In silico and pepscan analysis of African swine fever virus p54 protein

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Abstract

Due to the great importance and economic impact of African swine fever, great emphasis is placed on the diagnosis of its causative agent and the development of new types of vaccines. However, a prerequisite for the shift in these areas is a more thorough understanding of the structure and characteristics of virus proteins. The aim of this work was to analyze the location of B-cell epitopes in the structure of the African swine fever virus p54 protein, combining the results of in silico and pepscan analysis. In silico analysis revealed the presence of potential B-cell epitopes primarily in the C-terminal part of the protein, pepscan technique confirmed these immunogenic regions between amino acids 24-61 and 151-182.

ASFV, B-cell epitopes, serology, peptides

African swine fever virus (ASFV) is a cytoplasmic, enveloped dsDNA virus belonging to the family *Asfarviridae*, genus *Asfivirus*. Virus genome consists of a linear dsDNA molecule with a length of approximately 170–193 kpb, containing 150–167 open reading frames (ORFs). It is the only nucleocytoplasmic DNA virus transmitted by insects. The genome of ASFV is very complex, which may play a role in the virus' ability to effectively regulate the host's immune response. The target host cells of ASFV are monocytes and macrophages (Alcami et al. 1990). The genome of the virus encodes 150–200 proteins, of which about 68 are structural proteins (Wang et al. 2021). Understanding the role of these viral proteins in the virus life cycle and their antigenic or immunogenic properties is necessary for the development of future vaccines (Galindo and Alonso 2017; Alonso et al. 2018). Although the role of some viral proteins in viral infection has been described, the exact mechanism of action in host cells at the molecular level is unknown (Arias et al. 2017).

Protein p54 is a membrane protein type II, found in the inner shell of the virion with the inner N-terminal 60 amino acids followed by a transmembrane domain and a 131 amino acid C-terminal ectodomain (Alcaraz et al. 1992; Rodriguez et al. 1994). The ectodomain is predicted to possess several glycosylation and phosphorylation sites (Sun et al. 1995; Mima et al. 2015) as well as both conserved and variable peptide regions (Rodriguez et al. 1994).

The p54 protein has a significant role in the adsorption of the virus to the receptors of the host cell and the subsequent replication of the virus. Interaction between p54 and host dynein protein allows the transport of virion to the perinuclear region of the host cell (Jia et al. 2017). The binding site of the p54 protein for dynein is also involved in the activation of caspase-3 and the induction of apoptosis (Alonso et al. 2001; Hernáez et al. 2010). Recently, epitope mapping of the protein was performed (Petrovan et al. 2020) demonstrating several p54 epitopes recognized by monoclonal antibodies as well as by ASFV positive swine sera.

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Due to the mentioned properties and the potential role of the p54 protein in the development of diagnostic tests or vaccines, the aim of our work was to localize B-cell epitopes in the structure of the p54 protein. Their position was first determined by in silico analysis of the p54 amino acid sequence and subsequently confirmed by pepscan analysis using ASFV positive swine sera.

Materials and Methods

In silico analysis

To predict the location of B-cell epitopes, the Bepipred Linear Epitope Prediction 2.0 algorithm (Jespersen et al. 2017) combined with algorithms for the prediction of antigenic regions (Kolaskar and Tongaonkar 1990), surface availability (Emini et al. 1985) and hydrophilicity (Parker et al. 1986) available at www.iedb.org was used. The analysis was performed on ORF E183L encoding p54 protein of ASFV (GenBank: EU874362.1).

Pepscan analysis

Synthetic peptides

For serological testing, the p54 protein (ORF E183L, GenBank: EU874362.1) was synthesized as six peptides covering its entire length. Peptides were prepared by chemical synthesis (Genecust, Boynes, France). The composition, length, and location in the p54 protein are shown in Table 1. Peptides were diluted to a concentration of 1 mg/ml in distilled water or dimethyl sulphoxide (Sigma-Aldrich, St. Louis, USA).

Table 1. Amino acid composition and location of synthetic peptides.

