Journal of Genetic Engineering and Biotechnology (2011) **9**, 1–8

Academy of Scientific Research & Technology and
National Research Center, Egypt

Journal of Genetic Engineering and Biotechnology

www.elsevier.com/locate/jgeb

ARTICLE

Identification of Cu/Zn superoxide dismutase in cattle and river buffaloes

Ahlam Ahmed Abou Mossallam

Cell Biology Department, Genetic Engineering and Biotechnology Division, National Research Center, Dokki, Egypt

Available online 15 June 2011

KEYWORDS

Cattle;
Buffalo;
SOD1;
Processed pseudogene

Abstract All air-living organisms produce superoxide dismutase (SOD) and several antioxidant enzymes that limit oxidative stress by detoxifying reactive oxygen species. SOD1 gene has been investigated in Egyptian native cattle and buffalos at the level of genomic DNA and cDNA that were extracted from leucocytes. An unexpected band at approximately 370 bp was obtained in cattle genomic DNA and cDNA as well as in buffalo cDNA. SOD1 amplified sequence of native cattle genomic DNA and cDNA showed a 93% alignment. Native cattle genomic DNA SOD1 amplicon shares sequence homology with mRNAs of *Bos taurus* "similar to superoxide dismutase" (SOD1) sequence of the GeneBank database. It also shares sequence homology with "similar to superoxide dismutase" on *B. taurus* chromosome BTA13. The results indicate that the genomic DNA of Egyptian native cattle contains SOD1 processed pseudo gene. SOD1 primers amplified three fragments in buffalo genomic DNA which indicates that buffalo genome has different copies of SOD1 due to alternative splicing. It failed to produce the 370 bp fragments found in cattle DNA. The protein analysis revealed no differences between Egyptian native cattle and *B. taurus* SOD1 mRNA. However, one amino acid, aspartic acid (Asp), in Egyptian native cattle and *B. taurus* SOD1, is substituted with asparagine (Asn) (D26N) in buffaloes. This amino acid substitution may be due to non-synonymous single nucleotide polymorphisms (nsSNPs). The nsSNPs detected in buffaloes may affect the function of the encoded protein. This study is the first investigation reporting that the resistance of the buffalo to diseases and parasites that afflict cattle may not be acquired but may have a genetic basis.

E-mail address: ahlammasry@yahoo.com

Peer review under National Research Center, Egypt.
doi:10.1016/j.jgeb.2011.05.002

Production and hosting by Elsevier

1. Introduction

The water buffalo is vital to the lives of small farmers; it is economical too for many countries worldwide. Despite their general good health, buffaloes are probably as susceptible as cattle to most infections. However, buffaloes seem to be peculiarly sensitive to a few cattle diseases and resistant to a few others. The reactions to some diseases seem to vary according the region, environment, and breed, but the differences are not well understood [31].

It is well established that oxidative stress is an important cause for cell damage that is associated with the initiation and progression of many diseases. Consequently, all air-living organisms produce antioxidant enzymes that limit oxidative stress by detoxifying reactive oxygen species, including hydrogen peroxide [3,36]. The past decade has brought us new evidence of SOD1 gene involvement in a number of diseases and pathologies that develop due to altered SOD1 activity and reactive oxygen species (ROS) [37]. A cationic and hydrophilic heparin-binding peptide corresponding to the C-terminal region of Cu/Zn superoxide dismutase (SOD-1) exerts antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* and *Candida albicans* [30].

High genetic conservation has been reported between cattle and river buffaloes [11]. The comparison between the genome sequences of the closely related species such as buffaloes and cattle provided an opportunity to search for genes whose exon–intron structure had changed. For closely related species a signature of the molecular mechanism of intron loss or gain should be identifiable, since insufficient time will have passed to obscure the signals through accumulation of random mutations [34].

The mechanism and implications of the changing in the exon intron structure of genes are a poorly understood aspect of genome evolution [34]. Homologous recombination of a reverse transcribed transcript has been previously proposed as a model of intron loss [21], but whether this occurs through direct recombination of the transcript with the locus or through integration of a transcript into the genome to form a pseudogene [10], this will be followed by gene conversion [13]. In addition to intron loss, several molecular mechanisms for intron gain have been proposed. These mechanisms include transposition of introns [25], transposon insertions that mutate to become introns [7], and tandem duplication of sequences where one copy mutates to become an intron [8].

