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Antigenic structure analysis of VP3 of infectious bursal disease virus

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Abstract

VP3 is one of the major structural proteins of infectious bursal disease virus (IBDV), but the epitopes of VP3 have not been precisely identified. To further identify its epitopes, VP3 of Gx strain was cloned and expressed as a recombinant protein in *Escherichia coli* BL21 (DE3). Female BALB/c mice were immunized with the purified VP3 and then four VP3-specific monoclonal antibodies (MAbs) were developed. The MAbs specifically reacted with chicken embryo fibroblasts (CEF) infected with IBDV. A set of 17 partially overlapping or consecutive peptides (P1–P17) spanning VP3 were expressed for epitope screening by pepscan. Through Western blot and enzyme-linked immunosorbent assay (ELISA), two epitopes of VP3, 109–119aa (864–874aa of polyprotein) and 177–190aa (932–945aa of polyprotein), were identified. The two epitopes are totally homologous in many vvIBDV, classical strains, attenuated strains and serotype 2. Both peptides have good immunogenicity and could induce antibodies against IBDV in BALB/c mice. In addition, the two epitope peptides could react with IBDV positive chicken serum and IBDV VP3 positive mice serum. This is the first time that the linear B cell epitopes on VP3 of IBDV have been identified in such a precise location, which may be a benefit to further understanding VP3 of IBDV.

Keywords: Infectious bursal disease virus (IBDV); VP3; Pepscan; Epitope

1. Introduction

Infectious bursal disease (IBD), caused by infectious bursal disease virus (IBDV), is a highly contagious disease in young chickens. It poses a considerable threat to the poultry industry worldwide. IBDV has two different serotypes, serotypes 1 and 2 (McFerran et al., 1980). Serotype 1 is subdivided into classical, antigenic variant and very virulent strains. IBDV belongs to the *Birnaviridae* family, which is composed of non-enveloped viruses containing a double-stranded RNA genome formed by two segments, segment A (3.2 kb) and segment B (2.9 kb) (Dobos et al., 1979; Hudson et al., 1986; Muller and Nitschke, 1987). Segment B encodes the VP1, the viral RNA-dependent RNA polymerase (RdRp) (von Einem et al., 2004), while segment A contains two partially overlapping open reading frames (ORFs). The smaller ORF encodes the VP5 (17 kDa) (Mundt et al., 1995). Although VP5 is dispensable for virus replication *in vitro* (Mundt et al., 1997; Yao et al., 1998), it plays an important role in virus egress and virulence (Lombardo et al., 2000; Yao et al., 1998). The larger ORF encodes a polyprotein (105 kDa) precursor in the order NH_2–pVP_2–VP_4–VP_3–COOH (Azad et al., 1985; Hudson et al., 1986; Kibenge et al., 1997). VP2 is the only structural component of the IBDV icosahedral capsid (Coulibaly et al., 2005; Lee et al., 2006; Garriga et al., 2006). VP3 gene is 774 bp (2396–3169 bp) encodes a 29 kDa protein corresponding to 756–1012aa of polyprotein, and is very similar among serotype, strains and isolates of the virus (Pitcovski et al., 1999). VP3 accomplishes numerous roles during viral cycle, acts as a scaffolding protein required for assembly control (Luque et al., in press).

Many efforts have been made into mapping the epitopes of VP2 (Azad et al., 1987; Jagadish and Azad, 1991; Cui et al., 2003; Heine et al., 1991; Wang et al., 2005; Yamaguchi et al., 1996). However, there have been few reports about the epitopes of VP3. A short fragment between amino acid 858 and 922 was located as one of the epitopes of VP3 (Jagadish and Azad, 1991). Also, serotype-specific and cross-reactive epitopes were found and mapped on VP3 (Mahardika and Becht, 1995; Oppling et

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al., 1991; Yamaguchi et al., 1996), but the antigenicity and epitope information of VP3 is not definite and precise. In the present study, using pepscan induced by monoclonal antibodies (MAbs), two linear epitopes of VP3, 864–874aa and 932–945aa, were identified. The two epitopes have good immunogenicity and reactogenicity.

2. Materials and methods

2.1. Cell lines, virus and serum

The SP2/0 cells were cultured in a 5% CO_2 atmosphere at 37 °C in Dulbecco's modified Eagle medium (DMEM) (Invitrogen, Carlsbad, CA, USA) supplemented with 10% heat-inactivated fetal bovine serum (PAA, Somerset, UK). Primary chicken embryo fibroblasts (CEF) were prepared from 10-day-old specific-pathogen-free (SPF) chicken embryos. The vvIBDV Gx strain was isolated by our laboratory (Wang et al., 2003). The IBDV Gx positive chicken serum and IBDV-VP3 positive mice serum were developed by us.