Peptide	Amino acid composition	Location in the p54 protein (aa residues)
p54-1	MDSEFFQPVYPRHYGECLSPTST	1–23
p54-2	PSFFSTHMCTILVAIVVLIIIIIVLIYLFSSRKKKAAA	24–61
p54-3	PAIEEEDIQFINPYQDQQWAGATPQPGTS	62–90
p54-4	KPAGATTGNVGKPITDRPVAMNRPVTNSSV	91–120
p54-5	ADRPVMNNPVTDRLIMATGGPAAASAPSDE	121–150
p54-6	LYTTATTQNTASQTMPAVEALRQRSTYTHKDLENSL	151–182

Serum samples

The testing of immunogenic properties of the synthesized peptides was performed on a panel of 10 ASFV positive and 10 negative pig sera. Positive sera (n = 5) were obtained from the EU reference laboratory for ASF (Valdeolmos, Spain), and field sera from wild boars (n = 5) were provided by the Veterinary Research Institute (Pulawy, Poland). The negative sera (n = 10) came from pig farms in the Czech Republic. The serological status of the sera was verified by commercially available test INGEZIM ASFV-R, 11.ASF.K.1 (Ingenasa, Madrid, Spain).

Pepscan analysis

Enzyme-linked immunosorbent assay (ELISA) plates were coated overnight with peptides dissolved in carbonate buffer, pH 9.6 at 2 µg/well (determined by checkerboard titration). After peptide coating, the wells were rinsed with phosphate buffered saline (PBS), then blocked with 2% bovine serum albumin (BSA; Sigma-Aldrich, St. Louis, USA) diluted in PBS, Tween 20 (T-PBS; Sigma-Aldrich) at room temperature for 60 min. Sera were diluted 1:50 in T-PBS with 2% BSA. One hundred microlitres of serum were added in peptide-coated wells and incubated for 60 min at room temperature. Subsequently, the plates were washed three times with T-PBS and 100 µl of the anti-pig IgG peroxidase conjugate (Sigma-Aldrich) diluted 1:30000 in T-PBS with 2% BSA were added into each well. The plates were incubated for 60 min at room temperature. After washing the plates, 100 µl per well of the TMB – complete substrate (TestLine Clinical Diagnostics s.r.o., Prague, Czech Republic) were added. The optical density (OD) was measured at 450 nm. Peptides were identified as positive if the absorbance was greater than twice the average of the absorbance of negative sera.

Results

In silico analysis

The result of prediction of B-cell epitopes by Bepipred Linear Epitope Prediction 2.0 is shown in Fig. 1 (Plate III). The areas with the highest probability of epitope presence

are located in the region encompassed by aa 50–180, with peaks in the positions of aa55, aa95, aa160 and aa175.

The result of surface accessibility prediction is shown in Fig. 2 (Plate III). The areas with the highest values of surface accessibility are represented as peaks in the positions of aa55, aa75, and aa170.

The result of antigenicity prediction is shown in Fig. 3 (Plate III). The area with the highest probability of antigenic sites is represented as peaks in the positions of aa117, aa122, and aa170.

The result of hydrophilicity prediction is shown in Fig. 4 (Plate IV). The area with the highest level of hydrophilicity is represented as peaks along the whole extracytoplasmatic domain of the protein.

Pepscan analysis

Results of pepscan analysis with ASFV positive and negative sera are shown in Fig. 5 (Plate IV).

Discussion

To achieve the goal of eliminating ASFV, novel antigens must be identified, and new vaccination strategies must be developed. Unfortunately, the most promising candidate vaccine did not confer significant protection against heterologous ASFV infection (Sánchez et al. 2019). Moreover, there are numerous unsolved problems related to the development of a new ASFV vaccine. To identify new antigens, we examined p54 ASFV protein with regard to the location of B-cell epitopes.

The ASFV p54 protein is considered a potential target of the host's immunological response to virus infection. A number of properties of the p54 protein have already been elucidated, including its structure (Rodriguez et al. 1996), variable regions (Rodriguez et al. 1994) and partially also immunologically significant regions (Petrovan et al. 2020). However, the use of this protein as a potential antigen for vaccines or as an antigen for serological tests requires sufficiently accurate identification of B-cell or T-cell epitopes in its structure. In this work, we attempted to identify B-cell epitopes by combining two approaches, namely in silico analysis followed by serological testing of synthetically prepared peptides covering the entire length of the p54 protein.

