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Identification of a mimotope of an infectious bronchitis virus S1 protein

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ABSTRACT

The S1 protein of the infectious bronchitis virus (IBV) is a major structural protein that induces the production of the virus-neutralization antibodies. The monoclonal antibody against the IBV M41 S1 protein was used as a target for biopanning. After three rounds of biopanning, randomly selected phages bound to the monoclonal antibody. Sequence analysis showed that the dominant sequence was SFYDFEMQGFFI. Indirect competitive enzyme-linked immunosorbent assay showed that SFYDFEMQGFFI is a mimotope of the S1 protein that was predicted by PepSurf. The mimotope may provide information for further structural and functional analyses of the S1 protein.

Keywords: IBV; S1 protein; mimotope

INTRODUCTION

The infectious bronchitis virus (IBV) is a serious and highly infectious pathogen that causes severe economic losses in the poultry industry. The IBV is an enveloped, single-stranded RNA(+) virus belonging to the order *Nidovirales*, family *Coronaviridae* and genus *Gammacoronavirus* [1,2]. This virus has multiple serotypes and genotypes; cross-protection between different serotypes is limited [3]. Therefore, it often leads to immune failure, making it extremely difficult to control the disease [4].

The S protein, the major surface protein of the IBV, is cleaved into the S1 subunit and S2 subunit by the host serine protease furin. The S1 protein determines the antigenicity and tissue tropism of the virus [5] and plays a vital role in the induction of neutralizing antibodies and attachment to the host cell receptors [6].

As an important structural protein, determining the epitope of S1 protein will help better understand its structure and function. The S1 protein contains the neutralizing epitopes for the IBV, but most of the neutralizing epitopes are conformational epitopes [7]. Traditional methods for determining epitopes have been used to determine linear epitopes. On the other hand, phage display technology was used to map both the linear and conformational epitopes. A mimotope displayed by phage could mimic the conformational epitope [8]. The mimotope recognized by the monoclonal antibody against the IBV M41 S1 protein used in

Conflict of Interest

The authors declare no conflicts of interest.

Author Contributions

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the study was biopanned and identified. In addition, the position of the mimotope in the S1 protein was predicted by bioinformatics.

MATERIALS AND METHODS

Phage-display peptide library and monoclonal antibody

The Ph.D.-12 Phage Display Peptide Library Kit was purchased from New England Biolabs Company. The S1 protein was expressed in prokaryotic cells. The monoclonal antibody named 3D9 was prepared by hybridoma cell technology and confirmed by IPMA to recognize the IBV M41 strain.

Biopanning the phages from the Ph.D.-12 phage display peptide library

A standard biopanning procedure was carried out according to the manufacturer's instructions. Briefly, one well of a 96-well microtiter plate was coated with 100 μ L of mAb 3D9 with a final concentration of 100 μ g/mL. After blocking, 100 μ L of the phages (about 2×10^{11} pfu/mL) from the Ph.D.-12 phage library was added to the wells and incubated for 1 h at room temperature. The unbound phages were removed, and the wells were washed with 0.1% TBST. An elution buffer was added to each well with gentle shaking for 1 h at room temperature. The eluent containing the phages was mixed with a neutralization buffer to determine the titer of the phages and for amplification. The coated mAb 3D9 concentration (100 μ g/mL, 75 μ g/mL, and 50 μ g/mL) was reduced and the concentration of Tween-20 in TBST buffer (0.1%, 0.2%, and 0.3%) was increased according to the number of biopanning steps.

Identification of positive phages

After three rounds of biopanning, fifteen phage clones were selected randomly, amplified, and purified. Ninety-six-well microplates were coated with 10 μ g/mL of mAb 3D9 and incubated overnight at 4°C. After blocking, a 1:100 dilution of the purified phages or the Ph.D.-12 phage library (as a negative control) was added to each well and incubated for 1 h at 37°C. The wells were washed 5 times with TBST and incubated with horse-radish peroxidase (HRP)-conjugated anti-M13 mAb diluted 1:5,000 in TBST for 1 h at 37°C. After washing 7 times in TBST, 3, 3', 5, 5'-tetramethylbenzidine (TMB) was used as a substrate, and the reaction was ended by adding 2 mol/L H₂SO₄. The absorbance at 450 nm was measured using a Bio-Rad Microplate Reader. The positive phage clones were sent to GENEWIZ Inc. for sequencing using the -96 gIII sequencing primer 5'-CCC TCA TAG TTA GCG TAA CG-3'.

Identification of the positive phage by indirect competition ELISA

Indirect competitive ELISA was used to confirm whether the mimotope could effectively mimic the epitope of the S1 protein. The wells were coated with the S1 protein and incubated overnight at 4°C. After blocking, the phage or the Ph.D.-12 phage library (as a negative control) was diluted serially and added to the wells together with the mAb 3D9 at 37°C for 1 h. The well with only mAb 3D9 was used as the positive control. After washing five times in PBST, the wells were incubated with the HRP-goat anti-mouse antibody diluted 1:5,000 for 1 h at 37°C. The wells were washed with PBST. TMB was used as a substrate for HRP and the reaction was quenched by the addition of 2 M H₂SO₄. The absorbance of each well was read at 450 nm.