2.2. Animals

Six- to eight-week-old female BALB/c mice were purchased from the Experimental Animal Center of Harbin Veterinary Research Institute, the Chinese Academy of Agricultural Sciences, China. Experiments were approved by the Animal Ethics Committee, Harbin Veterinary Research Institute, the Chinese Academy of Agricultural Sciences.

2.3. Production of recombinant VP3

The gene VP3 from IBDV Gx strain gene was acquired by reverse transcriptase polymerase chain reaction (RT-PCR), using the following primers designed according to the Gx strain sequence: P3U, 5′-GACGAATTCATGGCCGCTTCAGAGTTCA-3′; P3L, 5′-CCAAAGCTTTCACTCAAGGTCCTCATCA-3′. Purified with the Biospin Gel Extraction kit (BioFlux, Hangzhou, China), the PCR products were digested with EcoRI and HindIII and subsequently cloned into pET-30a (Novagen, Darmstadt, Germany). The recombinant plasmid, named pET-30a-VP3, was verified by PCR, restriction enzyme digestion and nucleotide sequencing. The positive plasmid was transformed into *Escherichia coli* BL21 (DE3) (Novagen) for expression. The recombinant VP3 was induced to express by 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) at mid-exponential phase and incubated at 37 °C for a further 3 h with agitation. Then the bacteria cells were harvested by centrifugation at 10 000 × *g* at 4 °C for 5 min. After analysis by SDS-PAGE and Western blot, the recombinant protein was purified by Glutathione Sepharose 4B RediPack column affinity chromatography (Amersham Life Sciences) according to the manufacturer's instructions.

2.4. Generation of monoclonal antibodies against VP3

The BALB/c mice were immunized with purified recombinant VP3, and VP3 monoclonal antibodies were developed according to the method of Zhang and Rong (1987). Hybridoma culture supernatants were screened by indirect enzyme-linked immunosorbent assay (ELISA) and cloned three times by the limiting dilution method. Monoclonal antibody isotypes were determined by ELISA using horseradish peroxidase-conjugated IgG1 and IgG2a (Southern Biotechnology Associates, Birmingham, AL, USA). Large quantities of the monoclonal antibodies were produced by intraperitoneal injection of hybridoma cells into liquid paraffin-treated BALB/c mice. The ascites containing high concentrations of antibodies were harvested 7–14 days post-injection. The specificities and reactivities of the MAbs against IBDV were tested by indirect immunofluorescence assay (IFA) with CEF infected with IBDV. Smears of IBDV-infected and mock-infected CEF were prepared on glass slides, air-dried and fixed in 100% acetone for 10 min. The slides were washed three times with PBS. Fifty microlitres of MAbs were added to the slides and they were incubated for 1 h in a moist chamber. After incubation, the slides were gently washed three times with PBS, then 50 μl fluorescein isothiocyanate (FITC)-conjugated rabbit anti-mouse secondary antibody (Sigma, St. Louis, MO, USA) was added to the slides and they were again incubated for 30 min in a moist chamber. The slides were washed as described above, mounted in fluorescent mounting medium and then examined under a fluorescence microscope (IMT2 Olympus, Tokyo, Japan).

2.5. Broad epitope mapping with overlapping VP3 fragments

Based on the analysis by DNAStar Protean, four partially overlapping fragments (expressed proteins named P1–P4) spanning the VP3 gene were amplified from pET-30a-VP3. By the restriction enzyme EcoRI and SalI sites, which were introduced into the corresponding primers (Table 1), the four fragments were cloned into the pET-32a, respectively. The recombinant plasmids were transformed into *E. coli* BL21 (DE3) (Novagen) for expression. The recombinant proteins P1–P4 were purified, as described above.

Using the MAbs HRB-3F, HRB-7B, HRB-7C and HRB-10E, the purified recombinant proteins were screened by Western blot and ELISA. In Western blot, protein samples were mixed with an equal volume of 2× sodium dodecyl sulfate (SDS) sample buffer, boiled for 5 min, separated on 5% stacking/12% separating SDS-polyacrylamide gels and electroblotted to nitrocellulose membrane (Millipore Corporation). Each membrane was washed three times with phosphate-buffered saline/Tween 20 (PBST) (Tianxiang, Jiangsu, China) for 5 min and blocked with 5% skimmed milk in PBST at 4 °C overnight. The membrane was then incubated with MAbs at room temperature for 1 h. After washing three times in PBST, the blot was incubated with horseradish peroxidase (HRP)-conjugated rabbit anti-mice IgG (Sigma, St. Louis, MO, USA). The substrate, 3,3-diaminobenzidine tetrahydrochloride (DAB), was used for color development.

