



Short Communication

Molecular characterization of MHC-DRB cDNA in water buffalo (*Bubalus bubalis*)

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Abstract

In the present study, water buffalo MHC (*Bubu*)-DRB cDNA was cloned and characterized. The 1022 base long-amplified cDNA product encompassed a single open reading frame of 801 bases that coded for 266 amino acids. The *Bubu*-DRB sequence showed maximum homology with the *BoLA-DRB3*0101* allele of cattle. A total of seven amino acid residues were found to be unique for the *Bubu*-DRB sequence. The majority of amino acid substitutions was observed in the β_1 domain. Residues associated with important functions were mostly conserved. Water buffalo DRB was phylogenetically closer to goat DRB*A.

Key words: water buffalo, cDNA, DRB, MHC, phylogeny.

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The major histocompatibility complex (MHC) plays a central role in the processing and presentation of antigens, and in discriminating between self and non-self. MHC class II molecules, after selectively binding different peptides derived from exogenous pathogens, present them to helper T cells (Brown *et al.*, 1993). MHC class II molecules, located primarily on the surface of antigen-presenting cells, are heterodimer glycoproteins composed of two polymorphic transmembrane polypeptide chains, termed α and β . The DRB genes under the DR region of the MHC complex encode for the highly polymorphic β -chain (Ellis and Ballingall, 1999). Three bovine DRB genes have been reported. Of these, DRB3 appears to be highly expressed, DRB1 is a pseudogene, and DRB2 is transcribed at a very low level in lymphocyte tissues (Lewin *et al.*, 1999). An extremely polymorphic second exon of the DRB gene encodes those peptide binding sites (PBS) responsible for antigen binding (Rask *et al.*, 1985). Currently, more than 100 alleles of the DRB3 gene have been described (BoLA

Nomenclature, ISAG; EMBL-EBI) in *Bos taurus* and *Bos indicus* cattle. Importantly, besides its association with several diseases, polymorphism at the DRB exon 2 region is also considered a potential candidate-marker gene.

Riverine buffalo (*Bubalus bubalis*), well adapted to a hot and humid tropical climate, seems to tolerate many common infectious diseases. Although already mapped on chromosome 2 (Rodrigues *et al.*, 2008), water-buffalo MHC has been little studied, with the scarcity of data regarding diversity (De *et al.*, 2002, Sena *et al.*, 2003, Niranjana *et al.*, 2009). Herein, DRB genes expressed in water buffalo were cloned and characterized.

Venous blood was collected from a randomly selected and healthy Indian Murrah water buffalo. Total RNA was extracted from a lymphocyte culture with total RNA minipreps super kit (Biogene, USA). RT-PCR was carried out with a RevertAid first strand cDNA kit (MBI Fermentas). Both strands of DRB cDNA were produced by PCR, using Forward - 5'-GACTTGCCTGCTCCTCTCAC TCTC-3' and Reverse - 5'-AGGGTGGGGGTGACTGAG AAAAC-3' primers, with design based on the cattle DRB3 sequence (Accession No. AY125893). PCR amplification was in a total volume of 25 μ L with 6 μ L of first strand cDNA, 1x PCR buffer, 1.5 mM of MgCl₂, 200 μ M of each dNTPs, 30 pmol of each primer and 1 unit of Taq DNA polymerase. The polymerase chain reaction (PCR) was carried out in a thermal cycler (DNA Engine PTC200, M.J. Research) under the following conditions: initial denaturation at 94 °C for 3 min, followed by 35 cycles of 94 °C for 1 min, 60 °C for 1 min and 72 °C for 1.5 min, and a final extension at 72 °C for 10 min. The amplified cDNA product

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showed 22, 26, 57 and 51 residue-differences with those of sheep, goats, pigs and humans, respectively. As expected, most of the substitutions were observed in the β_1 domain when compared across species. Alleles at the *BoLA-DRB3* locus gave evidence of over-dominant selection, with the peptide binding site (PBS) region presenting a higher proportion of mutations compared to non-PBS regions (Sena *et al.*, 2003).

169 residues (SP-13, β_1 -49, β_2 -70, CP-8, TM-15 and CY-14) were noted to be completely conserved across the species. Among these, 49 were found to be specific to ruminants, but not to pig and human. Out of the 24 residues associated with ABS (Brown *et al.*, 1993), in water buffalo, 17 were found to be conserved, and those at positions 40, 42, 61, 66, 100, 107 and 115 to be different. Valine (115), essential in creating a deep pocket at PBS surrounded by predominantly nonpolar residues of both the α_1 and β_1 domains, was found substituted by phenylalanine in water buffalo. Interspecies variability of this residue is great. The dominant 'anchoring' peptide residue binds to the hydrophobic pocket of Phe (115), along with α_{26} and α_{31} . One of the residues (84) associated with CD4 binding (Brown *et al.*, 1988), was found to be substituted in water buffalo *DRB*. Highly conserved Asn (111), which supposedly forms the hydrogen bond through its γ amide group with the main chain peptide, was invariant in water buffalo. Mutation at this position affects intracellular transport and cell-surface expression of class II molecules in mice. Similarly, the residue Trp (90), apparently conserved across species, is placed in such a way that its indole nitrogen is capable of donating a hydrogen bond to the main chain carbonyl group of a bound peptide, in exact homology to the location and function of Trp (147) of class I. Polymorphic residue Asp (86) forms a salt bridge with a conserved Arg (76) of the α chain under the existing peptide (Nydham *et al.*, 1998). This residue (β_{57}) of *HLA-DQ* has been found associated with insulin-dependent diabetes mellitus (IDDM) in humans (Brown *et al.*, 1993). In addition to any possible effect on peptide binding, polymorphism at this residue may affect either the stability or structure of the $\alpha\beta$ heterodimer, since nearby residues Glu (81) and Arg (84) form part of the interface of the dimer (Brown *et al.*, 1988).

