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# Identification of Bovine Invariant Chain (Ii) Gene by Nucleotide Sequencing[1]

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Four overlapping cDNA clones encoding the bovine invariant chain (Ii) were isolated and characterized. The bovine Ii cDNA clone, NI3 with a 1,381-bp insert, encoded a translated product of 204 amino acids. The amino acid sequences deduced from this clone revealed that the bovine Ii gene is more closely related to human Ii gene than to genes for rodents, such as mouse and rat, but lacked 10 amino acids of the 3′ end of the extracellular domain comparable to those in proteins from other species. Of interest is that the class-II-associated invariant chain peptide (CLIP) segment is present in the protein encoded by NI3, and this region exhibits a high degree of overall similarity to sequences encoded by human, mouse and rat, suggesting that bovine CLIP may have the biological function of CLIP binding to MHC class II molecules. Thus, it seems likely that this cDNA clone encodes a functional product which might perform an important function in MHC class II antigen presentation, as previously established in studies in mouse and man. 

The invariant chain (Ii), nonpolymorphic type II intracellular membrane glycoprotein, binds major histocompatibility complex (MHC) class II glycoprotein, probably via its class II-associated Ii peptide (CLIP) segment (1–4). Ii and MHC class II molecules make a stable nine subunit [($\alpha\beta$-Ii)3] complex composed of three Ii and three $\alpha\beta$ heterodimers before their exit from the endoplasmic reticulum (ER) (5). This complex is then transported into MHC class II compartments (MIIC) by an endosome sorting signal in the cytoplasmic domain of Ii chain (6–8). In this process, Ii is degraded by protease and forms $\alpha\beta$-CLIP complex (9–11), which is intermediate in the mature MHC class II molecule. In the MIIC, MHC class II DM molecules interact with $\alpha\beta$-CLIP complex, catalyze the dissociation of CLIP and facilitate the binding of exogenous antigenic peptide (1, 2, 12–14). Thus, since Ii prevents peptides from binding to class II molecules it plays a regulatory role in MHC class II expression and its function.

Analysis of restriction fragment length polymorphism (RFLP) and characterization of cloned bovine class II genes have provided evidence for the existence of one *DRA* gene and at least three *DRB* genes, one or two *DQA* and *DQB* genes, one *DOA* and one *DNA* gene, as well as three novel genes, namely, *DYA*, *DYB* and *DIB* (15–20). Whereas the presence of bovine *DMA* and *DMB* genes, which differ from conventional bovine class II genes, was also confirmed (21), there is yet no evidence, as yet, for Ii gene in the bovine. To understand the biological function and the mechanisms of bovine Ii binding to bovine MHC class II molecules, we isolated and characterized cDNA clones that correspond to bovine Ii.

## MATERIALS AND METHODS

*Preparation of a cDNA library.* A cDNA library from the bovine lymphoid cell line BLSC KU-1 was constructed in the mammalian expression vector pCDM8 as described previously, and introduced into *E. coli* MC1061/P3 (16).

*Isolation and characterization of bovine Ii chain cDNAs.* To obtain a partial bovine Ii chain cDNA clone, plasmid DNA prepared from the cDNA library was amplified by polymerase chain reaction (PCR) with 5′-GCT-TCT-AGA-ATG-GAT-

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GAC-CAG-CGC-GAC-CT-3′ and 5′-CTG-TCT-AGA-CAC-TCC-CAG-GCC-AGA-AGA-TG-3′ as primers. These sequences were derived from exons 1 and 7 of human Ii gene (22). Amplification was achieved with 35 cycles at 94°C for 1 min, at 61°C for 2 min, and at 72°C for 2 min, followed by a 10 min extension at 72°C. Next, to obtain the sequences of a 5′- or 3′-untranslated (UT) region of bovine Ii chain cDNA clone, we generated amplified fragments from the cDNA library by PCR using the following primers: for 5′-UT region, 5′-ACC-TGC-AGG-CGC-AGA-ACT-GGT-A-3′ and 5′-TGC-TCT-AGA-GGA-GCA-GAG-CCA-CCA-AGA-CG-3′, which were derived from the stuffer sequences of the pCDM8 vector and sequences of 5′ end of a partial bovine Ii cDNA clone; for 3′-UT region, 5′-CGG-AGG-CTT-CTA-GAG-ATC-CCT-3′ and 5′-TTT-TCT-AGA-TGG-CTG-CAT-CAG-TGG-CTC-TTG-3′, which were derived from the stuffer sequences of the pCDM8 vector and sequences of 3′ end of a partial bovine Ii chain cDNA clone. Amplification was achieved with 35 cycles at 94°C for 1 min, at 55°C for 1 min, and at 72°C for 2 min, followed by a 10 min extension at 72°C. Finally, a cDNA clone that contained the full-length coding region of bovine Ii gene was amplified by PCR with an annealing temperature at 61°C using the primers, 5′-CGG-AAG-CTT-CTA-GAG-ATC-CCT-3′ and 5′-TGC-TCT-AGA-GGT-GGC-CTC-CTG-TAG-ACG-AAT-3′, which were derived from sequences of a 5′-UT region of a bovine Ii cDNA clone and the stuffer sequences of the pCDM8 vector.