For ELISA, ELISA plates (Invitrogen, Carlsbad, CA, USA) were coated overnight with recombinant protein (30 ng/well) and blocked for 2 h with 4% skimmed milk at 37 °C, fol-

Table 1
Position and primers of peptides of IBDV VP3

Name of peptide	Sequences of primers and synthesized oligonucleotides	Position in polyprotein (aa)	Position in VP3 (aa)
P1	5′-GGGAATTCGCTTCAGAGTTCAGAGTT-3′ 5′-ATGTCGACGTCGCTGAGTGCGAAGTT-3′	756–808	1–53
P2	5′-TAGAATTCCTCAGCGACCCGAACGCC-3′ 5′-GCGTCGACGTAGTCCTCGTTTGGATC-3′	806–904	51–149
P3	5′-GGGAATTCGACTACCTAGACTACGTG-3′ 5′-ATGTCGACGATTTCATAGACTTTGGC-3′	903–951	148–196
P4	5′-CCGAATTCGCCAAAGTCTATGAAATC-3′ 5′-ATGTCGACTCACTCAAGGTCCTCATC-3′	946–1012	191–257
P5	5′-GCGAATTCACTATGGGCATCTACTTT-3′ 5′-TAGTCGACTCACTGCCCTTAGGATTTG-3′	864–924	109–169
P6	5′-TAGAATTCCTCAGCGACCCGAACGCC-3′ 5′-GCGTCGACTCACCATCTTCTTTGAGATCC-3′	806–863	51–108
P7	5′-GCGAATTCCTACGTCGATCTACGG-3′ 5′-ATGTCGACGATTTCATAGACTTTGGC-3′	925–951	170–196
P8	5′-AATTCACTATGGGCATCTACTTTGCAACACCAGAATGGGTAGCACTCAATG-3′ 5′-TCGACATTGAGTGCTACCCATTCTGGTGTTGCAAAGTAGATGCCCATAGTG-3′	864–878	109–123
P9	5′-AATTCGGGCACCGGGGGCCAAGCCCCGGCCAGCTGAAGTACTGGCAGAACG-3′ 5′-TCGACGTTCTGCCAGTACTTCAGCTGGCCGGGGCTTGGCCCCCGGTGCCCG-3′	879–893	124–138
P10	5′-AATTCACACGAGAAATACCTGATCCAAACGAGGACTACCTAGACTACGTGG-3′ 5′-TCGACCACGTAGTCTAGGTAGTCCTCGTTTGGATCAGGTATTTCTCGTGTG-3′	894–908	139–153
P11	5′-AATTCCATGCAGAGAAGAGCCGGTTGGCATCAGAAGAACAAATCCTAAGGGCAG-3′ 5′-TCGACTGCCCTTAGGATTTGTTCTTCTGATGCCAACCGGCTCTTCTCTGCATGG-3′	909–924	154–169
P12	5′-AATTCGTAGCACTCAATGGGCACCGGGGGCCAAGCCCCGGCCAGCTGAAGG-3′ 5′-TCGACCTTCAGCTGGCCGGGGCTTGGCCCCCGGTGCCCATTGAGTGCTACG-3′	875–889	120–134
P13	5′-AATTCTACTGGCAGAACACACGAGAAATACCTGATCCAAACGAGGACTACG-3′ 5′-TCGACGTAGTCCTCGTTTGGATCAGGTATTTCTCGTGTGTTCTGCCAGTAG-3′	890–904	135–149
P14	5′-AATTCCTAGACTACGTGCATGCAGAGAAGAGCCGGTTGGCATCAGAAGAAG-3′ 5′-TCGACTTCTTCTGATGCCAACCGGCTCTTCTCTGCATGCACGTAGTCTAGG-3′	905–919	150–164
P15	5′-AATTCGCTACGTCGATCTACGGGGCTCCAGGACAGGCAGAGCCACCCG-3′ 5′-TCGACGGGTGGCTCTGCCTGTCCTGGAGCCCCGTAGATCGACGTAGCG-3′	925–938	170–183
P16	5′-AATTCCCAGGACAGGCAGAGCCACCCCAAGCCTTCATAGACGAAGTCG-3′ 5′-TCGACGACTTCGTCTATGAAGGCTTGGGGTGGCTCTGCCTGTCCTGGG-3′	932–945	177–190
P17	5′-AATTCCAAGCCTTCATAGACGAAGTCGCCAAAGTCTATGAAATCG-3′ 5′-TCGACGATTTCATAGACTTTGGCGACTTCGTCTATGAAGGCTTGG-3′	939–951	184–196

