Infectious Bronchitis Virus S2 Gene Sequence Variability May Affect S1 Subunit Specific Antibody Binding

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Abstract. The S2 gene of several strains of infectious bronchitis virus (IBV) belonging to the Arkansas, Connecticut, and Florida serotypes was sequenced. Phylogenetic analysis of the S2 gene nucleotide and deduced amino acid sequence data resulted in groups of strains that were the same as groupings observed when S1 sequence data was used. Thus, it appears that S2 subunits are conserved within a serotype but not between serotypes. Although the sequence differences were small, we found that only a few amino acid differences were responsible for different secondary structure predictions for the S2 subunit. It is likely that these changes create different interactions between the S1 and S2 subunits, which could affect the conformation of the S1 subunit where serotype specific epitopes are located. Based on this sequence data, we hypothesize that the S2 subunit can affect specific antibody binding to the S1 subunit of the IBV spike glycoprotein.

Key words: S2, IBV, SI antibody binding, Coronavirus

Introduction

Infectious bronchitis (IB) is an acute, highly transmissible, upper respiratory tract disease in chickens. Clinical signs include tracheal rales, nasal exudate, coughing, and sneezing. Infectious bronchitis affects both sexes and the disease may spread to the reproductive and renal systems (1). It is of economic importance because it can cause poor weight gain and reduced feed efficiency in broilers and a decline in egg production and egg quality in layers (2).

Infectious bronchitis virus (IBV), the causal agent of IB, is a member of the Coronaviridae family. The virion is pleomorphic (diameter 90–200 nm) and enveloped with club-shaped surface projections (spikes) on the surface of the virion. It contains a single stranded, positive-sense RNA genome approximately 27.5 kb in length (3). The virion contains four major structural proteins: a nucleocapsid (N) protein associated with the viral RNA, the integral membrane

(M) glycoprotein, a small membrane (sM) protein, and the spike (S) glycoprotein. The S glycoprotein is a polypeptide of approximately 1200 amino acids. It is proteolytically cleaved after translation into two subunits, S1 and S2 (4). Both subunits are glycosylated with high mannose, N-linked oligosaccharides (5).

The virion spike is thought to be an oligomeric protein composed of two polypeptides each of the S1 and S2 subunits. The two subunits associate by noncovalent forces and retain their three-dimensional shape by way of intrapeptide, but not interpeptide, disulfide bridges (5). The S2 subunits, which form the stalk portion of the spike, anchor it in the membrane, whereas the S1 subunits form the globular head of the spike glycoprotein (5). The S1 subunit encodes amino acids involved in the induction of neutralizing, serotype specific, and hemagglutination inhibiting antibodies (6,7).

Although the S1 subunit of IBV has been examined extensively, the S2 subunit remains enigmatic. Based on the highly conservative nature of the S2 subunit among different members of the *Coronavirus* genus

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and different strains of IBV, it would appear that it plays little or no role in the induction of a host immune response (8). However, it has been shown for IBV that an immunodominant region localized in the N-terminal half of the S2 subunit can induce neutralizing, but not serotype specific antibodies (9). A DNA-binding protein region or leucine zipper motif has also been identified in the S2 subunit of other coronaviruses (10). Leucine zipper motifs are thought to be involved in transcriptional activation.

Furthermore, site-directed mutagenesis of the S2 subunit of another *Coronavirus*, mouse hepatitis virus (MHV), inhibited the binding of a virus neutralizing monoclonal antibody to the unchanged S1 subunit (11). Last, a monoclonal antibody neutralization resistant mutant was reported to have an S1 gene sequence identical to the parental virus, suggesting that the mutant escapes neutralization due to changes in the S2 gene sequence (12).

Thus, we are interested in examining the S2 gene and its deduced amino acid sequence of IBV strains in an attempt to determine if it plays a role in the binding of S1 subunit specific antibodies to the virus. We selected four strains belonging to the Arkansas serotype, Ark 99, Ark DPI, 3668-4, and GAV 92 because their S1 deduced amino acid sequences were very similar, > 90%. Strains 3668-4 and GAV 92 were determined to be Ark-''like'' strains by restriction fragment length polymorphism (RFLP) analysis and later confirmed by serology studies. (13). We also selected Connecticut 46 and Florida 18288 for S2 gene sequencing because these strains are known to share 96.6% deduced amino acid identity for their S1 subunits, yet remain serologically distinct (14,15).

Materials and Methods

Virus Strains

Infectious bronchitis virus strains used in this study are listed in Table 1. Viruses were inoculated into embryonating eggs for propagation (16). The allantoic fluid was harvested and stored at $-70°C$ until needed.

