexp-91 [d]	LDV-P [e]	LDV-C [f]	EAV [g]
LV-ORF2	99	63	62	ND	38	32	NS
LV-ORF3	99	60	57	54	31	28	NS
LV-ORF4	99	70	69	68	29	30	NS
LV-ORF5	99	55	54	52	47	47	NS
LV-ORF6	100	79	78	81	53	53	23
LV-ORF7	100	64	57	59	41	44	20

[a] Data derived from Conzelmann et al. (1993).
[b] Data derived from Murtaugh et al. (1993).
[c] Data derived from Meng et al. (1994).
[d] Data derived from Mardassi et al. (1994).
[e] Data derived from Chen et al. (1993).
[f] Data derived from Godeny et al. (1993).
[g] Data derived from den Boon et al. (1991).
ND = not determined. NS = not significant.

1993; Godeny et al., 1993) and EAV (den Boon et al., 1991). These data and the identity observed between the amino acid sequences of ORFs 2 to 7 of these viruses (Table 1) indicate that LV is more related to LDV than to EAV. Unfortunately the sequences of ORFs 1a and 1b of LV could not be compared with those of other PRRSV isolates since these have not yet been determined. However, the nucleotide sequence has been determined for ORFs 2 to 7 of another European isolate, PRRSV-10 (Conzelmann et al., 1993), two U.S. isolates, VR2332 (Murtaugh et al., 1993) and VR2385 (Meng et al., 1994) and a Canadian isolate IAFexp-91 (Mardassi et al., 1994). The amino acid sequences of ORFs 2 to 7 of LV are highly identical with those derived from the nucleotide sequences of the European isolate PRRSV-10, but vary widely with those derived from the nucleotide sequences of North American isolates VR2332, VR2385 and IAFexp-91 (Table 1). This variation is in line with the antigenic differences observed in the reactivity of European and U.S. isolates with polyclonal pig sera as well as mouse monoclonal antibodies (Wensvoort et al., 1992; Nelson et al., 1993; Drew et al., 1995).

4. Structural proteins

In cells infected with LV, or the North American isolate VR2332, at least three viral proteins of 15, 18 and 25 kDa have been detected by Western blotting with polyclonal pig sera directed against the respective strains (Table 2; Meulenberg et al., 1995; Nelson et al., 1993). The corresponding ORFs from which these proteins are expressed were identified with gene-specific anti-peptide sera raised in rabbits. It was shown that the 15 kDa nucleocapsid protein N is encoded by ORF7, the 18 kDa M protein is encoded by ORF6 and the 25 kDa protein is encoded by ORF5 (Table 2; Meulenberg et al., 1995). Using the cell line CL2621, which is susceptible for LV, we have been able to improve the virus growth and purification methods. Hence, using more viral antigen and LV-specific monoclonal antibodies, recently developed by van Nieuwstadt et al. (1996),

Table 2
Characteristics of proteins encoded by ORFs 2 to 7 of LV

ORF	Protein	Calculated [a] Mw	*N*-glycosylation [b] sites	Signal [c] sequence	Putative transmembrane region	Mw on SDS-PAGE [d]
2	GP_2	28.4	2	37–38	210–228	29–30
3	GP_3	30.6	7	41–42	—	45–50
4	GP_4	20.0	4	—	1–17/165–183	31–35
5	GP_5	22.4	2	32–33	108–131	25
6	M	18.9	2	—	17–88	18
7	N	13.8	1	—	—	15

[a] Calculated Mw (kDa) on the basis of the amino acid sequence.
[b] Number of putative *N*-glycosylation sites.
[c] Amino acid position of the putative signal sequence cleavage site.
[d] Mw (kDa) on SDS-PAGE as determined by Western blot analysis of purified LV virions.

we have identified three additional structural proteins in Western blot analysis. These were a 29–30 kDa protein encoded by ORF2, a 45–50 kDa protein encoded by ORF3 and a 31–35 kDa protein encoded by ORF4. Earlier findings of Drew et al. (1995) also indicated that the 45–50 kDa ORF3 protein is a structural protein. Endoglycosidase treatment of the LV proteins has shown that the 25, 29–30, 31–35 and 45–50 kDa proteins are *N*-glycosylated proteins whereas the N and M protein are not (Meulenberg et al., 1995; van Nieuwstadt et al., 1996). Therefore, we may conclude that LV contains six structural proteins, four glycoproteins encoded by ORFs 2 to 5, an unglycosylated membrane protein M encoded by ORF6 and the nucleocapsid protein N encoded by ORF7.

5. Nomenclature

Since we have now identified six structural proteins of LV, we would like to propose a nomenclature for these proteins. In line with the nomenclature for coronaviruses, which are distantly related to arteriviruses, general concensus exists to also use N for the nucleocapsid protein encoded by ORF7 and to use M for the nonglycosylated membrane protein M encoded by ORF6 of arteriviruses. The other four structural proteins, the 29–30 kDa protein encoded by ORF2, the 45–50 kDa protein encoded by ORF3, the 31–35 kDa protein encoded by ORF4 and the 25 kDa protein encoded by ORF5 are glycoproteins and therefore we propose to name these GP_2, GP_3, GP_4, and GP_5, respectively. In this way the proteins are linked to the ORFs, from which they are expressed and no confusion exists about their molecular weights. Two structural *N*-glycosylated proteins were identified in virions of EAV. These were a 30–42 kDa glycoprotein designated $G_{l(large)}$ encoded by ORF5 and a 25 kDa glycoprotein, designated $G_{s(small)}$ encoded by ORF2 (De Vries et al., 1992). Since the glycoprotein encoded by ORF2 of LV is larger than the glycoprotein encoded by ORF5 of LV, the names G_s and G_l used for EAV are not appropriate for LV. The major envelope glycoprotein of 24–44 kDa of LDV is expressed by ORF5 and named VP3 (Faaberg et al., 1995). In our opinion the nomenclature proposed here for the structural glycoproteins of LV may also be applied for proteins expressed by the corresponding ORFs of other arteriviruses, although their molecular masses may be different.

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