Note: The primers for P1–P7 and oligonucleotide pairs for P8–P17 are in accordance with the published sequence of IBDV strain Gx (GenBank accession no. AY444873). For oligonucleotide pairs of P8–P17, there is a sequence of AATTC and G at the 5′ and 3′ termini of each sense strand, respectively. There is also a sequence of TCGAC and G at the 5′ and 3′ termini of the antisense strand. When the sense and antisense oligonucleotides annealed, a cohesive EcoRI site at the 5′ terminus and a cohesive SalI site at the 3′ terminus would be formed. The nucleotides about the introduced restriction enzyme sites are underlined.

lowed by three washes with PBST. The plates were then incubated with hybridoma supernatant at 37 °C for 1 h. The bound MAbs were detected with HRP-conjugated goat anti-mice IgG (Sigma–Aldrich, St. Louis, MO, USA), and developed with *o*-phenylenediamine dihydrochloride (OPD, Sigma–Aldrich).

2.6. Further limit the epitopes

Based on the epitope analysis using four peptides, P1–P4, the 51–196aa of VP3 was divided into three consecutive non-overlapping fragments (expressed proteins named P5–P7) (Table 1) and cloned in pET-32a, respectively. The recombinant proteins were expressed and then were screened by Western blot and ELISA, as described above.

2.7. Precise location of the epitopes

To further map epitopes of VP3 precisely, two sets of seven and three partially overlapping peptides spanning 109–169aa and 170–196aa of VP3 were designed, respectively. The complementary oligonucleotide pairs encoding each peptide were synthesized. After directly annealing, the cohesive ends of

EcoRI and SalI sites were formed at the termini of the nucleotide segments. Then the nucleotide segments were cloned into pET-32a digested by the same restriction enzyme. The vector construction, expression of the recombinant peptides, Western blot and ELISA detection were performed according to the descriptions above. The positions of the 10 peptides and the synthesized complementary oligonucleotide pairs are listed in Table 1.

2.8. Detection of the immunogenicity of epitope peptides

Female BALB/c mice were immunized subcutaneously on 1st and 15th day with 50 μg purified epitope peptides emulsified with complete and incomplete Freund's adjuvant (Sigma, St. Louis, MO, USA), respectively. Ten days after the second injection, the mice were mercifully killed and the sera were collected to assay the antibody levels by ELISA and Western blot according to the method described above. In Western blot and ELISA, VP3 and peptides were used. The anti-epitope serum was also confirmed by IFA with CEF infected with IBDV.

2.9. Detection of the reactogenicity of the epitope peptides

To investigate whether the epitopes could be recognized by IBDV positive chicken serum and IBDV VP3 positive mice serum, the epitope peptides P8 and P16 were used to coat the ELISA plates (Invitrogen) (30 ng/well) to detect the corresponding antibody. ELISA was performed according to the method described above. Transformed into *E. coli* BL21 (DE3), the induced pET-32a empty vector was taken as negative control.

2.10. Homology analysis of the epitopes

Compared with the amino acid sequence of the same domain of other strains of IBDV, the homology of the two epitopes of VP3 was analysed. The IBDV strains used were as follows, very virulent strain, D6948 (AF240686), HK46 (AF092943), UK661 (X92760), BD399 (AF362776); classical strain, GLS (AY368653), STC (D00499); attenuated strain, Gt (DQ403248), CEF94 (AF194428), CU1 (X16107), D78 (AF499929); serotype-2, OH (U30818).

3. Results

3.1. Development of MAbs against VP3

The recombinant VP3 was expressed in *E. coli* BL21 (DE3) after induction by IPTG, which existed predominantly in the supernatant. After purification by Ni^{2+}-affinity chromatography, the recombinant VP3 was immunized into BALB/c mice, and four MAbs, HRB-3F, HRB-7B, HRB-7C and HRB-10E were obtained. IFA showed that the four MAbs could react positively with CEF infected with IBDV (data not shown). Also, the isotypes of all of the MAbs were IgG_1, κ strains.