Amplified PCR products were isolated and subcloned into pBluescript II SK(+) (Stratagene, Heidelberg, Germany), and the nucleotide sequences of both strands were determined by the dideoxy chain termination method with a *Bca*BEST sequence kit (Takara Shuzo Co., Shiga, Japan) (23). Computer analyses were performed with the program package from the Wisconsin Genetics Computer Group, which included FASTA and TFASTA (24).

## RESULTS AND DISCUSSION

### *Characterization of the Bovine Ii Chain cDNA Clone*

A partial bovine Ii cDNA clone, I-600, with a 572-bp insert was isolated from the bovine lymphoid cell line BLSC KU-1 cDNA library by PCR (Figure 1A). The sequence of this clone was similar to the sequences of part of the cytoplasmic (CY) domain, the transmembrane (TM) domain and part of the extracellular (EC) domain of a human Ii cDNA clone, reported by Strubin *et al.* (22). The I-600 cDNA clone did not, however, contain the sequences of the 3′ and 5′ portions of the gene. Therefore, two clones containing the sequences of the 5′-UT region or 3′-UT region were isolated by PCR amplification using primers designed on the basis of sequences of I-600 clone and stuffer sequences of CDM8 vector; they were designated 3-1 (736 bp) and 5-6 (242 bp). Next, we generated a 1,381-bp amplified fragment that contained the full-length coding region of bovine Ii from the cDNA library by PCR using two primers designed on the basis of sequences of clone 5-6 and stuffer sequences of pCDM8 vector; the new clone was designated NI3. Analysis of the nucleotide sequence of NI3 demonstrated that the NI3 and I-600, 3-1 or 5-6 were identical throughout the sequences, and that the NI3 clone was an analogue of the bovine Ii gene. We also obtained three other clones which are identical to the NI3.

The complete nucleotide sequences and deduced amino acid sequences of the NI3 clone are shown in Figure 1B. This clone contains a single open reading frame that begins with an ATG initiation codon [nucleotide (nt) 130 to 132] and ends with a TAA termination codon (nt 742 to 744), predicting a coding sequence of 204 amino acids. The bovine Ii polypeptide contains a CY domain (positions 1 to 30), a TM domain (positions 31 to 56), and a EC domain (positions 57 to 204). There is a 3′-untranslated region of 620 nucleotides with a typical polyadenylation signal (AATAAA; nt 1,348 to nt 1,353) located 16 nt upstream from a short poly(A) tail. Thus, NI3 encoded the complete molecule encoded by bovine Ii gene.

Figure 2 shows an alignment of predicted amino acid sequences encoded by the NI3 clone and Ii genes from human (22), mouse (25) and rat (26). In contrast to the Iis of human, mouse and rat, the NI3 clone lacked 10 amino acids of the 3′ end of EC domain, suggesting that the deletion at these positions in bovine Ii might be one of the critical features distinguishing it from other species. Indeed, the sequencing of other clones isolated from mRNA of bovine lymphoid cell line BL312 (27) by reverse transcription-PCR shows the same tendency as the NI3 clone (data not shown). The sizes of all other functional domains (CY and TM) encoded by NI3 are identical to Ii chain of human and rat. The deduced amino acid sequence of NI3 indicates the presence of two *N*-linked glycosylation sites, at positions 112 to 114 and 118 to 120, respectively. Other conserved features

