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Identification of Novel Linear Epitopes Located in the Infectious Bronchitis Virus Spike S2 Region

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SUMMARY. We identified novel linear epitopes on the infectious bronchitis virus (IBV) spike S2 region. The conformational structure of the IBV spike protein was predicted from a homologous protein, human coronavirus NL63 spike. Although the obtained structure was incomplete, most of the IBV spike protein structure was predicted; the N-terminus of the S1 region could not be predicted due to its variability. In the model, the region located in the proximity of the fusion peptide appeared to be well conserved, and we evaluated the antigenicity of these domains, which are involved in the membrane fusion machinery. Western blotting revealed that IBV TM86 spike residues 686–723 were antigenic. Epitope mapping analysis using synthesized peptides revealed that IBV TM86 spike 669–685 (SNFSTGAFNISLLLTPP), 686–697 (SNPRGRSFIEDL), and 692–703 (SFIEDLLFTSVE) residues were major linear epitopes; two identified epitopes (686–697 and 692–703) were covered by the fusion peptide, and the other epitope (669–685) was adjacent to the fusion peptide. Although the identified epitopes are identically located as the neutralizing epitope in severe acute respiratory syndrome coronavirus, the recombinant protein that includes those epitopes could not elicit neutralizing antibodies against IBV. This is the first report describing IBV spike S2 epitopes located in the proximity of the fusion peptide, and it is suggested that the spike fusion machinery of IBV may differ from that of severe acute respiratory syndrome coronavirus, or, alternatively, IBV may have another mechanism to penetrate the cell membrane.

RESUMEN. Identificación de nuevos epítopes lineales localizados en la región S2 de la espícula del virus de la bronquitis infecciosa.

Se identificaron unos epítopes lineales nuevos en la región S2 de la espícula del virus de la bronquitis infecciosa (IBV). La estructura conformacional de la proteína de la espícula del virus de la bronquitis se predijo a partir de una proteína homóloga, la espícula NL63 del coronavirus humano. Aunque la estructura obtenida resultó incompleta, se predijo la mayor parte de la estructura de la proteína de la espícula del virus de la bronquitis; el extremo N de la región S1 no pudo predecirse debido a su variabilidad. En el modelo, la región situada en las proximidades del péptido de fusión parece estar bien conservada, y se evaluó la antigenicidad de estos dominios, que están implicados en la maquinaria de fusión de la membrana. Mediante la prueba de inmunotransferencia se reveló que los residuos 686-723 de la espícula del virus de bronquitis TM86 eran antigénicos. El análisis de mapeo de epítopes usando péptidos sintéticos reveló que los residuos 669-685 (SNFSTGAFNISLLLTPP), 686-697 (SNPRGRSFIEDL) y 692-703 (SFIEDLLFTSVE) del virus de bronquitis TM86 eran epítopes lineales importantes; dos epítopes identificados (686-697 y 692-703) estuvieron cubiertos por el péptido de fusión, y el otro epítope (669-685) era adyacente al péptido de fusión. Aunque los epítopes identificados están ubicados idénticamente como el epítope neutralizante en el coronavirus que induce el síndrome respiratorio agudo severo, la proteína recombinante que incluye esos epítopes no pudo inducir anticuerpos neutralizantes contra el virus de la bronquitis infecciosa. Este es el primer informe que describe los epítopes S2 de la espícula del virus de la bronquitis infecciosa localizados en las proximidades del péptido de fusión y se sugiere que la maquinaria de fusión de las espículas del virus de la bronquitis infecciosa puede diferir del coronavirus del síndrome respiratorio agudo severo o, alternativamente, el virus de la bronquitis infecciosa puede tener otro mecanismo para penetrar la membrana celular.

Key words: coronavirus, epitope, fusion peptide, infectious bronchitis virus, S2, vaccine

Abbreviations: GST = glutathione S-transferase; IBV = infectious bronchitis virus; PBS = phosphate-buffered saline; RT = room temperature; SARS-CoV = severe acute respiratory syndrome coronavirus; SDS-PAGE = sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SPF = specific-pathogen-free; VN = virus neutralization

Infectious bronchitis virus (IBV) belongs to the order *Nidovirales*, family *Coronaviridae*, genus *Gammacoronavirus*, and causes respiratory disease and pathology in the kidneys and gonads of chickens (5,6). IBV is an economically important disease in the poultry industry, and several hygiene management practices have been used to prevent the spread of IBV. IBV has extensive antigenic variation, reflecting mutations in the spike protein gene (8,9,31). Vaccines targeting individual IBV serotypes yield poor cross-protection; therefore, various attenuated and inactivated multivalent vaccines (derived from several different serotypes) are used (12,26). The spike protein is an envelope glycoprotein that forms a trimer and has been shown to play an important role in viral infection (7,14,33). The spike protein is highly glycosylated, and based on its amino acid sequence, is predicted to contain 21 to 35 N-glycosylation sites. The spike protein has two main functions: to attach the virus to the host cell receptor and to activate the fusion of the virion membrane with the host cell membrane (10,33). The spike protein is the major antigenic determinant in inducing neutralizing antibodies against IBV, and the N-terminal S1 region is especially important (7,14,16,19,24). The S1 domain forms the bulbous head of the spike protein, and several virus neutralization (VN) epitopes have been reported to reside within the first and third quarter of the S1 sequence (8,16,19,26). Thus, analysis of the antigenicity of the S1 domain is critical to the development of effective anti-IBV vaccines. On the other hand, the C-terminal S2 region is anchored to the membrane and plays an important role in the membrane fusion step

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Table 1. Primers used in this study.

