ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 5 (1): 58-61, 2006
ISSN 1682-296X

A PCR-RFLP Method for the Analysis of Egyptian Goat MHC Class II DRB Gene

Sahar Ahmed and Othman E. Othman
Department of Cell Biology, National Research Center, Dokki, Egypt

Abstract: This study aimed to analysis the genetic polymorphisms of MHC class II DRB gene in the Egyptian goat using PCR-RFLP method. The amplified fragment with size of 285-bp was digested by two restriction enzymes *Taq*I and *Pst*I. Restriction digestion of PCR product by *Taq*I enzyme represented two digested fragments at 122- and 163-bp (T restriction pattern) or undigested fragment at 285-bp (t restriction pattern). The frequencies of TT, Tt and tt patterns of MHC class II DRB in the Egyptian goats were 29.5, 61.4 and 9.1%, respectively. After the *Pst*I digestion of the 285-bp PCR products amplified by Egyptian goat DNA, the results showed that the frequency of pp pattern (270- and 15-bp restricted fragments) was 29.5% and the frequency of heterozygous Pp pattern (270-, 226-, 44- and 15-bp restricted fragments) was 70.5%, while the PP pattern (226-, 44- and 15-bp restricted fragments) was not displayed in tested goat animals. The results declared that the p pattern (64.8%) is dominant over the P pattern (35.2%) in Egyptian goat.

Key words: Goat, major histocompatibility complex, PCR, RFLP

INTRODUCTION

Cell surface proteins coded for gene families in the Major Histocompatibility Complex (MHC) are classified as class I or class II based on differences in cellular distribution, molecular weight and function. Class I genes of the MHC code for proteins found on almost all nucleated cells of the body. Class II proteins are primarily restricted to the surface of immune cells and are responsible for immune regulation (Andersson *et al.*, 1986). The characteristic feature of some class I and II genes is the very high degree of genetic polymorphism which has been documented in a number of vertebrate species (Klein, 1975; Zinkernagel and Doherty, 1979; Nagy *et al*., 1981).

There are different kinds of MHC class II molecules, where the DQ and DR subtypes are the most polymorphic both in man and domestic species and probably play a major role in the development of MHC restricted immune responses (Amills *et al*., 1996).

In cattle, two DQA genes, two DQB genes, three DRB genes and one DRA gene have been cloned and sequenced (Groenen *et al.*, 1990 and Van der Poel *et al.*, 1990). In the goat, one DRB gene with 22 different sequences has been described (Schwaiger *et al*., 1993). The resemblance between MHC polymorphisms in different species has been reported by Hoang-Xuan *et al.* (1982); Chardon *et al.* (1983) and Cameron *et al.* (1990), which is related to the persistence of short ancestral motifs, convergent evolution and overdominant selection (Klein, 1980, 1987; Hughes and Nei, 1989; Andersson *et al*., 1991).

The present research focused on the analysis of the genetic polymorphisms for MHC class II DRB gene in the Egyptian goat using *Taq*I- and *Pst*I-RFLP method.

MATERIALS AND METHODS

Genomic DNA extraction: Genomic DNA was extracted from whole blood of 44 goat animals by phenol-chloroform method (John *et al*., 1991). Ten ml of blood taken on EDTA was mixed with 25 ml cold sucrose-triton and the volume was completed to 50 ml by autoclaved double distilled water. The nuclear pellet was suspended in lysis buffer with sodium dodecyl sulfate and proteinase K and incubated overnight in a shaking water-bath at 37°C.

Nucleic acids were extracted once with phenol, saturated with Tris-EDTA buffer followed by extraction with phenol-chloroform-isoamyl alcohol (25:24:1) and this was followed by extraction with chloroform-isoamyl alcohol (24:1). To the final aqueous phase, sodium acetate and cold 95% ethanol were added. The tubes were agitated gently to mix the liquids and a fluffy white ball of DNA was formed. The DNA was finally dissolved in an appropriate volume of 1X TE buffer. DNA concentrations were determined and diluted to the working concentration of 50 ng μL^{-1}, which is suitable for polymerase chain reaction.