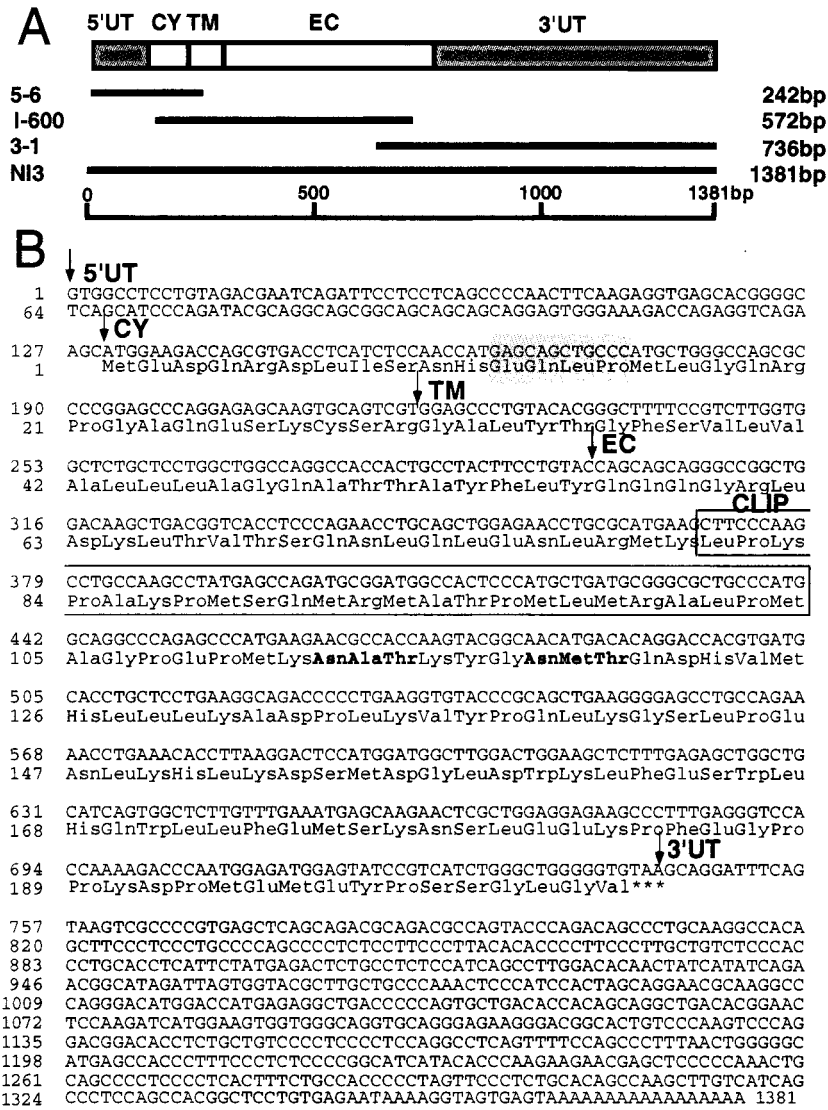


**FIG. 1.** (A) Physical maps of the four overlapping cDNA clones isolated from the BLSC-KU-1 cell cDNA library. The 5′ untranslated region (5′UT), cytoplasmic domain (CY), extracellular domain (EC), and 3′ untranslated region (3′ UT) are indicated. (B) Complete nucleotide and predicted amino acid sequences of bovine Ii cDNA clone N13. Arrows designate the putative Ii domains: 5′UT, CY, EC and 3′UT. Two putative sites of *N*-linked glycosylation are indicated in boldface. Putative sorting signal for endosomal compartments is indicated by shaded boxes. Class II associated invariant chain peptide (CLIP) segment is boxed.

of Ii found in NI3 can also be recognized, for example, a Glu-Gln-Leu-Pro motif at positions 12 to 15 in the CY domain, which is believed to be a sorting signal for endosomal compartments (6).

The NI3-encoded protein exhibits a high degree of overall identity (approximately 80% protein and 85% nucleotide identities) with sequences encoded by the human, mouse and rat Ii cDNA clones. The extent of the identity between the putative NI3 protein and putative human is also somewhat higher than the identities between the putative NI3 protein and the putative mouse or rat proteins. It appears, therefore, that the bovine Ii gene is more similar to its counterpart in human than to that of the rodent.