Primer	Sequence[A]
TM 686 forward	aaaa<u>ACTAGT</u>AGTAATCCTAGAGGGCGTTC
TM 724 forward	aaaa<u>ACTAGT</u>CTTAAAGATCTTATCTGTGCTAGGG
TM 669 forward	aaaa<u>ACTAGT</u>AGTAATTTTAGTACTGGTGC
TM 762 reverse	aaaa<u>CTCGAG</u>**CTA**ACCAAAAGCCATAGCACCTAC
TM 723 reverse	aaaa<u>CTCGAG</u>**CTA**AGTGCCCAAAGGTCCCGCTG
TM 703 reverse	aaaa<u>CTCGAG</u>**CTA**TTCAACACTTGTAAATAACAAATC

[A]Underlining indicates restriction enzyme recognition sequences; bold triplets indicate stop codons.

(4,25). In contrast to the S1 domain, further discussion of the S2 domain is needed; the S2 domain has been reported to have no neutralizing epitope(s) (7) or to have linear neutralizing epitope(s) (20,34). Although some researchers have reported epitopes located in the IBV S2 region and developed recombinant vaccines (15,27,34), information about IBV S2 epitopes is still limited. In other coronaviruses, however, it has been reported that S2 epitopes induce neutralizing activity, and the S2 region is an important target to establish effective vaccines (11,28,30,36,38). Given this background, it is worthwhile to analyze IBV S2 epitopes.

Recent studies have revealed conformational information of coronavirus spike proteins (13,18,28,29,30,35). Although the structure of the IBV spike has not been elucidated, it can be predicted by homology modeling. In the present study, we identified novel linear epitopes located in the IBV spike S2 region by using protein structure modeling and epitope mapping.

MATERIALS AND METHODS

Virus. The IBV TM86 strain, an isolate of the S1 genotype JP-II that was originally recovered from a chicken in Japan, was used in this study; this strain has been used as a vaccine strain (2,23). The virus was propagated in specific-pathogen-free (SPF) chicken embryonated eggs. The SPF chickens (layer-type) used for this study were maintained at our institute.

Construction of the expression plasmid. Partial sequences of the IBV spike gene were amplified from cDNA of the IBV strain TM86, which had been previously cloned (1). PrimeSTAR Max DNA Polymerase (Takara, Shiga, Japan) was used for the PCR. The reaction was performed as follows: 94 C for 1 min followed by 30 cycles at 98 C for 10 sec, 55 C for 5 sec, and 72 C for 30 sec. PCR products were digested by the restriction enzymes *Spe*I and *Xho*I, followed by insertion into the expression plasmid pET41a. Constructed plasmids were designated as pET41a/IBV/S/686-762, pET41a/IBV/S/686-723, pET41a/IBV/S/724-762, and pET41a/IBV/S/669-703. PCR primers used in this study are listed in Table 1.

Peptide synthesis. Eight peptides were synthesized by Genscript Biotech (Tokyo, Japan). Synthesized peptides were dissolved in dimethyl sulfoxide at 10 mg/ml final concentration and kept at −80 C until used. Synthesized peptides used in this study are listed in Table 2.

Expression of recombinant protein. Various spike fragments were expressed as fusion proteins with glutathione S-transferase (GST). *Escherichia coli* BL21 (DE3) containing the recombinant plasmid was inoculated into circle-grow medium (MP Biomedicals, Tokyo, Japan) containing 50 µg/ml kanamycin and incubated 3–4 hr at 37 C, and then isopropyl b-D-1-thiogalactopyranoside was added at the 10 µM final concentration. After induction, cells were incubated overnight at 30 C, followed by harvesting, resuspension in phosphate-buffered saline (PBS), and disruption by sonication. After sonication, inclusion bodies were subjected to immunoblot analysis.

For the immunization test, the recombinant protein was purified from the soluble supernatant by using Glutathione Sepharose 4B (GE Healthcare, Tokyo, Japan). The purified protein was mixed with oil adjuvant to make a recombinant protein suspension at a concentration of 50 µg/dose. Recombinant protein, light liquid paraffin, sorbitan monooleate, and polysorbate 80 in a volume ratio of 9:36:4:1 was used as oil adjuvant (1).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and western blotting. The recombinant protein sample was mixed with 2× sample buffer (100 mM Tris-HCl [pH 6.8], 4% SDS, 20% glycerol, and 0.2% bromophenol blue) containing 200 mM dithiothreitol and boiled for 5 min at 95 C. The protein was separated by a 5%–20% polyacrylamide gradient gel (e-PAGEL; ATTO, Tokyo, Japan) and transferred to a polyvinylidene difluoride membrane (Millipore, Tokyo, Japan). The membrane was incubated in 5% skim milk (Wako, Osaka, Japan) in T-PBS buffer (PBS [pH 7.2] containing 0.05% Tween 20) for 1 hr at 37 C, and then incubated with a monoclonal antibody against the GST protein or anti-IBV immunoserum in 5% skim milk in T-PBS buffer. Next, the membrane was incubated with a peroxidase-conjugated second antibody, goat anti-mouse IgG (H+L) (Jackson, PA, USA) or donkey anti-chicken IgY (H+L) (Jackson). The reacted protein was visualized using a TMB substrate kit (Invitrogen, Tokyo, Japan).