Single nucleotide polymorphisms (SNPs) are an abundant form of genetic variation in every species. SNPs are more stable than other genetic markers. They are distributed throughout the genome and in the genome of *Bos taurus*, one putative SNP was detected approximately every 716 bp [22] making them the most common form of genetic variation. Galloway et al. [16] reported that SNPs are extremely useful for association studies, gene mapping and phylogenetic studies. In species of agricultural importance, SNPs can be used to identify regions influencing important economic traits. SNPs can be identified in coding regions (cSNPs) or non-coding regions. A subset of SNPs in coding regions give rise to variation in the amino acid sequence of the encoded protein and are known as non-synonymous SNPs (nsSNPs). Such SNPs have been reported to occur less frequently than synonymous SNPs, presumably due to evolutionary constraints as selection eliminates deleterious substitutions from the population [6].

In the course of studying the natural immunity of the native cattle, buffaloes and other rural ruminants in Egypt, SOD1 gene was one of the genes under investigation.

2. Materials and methods

Five blood samples of each Egyptian native cattle and river buffaloes were collected in ethylene diamine tetra acetic acid (EDTA). Genomic DNA was extracted from leucocytes following the established protocols [4], modified by Shih and Weinberg [33]. Total RNA from blood was extracted using TRIFAST reagent [19]. First Strand cDNA synthesis has been conducted using READY TO GO YOU prime-First Strand Beads kit (Amersham).

The PCR primers were selected in such a way that the 5′ and the 3′ primers span different exons, so that the amplification product obtained from the cDNA would be of different length from that obtained from genomic DNA comprising with intronic sequences. The SOD1 primers were 5-GTG CTG AAG GGC GAC G-3 for sense and 5-TTT CCA CCT TTG CCC AAG-3 for antisense [26]. The primers were synthesized by Amersham Pharmacia Biotech. The PCR amplification was carried out at 94 °C for 1 min, 58 °C for 2 min, and 72 °C for 2 min and repeated in 35 cycles. The PCR and RT-PCR products of the five blood samples of each Egyptian native cattle and river buffaloes were analyzed on a 1.5 agarose gels [24]. The gels were examined with a UV lamp at a wave length 312 nm. The gels were photographed using an Mp4 plus Polaroid Camera.

The internal sequencing of all tested SOD1 samples from genomic DNA and cDNA has been performed using ABI PRISM version 3.7. Sequence alignment was carried out using the *Ex*pert *P*rotein *A*nalysis *Sy*stem (CLUSTAL-W) server of the Swiss Institute of Bioinformatics [18] and NCBI-BLASTN 2.2.13 version [2]. Six Frame Translation of Sequence and protein alignment were carried out using http://searchlauncher.bcm.tmc.edu/seq-util/Options/sixframe.html and http://msa.cgb.ki.se/cgibin/msa.cgi, respectively.

3. Results

A pair of primers specific for SOD1 was selected to identify SOD1 in the native cattle and buffalo genomic DNA and cDNA extracted from leucocytes using PCR and RT-PCR techniques, respectively. A single sharp band of 370 bp was obtained in all the five blood samples of the native cattle genomic DNA and cDNA as well as buffalo cDNA, while the buffalo genomic DNA yielded three fragments: 450, 700 and 1200 bp (Fig. 1).

The amplified PCR products (amplicon) obtained from native cattle genomic DNA, cDNA and buffalo cDNA were sequenced, while the comparison between each of the individual five sequences of native cattle genomic DNA, cDNA as well as buffalo cDNA, showed no relevant differences, therefore, one representative sequence was used for multiple alignment.

Sequences of native cattle genomic DNA and cDNA and buffalo cDNA were submitted to the DDBJ/EMBL/GeneBank nucleotide sequence database with Accession Nos. EF471302, EF471304 and EF471303, respectively.

Different segments of SOD1 native cattle genomic DNA sequence showed high homology with two ‘‘similar superoxide dismutases’’ (SOD1) mRNA in *B. taurus*. The segment from 1 to 275 nt shares sequence homology (100%) with *B. taurus* ‘‘similar superoxide dismutase’’ (SOD1) mRNA from 287 to 561 nt ref|XM_584414.3| (LOC507743) (Fig. 2). A second segment from 45 to 271 nt shares sequence homology (98%) with another mRNA of *B. taurus* ‘‘similar superoxide dismutase’’ (SOD1) sequences from 1 to 227 nt of the GeneBank database: Accession Nos. dbj|AB099022.1| and dbj|AB099025.1| (Fig. 3).