Viral RNA Extraction

The Boehringer Mannheim (BM) High Pure PCR Template Preparation Kit (Indianapolis, IN) was used to extract viral RNA from allantoic fluid per the manufacturer's directions.

S2 Gene RT-PCR

The S2 gene of the IBV strains was amplified using primers that flanked both sides of the entire S2 gene. The 3′ PCR primer (5′-TTGAATCATTAAACAGAC-3′) was designated S2-3′ Ark, and the 5′ PCR primer (5′-GTAGGTATTCTTACTTCACGTA-3′) was designated S2-5′ Ark. The relative primer positions using the ATG start site for the Beaudette strain S1 gene (M95169) as 1, were $+1516$ to 1537 for S2-5′ Ark and $+3480$ to $+3497$ for S2-3′ Ark. The reverse transcriptase (RT) and polymerase chain reaction (PCR) were conducted as previously described (17). The amplicon was purified and concentrated using GenElute™ spin columns (Supelco, Bellefonte, PA 16823-0048) and Microcon™ 30 columns (Amicon, Beverly, MA 01915), respectively.

Table 1. Virus strains used in this study

Virus	Origin	Bird Type	Type	Source	Serotype
Ark 99	Arkansas	Broiler	Vaccine	Schering-Plough	Arkansas
Ark DPI	Delmarva	Broiler	Vaccine	SELECT	Arkansas
3668-4	Delmarva	Broiler	Pathogenic field isolate	Dr. Jack Gelb, Jr.	Arkansas
GAV 92	Georgia	Broiler	Pathogenic field isolate	Dr. Mark Jackwood	Arkansas
Florida 18288	Florida	Layer	Pathogenic field isolate	Dr. Pedro villegas	Florida
Connecticut 46	Connecticut	Broiler	Pathogenic field isolate	IBV repository	Connecticut

S2 Gene Sequence Analysis and Secondary Structure Predictions

The purified S2 DNA was sent to the Molecular Genetics Instrumentation Facility (UGA, Athens, GA) for sequencing with the Prism^TM DyeDeoxy terminator cycle sequencing kit as directed by the manufacturer (Perkin Elmer, Foster City, CA). The entire S2 gene sequence and the deduced amino acid sequence were analyzed by the Clustal method and the pair distances calculated using Lasergene V 3.12 (DNASTAR, Inc., Madison, WI).

Sequence data for the S2 genes sequenced in our laboratory and of other IBV strains were also used for phylogenetic analysis by the Higgins-Sharpe (18) unweighted pairgroup method with arithmetic mean algorithm (UPGMA) using MacDNASIS Pro V3.5 (Hitachi Software Engineering Corp., San Bruno, CA). The strain names and GenBank accession numbers are as follows: Arkansas 99, AF094814; Arkansas DPI, AF094815; 3668-4, AF094816; GAV 92, AF094817; Connecticut 46, AF094818; Florida 18288, AF094819; D1466, X58001; D207, X58003; 6/82, X04723; D274, X15832; Beaudette, X02342; Mass 41, M21883; CU-T2, U49858.

Secondary structure predictions (Chou and Fasman (19), Garnier et al. (20)) and hydrophobicity plots (Hopp and Woods (21) and Kyte and Doolittle (22)) using S2 deduced amino acid sequence data were done with computer algorithms using MacDNASIS Pro V3.5 and Lasergene V 3.12.

Results

There was a high nucleotide similarity for the S2 genes from the IBV strains used in this study (Table 2). The S2 gene sequence for the related Arkansas serotype strains Ark 99 and Ark DPI were identical, while 3668-4 and GAV 92 were respectively 98.9% and 98.6% similar to both Ark 99 and Ark DPI. The S2 gene nucleotide sequences of the Florida 18288 and Connecticut 46 strains were 99.8% similar.

The deduced amino acid sequence of the S2 subunit was also compared (Table 2). The Ark 99 and Ark DPI strains were identical. We observed that 3668-4 and GAV 92 were respectively, 98.7% and 98.1% similar to both Ark 99 and Ark DPI, whereas they were 98.9% similar to each other. The Florida 18288 and Connecticut 46 strains were 99.5% similar to each other and less than 93.7% similar to strains in the Arkansas serotype.

There were few amino acid differences among all the IBV strains (Fig. 1). The strains 3668-4 and GAV 92 had 7 and 11 amino acid substitution differences, respectively, when compared with the Ark 99 and Ark DPI strains. The Florida 18288 and Connecticut 46 strains had only two differences between themselves, and both were nonconservative.

