COMPARATIVE ANALYSIS OF INTERFERON ALPHA GENE IN SOKOTO GUDALI AND WHITE FULANI CATTLE BREEDS IN NIGERIA

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Abstract

Interferons (IFNs) are multifunctional cytokines that confer antiviral responses in different types of cells. Interferon alpha (IFNα) possesses various biological roles which include the activation of the immune system and modulation of cell differentiation. The physico-chemical properties (Molecular weight, instability index, Grand average of hydropathicity, aliphatic index, total number of positively charged residues (Arg + Lys) and isoelectric point) The IFNα sequences of both Sokoto Gudali (SG) and White Fulani (WF) were carried out after DNA extraction. The results of physico-chemical properties revealed that the IFNα had high molecular weights which classified it as immunogenic in nature. The instability index revealed that the gene is unstable while the negative grand average of hydropathicity (-0.601 to -0.200) values defined it as hydrophilic. Antigenic determinants predicted revealed that it contained four antigenic residues each with propensity greater than 1.0 making it potentially antigenic and might play a crucial role in immunological processes modulating disease pathogenesis.The population genetic diversity revealed that polymorphism within WF IFNα was very high. This study is useful in gaining knowledge of the immunological response of trypanosusceptible cattle breeds of Nigeria.

Key words: Interferonalpha, Sokoto Gudali, White Fulani, physico-chemical

Introduction

The White Fulani breed are found from Lagos to Sokoto, Katsina and Kano States and spread across the Nigerian middle belt while the Sokoto Gudali breed, stereotypically occurs mainly in the North-western part of Nigeria, in reality it is now distributed widely throughout the country (Blench, 1999). Interferons (IFNs) are multifunctional cytokines that confer antiviral responses in many types of cells. In addition, they are involved in antiproliferative responses, tumour suppression, and apoptosis in a wide range of cells. Their biological function include; inhibition of proliferation, induction of differentiation, modulation of immune system, and inhibition of angiogenesis, that is the formation of new blood cells essential for the development of a tumour (Uddin et al., 1999; Brierley and Fish, 2002). The presence of animal trypanosomosis is a major constraint to the introduction of highly productive exotic dairy animals and draught oxen to lowland settlement and resettlement areas for the utilization of large land resources (Getachew, 2005; Cherenet et al., 2006). Trypanosomosis is a serious constraint to livestock production in sub-Saharan Africa. The important species causing the diseases in cattle are T. congolense, T. vivax and T. brucei (Clarkson, 1976; Obidike et al., 2005). Comparative analyses of interferon alpha gene in Sokoto Gudali and White Fulani cattle breeds in this study will contribute to the characterization of the IFNα gene in these breeds which can be used for disease association studies and to provide insights to sub-regions within the cattle Interferons.

Materials and Methods

The sampling of both SG and WF were carried out in the Northern part of Nigeria (Bauchi state) and twenty samples of each breed were used for the study. DNA was extracted from the blood using Quick-Gdna™ Mini-Prep (Zymo Research Corporation, Irvine, CA. USA) as described by the manufacturer protocol. Polymerase chain reaction (PCR) amplification was performed in 20 µl final reaction volume containing equivalent of 20 ng of genomic DNA, 10 nM Tris- HCl, pH 8.3, 1.5 mM MgCl2, 501M KCl, 200 lM each of dNTPs, 40 ng of each of the primers and 1 unit of Taq DNA polymerase (Bioneer, Inc. Alameda, CA USA). The reactions were placed in a C-1000 series thermocycler (Biorad, Hercules, CA, USA). The reaction conditions were as follows: Initial denaturation at 94°C for 4 min followed by 35 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min; and final extension at 72°C for five minutes. Ten microlitres of the PCR products were electrophoresed through 1% agarose gel in 1% TBE (89 mM Tris, 89 mM boric acid 1 mM EDTA) at 90 V for 80 min. along with 10 µl of GENEMate Quanti-Marker 100 bp DNA ladder

(BioExpress, Kaysville, UT,USA). Gels were stained with GelRed. Nucleic Acid Stain (Phenix Research Products Candler, NC, USA) at 5 µl /100 ml of the agarose gel suspension. After electrophoresis, PCR products were visualized on a UV transilluminator and were photographed using an Alphalmager Hp system (Protein Simple, Santa Clara, CA, USA). PCR products were cleaned using the Zymo DNA Clean and Concentrator kit (Zymo Research Corp., Irvine, CA, USA) and directly sequenced using the same PCR primers with an Applied Biosystems Automated 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA), Big DyeTerminator chemistry, and AmpliTaq-FS DNA polymerase. DNA Polymorphism parameters that were considered include; Number of Haplotypes, Haplotype diversity, Nucleotide diversity and Average number of nucleotide differences, Number of polymorphic sites, Singleton variable sites and Parsimony informative site. A total of 108 amino acids of both Sokoto Gudali and White Fulani *IFNα* were analysed respectively. The physico-chemical properties of the *IFNα* from both White Fulani and Sokoto Gudali were determined using the ProtParam tool (Gasteiger *et al.*, 2005) available at http://web.expasy.org/tools/protparam. Antigenic determinants also known as epitope were predicted using the online tool BepiPred 1.0 server (Larsen, *et al.*, 2006) available at http://www.cbs.dtu.dk/services/BepiPred. Polymorphism and Divergence within the sequences of the interferon alpha gene of the Zebu breeds were determined using the DNASP v5 software (Librado and Rozas, 2009).