ELISA. For the epitope mapping experiment, synthesized peptides were diluted at concentrations of 100 to 0.001 µg/ml in PBS, and 50 µl was added per well in 96-well microplates (Maxisorp; Nunc, Tokyo, Japan). For the evaluation of the peptide ELISA, synthesized peptides were diluted at concentrations of 10 µg/ml. After incubation overnight at 4 C, plates were washed three times with T-PBS. Next, 300 µl of T-PBS containing 5% skim milk was added and incubated at room temperature (RT) for 1 hr. After washing three times with T-PBS, 50 µl of anti-IBV immunoserum (diluted 1:500 with dilution buffer) was added to each well and incubated at RT for 1 hr. The contents of the wells were washed with T-PBS; 50 µl of peroxidase-conjugated anti-chicken immunoglobulin (donkey anti-chicken IgY [H+L]; Jackson) was dispensed to each well, and plates were incubated at RT for 30 min. The contents of the wells were washed with T-PBS; an aliquot (100 µl) of TMB substrate (Dako, Tokyo, Japan or Sigma Chemical Co., St. Louis, MO) was dispensed to each well, and plates were incubated at RT

Table 2. Synthesized peptides.

Peptide number	Amino acid sequence	Position in the IBV TM86 spike protein
1	SNPRGRSFIEDL	686–697
2	SFIEDLLFTSVE	692–703
3	LFTSVETVGLPT	698–709
4	TVGLPTDSEYKK	704–715
5	SEYKKCTAGPLGT	711–723
6	RGFDTPVLSNFSTGAFN	661–677
7	SNFSTGAFNISLLLTPP	669–685
8	LLLTPPSNPRGR	680–691

for 15 min. After incubation, the enzymatic reaction was stopped by the addition of 100 µl/well of 1 M sulfuric acid. The absorbance was measured using a spectrophotometer (Tecan, Kanagawa, Japan) with a 450-nm filter.

Immunization experiment. Chickens were divided into two groups. Five chickens were immunized with the recombinant protein and three chickens were immunized with an inactivated vaccine. The IBV TM86 strain was inactivated using formaldehyde and mixed with oil adjuvant to generate a suspension containing inactivated virus at a virus concentration of $10^{7.0}$ EID_{50}/dose. Four, five, and six weeks after immunization, blood samples were collected from each animal and the resulting sera were used to determine antibody titers.

Animal experiments in this study were performed according to the regulations and guidelines for animal ethics of the Chemo-Sero-Therapeutic Research Institute, with prior approval from the institute's Animal Experimentation Committee.

Immunosera. Immunosera against IBV were obtained from SPF chickens that had been experimentally immunized with the inactivated IBV TM86 vaccine. For the epitope mapping ELISA, a single immunoserum obtained from the SPF chicken that had repeatedly been immunized with the inactivated IBV vaccine was used. For the evaluation of peptide ELISA, 63 samples from 48 chickens were used. These sera were obtained as follows: chickens 4–5 weeks of age were intramuscularly injected with the inactivated IBV vaccine, and sera were collected at 4–12 weeks postimmunization.

VN test. The VN test was performed as previously described (1). Briefly, sera were serially diluted two-fold with Eagle's minimum essential medium in a microplate and mixed with 200 median tissue culture infective dose ($TCID_{50}$) of the chicken kidney-adapted IBV TM86 strain. After incubation for 1 hr at 37 C, chicken kidney cells were inoculated with IBV and incubated at 37 C in 5% CO_2 incubators. VN titer was defined as the reciprocal of the highest dilution showing no cytopathic effect.

RESULTS

***In silico* protein-folding analysis of the IBV TM86 spike protein.** The complete amino acid sequence of the IBV TM86 spike protein was subjected to protein-folding analysis with the PHYRE2 Protein Fold Recognition Server (17). The amino acid sequence of IBV TM86 spike protein is shown in Supplemental Fig 1. The predicted conformational structure of the IBV TM86 spike protein showed high homology with that of human coronavirus NL63 (PDB accession no. 5SZS; Fig. 1). The conformational structure of the S1 N-terminal domain could not be predicted because this region has extensive variation. The conformational structure of the NL63 spike protein has been reported by Walls *et al.* (28), and they described the structure of the fusion peptide and the region located in the proximity of fusion peptide. The fusion peptide of the IBV M41 spike protein has been reported as amino acids 691–708 (SFIEDLLFTSVESVGLP), and it was reported that this sequence is homologous with that of NL63 sequence 871–889 (SALEDLLFSKVVTSGLGTV) (22). In the predicted structure model, the fusion peptide of the IBV TM86 spike protein was located in the helix domain of the S2 region, and its peripheral region appeared to be identical to that of NL63 (Fig. 1B, 1D). To elucidate the importance of the fusion peptide and its peripheral regions, we evaluated the antigenicity of these domains.

Identification of immunogenicity in the IBV TM86 spike S2 region by immunoblotting. To test the antigenicity of the region located in the proximity of fusion peptide, we constructed GST-fusion recombinant proteins containing the fusion peptide and its peripheral regions, residues 686–762 (Fig. 2A). Objective domains were flanked at the position of 723 and divided into two fragments, 686–723 and 724–762 (Fig. 2A). As shown in Fig. 2B, recombinant proteins containing residues 724–762 were expressed as inclusion bodies. Recombinant proteins containing 686–723, but not 724–762, were expressed both in soluble and inclusion body form. Western blotting indicated that recombinant proteins containing residues 686–723 reacted with anti-IBV immunoserum, indicating that IBV TM86 spike residues 686–723 are antigenic (Fig. 2D).