Sequence data for the S2 genes of other IBV strains was used to construct a phylogenetic tree for the deduced amino acid sequence of the S2 subunit (Fig. 2). In the alignment, members of the U.S. serotypes Arkansas, Mass, Connecticut, Florida, and foreign

Table 2. S2 gene nucleotide and deduced amino acid sequence alignment pair distances

	Percent Similarity—Nucleotide														
	1	2	3	4	5	6	7	8	9	10	11	12	13		
1		100.0	99.1	98.6	97.6	92.8	92.6	91.7	92.0	90.0	89.9	88.6	74.9	1	Ark 99 S2
2	100.0		99.1	98.6	97.6	92.8	92.6	91.7	92.0	90.0	89.9	88.6	74.9	2	Ark DPI S2
3	98.7	98.7		98.8	97.3	92.4	92.2	91.1	91.6	89.8	89.7	88.4	74.9	3	3668-4 S2
4	98.1	98.1	98.9		96.8	92.4	92.2	91.0	91.5	89.6	89.5	88.4	74.5	4	GAV 92 S2
5	96.8	96.8	96.2	95.4		92.4	92.2	90.6	91.1	89.0	88.9	87.7	74.7	5	CU-T2 S2
6	93.7	93.7	93.0	92.4	92.4		99.8	95.0	95.1	92.6	92.6	91.3	74.6	6	Connecticut 46 S2
7	93.3	93.3	92.7	92.1	92.1	99.5		94.8	94.9	92.5	92.5	91.2	74.5	7	Florida 18288 S2
8	90.3	90.3	89.7	89.0	88.9	93.8	93.5		98.1	91.6	91.5	90.3	73.4	8	Beaudette S2
9	91.1	91.1	90.5	89.8	89.7	94.3	94.1	97.1		91.9	91.8	90.6	73.6	9	Mass 41 S2
10	91.9	91.9	91.6	91.0	90.5	94.9	94.6	93.0	93.0		99.4	97.4	74.1	10	D207 S2
11	91.4	91.4	91.1	90.5	89.8	94.4	94.1	91.9	92.5	98.1		97.8	74.3	11	D274 S2
12	91.9	91.9	91.6	91.0	90.5	95.1	94.8	92.4	92.9	98.3	97.6		75.2	12	6/82 S2
13	74.8	74.8	74.8	74.5	74.8	76.1	75.9	73.7	74.1	74.3	73.6	75.6		13	D1466 S2
	1	2	3	4	5	6	7	8	9	10	11	12	13		

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     H R S R R S V N E N V T N C P Y V S Y G K F C I K P D G S V S P I V P K E L E Q  Majority
                     10              20              30              40
1    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  Ark 99 S2
1    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  Ark DPI S2
1    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  3668-4 S2
1    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  GAV 92 S2
1    R . . . . . I T A . . . . . . . . . . . . . . . . . . . . . . . . A . . . . . . .  Conn 46 S2
1    R . . . . . I T A . . . . . . . . . . . . . . . . . . . . . . . . A . . . . . . .  Fla 18288 S2

     F V A P L L N V T E N V L I P N S F N L T V T D E Y I Q T H M D K I Q I N C L Q  Majority
                     50              60              70              80
41   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  Ark 99 S2
41   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  Ark DPI S2
41   . . . . . . . . . G . . . . . . . . . . . . . . . . . . N . . . . . . . . . . .   3668-4 S2
41   . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   GAV 92 S2
41   . . . . . . . . . . . . . . . . . . . . . . . . . . . . R . . . . . . . . M .     Conn 46 S2
41   . . . . . . . . . . . . . . . . . . . . . . . . . . . . R . . . . . . . . M .     Fla 18288 S2

     Y V C G N S L A C R K L F Q Q Y G P V C D N I L S V V N S V G Q K E D M E L L S  Majority
                     90              100             110             120
81   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  Ark 99 S2
81   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  Ark DPI S2
81   . . . . . . . . K . . . . . . . . . . . . . . . . . . . . . . . . . . F . .       3668-4 S2
81   . . . . . . . . K . . . . . . . . . . . . . . . . . . . . . . . . . . F . T       GAV 92 S2
81   . . . . . . . D . . . . . . . . . . . . . . . . . . . . . . . . . . . . N         Conn 46 S2
81   . . . . . . . D . . . . . . . . . . . . . . . . . . . . . . . . . . . . N         Fla 18288 S2