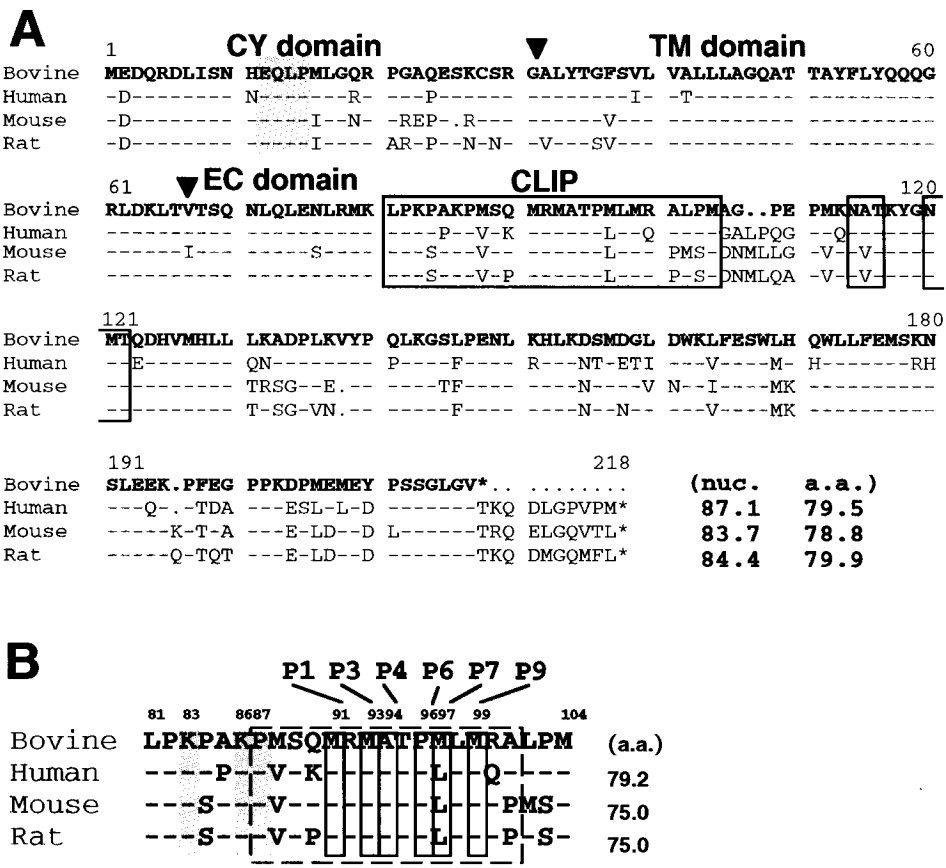


**FIG. 2.** (A) Alignment of the amino acid sequence predicted from bovine Ii cDNA clone, N13, with those predicted from the human (22), mouse (25) and rat (26) Ii genes. The numbers denote positions of amino acids. Two putative sites of *N*-linked glycosylation and class II associated invariant chain domain (CLIP) domain are boxed. Putative sorting signal for endosomal compartments is indicated by shaded boxes. Total percentages of amino acids (right) or nucleotides (left) are homologous among N13 and Ii chain genes of other species. (B) Alignment of the amino acid sequence of CLIP segment predicted from the bovine Ii gene with those predicted from the human, mouse and rat Ii genes. Putative site of rapid dissociation of CLIP is indicated by shaded boxes. Amino acid residues making hydrogen bonds between CLIP and DR are dotted boxed. Amino acid residues corresponding to CLIP side chains which extend into peptide-binding pockets are boxed. P1, 3, 4, 6, 7 and 9 showed peptide-binding pockets of DR which bind to CLIP. Total percentages of amino acids are homologous among CLIP segments encoded by Ii genes of bovine and other species.

### *Characterization of CLIP in Bovine Ii Chain*

Figure 2A shows a comparison of amino acid sequences encoded from the bovine Ii cDNA clone NI3 and from genes for Ii from human, mouse and rat. It is of interest here that the 24mer CLIP segment (positions 81–104) necessary for the Ii activity of inhibiting antigen peptide binding to MHC class II molecules is present in the protein encoded by NI3. As shown in Figure 2B, this region exhibits approximately 79.2%, 75% and 75% overall similarities to sequences encoded by human, mouse and rat, respectively. The X-ray crystal structure of HLA-DR3-CLIP indicated that 15 residues (positions 87–101) of the 24 residues in CLIP forms a nearly identical extensive network of hydrogen bonds to class II MHC residues and the side chains (positions 91–99) extend into the same pockets in the HLA-DR3 peptide-binding sites (28). Four differences in these 15 residues of CLIP making 17 hydrogen bonds between CLIP and HLA-DR3 (28) were identified between bovine and human, suggesting that bovine Ii differs more from the Ii gene of human than