Corresponding Author: Dr. Sahar Ahmed Department of Cell Biology, National Research Center, Dokki, Egypt
Fax: 202 33370931

Polymerase chain reaction (PCR): The amplification of the second exon of the caprine DRB gene was achieved using primers DRB1.1: TAT CCC GTC TCT GCA GCA CAT TTC and DRB1.2: TCG CCG CTG CAC ACT GAA ACT CTC. A PCR cocktail consists of 1.0 μM upper and lower primers and 0.2 mM dNTPs, 10 mM Tris (pH 9), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01 % gelatin (w/v), 0.1% Triton X-100 and 1.25 units of Taq polymerase. The cocktail was aliquot into tubes with 100 ng DNA of goat. The reaction ran in a Perkin Elmar apparatus. The reaction was cycled for 1 min at 94°C, 1.30 min at 60°C and 2 min at 72°C for 30 cycles.

RFLP and agarose gel electrophoresis: Twenty microliter of PCR product were digested with 10 units of each restriction enzyme used in this study in a final reaction volume 25 μL. The reaction mixture was incubated at 37°C for *Pst*I and at 65°C for *Taq*I in water bath over night. The restricted fragments were analyzed by electrophoresis on 2.5% agarose/1X TBE gel stained with ethidium bromide. The 100 bp ladder was used as molecular size marker.

RESULTS AND DISCUSSION

MHC class II molecules are glycoproteins composed of two non-covalently linked α and β chains. Several domains can be distinguished in each one: two extracellular domains (α_1, α_2 and β_1, β_2), a transmembrane segment and a cytoplasmatic tail (Amills *et al*., 1995). MHC class II molecules are mainly found at the surface of B cells and antigen presenting cells such as macrophages, dendritic cells and Langerhans cells. Their molecules are involved also in antigen presentation to $CD4^+$ T cells which help B cells to produce appropriate immunoglobulins (Andersson, 1990).

The second exon of the caprine MHC class II DRB gene encodes the β1 domain of DR molecule which is in close contact with foreign antigen, displaying a very high degree of polymorphism with 22 different sequences identified (Schwaiger *et al*., 1993). This highly polymorphism correlated the functional ability of MHC II molecules to form complexes with very large array of different antigen derived peptides.

Our PCR product amplified the caprine MHC class II DRB23 allele (Caae DRB23) containing a 285-bp which is very similar to the caprine DRB5 allele with 96.5% nucleotide identify (Amills *et al*., 1995). Caae-DRB5 and caae-DRB23 diverged in five amino acid substitutions that are very polymorphic in goat. Four amino acids substitutions have been found at position 70 (Glu, Ser, Arg and Asp), three at position 71 (Arg, Ser and Lys), two at position 73 (Ala and Thr), three at position 74 (Ala, Glu and Asn) and four at position 78 (Tyr, Val, Cys and Phe) (Schwaiger *et al*., 1993).

Restriction analysis of the caprine second exon of MHC class II by *Taq*I enzyme represents two digested fragments at 122- and 163-bp (T restriction pattern) or undigested fragment at 285-bp (t restriction pattern). Also, the presence of the *Taq*I site could be associated with TTC codon (Phe) at position 40 which its absence associated with TAC codon (Tyr) at the same position (Amills *et al*., 1995).

Our results showed that the appearance of the TT homozygous restriction pattern in 13 of 44 tested animals (29.5%), the Tt pattern was displayed in 27 animals (61.4%) and the homozygous tt pattern was found in 4 animals (9.1%) (Fig. 1). This result declared that the T pattern is dominant over the t pattern in the Egyptian goat breeds where the frequency of T pattern was 60.2%, while the frequency of t pattern was 39.8% in tested animals.

Also, the results declared the absence of *Taq*I restriction pattern at position 40 bp, indicating the presence of the TAC codon (Tyr) at this position. Tyr is considered as one of the amino acids play an important role in the HLA-DR molecule, which involved in the formation of the antigen-binding site (Brown *et al*., 1993). In cattle, Tyr substitution at position 78 of the bovine DR molecule has been correlated with a tendency towards susceptibility to persistent lymphocytosis in cattle infected with bovine leukemia virus (Lewin, 1994).