Prediction of the mimotope by PepSurf

PepSurf (<http://pepitope.tau.ac.il/index.html>) is a web tool for epitope mapping based on the peptides extracted from a phage display library and can map the peptides displayed on the phages to the surface of the protein.

The structure of the IBV S1 protein (PDB ID: 6CV0) was derived from the Protein Data Bank, and the mimotope was entered into the web. BLOSUM62 is the default matrix. The Gap penalty chooses the -0.5. NNK library type and UAG stop codon modification was selected.

RESULTS

Biopanning and enrichment of phage

For each round of biopanning, the titer of the phages was measured, and the recovery rate was calculated (Recovery rate = Output phages/input phages). As the coating concentration decreased and the concentration of Tween-20 in TBST buffer increased, the output of the phages that bind specifically to mAb 3D9 increased, confirming that the specific phages were effectively enriched (**Table 1**).

Identification of phage clones and sequence analysis

After the third round of biopanning, 15 phage clones were selected randomly to assess their reactivity with mAb 3D9 by indirect ELISA. The Ph.D.-12 phage library was used as a negative control (NC). The results suggested that 15 phage clones could react with mAb 3D9, and these phages were considered as positive phages ($OD_{450(\text{phages})}/OD_{450(\text{NC})} > 2.1$) (**Fig. 1A**). Eleven phages from the fifteen positive phage clones were sequenced successfully. The dominant sequence of the peptide sequences displayed by the phages was SFYDFEMQGFFI (**Table 2**).

Identification of positive phage by indirect competition ELISA

Phage P9 was selected for indirect competitive ELISA because P7, P9, P12, and P14 displayed the same peptide sequences as the dominant sequences. The inhibition rate also decreased gradually with decreasing phage concentration. The inhibition rate of phage P9 could reach 65% when the concentration of phage P9 was 10^{10} pfu/mL, indicating that the peptide SFYDFEMQGFFI displayed by phage P9 can mimic the epitope of the S1 protein (**Fig. 1B**).

Prediction of the mimotope

The 3D structure of IBV S1 protein was derived from the Protein Data Bank (**Fig. 1C**). The mimotope was predicted to be a conformational epitope candidate using PepSurf and is shown as red balls. Each amino acid of the mimotope corresponded to the S1 protein sites. The predicted positions of the mimotope were 313-316aa, 325-326aa, 354aa, 363-364aa, 375aa, 377aa, and 394aa exposed on the surface of the S1 protein (**Fig. 1D**).

Table 1. Enrichment of a specific phage by biopanning

Round	Coating mAb ($\mu\text{g/mL}$)	Tween-20 in TBST (%)	Input phage (pfu)	Output phage (pfu)	Recovery rate
1	100	0.1	2×10^{11}	5×10^4	2.5×10^{-7}
2	75	0.2	2×10^{11}	1.5×10^6	7.5×10^{-6}
3	50	0.3	2×10^{11}	9×10^7	4.5×10^{-4}

pfu, plaque-forming unit.