In silico analysis of the p54 protein allowed us to predict the localization of immunogenic regions based on the primary amino acid structure combined with the prediction of the localization of antigenic regions, surface accessible and hydrophilic regions. A similar approach was used to detect immunogenic peptides in the case of other virus or bacterial proteins (Nas 2020; Khanum et al. 2022). In our work, the position of immunologically reactive epitopes was predicted to the region encompassed by aa 50-60 and aa 170-180, in which majority of all analysed indices were significantly above the threshold. Serological testing using synthetic peptides as antigens confirmed antigenically significant regions in peptides 2 (aa 24-61) and 6 (aa151-182). Both peptides reacted with all ASFV positive sera.

The reactivity in the area of the peptide 6 was not surprising, given that it is a part of the protein that is exposed to the external environment and therefore exposed to the activities of the immune system. In silico analysis of this region also confirmed the presence of immunoreactive epitopes, especially in the prediction of B-cell epitopes, surface availability and hydrophilicity.

However, our work identified another, unexpected immunogenic region of the protein bounded within amino acids 24-61 (peptide 2). This region is located in the transmembrane domain of the protein, which is usually not exposed to the external environment, and therefore was probably not included in previous studies. However, in silico analysis in our

work identified a very short region in the range of amino acids 50–60 with a significant combination of potential presence of B cell epitopes, surface availability, antigenicity and hydrophilicity. This prediction was also confirmed by the reactivity of peptide 2, which includes this region, with all positive sera. Although the transmembrane region of proteins is usually hidden from the immune system, in the case of the p54 protein, a small part of it, approximately 10 amino acids in length, is likely accessible extracellularly.

Interestingly, the region defined by peptide 6 (aa151–182) was not found to be antigenic in the work of Petrovan et al. (2020), where epitopes reacting with monoclonal antibodies raised against the recombinant protein were in the areas of aa 65–75, 93–113, and 118-127.

In our work, we identified the presence of two B-cell epitopes in the ASFV p54 protein structure, which may be utilized in the development of new diagnostic tests, or in the development of vaccines.

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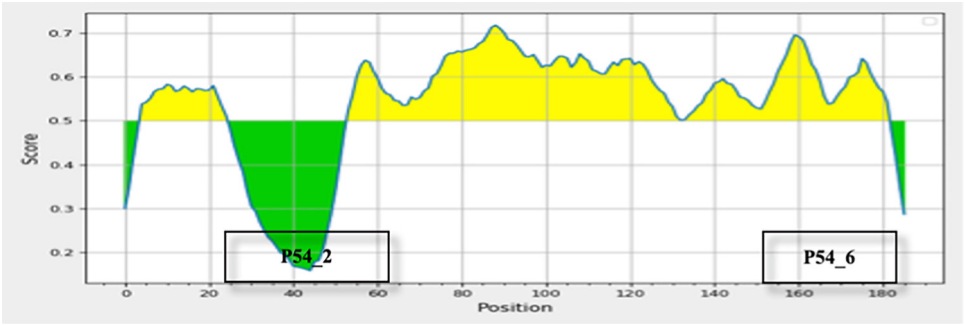

Fig. 1. Prediction of B-cell epitopes based on algorithm: Bepipred Linear Epitope Prediction 2.0. Yellow areas represent amino acid positions above the threshold (X axis) indicating areas with possibility of B-cell epitopes presence. The area of the protein covered by peptides 2 and 6 is shown as boxes along the X axis.

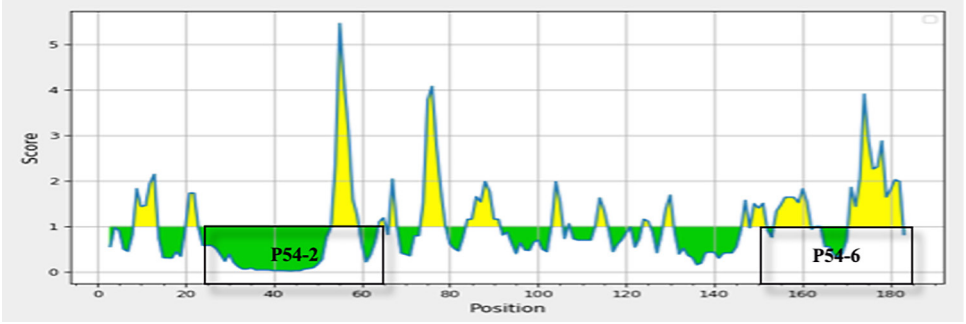

Fig. 2. Prediction of p54 protein surface accessibility. Yellow peaks above the threshold (X axis) indicate areas with surface accessibility. The area of the protein covered by peptides 2 and 6 is shown as boxes along the X axis.