Two salt bridges from Glu (81) to Arg (84) on the neighboring molecule are conserved in *HLA-DR1*, which forms the first of four interfaces between DR1 molecules in dimers. However, residue Arg (84) was found to be substituted by Gln in water buffalo. Three more salt bridges, *i.e.*, His (140), Glu (191) and His (141) of the β_2 domain, with the α_2 domain residues Glu (158), His (177) and Glu (179), respectively, were found to be conserved across the species. The T-cell coreceptor molecule, CD4, is thought to bind to the residues, Gln (139), Glu (166), Ala (169), Gly (170) and Val (171). With the exception of residue Ala (169) in humans (Ala \rightarrow Thr), total conservation across

species was observed in these positions. Apart from a partial affinity for Gln (139), residues Gly (170) and Val (171) are very important for CD4 binding.

In the mechanism by which MHC molecules tightly bind several peptides, the role of clusters of conserved residues in the class II molecules, Trp (91) (except Arg in pig) and Asn (111) (along with α_{62} Asn, α_{69} Asn and α_{76} Arg), are spaced so as to bind at intervals to the extended main chain of bound peptides, thereby providing an independent peptide side chain component to the affinity (Brown *et al.*, 1988). The disulphide linked Cys residues at positions 44 and 108 of the β_1 domain, and at positions 146 and 202 of the β_2 , were conserved across species. The N-glycosylation region, or the -CHO site at residue positions 48-50 (Asn-Gly-Thr), was completely conserved in all the species compared.

Phylogenetic analysis based on complete cDNA using the Neighbor-Joining method (Nucleotide: Kimura 2-parameter model), revealed the gathering of several ruminant *DRB* alleles into a single group (Figure 2). The clustering of different ruminant sequences along with those of water buffalo seems to have arisen from trans-species evolution, a feature common to MHC genes, where the clusters had possibly ensued from primordial sequences in a common ancestor (Klein, 1986). Similarly, the sharing of common types of pathogens among the ruminants is also indicated (Hickford *et al.*, 2004). Nevertheless, *Bubu-DRB* seems to be closer to the goat *DRB* sequence than to those of cattle. Evolutionary divergence of *Bubu-DRB* based on base substitution per site was the lowest (0.036) with *BoLA-DRB3*0101* (0.036) and the highest (0.139) in pig (Table 1).

Thus, it is apparent that *Bubu-DRB* is expressed, and that there is considerable similarity with other ruminant

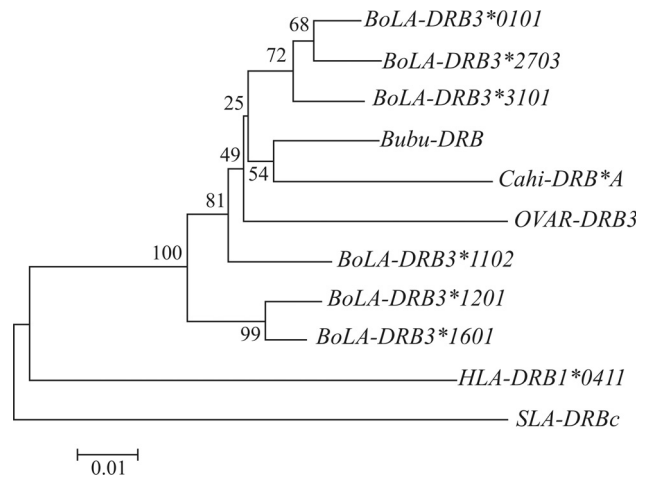


Figure 2 - Phylogenetic tree, compiled by using the Neighbor-Joining method (p-distance model: Kimura 2-parameter method) based on cDNA sequences of *DRB* genes from different species (as described in Figure 1). Branch lengths are in the same units as those used for evolutionary distances. The number at each branch-point indicates the bootstrap (500) confidence level.

Table 1 - Evolutionary divergence of the buffalo (*Bubu*)-*DRB* cDNA sequence from those of other species (base substitution/site) computed by the bootstrap procedure (500 replicates), using the Kimura 2-parameter method, based on the nucleotide model in the MEGA 4 programme (Tamura *et al.*, 2007). Accession No(s). for the *DRB* sequences from different species are described in Figure 1.

Sequence	Base substitution/site
<i>BoLA-DRB3*0101</i>	0.036 ± 0.007
<i>BoLA-DRB3*1102</i>	0.044 ± 0.008
<i>BoLA-DRB3*1201</i>	0.050 ± 0.008
<i>BoLA-DRB3*1601</i>	0.052 ± 0.008
<i>BoLA-DRB3*2703</i>	0.039 ± 0.007
<i>BoLA-DRB3*3101</i>	0.047 ± 0.008
<i>OVAR-DRB3</i>	0.065 ± 0.009
<i>Cahi-DRB*A</i>	0.053 ± 0.009
<i>SLA-DRBc</i>	0.139 ± 0.014
<i>HLA-DRB1*0411</i>	0.128 ± 0.014

species, not only as to cDNA sequences, but also functionally important conserved residues. Nevertheless, high genetic variation corresponding to the exon 2 region of cDNA, possibly supplementary to antigen repertoire properties, was revealed.

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Internet Resources

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<http://www.projects.roslin.ac.uk/bola/bolahome.html> (June 25, 2011).
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