The *Pst*I polymorphism sites expressed at bands 226-, 44- and 15-bp (P restriction pattern) or 270- and 15-bp (p restriction pattern). The *Pst*I site at position

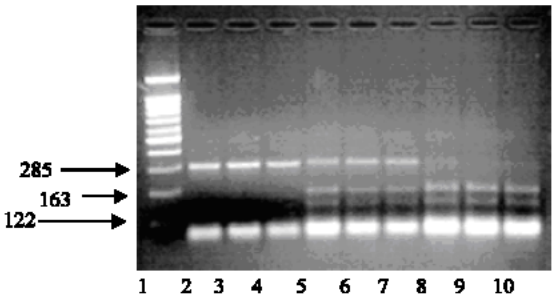

Fig. 1: DNA electrophoretic pattern obtained after digestion of PCR amplified goat MHC class II products with *Taq*I
Lane 1 : 100 bp ladder marker
Lanes 2-4 : tt homozygous genotype showed undigested fragment at 285-bp.
Lanes 5-7 : Tt heterozygous genotype showed three fragments at 285-bp, 163-bp and 122-bp
Lanes 8-10 : TT homozygous genotype showed two digested fragments at 163-bp and 122-bp

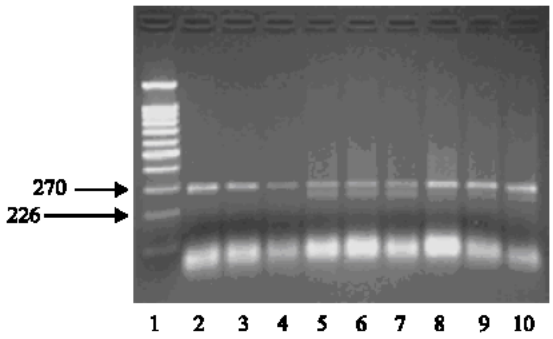

Fig. 2: DNA electrophoretic pattern obtained after digestion of PCR amplified goat MHC class II products with *Pst*I
Lane 1 : 100 bp ladder marker
Lanes 2-4 and 8-10 : pp homozygous genotype showed two fragments at 270-bp and 15-bp (not demonstrated).
Lanes 5-7 : Pp heterozygous genotype showed four fragments at 270-bp, 226-bp, 44-bp (not demonstrated) and 15-bp (not demonstrated).

15-bp reported as a non-polymorphic site. The presence of *Pst*I site could be associated with TTC or TAC codon at position 78, while its absence associated with GTG (Val) or TGT (Cys) codon at the same position (Amills *et al*., 1995).

After the *Pst*I digestion of PCR, we found the appearance of homozyogous pp pattern in 13 animals (29.5%). The heterozygous Pp pattern was displayed in 31 animals (70.5%) whereas PP pattern was not displayed in tested animals (Fig. 2). Present results showed that the frequency of P allele in Egyptian goat was 35.2% and the allele p was predominant in our breeds where its frequency was 64.8%. Also, the result revealed the absence of *Pst*I restriction site at position 78 bp, indicating the presence of GTG (Val) or TGT (Cys) codon at this position.

The genetic polymorphisms of the caprine MHC class II DRB gene were studied in three Spanish breeds (Amills *et al*., 1995). Their frequencies patterns were 65%, (T), 35% (t), 41% (P) and 59% (p). This result nearly close to our results where the pattern frequencies in the Egyptian goats were 60.2, 39.8, 35.2 and 64.8% for T, t, P and p patterns, respectively.

In conclusion, the structural organization and polymorphisms of the caprine MHC provided the possibility for existence of close associations between restriction sites and amino acid substitutions at position with disease resistance. Therefore, improving of the caprine quantitative traits in different breeds require further studies for detecting the polymorphisms of the Antigen-Recognition Site (ARS) of the caprine MHC molecules and their values in disease resistance.

REFERENCES

Amills, M., O. Francino and A. Sanchez, 1995. Nested PCR allows the characterization of *Taq*I and *Pst*I RFLP in the second exon of the caprine MHC class II gene. Vet. Immunol. Immunopathol, 48: 313-321.

Amills, M. O. Francino and A. Sanchez, 1996. A PCR-RFLP typing method for the caprine MHC class II DRB gene. Vet. Immunol. Immunopathol. 55: 255-260.