     F Y S S T K P S G F N T P V F S N L S T G E F N I S L L L T T P S S P R G R S F  Majority
                     130             140             150             160
121  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  Ark 99 S2
121  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  Ark DPI S2
121  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  3668-4 S2
121  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  GAV 92 S2
121  . . . . . . . . . . . . . . . . . . . . . . . D . . . . . . . . P . . . T T . . . . .  Conn 46 S2
121  . . . . . . . . . . . . . . . . . . . . . . . D . . . . . . . . P . . . T T . . . . .  Fla 18288 S2

     I E D L L F T S V E S V G L P T D E A Y K K C T A G P L G F L K D L A C A R E Y  Majority
                     170             180             190             200
161  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  Ark 99 S2
161  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  Ark DPI S2
161  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  3668-4 S2
161  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  GAV 92 S2
161  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  Conn 46 S2
161  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  Fla 18288 S2
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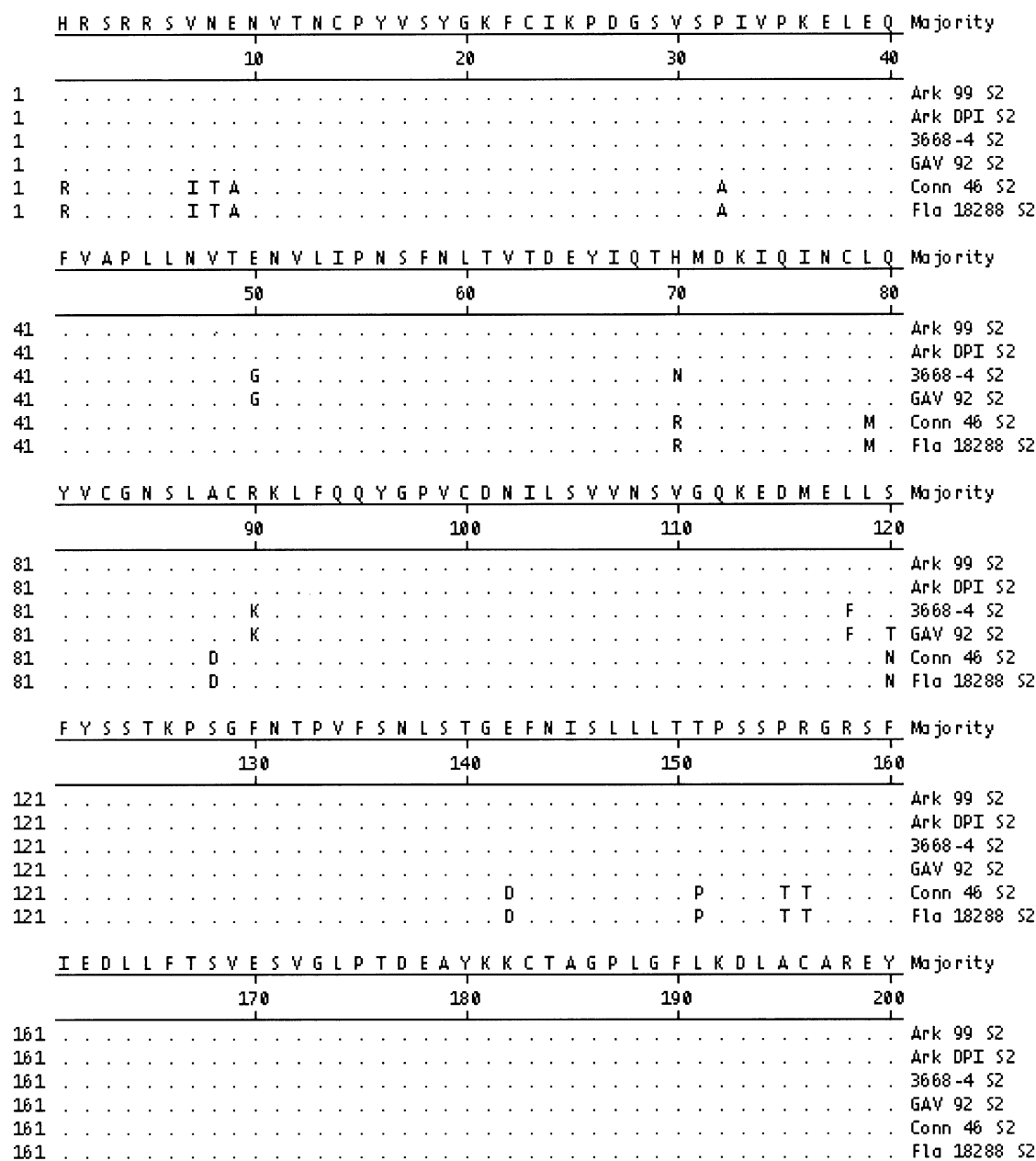
Fig. 1. Deduced amino acid sequence of the S2 glycoprotein showing differences from the consensus for each of the virus strains listed. Dashes were introduced to align the sequences. "X" indicates unavailable sequences. Dots indicate amino acid residues that match the consensus exactly.

serogroups B and C, fall into the same groupings as observed when deduced amino acid sequence data for the S1 subunit is used for phylogenetic analysis (Fig. 3). However, the range of percent similarities was much less for the S2 subunit sequence than that observed for the S1 subunit sequence data.