Results and Discussion

Table 1 shows the Population genetic parameters of *IFNα* in Sokoto Gudali and White Fulani cattle breeds. In Sokoto Gudali *IFNα*, 16 nucleotide sequences were used. The selected region was 1-337 while the number of sites (excluding fixed gaps / missing data) is 335 and the total number of sites (excluding sites with gaps /missing data) is 317. A total of 16 sequences were used to detect the population parameters of White Fulani *IFNα*. The selected region was 1-340 while the number of sites (excluding fixed gaps / missing data) is 339 and the total number of sites (excluding sites with gaps / missing data) is 317. The estimated population genetic parameters in both breeds of cattle showed that the *IFNα* in WF had higher values which implied that genetic diversity is higher in WF *IFNα* compared to that of SG.

The protein molecular weight, isoelectric potential, instability index, aliphatic index and **Grand average of hydropathicity** observed are shown in Table 2. Sokoto Gudali *IFNα* **has an average antigenic propensity of 1.0555** while that of **White Fulani *IFNα* was observed to be 1.0498. The antigenic residues and the positions (4-17, 20-41, 57-78 and 88-103) of both** Sokoto Gudali **and White Fulani are as shown in Table 3.**The molecular weight of *IFNα*for both Sokoto Gudali and White Fulani were higher than 6000Daltons depicting them to be immunogenic in nature (Benjamini *et al.*, 2000). The total number of positively (Arg + Lys) and negatively (Asp + Glu) charged residues of both *IFNα* in Sokoto Gudali and White Fulani showed that the total number of negatively charged residues exceeded the total number of positive charged residues. This possible variation might be due to their isoelectric point in acidic range (Rani *et al,* 2013). This indicates the proteins to be intracellular as intracellular proteins have higher fraction of negatively strand residues. Negatively charged residues like the ones from aspartate and glutamate play a key role in transmembrane ion transport (Lutsenko and Kaplan 1995).

The grand average of hydropathicity of*IFNα* in Sokoto Gudali and White Fulani were all negative values which indicates that the proteins are hydrophilic, that is, interact well with water (Kyte and Doolittle, 1982). The instability index computed for both residues of *IFNα* in Sokoto Gudali and White Fulani classified the proteins as unstable as they were above 40. According to the instability index, these proteins are unstable but their aliphatic index is high enough to say that they are stable. These findings are consistent with earlier researches by Giroux *et al.* (1996) and Rani *et al.* (2013).

Conclusion

The detection of antigenic residues and the physico-chemical properties in the *IFNα* of both Sokoto Gudali and White Fulani cattle breeds classify them as immunogenic in nature and can be useful in gaining knowledge of the immunological response of trypanosusceptible cattle breeds of Nigeria to trypanosomosis and other prevalent diseases.

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Table 1: Population genetic parameters of *IFNα* in Sokoto Gudali and White Fulani

Sequence	H	Hd	Pi	K	S	SVS	PIS
Sokoto Gudali *IFNα*	9	0.867	0.00574	1.808	7	3	4
White Fulani *IFNα*	14	0.983	0.02205	6.833	30	18	12

Number of haplotypes (h), Haplotype (gene) diversity (Hd), Nucleotide diversity (Pi), Average number of nucleotide differences (K), Number of polymorphic sites (S), Singleton variable sites (SVS) and Parsimony informative site (PIS).

Table 2: Physico-chemical properties of both *IFNα* of White Fulani and Sokoto Gudali

Physico-chemical Properties	Sokoto Gudali *IFNα*	White Fulani *IFNα*
Number of amino acids	108	108
Molecular weight	12022.6	11973.5
Theoretical pI (Isoelectric point)	6.54	6.63
Total number of positively charged residues (Arg + Lys)	9	9
Total number of atoms	1691	1677
The N-terminal of the sequence considered	Valine	Glycine
The instability index (II)	67.67	68.13
Aliphatic index	98.52	92.22
Grand average of hydropathicity	0.200	0.290

Table 3: Antigenic determinants in Sokoto Gudali *IFNα* and *IFN*

Sokoto Gudali *IFNα* antigenic residue	Position	White Fulani *IFNα* antigenic residue	Position
QAICSLGCHLPHTH	4-17	HAICSLGCHLPHTH	4-17
ANRRVLMLLQQLRRVSPSSCLQ	20-41	ANRRVLMLLQQLRRVSPSSCLQ	20-41
QLQKAQAISVLHEVTQHTFQLF	57-78	QLQKAQAISVLHEVTQHTFQLF	57-78
DKSLLDKLRAALDQQL	88-103	DKSLLDKLRAALDQQL	88-103