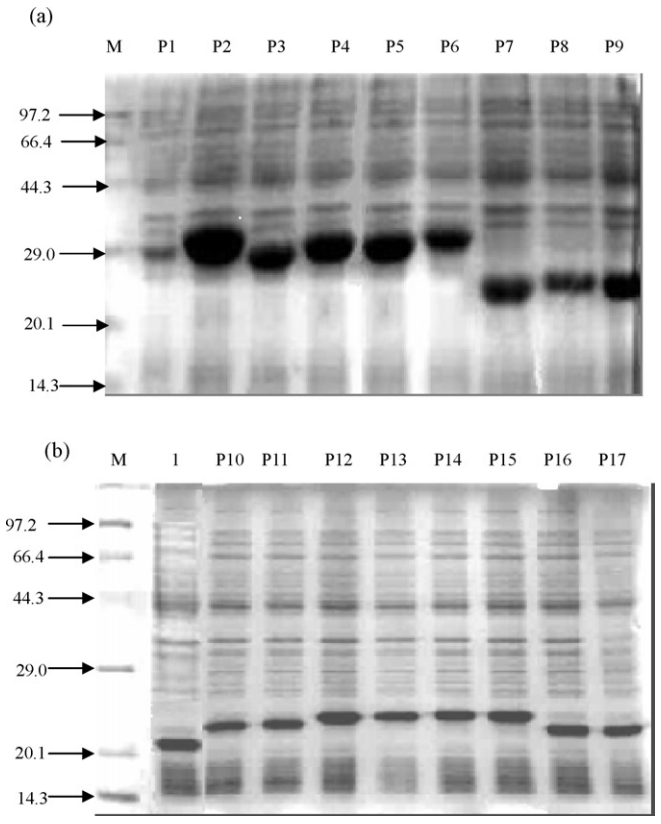

Fig. 1. SDS–PAGE analysis of recombinant peptides of IBDV VP3: (a) P1–P9 are shown in Lanes 2–10 and (b) P10–P17 are shown in Lanes 3–10. The negative control of the empty pET-32a is also shown in Lane 2 in (b). The sizes of protein molecular weight markers in Lane M are indicated in kilodaltons.

3.2. Expression of the recombinant peptides

In order to map the epitopes of VP3, 17 peptides (P1–P17) were successively and successfully obtained and purified (Fig. 1).

3.3. Localization of the two epitopes of VP3

Scrutinizing the immunoreactivity of the peptides by Western blot and ELISA, we found that P2 (51–149aa) was recognized by MAbs HRB-3F and HRB-7B (data not shown), and P3 (148–196aa) was recognized by MAbs HRB-7C and HRB-10E (data not shown). Therefore, we deduced that the 51–196aa of IBDV VP3 might be the dominant antigen region of VP3. Furthermore, the screening results showed that P5 (109–169aa) was recognized by MAbs HRB-3F and HRB-7B (data not shown), and P7 (170–196aa) was recognized by MAbs HRB-7C and HRB-10E (Fig. 2). To define the precise epitopes of VP3, the domains of 109–169aa and 170–196aa were further divided into seven and three partially overlapping fragments, respectively. The results of Western blot showed that only P16 (177–190aa) reacted with HRB-7C and HRB-10E (Fig. 2), P15 (109–169aa) and P17 (170–196aa) were not recognized by the two MAbs. Furthermore, MAbs HRB-3F and HRB-7B reacted with P8 (109–123aa) but did not react with the neighboring fragment P12 (120–134aa) (Fig. 3), so MAbs HRB-3F and HRB-7B only