There were no amino acid differences within the immunodominant region of the S2 subunit for the Arkansas serotype strains (approximately the first 30 residues). There were also no differences between the Connecticut 46 and Florida 18288 strains in the immunodominant region, however, there were differ-

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    N G L L V L P P I I T A E M Q T L Y T S S L V A S M A F G G I T S A G A I P F A  Majority
                    210             220             230             240
201 . . . . . . . . . . . . . . . . . . . . . . . . . . . . V . . . . . . . . . . . . . . .  Ark 99 S2
201 . . . . . . . . . . . . . . . . . . . . . . . . . . . . V . . . . . . . . . . . . . . .  Ark DPI S2
201 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  3668-4 S2
201 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  GAV 92 S2
201 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . . . . . . .  Conn 46 S2
201 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . A . . . . . . . . .  Fla 18288 S2

    T Q L Q A R I N H L G I T Q S L L L K N Q E K I A A S F N K A I G H M Q E G F R  Majority
                    250             260             270             280
241 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  Ark 99 S2
241 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  Ark DPI S2
241 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  3668-4 S2
241 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . R . . . . .  GAV 92 S2
241 . . . . . . . . . . . . . . . . . Q . . . . . . . . . . . . . . . . . . . . . . . . . .  Conn 46 S2
241 . . . . . . . . . . . . . . . . . Q . . . . . . . . . . . . . . . . . . Y . . . . . . .  Fla 18288 S2

    S T S L A L Q Q I Q D V V N K Q S A I L T E T M L A L N K N F G A I S S V I Q D  Majority
                    290             300             310             320
281 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  Ark 99 S2
281 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  Ark DPI S2
281 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  3668-4 S2
281 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  GAV 92 S2
281 . . . . . . . V . . . . . . . . . . . . . . . . . A S . . . . . . . . . . . . . . . . .  Conn 46 S2
281 . . . . . . . V . . . . . . . . . . . . . . . . . A S . . . . . . . . . . . . . . . . .  Fla 18288 S2

    I Y Q Q L D D I Q A D A Q V D R L I T G R L S S L S V L A S A K Q S E Y I R V S  Majority
                    330             340             350             360
321 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  Ark 99 S2
321 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  Ark DPI S2
321 . . . . . . . . . . . . . . . . . . I . . . . . . . . . . . . . . . . . . . . . . . . .  3668-4 S2
321 . . . . . . . . . . . . . . . . . . I . . . . . . . . . . . . . . . . . . . . . . . . .  GAV 92 S2
321 . . . . . . A . . . N . . . . . . . . . . . . . . . . . . . . . . . . . A . . . . . . .  Conn 46 S2
321 . . . . . . A . . . N . . . . . . . . . . . . . . . . . . . . . . . . . A . . . . . . .  Fla 18288 S2

    Q Q R E L A T Q K I N E C V K S Q S I R Y S F C G N G R H V L T I P Q N A P N G  Majority
                    370             380             390             400
361 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  Ark 99 S2
361 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  Ark DPI S2
361 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . S . . . . . 3668-4 S2
361 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . S . . . . . GAV 92 S2
361 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . Conn 46 S2
361 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . Fla 18288 S2
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Fig. 1. (Continued)

ences between the Arkansas serotype and the Connecticut/Florida serotypes. For all of the strains, the predicted membrane associated domain (anchor sequence) and the leucine zipper motif as defined by Britton et al. (10), were identical.