Identification of the major epitope in IBV TM86 686–723. To identify the major antigenic region in IBV TM86 residues 686–723, five overlapping synthetic peptides were analyzed by ELISA by using immunoserum against IBV. The details of the synthesized peptide (sequence and location) are shown in Table 2. As shown in Fig. 3A, two major antigenic sequences, peptide 1 (686–697: SNPRGRSFIEDL) and peptide 2 (692–703: SFIEDLLFTSVE), were identified. These epitopes overlapped each other, and peptide 1 (686–697) was more antigenic than peptide 2 (692–703).

Further identification of linear epitopes in IBV TM86 spike 661–685. As shown in Fig. 1D, the conformational structure of the IBV TM86 spike 661–685 domain (shown as gray in Fig. 1D) is located upstream of the fusion peptide and seems to be exposed to the surface. In addition, as shown in Fig. 4, the IBV TM86 spike has two N-linked glycosylation sites in this region, and N-linked glycosylation sites are also found in other coronaviruses. Walls *et al.* (30) reported that this region may be masked by glycan shields. Therefore, we hypothesized that this region is located on the surface of the spike protein and may contain an immunogenic epitope. To investigate the antigenicity of this region, we performed epitope mapping analysis. We analyzed three synthetic peptides by ELISA and confirmed that peptide 7 (669–685: SNFSTGAFNISLLLTPP) was also antigenic (Fig. 3B). This epitope contained two N-linked glycosylation sites.

Immunization experiment using identified epitopes. To evaluate the immunogenic character of the identified epitopes, we constructed a recombinant protein expression plasmid pET41a/IBV/S/669-703 containing three epitopes (peptide 1, 2, and 7) and conducted the immunization experiment. As a result, the recombinant protein could not elicit neutralizing antibodies against IBV. Nevertheless, antibodies against three epitopes were elicited (Table 3).

Evaluation of peptide ELISA. As described above, the identified epitopes could not elicit neutralizing antibodies. Next, immunosera against IBV were subjected to the VN test and peptide ELISA to evaluate the correlation between these epitopes and the VN titer. As shown in Fig. 5, the peptide ELISA titer did not correlate with the VN titer, and it was suggested that there are individual differences in the immunoreaction against these epitopes.

DISCUSSION

In the present study, we identified three novel linear epitopes in the IBV TM86 spike S2 region. We used the PHYRE2 program to predict the conformational structure of the IBV spike protein and produced a structural model of the S1 C-terminal and S2 domains. In a previous study, Leyson *et al.* (21) predicted the conformational structure of the IBV S1 domain by using the I-TASSER program (37). Although they analyzed and compared S1 structures of some serotypes, the S2 domain was not evaluated. In this study, we focused on the structure of the S2 domain. Although some epitopes located in the IBV S2 domain have been reported (15,27,34), this is
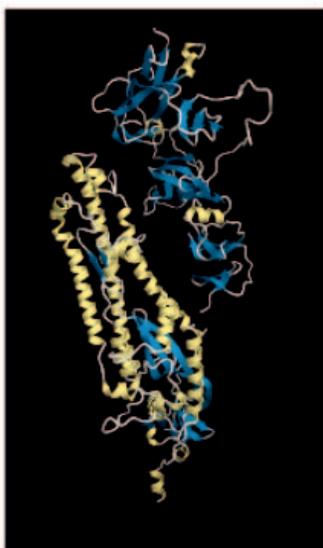
(A)

(B)
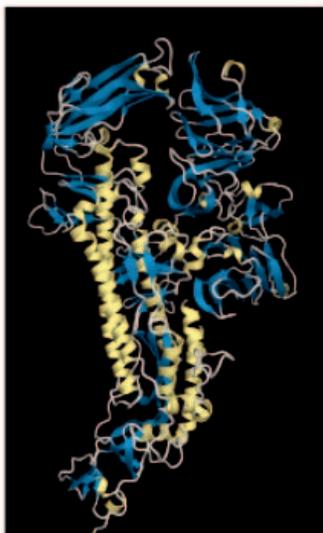
Human coronavirus NL63
(PDB : 5SZS)

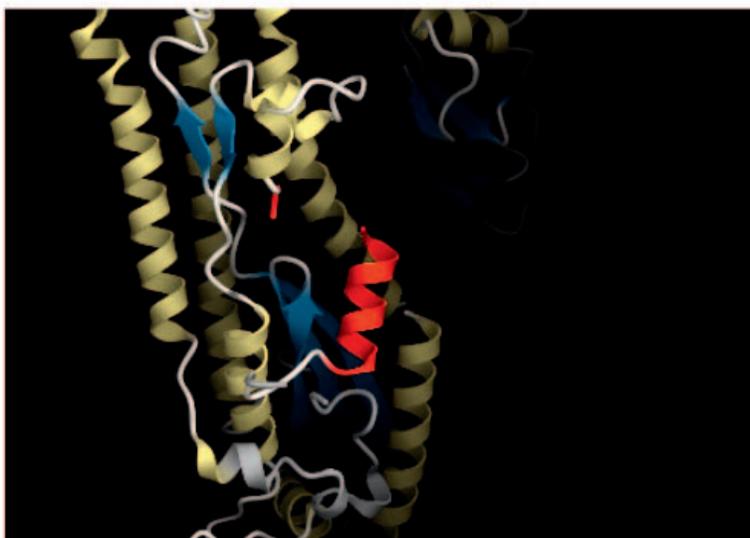(C)

