Preliminary screening of dominant epitopes of truncated p72 protein of African swine fever virus

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Abstract: African swine fever (ASF) causes huge economic losses in the absence of vaccines with protective efficacy. Diagnostic tools are critical for the early detection, prevention, and control of African swine fever virus (ASFV). The protein p72 is a good serological target for conducting ASF detection and surveillance. This study aimed to adopt a multiparameter analytic method to predict the dominant epitopes for p72 protein of ASFV based on a comprehensive analysis of the secondary structure, hydrophilicity, surface accessibility, and effects of the index of proteins using the software. The results showed that the area of the dominant epitope of protein p72 contained the N-terminal residues aa137–286. This study laid the foundation for developing vaccines and immunodiagnostic techniques.

1. Introduction
The African swine fever (ASF) caused by the African swine fever virus (ASFV) is an acute and hyperacute infectious disease of domestic pigs and wild boars with various clinical signs such as systemic hemorrhagic, respiratory disorders, and neurological symptoms. The morbidity and mortality rates of ASF acute forms can reach 95%–100%. ASFV is a large, enveloped, and double-stranded DNA virus that is also the only member of the family Asfarviridae, genus Asfivirus. The genomes of different isolates vary in length from ~170 to 190 kbp and encoded between 151 and 167 open reading frames that contained a conserved central region of ~125 kb and two variable ends. Early studies showed that the ASFV had a complex structure with multiple membrane and protein layers. The major components of the viral capsid are the protein p72, the two structural proteins p30(p32) and p54, and the polyprotein. The major capsid protein p72 is a very conservative antigenic protein that constitutes ~32% of the total mass of the virion. Therefore, p72 has been used as a target gene in enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR) assay, nested PCR assay, hot-start multiplex PCR, and real-time PCR. This study aimed to adopt the bioinformatics method to analyze the dominant epitopes of ASFV protein p72. The findings of this study would provide assistance for further research on protein p72 and promote the development of ASFV vaccine and detection technology.

2. Material and methods
2.1. Sequence of truncated p72 protein of ASFV
The sequence of ASFV p72 was AAN86724 obtained from the protein data bank of NCBI (National Center for Biotechnology Information), which was 396 amino acids in length (246 nucleotides downstream of the ATG start site in the p72 gene).

2.2. Analysis of the secondary structure of truncated p72 protein
Protean software of DNA package was used in this study to analyze the α-helix, β-sheet, corner region, hydrophilicity, surface accessibility, antigen index (AI), and so on. In the Protean software of DNA package, the prediction of surface accessibility, plasticity, and antigen possibility are the most referenced [1].

2.3. Analysis of antigen epitopes of truncated p72 protein
The average AI of dominant regions was calculated using the Wu Yuzhang's method, which could predict the antigen epitope of p72. The potential epitopes of p72 were predicted according to the comprehensive consideration of the aforementioned parameters. First, the predicted epitopes should avoid as much as possible the α-helix, β-sheet, and corner regions, which are prone to be present on the inside of the protein and are less likely to form antigenic epitopes. Second, the antigenicity index of predicted epitopes should be ≥0, the hydrophilicity index of predicted epitopes should be ≥0, and the surface accessibility index of predicted epitopes should be ≥1. According to the predicted epitope analysis, the dominant regions of p72 protein were determined, which were the regions with relatively high immunogenicity and suitable for use as a diagnostic test antigen [2].

3. Results and analysis

3.1. Secondary structure of truncated p72 protein
According to the analysis of the secondary structure of truncated p72 (tP72) protein shown in Figure 1, the distribution regions of the α-helix, β-sheet, and flexible region were determined using the software. The α-helix of the tP72 secondary structure was located at 6–11, 16–26, 49–66, 99–105, 107–113, 125–128, 133–136, 326–339, and 361–370. The β-sheet of the tP72 secondary structure was shown in 67–70, 90–94, 152–158, 163–165, 175–181, 190–192, 208–215, 226–235, 293–299, 304–311, 344–350, and 354–360. The flexible region of the tP72 secondary structure was predicted at 13–18, 24–55, 78–89, 95–98, 116–126, 133–141, 144–151, 159–171, 183–187, 196–210, 217–224, 229–241, 250–268, 270–275, 280–285, 287–301, 315–326, 361–367, and 377–390 (Table 1). The hydrophilicity, surface accessibility, and antigenic index of tP72 protein were predicted using the software and are shown in Table 2.

![Figure 1. Secondary structure of tP72 protein predicted using the Protean software of DNA package.](image-url)
3.2. Region and amino acid sequence of dominant antigen epitope of the tP72 protein

The dominant antigen epitope regions of the tP72 protein were finally obtained by integrating the data in the regions shown in Tables 1 and 2, superimposing their flexible regions, hydrophilicity, surface accessibility, and antigenic index (hydrophilicity ≥0, surface accessibility ≥1, and antigenic index ≥0), and deducting the α-helix and β-sheet regions. The analysis results are listed in Table 3. The numbers of predicted antigen epitopes and protein antigen's sequences were different when using different prediction methods. The aforementioned analysis showed that the dominant epitope of the ASFV tP72 protein was mainly concentrated between the 137th and 286th amino acids. The results provided a foundation for further study and development of detection strategies.