Hydrophobicity plots using the Hopp and Woods (21) algorithm gave identical values of -0.24 ± 0.01 for each strain. However, there were differences in the predicted secondary structures using the method of Chou and Fasman (19). The predicted secondary structure of the S2 subunit of the Ark 99 and Ark DPI strains were identical due to their identical protein sequence. The predicted secondary structure of the S2 subunit of the 3668-4 strain differed from that of the
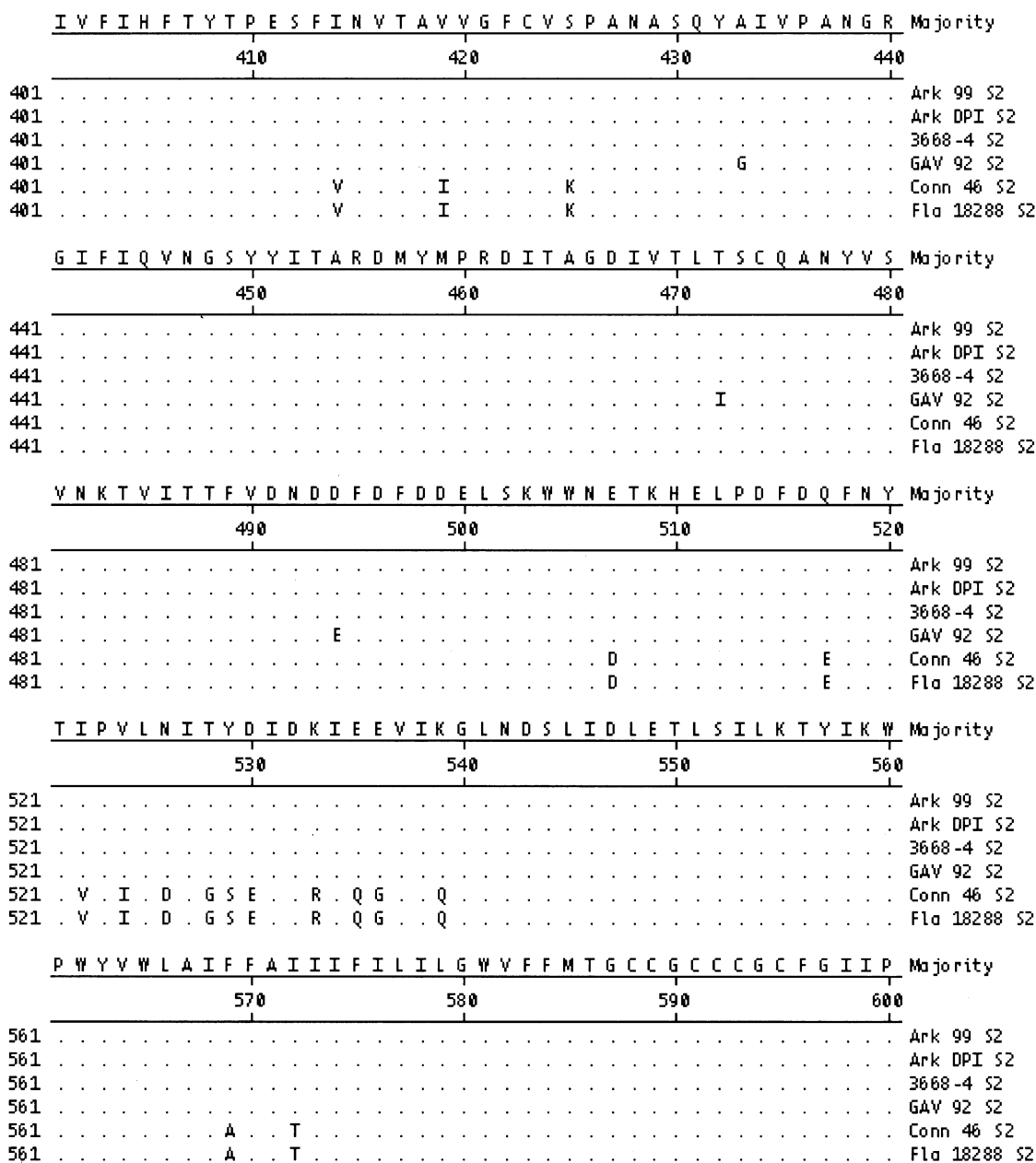
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I V F I H F T Y T P E S F I N V T A V V G F C V S P A N A S Q Y A I V P A N G R  Majority
              410              420              430              440
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401 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  Ark 99 S2
401 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  Ark DPI S2
401 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  3668-4 S2
401 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . G . . . . . . . .  GAV 92 S2
401 . . . . . . . . . . . . V . . . I . . . . K . . . . . . . . . . . . . . . . . .  Conn 46 S2
401 . . . . . . . . . . . . V . . . I . . . . K . . . . . . . . . . . . . . . . . .  Fla 18288 S2
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G I F I Q V N G S Y Y I T A R D M Y M P R D I T A G D I V T L T S C Q A N Y V S  Majority
              450              460              470              480
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441 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  Ark 99 S2
441 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  Ark DPI S2
441 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  3668-4 S2
441 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . I . . . . . . .  GAV 92 S2
441 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  Conn 46 S2
441 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  Fla 18288 S2
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V N K T V I T T F V D N D D F D F D D E L S K W W N E T K H E L P D F D Q F N Y  Majority
              490              500              510              520
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481 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  Ark 99 S2
481 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  Ark DPI S2
481 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  3668-4 S2
481 . . . . . . . . . . . . . E . . . . . . . . . . . . . . . . . . . . . . . . . .  GAV 92 S2
481 . . . . . . . . . . . . . . . . . . . . . . . . . D . . . . . . . . . E . . .  Conn 46 S2
481 . . . . . . . . . . . . . . . . . . . . . . . . . D . . . . . . . . . E . . .  Fla 18288 S2
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T I P V L N I T Y D I D K I E E V I K G L N D S L I D L E T L S I L K T Y I K W  Majority
              530              540              550              560
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521 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  Ark 99 S2
521 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  Ark DPI S2
521 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  3668-4 S2
521 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  GAV 92 S2
521 . V . I . D . G S E . . R . Q G . . Q . . . . . . . . . . . . . . . . . . . .  Conn 46 S2
521 . V . I . D . G S E . . R . Q G . . Q . . . . . . . . . . . . . . . . . . . .  Fla 18288 S2
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P W Y V W L A I F F A I I I F I L I L G W V F F M T G C C G C C C G C F G I I P  Majority
              570              580              590              600
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561 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  Ark 99 S2
561 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  Ark DPI S2
561 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  3668-4 S2
561 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  GAV 92 S2
561 . . . . . . . . A . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . .  Conn 46 S2
561 . . . . . . . . A . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . .  Fla 18288 S2
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Fig. 1. (Continued)