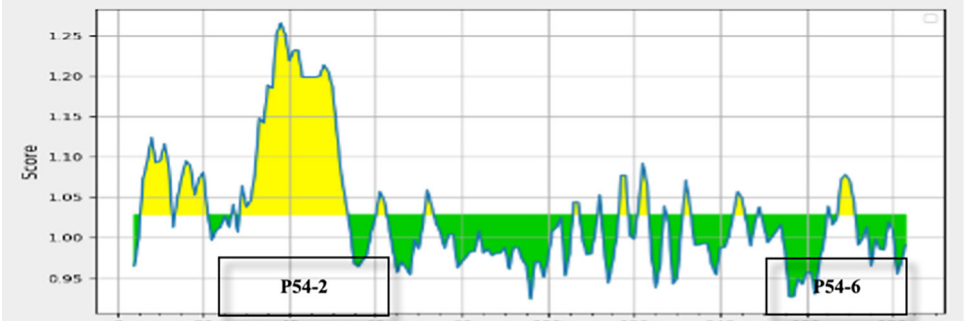

Fig. 3. Prediction of p54 protein antigenicity. Yellow peaks above the threshold (X axis) indicate possible antigenic regions. The area of the protein covered by peptides 2 and 6 is shown as boxes along the X axis.

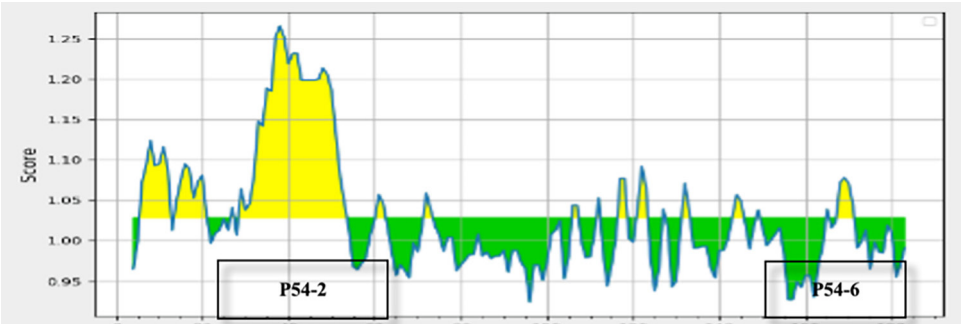

Fig. 4. Prediction of p54 protein hydrophilicity. Yellow areas represent amino acid positions above the threshold (X axis) indicate areas with possible presence of antigenic regions. The area of the protein covered by peptides 2 and 6 is shown as boxes along the X axis.

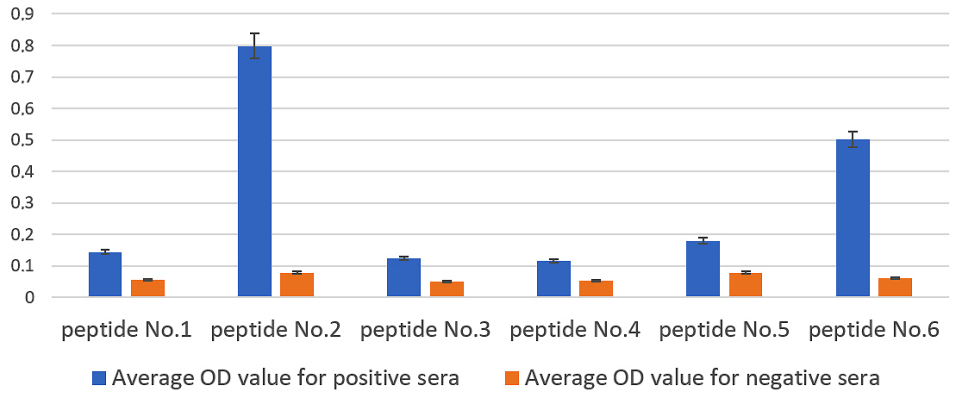

Fig. 5. Optical density values of African swine fever virus positive and negative swine sera with synthetic peptides as antigens. The error bars represent value of the standard deviation.