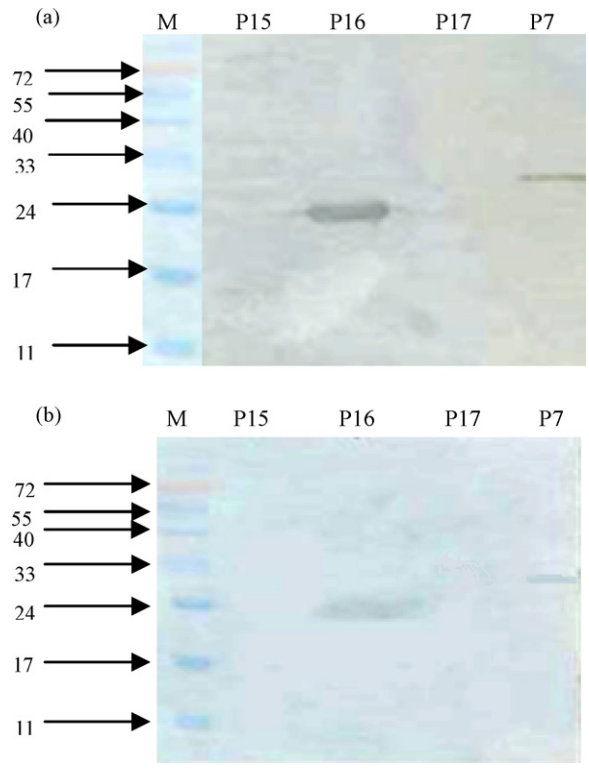

Fig. 2. Detection of P15–P17 by Western blot with HRB-7C and HRB-10E. In both parts, P15–P17 (Lanes 2–4) and P7 (Lane 5) were transferred from the gel to nitrocellulose membranes. Then the peptides were incubated with monoclonal antibodies (a) HRB-7C and (b) HRB-10E, respectively. Both MAbs only reacted with fragment P16. The PageRuler^{TM} prestained protein ladder in Lane M is indicated in kilodaltons.

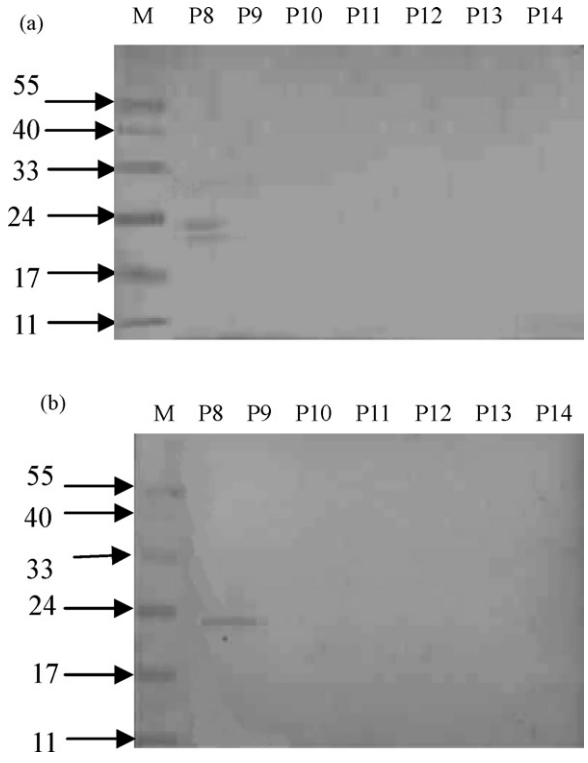

Fig. 3. Detection of P8–P14 by Western blot with HRB-3F and HRB-7B. In both parts, P8–P14 (Lanes 2–8) were transferred from the gel to nitrocellulose membranes. Then the peptides were incubated with monoclonal antibodies (a) HRB-3F and (b) HRB-7B. Both MAbs only reacted with fragment P8. The PageRuler^{TM} prestained protein ladder in Lane M is indicated in kilodaltons.

recognized the domain of 109–119aa. The results of ELISA were coincident with those of Western blot (Fig. 4). Obviously, two epitopes of IBDV VP3, 109–119aa (864–874aa of polyprotein) and 177–190aa (932–945aa of polyprotein), were identified.

3.4. Anti-epitope sera has good immunogenicity

The IFA experiment showed that both sera from the BALB/c mice immunized twice with the epitope peptides of P8 and P16 could react with CEF infected by IBDV, respectively (Fig. 5). Western blot and ELISA showed that anti-epitope serum reacted with VP3 and epitope peptides (data not shown), could not react with P9–P14 and P15, P17, respectively.

3.5. Epitope peptides have good reactogenicity

ELISA analysis indicated that both the epitope peptides, P8 and P16, were recognized by IBDV positive chicken serum and IBDV VP3 positive mice serum (Fig. 6).

3.6. Homology analysis of the epitopes

Sequence analysis of the 12 strains IBDV revealed that the epitope sequences (T^{864}MGIYFATPEW^{874}, P^{932}GQAEPPQAFIDEV^{945}) were totally conserved in the very virulent strain (D6948, HK46, UK661, BD399), the classical strain (GLS, STC), the attenuated strain (Gt, CEF94, CU1, D78) and serotype-2 (OH).

4. Discussion

VP3 might interact with the virus genome (Hjalmarsson et al., 1999; Hjalmarsson and Everitt, 1999), and it might play an important role in stabilizing the genomic RNA within the particle (Tacken et al., 2003). Also, a recent study suggested that virus assembly required screening of the negative charges at the C-terminus of VP3, so the last C-terminal residue of VP3, glutamic acid 257, controls capsid assembly of infectious bursal disease virus (Christophe et al., 2004).