(D)
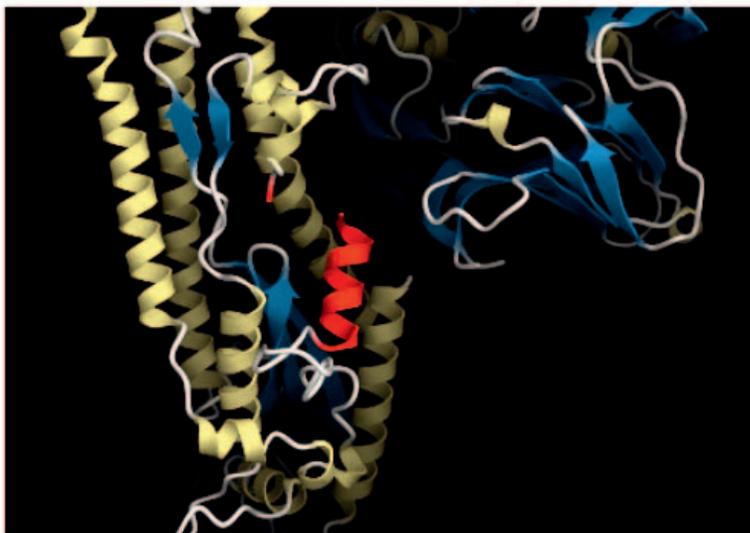
IBV TM86 predicted model

Fig. 1.   Conformational structure of human coronavirus NL63 spike protein (A, B) and the predicted structure of IBV TM86 spike protein (C, D). B and D focus on the fusion peptide and its peripheral region (fusion peptide is indicated as red color). The prediction model was obtained from the PHYRE2 server, and the conformational diagram was built by the structural biology modeling software CueMol.

the first report describing IBV S2 epitopes located in the proximity of the fusion peptide. We performed the BLAST analysis by using an epitope sequence of 35 amino acids (SNFSTGAF-NISLLLTPPSNPRGRSFIEDLLFTSVE) and confirmed that the top 100 hits, which contain several strains, possess 91%–100% homology. This indicated that the identified epitopes are highly conserved in IBV.

IBV spike epitopes reported by Ignjatovic et al. (15) and Yang et al. (34) are located at the proximity of the S1-S2 cleavage site. Therefore, an antibody against these epitopes would affect the protease attack and thus inhibit the conformational change of the spike protein. Ignjatovic et al. (15) concluded that the epitope peptide Sp7 (amino acid positions 566–584), located at the amino terminal end of the S2 glycoprotein, was consistently the most
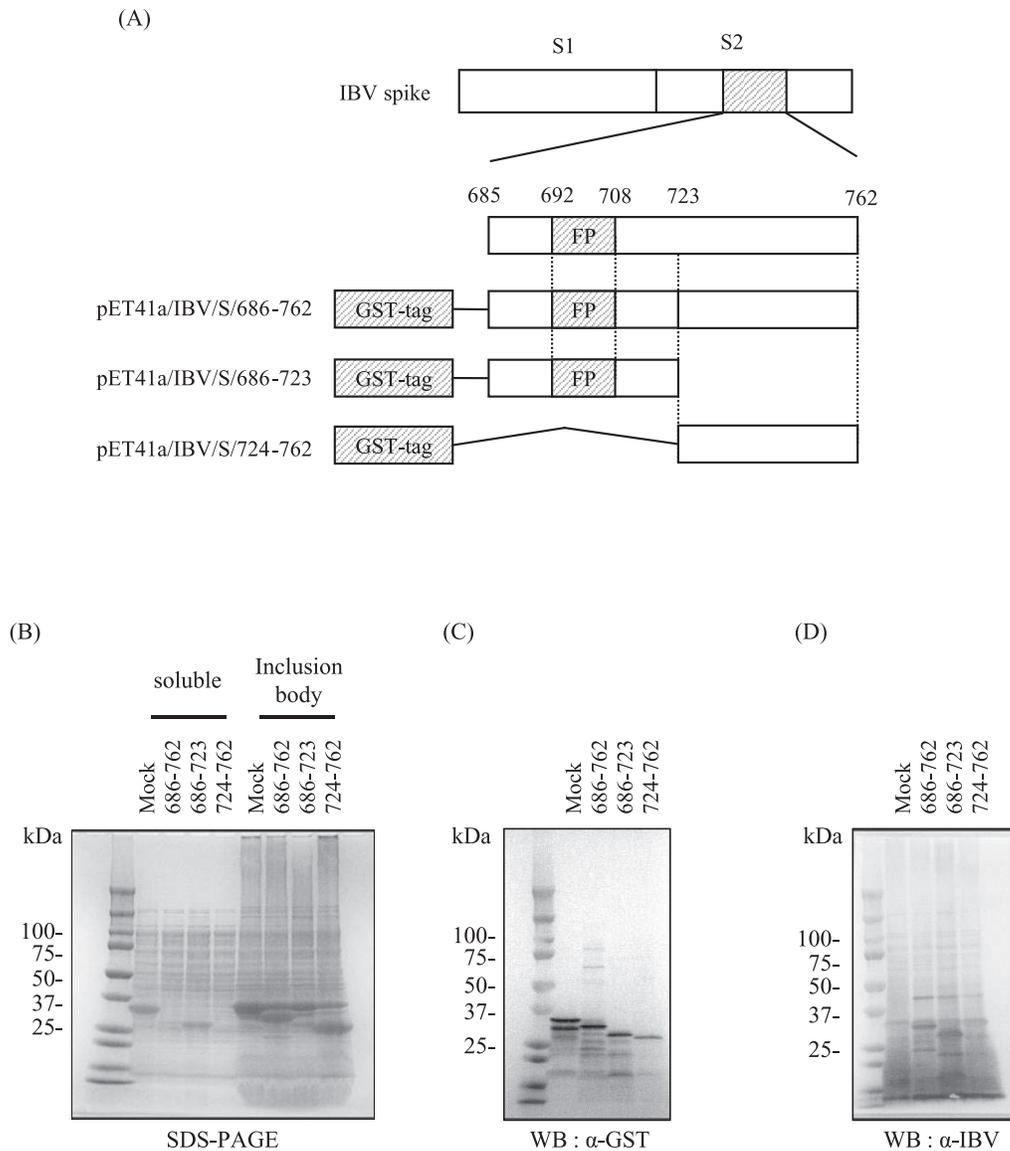
(A)