Ark 99 and Ark DPI strains due to amino acid substitutions at position 50 (E to G) and 70 (H to N) that resulted in the addition of two turns (Fig. 4). The GAV 92 strain differed tremendously due to an amino acid substitution at position 50 (E to G), resulting in an odd number of turns between amino acids 40 and 75. The odd number of turns resulted in a 180° flip in the middle of the predicted secondary structure.

The predicted secondary structure for the S2 subunit of the Florida 18288 and Connecticut 46 strains was remarkably different (Fig. 5). There were two nonconservative amino acid changes at positions

```
      L M S K C G K K S S Y Y T T F D N D V V T E Q Y R P K K S V -        Majority
                      610            620            630

601   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .       Ark 99 S2
601   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .       Ark DPI S2
601   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .       3668-4 S2
601   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .       GAV 92 S2
601   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .       Conn 46 S2
601   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .       Fla 18288 S2
```

Fig. 1. (Continued)

227 and 274. The alanine residue at position 227 for the Connecticut 46 strain was changed to a threonine residue for the Florida 18288 strain. This resulted in the changing of some amino acid residues from helix to sheet and the addition of a turn in the predicted secondary structure of the S2 subunit for the Florida 18288 strain. The histidine residue at position 274 for the Connecticut 46 strains was changed to a tyrosine

residue for the Florida 18288 strain. This resulted in the changing of some amino acid residues from helix to sheet and the addition of a coil in the secondary structure of the S2 subunit for the Florida 18288 strain.

Discussion

We analyzed six strains of IBV in the Arkansas, Connecticut, and Florida serotypes. Although S2 sequence data are more conserved among different strains of IBV than S1 sequence data, it appears that strains can be grouped into serotypes based on S2 gene nucleotide sequence data, as well as deduced amino acid sequence for the S2 subunit. This agrees with S1 gene phylogenetic trees for U.S. and international viruses. The only exception for grouping is between the Connecticut and Florida serotypes, which cannot be grouped into different serotypes using S1 gene or deduced amino acid sequence data, but can be separated serologically (14,15).