Regarding the epitope research of IBDV, much effort has been made with VP2. However, the epitopes of VP3 have not been precisely identified. A rapid immune response to linear VP3 epitopes was found after both vaccination and infection, which suggested that VP3 was one of the major immunogens of IBDV (Fahey et al., 1985). Formation of the common or serotype-specific epitopes was in agreement with homologous or mismatching amino acid sequences yielding hydrophilic segments on the VP3 polypeptide. These antigenic patterns obtained by immunoblotting could be verified by a competitive ELISA (Mahardika and Becht, 1995; Oppling et al., 1991; Yamaguchi et al., 1996). Jagadish and Azad (1991) thought that the peptide of 858–922aa was located as one of the epitopes of VP3. Another study suggested that VP3 had both group- and serotype-specific

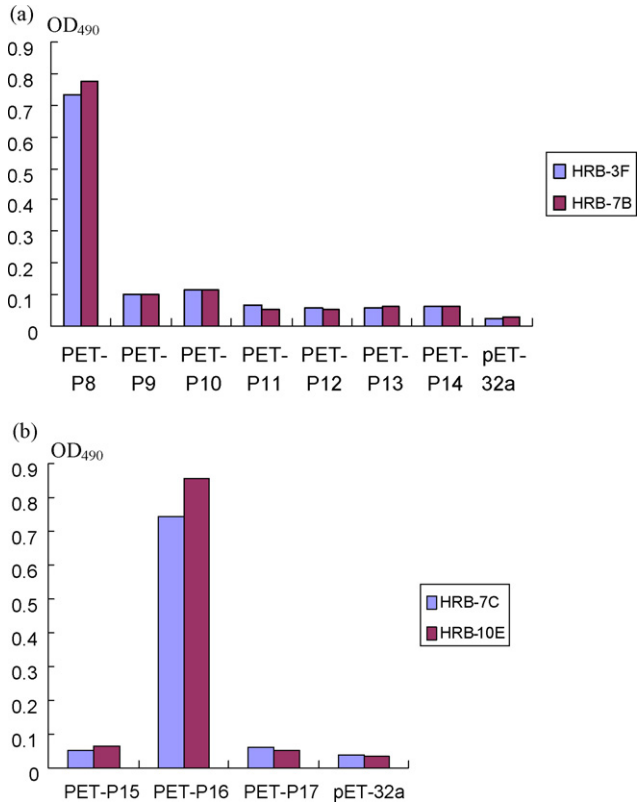

Fig. 4. Identification of the epitopes by ELISA induced by MAbs: (a) peptide P8 reacted with MAb HRB-3F and HRB-7B and (b) peptide P16 reacted with MAb HRB-7C and HRB-10E. No cross-reactivity was found with pET-32a control.

epitopes. Two of these antigenic domains (890–910aa and C-terminal 944aa) also carry epitopes, which are specific for one serotype only, the cross-reacting epitope is located in 764–885aa (Mahardika and Becht, 1995). Obviously, the identified epitopes of VP3 were too long.

To precisely locate the epitopes of VP3, the purified recombinant VP3 protein was expressed in *E. coli* BL21 (DE3) and then the four MAbs, HRB-3F, HRB-7B, HRB-7C and HRB-10E, were developed. These MAbs could react with recombinant VP3 and CEF infected with IBDV, which demonstrated good specificity.

Then a set of 17 partially overlapping or consecutive peptides (P1–P17) spanning VP3 were expressed. Using the four MAbs, HRB-3F, HRB-7B, HRB-7C and HRB-10E, the peptides of P1–P17 were screened by pepscan analysis. Finally, two antigenic epitopes, 109–119aa (864–874aa of polyprotein) and 177–190aa (932–945aa of polyprotein) of IBDV VP3, were identified by Western blot. The HRB-3F and HRB-7B reacted with P8 (109–123aa) but did not react with the neighboring fragments P12 (120–134aa), which has 4aa overlapping with P8. Besides, the dominant region of linear epitope is generally 6–10 amino acid (Wang et al., 2005; Zhou et al., 2006; Binder et al., 2007), so the epitope for HRB-3F and HRB-7B were located at 109–119aa of VP3, which corresponds with 864–874aa of polyprotein. Meanwhile, the HRB-7C and HRB-10E only bind to P16 (177–190) but not bind to P17 (184–196) and P15 (170–183aa), so the intact epitope for HRB-7C and