Table 1. Distribution of α-helix, β-sheet, and flexible regions of tP72 protein.

| α-Helix  | β-Sheet  | Flexible regions |
|----------|----------|-----------------|
| 6 - 11   | 67 - 70  | 13 - 18         |
| 49 - 66  | 152 - 163| 95 - 98         |
| 107 - 113| 175 - 190| 144 - 151       |
| 133 - 136| 208 - 226| 196 - 210       |
| 361 - 370| 291 - 304| 250 - 268       |

Table 2. Hydrophilicity, surface accessibility, and antigenic index for tP72 protein.

| Hydrophilicity ≥0 | Surface accessibility ≥1 | Antigenicity index ≥0 |
|-------------------|--------------------------|-----------------------|
| 13 - 17           | 12 - 18                  | 11 - 18               |
| 62 - 67           | 85 - 88                  | 136 - 151             |
| 115 - 123         | 182 - 189                | 215 - 224             |
| 181 - 206         | 234 - 239                | 279 - 295             |
| 246 - 298         | 289 - 294                | 383 - 396             |
| 362 - 379         | 383 - 396                |                       |

Table 3. Regions and sequences of antigenic epitope of tP72 protein.

| No. | Epitope position (AA) | Amino acid sequence of antigen epitope | Number of amino acids |
|-----|-----------------------|---------------------------------------|-----------------------|
| 1   | 13 - 17               | DGKAD                                 | 5                     |
| 2   | 34 - 48               | KNVKNKSYGKPDEPT                       | 15                    |
| 3   | 74 - 81               | YNKVRPH                                 | 8                     |
| 4   | 137 - 150             | FPRNGYDWDNQTPL                        | 14                    |
| 5   | 168 - 173             | GTKNAY                                | 6                     |
| 6   | 182 - 188             | YPGERLY                                | 7                     |
| 7   | 195 - 206             | VNGNLDEYSSD                           | 11                    |
| 8   | 216 - 224             | IPGDKMTGY                             | 9                     |
| 9   | 247 - 276             | DLHKPHPQSKPILTDEYTQRTCSHT             | 21                    |
| 10  | 280 - 286             | NPKF                                  | 7                     |
| 11  | 315 - 322             | FPENSHN                               | 8                     |
| 12  | 383 - 392             | NGPQTPKY                              | 10                    |
|     |                       | PGRPSRRNIR                            |                       |
4. Discussion
As a chemical group with special stereoscopic configuration and immune activity, antigen epitope (epitope) determines the ability of antigen–antibody interaction, which stimulates the body to produce antibodies or sensitized lymphocytes. Currently, the methods for screening antigen epitope mainly focus on B-cell epitopes, especially the linear B-cell epitopes\(^3\). Protein secondary structure has a fundamental influence on the antigen epitopes. The \(\alpha\)-helix and \(\beta\)-sheet chemical bonds play an important role in maintaining the advanced structure of proteins and are located in the interior of proteins so that it is difficult for proteins to chimerize with antibodies. Therefore, antigenic epitopes are not located in \(\alpha\)-helix and \(\beta\)-sheet regions. The corner of the protein and the flexible structure of the random curl are relatively loose and easy to twist, hover, and display on the surface of the protein. However, the structure is prominent and beneficial to chimerism with antibodies, so the corner of the protein and the flexible structure are more likely to become antigen epitopes\(^4\).

P72 proteins play an important role in virus binding and uptake into recipient cells, and antibodies against P72 protein can prevent the replication of the virus by stopping viral attachment. Previous studies indicated that the expression of P72 protein in the eukaryotic and prokaryotic expression systems could be used as the detection antigen for differential diagnosis. Currently, the common methods for detecting ASFV antigens are direct immunofluorescence and viral hemadsorption (HAD) method. The HAD method is a highly sensitive method for detecting ASFV, but the presence of nonhematocyte adsorption strain leads to a false-negative result. Indirect immunofluorescence (IIF) and ELISA are the commonly used methods for detecting antibodies. The IIF method is highly sensitive and fast, but it is difficult to automate and detect in large quantities. The ELISA method was first established with good sensitivity and specificity. Carmina Gallardo et al. established an ELISA method for detecting ASF with a variety of fusion proteins. The method was highly sensitive and specific in detecting antibodies to ASF in domestic and wart swine in Europe and West Africa\(^5,6\). At present, ELISA is the standard method for detecting ASFV in international trade\(^7\). OIE uses ELISA as the preferred serological method for the diagnosis of ASF, which can detect serum and tissue fluid\(^8\).

In terms of molecular biology diagnosis, the PCR technology is rapid, sensitive, and specific in detecting ASFV; It also has good biological safety and no risk of dispersal, thus achieving early diagnosis. In this study, the secondary structure of the ASF p72 protein was analyzed. First, the secondary structure of the p72 protein was predicted by the Chou–Fasman method, and then the B-cell antigen epitope of the P72 protein was predicted using the Emini surface accessibility scheme, Kyte–Doolittle hydrophilicity scheme, and Jameson–Wolf antigenic index scheme. The results showed that the main antigenic region of the P72 protein was concentrated in some regions of the N-terminal part of the p72 gene, and the P72 of ASFV antigen epitopes were screened out in the 137aa–286aa region, which was a strong antigenic region of ASFV. The predicted results were confirmed by a relevant experiment. Monoclonal antibodies were successfully prepared against a recombinant antigenic fragment, from amino acid (aa) 165–171 and 265–280, expressed in baculovirus\(^9\). The anti-p72 reactive monoclonal antibodies reacted with p72 in amino acids 180 and 250\(^10\). This predicted epitope region provided a new method for detecting the ASFV antibody, which would help in developing ASFV vaccines and diagnostic techniques.

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