Based on the secondary structure predictions using the Chou and Fasman (19) algorithm it appears that
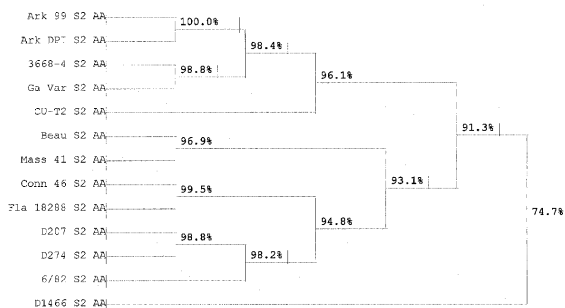
Fig. 2. Phylogenetic tree of deduced amino acid sequence similarity for the S2 glycoprotein of U.S. and international strains of IBV.
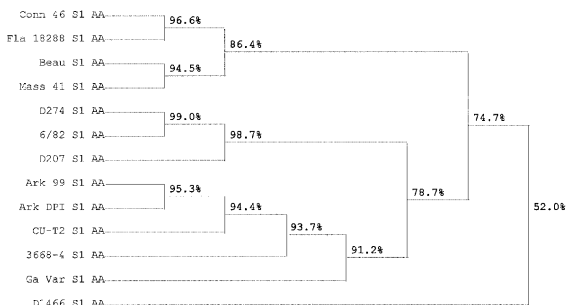
Fig. 3. Phylogenetic tree of deduced amino acid sequence similarity for the S1 glycoprotein of U.S. and international strains of IBV.
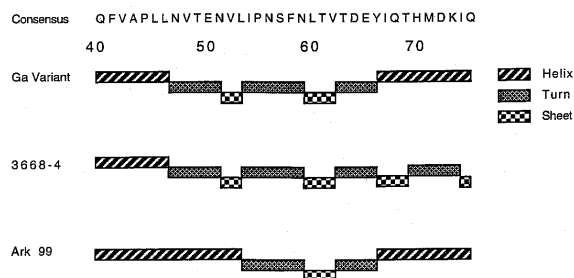
Fig. 4. Secondary structure predictions of amino acid residues 40 to 75 for the S2 glycoprotein of Ark-''like'' IBV strains using the Chou and Fasmon (18) algorithm.

File: Connecticut 46 S2 AA
Size: 631 aa
Seq: 1 -   631
Function: Chou and Fasman
████ : HELIX   ▥▥▥ : SHEET   ▨▨▨ : TURN   ▧▧▧ : COIL
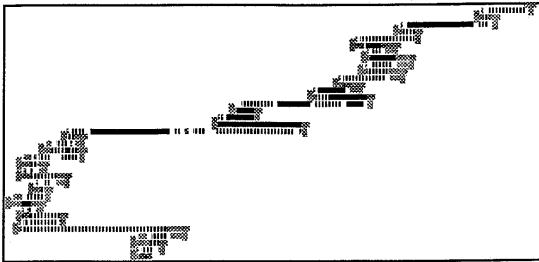
File: Florida 18288 S2 AA
Size: 631 aa
Seq: 1 -   631
Function: Chou and Fasman
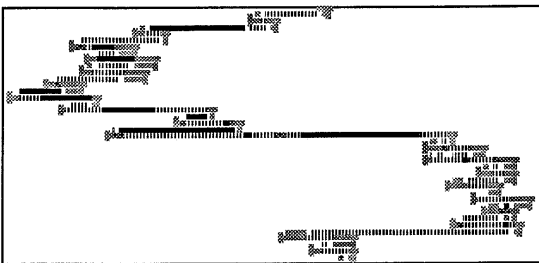████ : HELIX   ▥▥▥ : SHEET   ▨▨▨ : TURN   ▧▧▧ : COIL

Fig. 5. Secondary structure prediction of the S2 glycoprotein for the Connecticut 46 and Florida 18288 strains of IBV using the Chou and Fasman (18) algorithm.

only a few amino acid changes in the correct location can alter the shape of the S2 subunit. One change in the GAV 92 S2 deduced amino acid sequence (position 50 E→G) led to a 180° flip in the secondary structure prediction of the S2 subunit. The two nonconservative amino acid changes between the Florida 18288 and Connecticut 46 strains led to radically different secondary structure predictions. It is plausible that these S2 subunit secondary structure changes could affect the tertiary structure of the S2 subunit. Therefore, creating different interactions between the S1 and S2 glycoproteins that could change the quaternary structure of the spike glycoprotein. Such changes would affect antibody binding and therefore account for serologic differences between GAV 92, 3668-4, and Arkansas viruses as well as the serotype differences between the Connecticut and Florida strains.

The S1 and S2 subunits are known to interact by noncovalent attractive forces (5). Other research on a

different *Coronavirus*, mouse hepatitis virus, by Grosse et al., showed that a single amino acid change in the S2 subunit could create a S1 subunit specific monoclonal antibody resistant mutant (11). This suggests that the interaction between S1 and S2 subunits may determine the shape or availability of S1 subunit specific epitopes. Whether the S2 subunit is actually involved in S1 subunit specific antibody recognition, sterically hinders antibody from binding to the S1 subunit, or effects the presentation of S1 subunit epitopes is not known. However, from our sequence data we hypothesize that the S2 subunit can affect binding of S1 subunit specific antibody due to S2 gene variability and subsequent secondary structure differences.

Notes

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