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Characterization and antivirus activities of a novel bovine IFN-omega24

Xiuxin Luo[a], Yongli Guo[a], Jun Bao[b,c], Ying Liu[a], Dong An[a], Bo Ma[a], Mingchun Gao[a,*], Junwei Wang[a,c,*]

[a] Department of Preventive Veterinary Medicine, College of Veterinary Medicine, Northeast Agricultural University, Harbin, Heilongjiang 150030, PR China
[b] College of Animal Science and Technology, Northeast Agricultural University, Harbin, Heilongjiang 150030, PR China
[c] National Food Safety and Nutrition Collaborative Innovation Center, Wuxi, Jiangsu 214122, PR China

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ABSTRACT

A novel bovine interferon-ω (BoIFN-ω) gene, which encodes a protein of 195 amino acids with a 23-amino acid signal peptide, was amplified from bovine liver genomic DNA through PCR and named BoIFN-ω24 according to its position in the bovine genome. In this study, the recombinant protein was expressed in *Escherichia coli* and antiviral or antiproliferation activity was determined in vitro. Results showed that BoIFN-ω24 exhibits high antiviral activity, which can be abrogated using PAb against BoIFN-ω24, and inhibits cell proliferation. BoIFN-ω24 also presents high sensitivity to trypsin and stability at pH 2.0 or 65 °C, which are typical characteristics of type I IFN. This study revealed that BoIFN-ω24 is a potential novel effective therapeutic agent and provided a basis for further research on the BoIFN-ω multigene family.

1. Introduction

Interferons (IFNs) represent a large family of proteins that exhibit antiviral, cell growth regulatory, and immunomodulatory activities (Bonjardim, 2005; Tian et al., 2014). IFNs were initially described by Isaacs and Lindenmann (1987) as an antiviral protein synthesized by cells in response to viral infection. IFNs are a heterogeneous group of secretory proteins and classified into three structurally unrelated types. Type I IFNs include IFN-α, IFN-β, IFN-ω, IFN-τ, and IFN-κ (Cheng et al., 2006; Pitha and Kunzi, 2007), which exert biological effects through the common receptor IFNAR; type II IFN includes only one member, IFN-γ (Roberts et al., 1997); and type III IFNs consist of IFN-λ (IFNL) and interleukin-28/29 (IL-28/29) (Kotenko et al., 2003; Sheppard et al., 2003; Yao et al., 2014).

IFN-ω genes diverged from the IFN-α gene approximately 130 million years ago (Yang et al., 2007); and were thought to be produced predominantly by leukocytes. IFN-ω was first found in humans, and the human IFN-ω family comprises four pseudogenes and one full gene expressed in leukocytes (Adolf et al., 1991). The porcine IFN-ω (PoIFN-ω) family contains four pseudogenes and eight functional genes, among which PoIFN-ω7 is approximately 20 times more active than PoIFN-ω4 (Zhao et al., 2009). More than 13 different subtypes of IFN-ω have been reported in feline, two subtypes in equine, and eight or more subtypes in rabbit (Charlier et al., 1993; Himmler et al., 1986; Yang et al., 2007), whereas none was found in canines and mice (Himmler et al., 1987; Zhao et al., 2009).

BoIFN-ω was first classified as BoIFN-αII but was later recognized as a novel class because its C-terminal contains six additional amino acids compared with BoIFN-α (Capon et al., 1985). BoIFN-ω1 inhibits virus replication in many cell lines and exhibits an antiluteolytic effect in cyclic ewes (Boue et al., 2000; Rodriguez et al., 1998). A newly cloned subclass member of the BoIFN-ω family with unknown biological activities was detected. In this study, we identified and characterized this novel gene and named BoIFN-ω24 according to its position in the bovine genome (Zhao et al., 2009). Biological activities, such as antiviral and antiproliferation, and typical physicochemical characteristics were analyzed. The results showed that the identified gene is a functional member of the BoIFN-ω family.

* Corresponding authors at: College of Veterinary Medicine, Northeast Agricultural University, 59 Mu-cai Street, Harbin 150030, PR China. Tel.: +86 451 55190385; fax: +86 451 55191672.
E-mail addresses: gaomingchun@163.com (M. Gao), jwwang@neau.edu.cn (J. Wang).

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2. Materials and methods

2.1. Cells and viruses

Liver was collected from Holstein cows in a dairy farm in Harbin, Heilongjiang in Northeast China. Vesicular stomatitis virus (VSV) was purchased from the China Institute of Veterinary Drug Control. Madin–Darby bovine kidney (MDBK) cells, baby hamster Syrian kidney cells (BHK-21), porcine kidney cells (PK-15), and Madin–Darby canine kidney (MDCK) cells were obtained from our laboratory. Primary bovine testicular (BT) cells were provided by Dr. Li Yu.

2.2. Cloning of the gene encoding BoIFN-ω24

Genomic DNA was extracted from bovine liver (Sambrook et al., 2012). A pair of degenerate primers (BoIFNWS and BoIFNWA) was designed using bovine genomic DNA containing the BoIFN-ω sequence. Bovine IFN-ω and its signal sequences were amplified from bovine liver genomic DNA through PCR because type I IFNs are encoded by intronless genes (Wonderling et al., 2002). The PCR product was cloned into the pEASY Blunt-T vector (TransGen, Beijing, China) and then sequenced.