(B) (C) (D)

Fig. 2. (A) Schematic diagram of recombinant plasmid constructs. Amplified spike fragments were fused downstream of the GST. Recombinant proteins were subjected to SDS-PAGE (B) and western blotting (C and D). Inclusion bodies were subjected to the western blotting analysis. Expression of recombinant proteins were confirmed by SDS-PAGE and western blotting analysis using the anti-GST antibody. The antigenicity of recombinant proteins was evaluated by the reactivity against anti-IBV immunoserum. FP indicates fusion peptide.

antigenic. Further, Sp7 induced protection in immunized chicks (15). Yang *et al.* (34) also concluded that a recombinant protein including $SP_{540–564}$ elicited a neutralizing antibody response against IBV (34). Their conclusion contradicts a previous report by Cavanagh *et al.* (6). Although the influence of differences between their IBV strains cannot be ruled out, these results indicate the value of analyzing IBV S2 epitopes. In this study, we identified new S2 epitopes and showed that their location differs from previous reports, indicating that the antibody against these epitopes may have another character.

As shown in Fig. 4, there are some arginine (R) repeats adjacent to the fusion peptide. Belouzard *et al.* (3) and Watanabe *et al.* (32) reported that this region is cleaved by proteases and is involved in the membrane fusion machinery. Therefore, we hypothesized that identified epitopes may also block the protease attack and thus

inhibit the membrane fusion step. Zhong *et al.* (38) also reported that antibodies that recognize the severe acute respiratory syndrome coronavirus (SARS-CoV) spike fusion peptide play an important role in preventing SARS infection. They identified some epitopes located in the proximity of SARS-CoV spike fusion peptide, and the epitope peptide was designated as SL26 (QILPDPLKPTKRS-FIEDLLFNKVTLA) (38). They identified this peptide using a phage library display assay and concluded that antibodies that recognize this epitope have neutralizing activity. In the conformation model, the location of SL26 is similar to that of peptide 1, 2, and 7 in IBV (Fig. 6). Therefore, we hypothesized that antibodies that recognize these epitopes would affect the conformational change and block the membrane fusion step. In fact, however, these epitopes could not elicit neutralizing antibodies. These results indicate that the spike fusion machinery of IBV may differ from that of SARS-
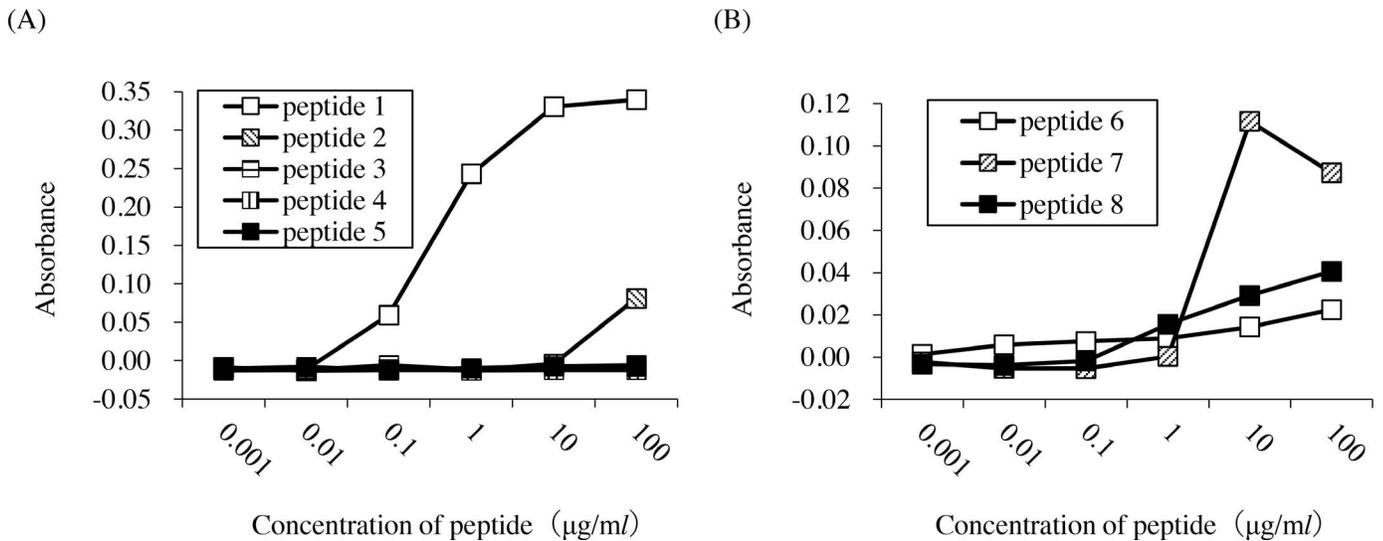
(A)