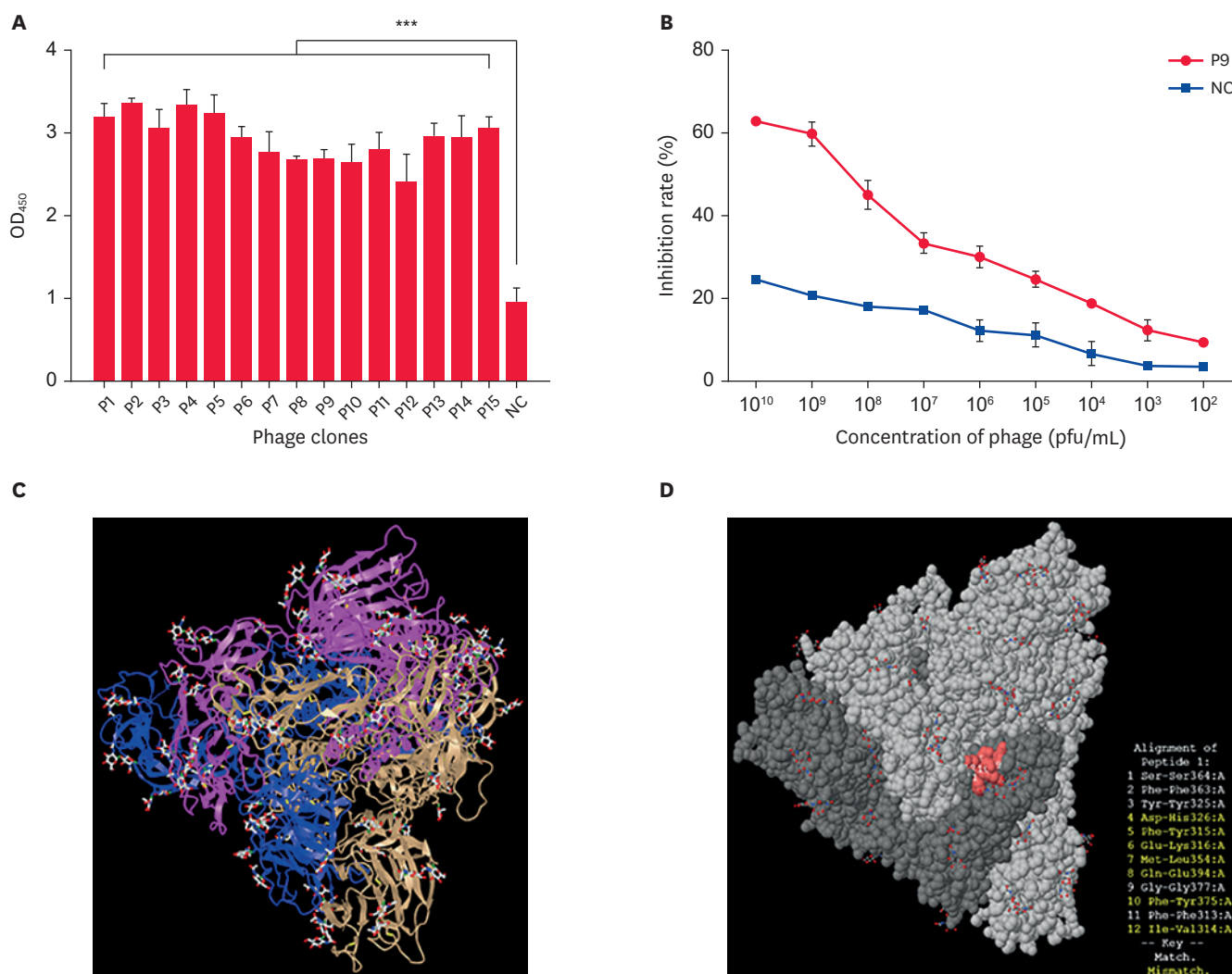


Fig. 1. (A) Identification of the selected phage clones for binding to mAb 3D9 by ELISA. Columns P1–P15 represent 15 phage clones, whereas the NC column means the negative control. (B) Phage P9 inhibits mAb 3D9 binding to the S1 protein. Indirect competition ELISA was conducted using the phage P9 as the competitor for the S1 protein. The inhibition rate of phage P9 decreased with decreasing concentration. (C) The 3D structure of IBV S1 protein. (D) The mimotope mapped to the IBV S1 protein. Statistically significant differences are indicated by $***p < 0.01$. ELISA, enzyme-linked immunosorbent assay.

Table 2. Peptide sequences of the positive phage clones

Phage clones	Sequences
P1, P3, P5, P10	S F Y D - E M - G F - I
P2, P4, P8	S - Y - F - M Q - F F -
P7, P9, P12, P14	S F Y D F E M Q G F F I
Dominant sequences	S F Y D F E M Q G F F I

DISCUSSION

The S1 protein is an important antigen that causes an organism to produce neutralizing antibodies and hemagglutination-inhibiting antibodies [9]. Identification of the conformational epitopes is meaningful for a study of the S1 protein. On the other hand, it is difficult to determine the conformational epitopes. The mimotope biopanned by phage display technology can effectively mimic the conformational epitope [10].

In the present study, phages capable of binding to the mAb 3D9 were enriched. The short peptides relevant to the S1 protein sequence in homology were not isolated by DNA sequencing and sequence homology analysis. Many group researchers have drawn similar conclusions using an antibody as a tool to biopan the mimotopes. An analysis of the sequencing results revealed a dominant sequence among the sequences displayed by the eleven phages.

The DNA vaccine containing the S1 protein (located at 24 to 150 aa and 290 to 400 aa) has the effect of protecting chickens against an IBV challenge [11]. Tan et al. [12] reported that a multi-epitope vaccine containing neutralizing epitope domains (located at 24 to 61aa, 132 to 149aa and 291 to 398aa) could induce a high level of immune responses and full protection of chickens against a lethal IBV challenge. In the present study, the dominant sequence displayed by the phage can inhibit the binding of the mAb 3D9 to the S1 protein. In other words, the dominant sequence can mimic the structure of IBV M41 S1 and can replace IBV M41 S1 antigenic to interact with mAb 3D9. The mimotope was mapped to the S1 protein using bioinformatics methods, which is more conducive to the precise positioning of the epitope. The predicted position is exposed to the surface of the antigen and can be bound by the antibody. A previous study reported a receptor-binding domain at the C-terminus (269-414aa) of the S1 protein, and the predicted amino acid sites of the mimotope were all located in the region [13].

In conclusion, a mimotope of the S1 protein was biopanned, identified, and analyzed, which might provide new insights for a diagnosis of the IBV and may be conducive to further understanding the structure of the S1 protein.

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