2.3. Sequence analysis of BoIFN-ω24

The BoIFN-ω24 DNA sequence was aligned using the DNAStar program (DNASTAR Inc., WI). Signal peptide was predicted with SignalP (http://www.cbs.dtu.dk/services/SignalP/). Putative N- and O-glycosylation sites were predicted with the NetNGlyc and NetOGlyc Websites, respectively (http://www.cbs.dtu.dk/services/NetNGlyc/ and http://www.cbs.dtu.dk/services/NetOGlyc/). Multiple alignments and phylogenetic tree were constructed with ClustalX and MEGA5.0 using UPGMA method, and the availability of the branch stem was verified with 500 bootstrap replicates.

2.4. Protein expression of BoIFN-ω24

pET30a (Invitrogen Corporation, CA, USA) was used as the expression vector. To obtain the BoIFN-ω24 protein, we amplified the mature peptide with the following primers: forward primer (BoIFN-ω24S) containing a *Bam*H I site and reverse primer (BoIFN-ω24A) containing a *Xho* I site. BoIFN-ω24 was cloned into the pET-30a vector between the *Bam*H I and *Xho* I sites to generate pET30a-BoIFN-ω24, which contains an N-terminal His tag for facilitating protein purification (Table 1).

The recombinant protein was expressed through transformation of the recombinant plasmid pET30a-BoIFN-ω24 into *Escherichia coli* Rosetta (DE3) Lys cells. The recombinant BoIFN-ω24 protein was induced with isopropyl β-D-thiogalactoside (Sigma, USA) until the optical density at 600 nm (OD_{600}) = 0.4–0.6. The expression products were analyzed through SDS-PAGE. A bacterial pellet was collected through centrifugation, and inclusion bodies containing the BoIFN-ω24 protein were solubilized in 8 M urea. The recombinant protein was purified using a nickel-chelated column (GenScript, Nanjing, China) according to the manufacturer's instructions. The purified protein was renatured through dialysis with TGE (50 mM Tris–HCl, 0.5 mM EDTA, 50 mM NaCl, 5% glycerinum, pH 8.0) under a urea gradient to reduce the concentration from 6 M to 0. After denaturation and renaturation, soluble homogeneous protein was obtained and biological activity was measured.

Table 1
Sequences of the primers.

Primer name	Primer sequences (5′–3′)
BoIFNWS	GATCCCTGGGCTGTGACYTGTCT
BoIFNWA	CTTCTCTTK CAGGTAGAYATGGAT
BoIFN-ω24S	TCGGGATCCTGTGACCTGTCTCAGAACCA
BoIFN-ω24A	AGGCTCGAGTCAAGGTGAGTTCAGATCTC

Note: the restriction enzyme sites that were introduced in primers are underlined.

2.5. Production of rabbit antiserum against BoIFN-ω24

New Zealand white (NZW) rabbits were immunized subcutaneously with 0.1 mg of the purified BoIFN-ω24 protein emulsified with 50% Freund's complete adjuvant (Sigma, USA). After 21 d, the rabbits were boosted twice every 14 d. After 10 d, antiserum against BoIFN-ω24 (PAb) was collected from the rabbit ear vessels.

2.6. Antiviral activity of BoIFN-ω24 in vitro

VSV titers were determined using an endpoint dilution assay, and the titers were expressed as the tissue culture infectious dose 50 ($TCID_{50}$) per milliliter by using the Reed–Muench method.

Antiviral activity was determined with a standard cytopathic effect assay (Rubinstein et al., 1981), which is similar to the previously described method (Guo et al., 2015; Rodriguez et al., 1998). Briefly, the monolayers of MDBK, MDCK, PK-15, BHK-21, or BT cells were seeded in 96-well plates, treated with 100 μL of four-fold serial dilutions of BoIFN-ω24 for 24 h, and challenged with VSV (100$TCID_{50}$/well). The capacity of BoIFN-ω24 to inhibit cytopathic effect was determined. Wells without viruses were designated as cell controls, and wells without IFN were used as virus controls. The plate was then incubated at 37 °C in a humidified 5% CO_2 atmosphere for 18–24 h. Antiviral activity was expressed in international units (IU). One antiviral activity unit is defined as a 50% reduction in destruction of the cell monolayer.

For neutralization of the BoIFN-ω24 antiviral activity, MDBK cells were treated with BoIFN-ω24 (100U) neutralized for 24 h by 2-fold serial dilutions of PAb against BoIFN-ω24 for 2 h at 37 °C. Then, all cells were challenged with 100$TCID_{50}$ of VSV. The preimmune rabbit serum was used as negative control.

2.7. Detection of primary physicochemical characteristics of BoIFN-ω24

2.7.1. Trypsin sensitivity assay of BoIFN-ω24

BoIFN-ω24 was combined with 1% trypsin to a final concentration of 0.25% and then placed in a water bath for 1 h at 37 °C. Antiviral activity was determined using the MDBK/VSV system and then compared between treated and untreated samples.

2.7.2. Stability assay of BoIFN-ω24 at pH 2

BoIFN-ω24 was added with HCl to obtain pH 2.0 for 24 h at 4 °C and then reverted to the original pH (7.0), as previously described (Cheng et al., 2006; Zhao et al., 2009). Antiviral activity was determined with the MDBK/VSV system and then compared between treated and untreated samples.

2.7.3. Temperature sensitivity assay of BoIFN-ω24

BoIFN-ω24 was placed in water bath at 42 °C, 56 °C, and 63 °C for 4 h and then rapidly cooled in an icebox. Antiviral activity was determined with the MDBK/VSV system.

2.8. Antiproliferative activity

MDBK cells were cultured in 96-well plates with DMEM supplemented with 10% FBS. After incubation for 24 h, the cells were treated with various concentrations of BoIFN-ω24 or BoIFN-α for 72 h at 37 °C. Briefly, 10 μL of MTT (5 mg/mL) was added to each