(B)

Fig. 3. ELISA using synthesized peptides 1–5 (A) and 6–8 (B). Synthesized peptides were serially ten-fold diluted and placed in coated 96-well plates, followed by reaction with anti-IBV immunoserum. The graphs were generated from the results of a single immunoserum.
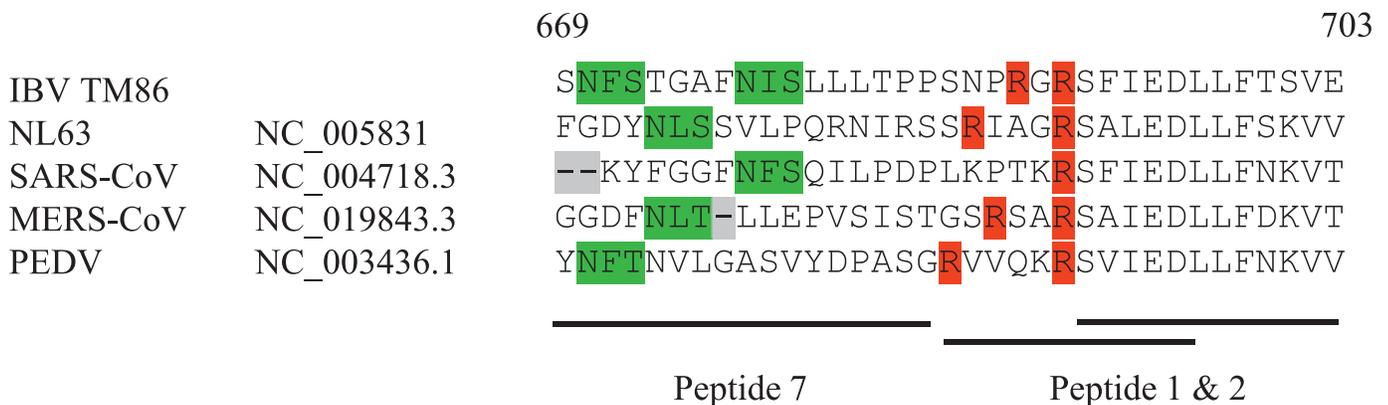

Fig. 4. Amino acid alignment of the fusion peptide and its peripheral regions in coronaviruses. Homologous regions of human coronavirus NL63 (NCBI reference sequence no. NC_005831), SARS-CoV (NCBI reference sequence no. NC_004718.3), Middle East respiratory syndrome coronavirus (NCBI reference sequence no. NC_019843.3), and porcine epidemic diarrhea virus (NCBI reference sequence no. NC_003436.1) were aligned. Green and red boxes indicate N-linked glycosylation sites and arginine residues, respectively.

Table 3. Results of the immunization experiment

Antigen	Chicken no.	Antibody titer (4 weeks postvaccination)				Antibody titer (5 weeks postvaccination)				Antibody titer (6 weeks postvaccination)			
		VN	ELISA 1	ELISA 2	ELISA 7	VN	ELISA 1	ELISA 2	ELISA 7	VN	ELISA 1	ELISA 2	ELISA 7
Recombinant protein	1	<2	0.13	0.04	0.02	<2	0.33	0.07	0.09	<2	0.39	0.07	0.12
	2	<2	0.05	0.04	0.02	<2	0.09	0.04	0.05	<2	0.11	0.05	0.06
	3	<2	1.45	0.20	0.58	<2	1.02	0.12	0.33	<2	0.85	0.12	0.16
	4	<2	0.14	0.08	0.02	<2	0.23	0.10	0.02	<2	0.23	0.10	0.02
	5	<2	1.88	0.35	1.39	<2	2.64	0.72	2.32	<2	2.20	0.80	1.96
Inactivated virus	1	128	0.66	0.04	0.02	1024	1.06	0.04	0.01	2048	1.18	0.03	0.01
	2	256	0.01	0.16	0.09	1024	0.01	0.10	0.08	512	0.01	0.05	0.05
	3	8	0.58	0.39	0.01	64	1.78	0.56	0.21	128	2.01	0.64	0.20

(A)

ELISA 1 (peptide 1)

(B)