Andersson, L., 1990. Major Histocompatibility Genes in Cattle and Their Significance for Immune Response and Disease Susceptibility. In: Geldermann, H. and F. Ellendorff, (Ed.). Genome Analysis in Domestic Animals. Vch, Weinheim, Germeny, pp: 213-223.

Andersson, L., J. Bohme, L. Rask and P.A. Peterson, 1986. Genomic hybridization of bovine class II major histocompatibility genes: 1. Extensive polymorphism of DQα and DQβ genes. Anim. Genet., 17: 95-112.

Andersson, L., S. Sigudardottir, C. Borsch and K. Gustafsson, 1991. Evolution of MHC polymorphism: extensive sharing of polymorphic sequence motifs between human and bovine DRB alleles. Immunogenet., 31: 188-193.

Brown, J.H., T.S. Jardetzky, J.C. Gorga, L.J. Stern, R.G. Urban, J.L. Strominger and D.C. Wiley, 1993. Three dimensional structure of the human class II histocompatibility antigen HLA-DR1. Nature, 364: 33-39.

Cameron, P.V., H.T. Tabarias, B. Pulendra, W.R. Robinson and R.L. Dawkins, 1990. Conservation of the central MHC genome: PGFE mapping and RFLP analysis of complement, HSP70 and TNF in the goat. Immunogenet, 31: 253-264.

Chardon, P., J. Kalil, H. Leveziel, J. Colombani and M. Vaiman, 1983. Monoclonal antibodies to HLA recognize monomorphic and polymorphic epitopes on BoLA. Tissue Antigens, 22: 62-71.

Groenen, M.A.M., J.J. Van der Poel, R.J.M. Dijkhof and M.J. Giphart, 1990. The nucleotide sequence of bovine MHC class II DQB and DRB genes. Immunogenet, 31: 37-44.

Hoang-Xuan, M., D. Charron, M.T. Zilber and D. Levy, 1982. Biochemical characterization of class II bovine major histocompatibility complex antigens using cross-species reactive antibodies. Immunogenet, 15: 621-624.

Hughes, A.L. and M. Nei, 1989. Nucleotide substitutions at major histocompatibility complex class II loci: evidence for overdominant selection. Proc. Natl. Acad. Sci. (USA), 86: 958-962.

John, S.W.M., G. Weitzner, R. Rozen and C.R. Scriver, 1991. A rapid procedure for extracting genomic DNA from leukocytes. Nucleic Acid Res., 19: 408-412.

Klein, J., 1975. Biology of the Mouse Histocompatibility-2 Complex. Springer Verlag, Berlin.

Klein, J., 1980. Generation of Diversity at MHC Loci: Implications for T Cell Repertoires. In: Fougereau, M. and D. Dausset (Eds.), Immunology 80. Academic Press, London, pp: 239-253.

Klein, J., 1987. Origin of the major histocompatibility complex polymorphism. The transspecies hypothesis. Hum. Immunol, 19: 155-162.

Lewin, H.A., 1994. Host genetic mechanism of resistance and susceptibility to a bovine retroviral infection. Anim. Biotechnol., 5: 183-191.

Nagy, Z.A., C.N. Boxevanis, N. Ishii and J. Klein, 1981. Ia antigens as restriction molecules in Ir-gene controlled T-cell proliferation. Immunol. Rev., 60: 59-83.

Schwaiger, F.W., E. Weyers, C. Epplen, J. Brun, G. Ruff, A. Crawford and J.T. Epplen, 1993. The paradox of MHC-DRB exon/intron evolution: α-helix and β-sheet encoding regions diverge while hypervariable intronic simple repeats coevolve with β-sheet codons. J. Mol. Evol., 37: 260-272.

Van der Poel, J.J., M.A.M. Groenen, R.J.M. Dijkhof, D. De Ruyter and M.J. Giphart, 1990. The nucleotide sequence of the bovine MHC class II alpha genes: DRA, DQA and DYA. Immunogenet, 31: 29-36.

Zinkernagel, R.M. and P.C. Doherty, 1979. MHC-restricted T cells: studies on the biological role of polymorphic major transplantation antigens determining T-cell restriction-specificity, function and responsiveness. Adv. Immunol., 27: 221-229.