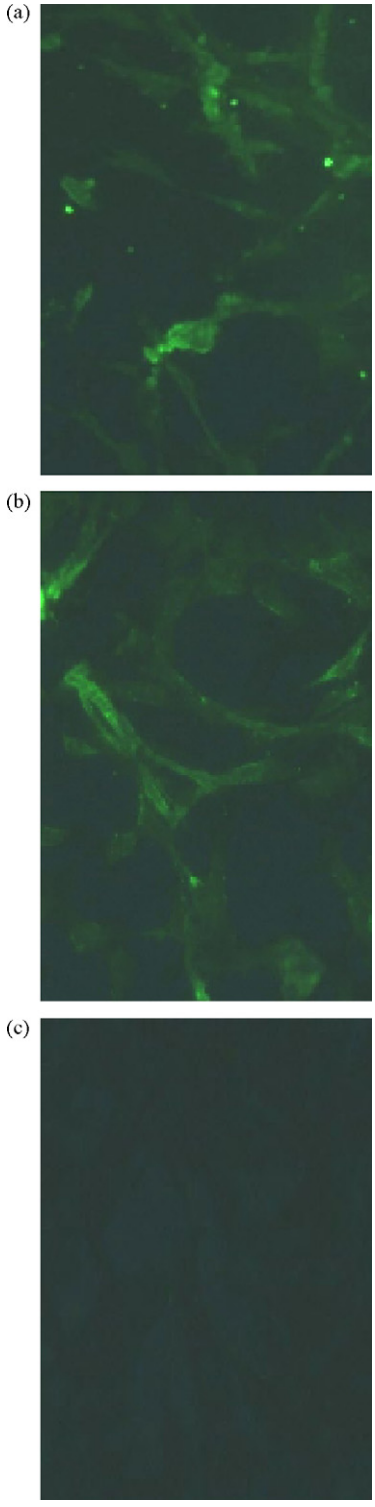

Fig. 5. Immunogenicity of epitopes was tested by indirect immunofluorescence assay (IFA) on CEF (400×) infected with IBDV. Both anti-P8 serum (a) and anti-P16 serum (b) reacted with CEF infected with IBDV. No special fluorescence was found on normal CEF (c).

HRB-10E were located at 177–190aa of VP3, which corresponds with 932–945aa of polyprotein. The results of ELISA also confirmed the two epitopes of VP3. Because only four MAbs were used in the pepscan and each two strains recognized the same

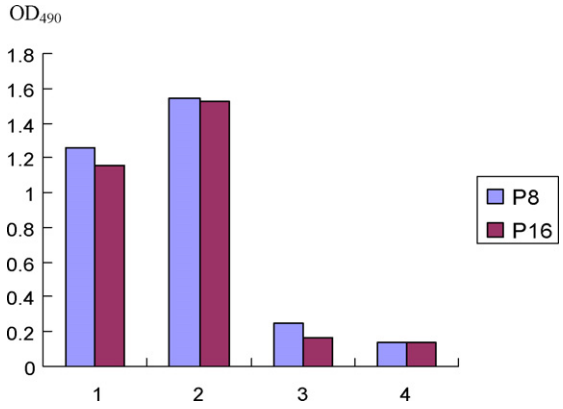

Fig. 6. Analysis of the reactogenicity of the epitope peptides. Both peptide P8 and peptide P16 reacted positively with IBDV positive chicken serum and IBDV VP3 positive mice serum in 1 and 2, respectively. While no cross-reactivity was found with induced pET-32a control in 3 and SPF chicken serum (negative control) in 4.

epitope, perhaps there are other epitopes of VP3, which have not been discovered.

The peptide of 109–119aa (864–874aa of polyprotein) is consistent with but more precise than the report of Mahardika and Becht (1995) who thought it was a cross-reacting epitope. Another peptide of 177–190aa (932–945aa of polyprotein) has only 2aa overlapped with one peptide of 944–1012aa reported by Mahardika and Becht (1995). They thought that the peptide of 944–1012aa was specific for serotype 1 (Mahardika and Becht, 1995). However, the peptide of 177–190aa (932–945aa of polyprotein) is conserved between serotype 1 and 2.

The epitopes have better immunogenicity. After immunization twice with P8 and P16, BALB/c mice could generate the corresponding antibody, which could be detected by IBDV, VP3 and the epitope peptides. In addition, the epitopes have better reactogenicity. Coated with peptides P8 and P16, respectively, the results of the detection of IBDV positive chicken serum and IBDV VP3 positive mice serum were positive. Compared with various IBDV strains, the two peptides are totally homologous in many classical strains, attenuated strains and vvIBDV.

As the conserved linear B cell epitopes on VP3 of IBDV, the two antigenic epitopes, 109–119 (864–874aa of polyprotein) and 177–190aa (932–945aa of polyprotein), have been identified, which may be a benefit to further understanding VP3 of IBDV.

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