TABLE 1
Comparison of DR$\alpha$ Amino Acid Residues of Pockets Involved in Binding CLIP

| | DR$\alpha$ amino acid residues making pockets | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | | | 3 | | | | 4 | | | 6 | 7 | | 9 | | |
| | 32* | 43 | 53 | 54 | 22 | 54 | 55 | 62 | 62 | 65 | 69 | 62 | 65 | 69 | 69 | 73 | 76 |
| Human | F | W | S | F | F | F | E | N | N | V | N | N | V | N | N | M | R |
| Bovine | F | W | S | F | F | F | E | N | N | V | N | N | V | N | N | M | R |

* The number indicates the position of each amino acid of DR$\alpha$ chains from bovine and human.

from the Ii genes of rodent. A comparison of the sequences also revealed that of the 6 amino acids corresponding to CLIP side chains which extended into peptide-binding pockets, 1, 3, 4, 6, 7 and 9, five residues (positions 91, 93, 94, 96 and 99) are conserved among bovine, human, mouse and rat homologues, as indicated by the box. In addition, all of 3 amino acids of CLIP at positions 83, 86 and 87, which increase CLIP rapid dissociation from the HLA-DR molecule proposed in the model of Kropshofer and co-workers (29), were found in the bovine. Thus, since the CLIP sequences of bovine Ii chain genes show evidence of strong conservation among the bovine, human, mouse and rat homologues, it seems likely that the NI3 cDNA clone encodes a functional CLIP which might perform an important function, as previously established in studies in human and mouse.

*Interaction between CLIP and DR Molecule*

On the basis of amino acid sequences of *HLA-DR* (30) and *BoLA-DR* (16), the formation of hydrogen bonds between CLIP and DR molecules are completely conserved in MHC class II residues (positions $\alpha$9, $\alpha$53, $\alpha$62, $\alpha$69 and $\alpha$76 in DR-$\alpha$ chains) of human and bovine, whereas the *HLA-DRA* genes exhibit approximately 80% identity in terms of being encoded to *BoLA-DRA* gene (Table 1). Likewise, all of the deduced amino acid sequences of pockets that accommodate the side chains of CLIP in the HLA-DR3 peptide binding site described by Ghosh *et al.* (28) are apparently monomorphic in MHC class II DR-$\alpha$ chains of human and bovine (Table 2). Thus, since the binding interactions of CLIP to DR observed in cattle are remarkably similar to those in human, bovine CLIP residues may bind to MHC class II DR molecules in the same way previously found in human. There is also a strong possibility that CLIP binds to DR molecule in a universal way without relation to species.

As part of an attempt to clarify the mechanism of antigen presentation of class II genes in cattle, we recently confirmed the presence of *BoLA-DMA* and *BoLA-DMB* genes, which are closely related to human and mouse *DM* genes (21). In this study, the presence of bovine Ii gene was also confirmed. The N13 cDNA clone is closely related to human, mouse and rat Ii genes, an observation that supports the hypothesis that the corresponding genes might be expressed and functional. Interestingly, bovine Ii gene contains CLIP show which sequences evidence of strong conservation among the human, mouse and rat homologues. These results suggested that, in the bovine system

TABLE 2
Comparison of DR$\alpha$ Residues Making Hydrogen Bonds between CLIP and DR Molecule

| | Amino acid position | | | | |
|---|---|---|---|---|---|
| Species | 9 | 53 | 62 | 69 | 76 |
| Human | Q | S | N | N | R |
| Bovine | Q | S | N | N | R |

as previously established in studies in mouse and man, the CLIP segment may bind to DR in a way almost identical to that in which antigenic peptides bind DR, and that, bovine DM may be required to remove CLIP from bovine MHC class II molecules in endosomes so that antigenic peptides are able to bind. Thus, informations on sequence and the reverse transcription-polymerase chain reaction (RT-PCR) obtained in our previous observations confirmed that the patterns of expression of conventional class II genes in human and cattle differ dramatically (15-20), whereas bovine CLIP residues may bind to MHC class II DR molecules in the same way show previously in human.

## ACKNOWLEDGMENTS

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