ELISA 2 (peptide 2)
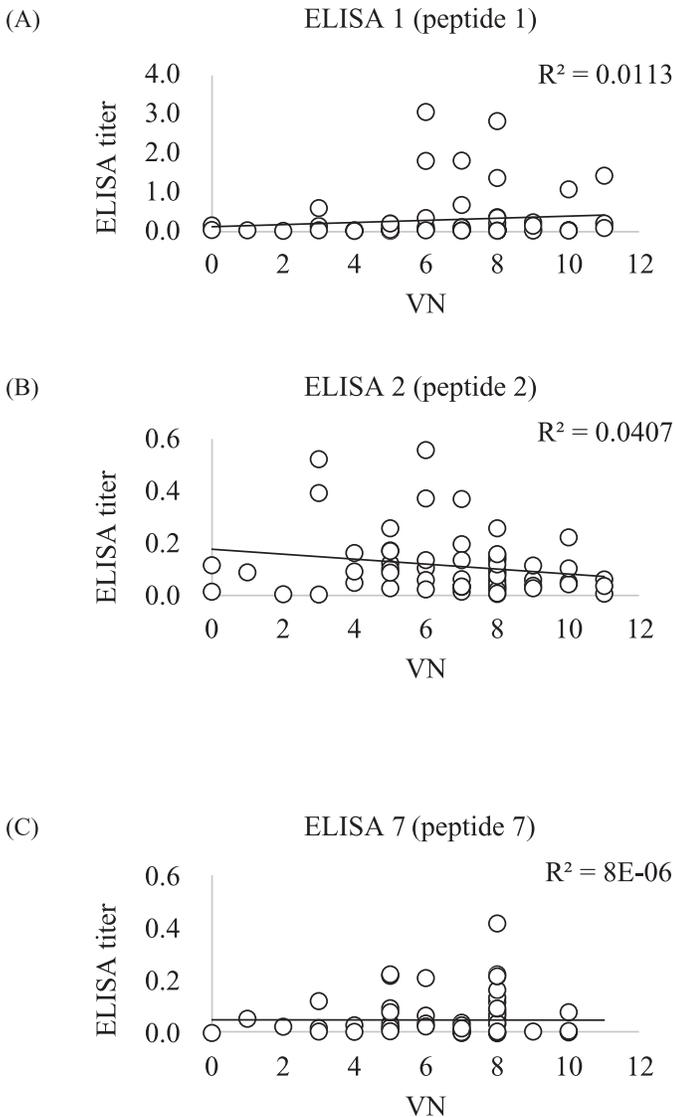

(C)

ELISA 7 (peptide 7)

Fig. 5.   Scatter plots of VN titer and peptide ELISA titer. Horizontal axis and vertical axis indicate ELISA titer and VN titer, respectively. ELISA titer was obtained as follows: ELISA titer = (absorbance value of sample well) − (absorbance value of blank well). R^2 indicates R-squared value.

CoV, or alternatively, IBV may have another mechanism to penetrate the cell membrane. The identically located epitope(s) in other coronaviruses should be evaluated to compare the coronavirus spike fusion machinery.

Peptide ELISA using identified epitopes could not detect VN antibody, and the ELISA titer was not correlated with the VN titer. This result is reasonable because almost all antibodies that have VN activity recognize the S1 region. The peptide ELISA described here displayed a lower sensitivity than the VN test and would be of less use than the other commercially available ELISA kits. Further refinement would be needed to use these epitopes.

In conclusion, we identified three linear epitopes in the IBV spike S2 region that may be common among all coronaviruses. Although the neutralizing activity of the antibody against these epitopes differs from that of other coronaviruses, further investigation is required to demonstrate the usefulness of these epitopes.

(A)
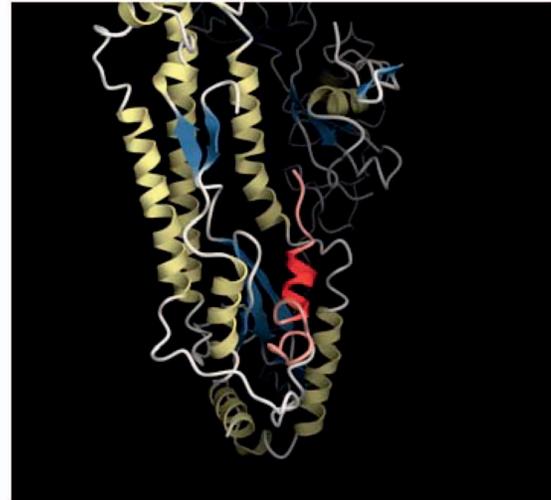
SARS-CoV
(PDB : 5WRG)

(B)
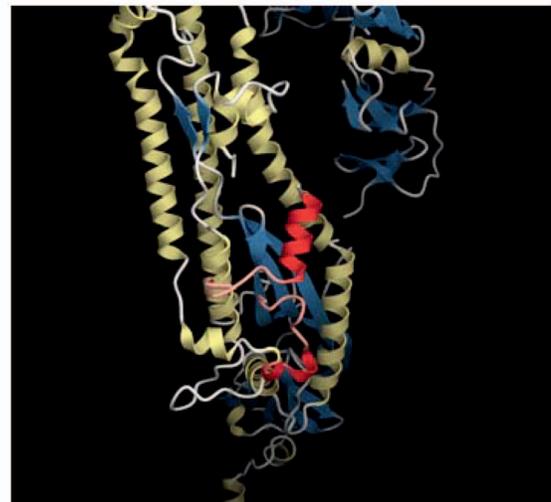
IBV TM86
predicted model

Fig. 6.   Conformational structures of SARS-CoV (PDB: 5WRG) and IBV (predicted model) spike protein. Red indicates SARS-CoV SL26 peptide and IBV epitope peptide (peptide 1, 2, and 7). The prediction model was obtained from the PHYRE2 server, and the conformational diagram was built using the structural biology modeling software CueMol.

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