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Comparative analysis of sialidase protein in velogenic and lentogenic strains of Newcastle disease virus

M. ESMAELIZAD, M.P. ASHTIANI

Biotechnology Department, Central Laboratory, Razi Vaccine and Serum Research Institute, Karaj, Alborz, Iran

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Summary. – The sialidase protein is a major part of hemagglutinin-neuraminidase (HN) protein of Newcastle disease virus (NDV), which is an important multifunctional envelope protein. This protein plays key roles in virus attachment to cells and virus-cell fusion. In this study, we compared the sialidase protein of Iranian virulent velogenic field strains with that of avirulent lentogenic strains. Six of seventeen variations in amino acid 395, 523, 550 432, 479 and 540 were observed near the catalytic and glycosylation sites in the sialidase protein. The obtained results showed fundamental differences in various biological parameters such as post-translational modification, antigenic index and electrostatic potential of tertiary structure of the sialidase protein. We suggest these six amino acids might play an effective role in the pathogenesis of NDV.

Keywords: Newcastle disease virus; sialidase; pathogenesis

Introduction

Newcastle disease is a highly contagious and lethal viral disease of birds. It is caused by the Newcastle disease virus (NDV), which has been categorized into three main pathotypes depending on the severity of the disease in chickens: the lentogenic, mesogenic and velogenic strains. Lentogenic strains are avirulent and may cause mild respiratory infection. Mesogenic strains are of intermediate virulence. Velogenic strains are highly virulent and cause high mortality. Velogenic strains cause hemorrhagic lesions in the digestive tract (Alexander, 1997). NDV is a member of the family *Paramyxoviridae,* in the order *Mononegavirales* (McGinnes *et al.,* 1995; Lamb *et al.,* 1996; Krishnamurthy *et al*., 1998). The velogenic strains of NDV have also been identified as potential animal pathogens, resulting in vast impacts on agricultural economy. Thus, it is important to understand the role of antigens for pathogenesis of Newcastle disease virus on the amino acids level and to explore technologies for designing alternative safe and efficient vaccines.

The HN protein plays an important role in the attachment of the virus to cells and cleaves sialic acid receptor from viral and cellular surfaces. HN also promotes cell fusion by interacting with the F protein of NDV. Thus, the HN has both hemagglutinating and neuraminidase activity (Lamb *et al.,* 1996). The HN protein is a type II glycoprotein containing six glycosylation sites. Glycosylation of proteins helps in protein folding, biological activities and influences antigenicity and immunogenicity (McGinnes, 1995). In this study, we attempted to compare the sialidase protein between the velogenic isolates from Iran and known lentogenic NDV strains and investigated the amino acid differences that might be involved in pathogenesis.

Materials and Methods

Virus samples. In this study, the 27 virulent NDV samples were isolated from Newcastle disease outbreaks in seven provinces of Iran (Tehran, Khorasan, Kerman Azerbaijan, Qazvin, Qom and

E-mail: m.Esmaelizad@rvsri.Ac.Ir; phone: +(98)2634570038-2266.
Abbreviations: hn = hemagglutinin-neuraminidase; ndv(s) = newcastle disease virus(es); spf = specific pathogen

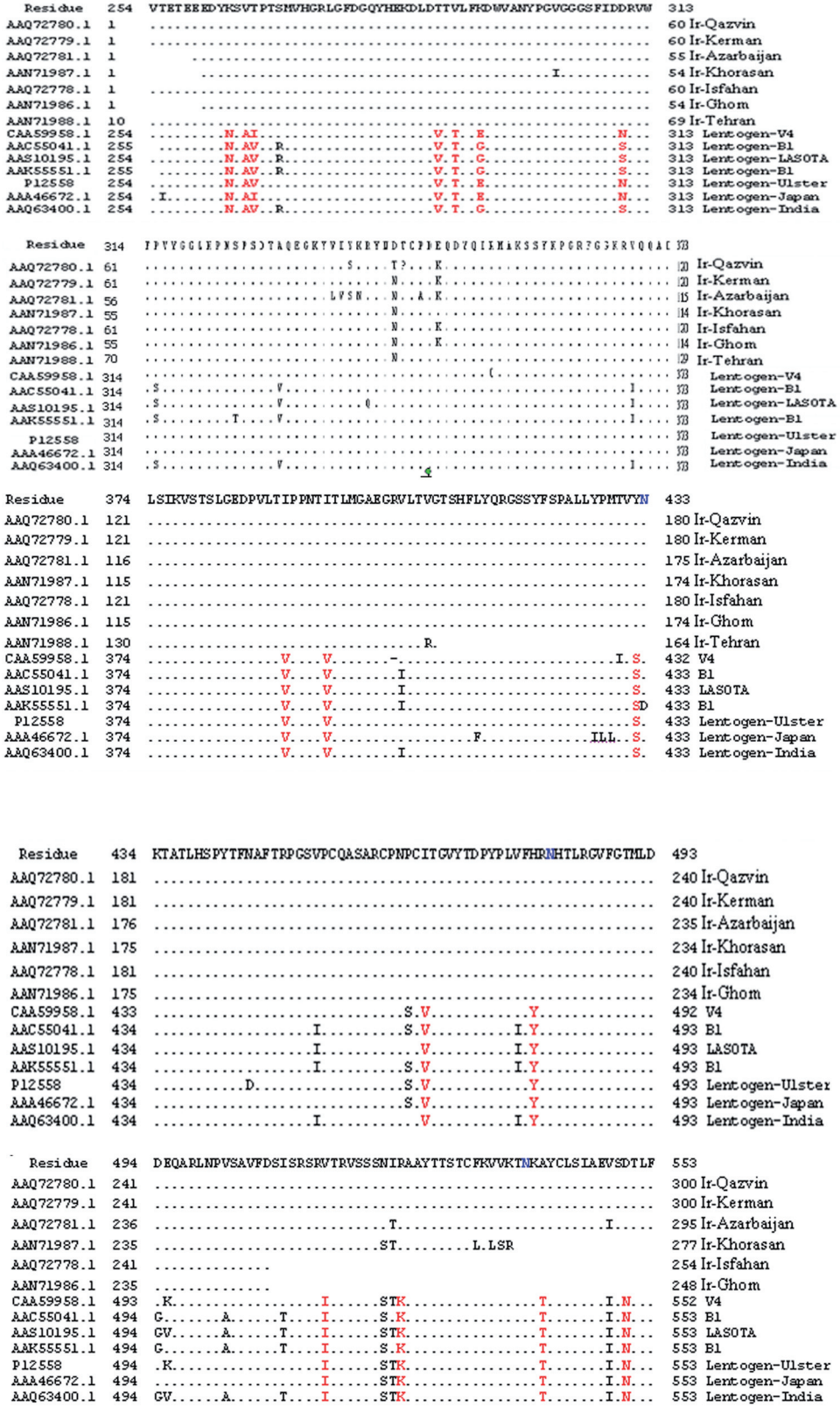

Fig. 1
Sialidase protein sequences of lentogenic and velogenic strains of NDVs

Table 1. Amino acid variations in the sialidase protein between virulent and avirulent NDV

Velogenic	Lentogenic	Residue	Velogenic	Lentogenic	Residue
I(Ile)	V(Val)	395	K(Lys)	N(Asn)	263
Y(Tyr)	S(Ser)	432	V(Val)	A(Ala)	265
I(Ile)	V(Val)	466	T(Thr)	I(Ile) /V(Val)	266
H(His)	Y(Tyr)	479	T(Thr)	V(Val)	288
V(Val)	I(Ile)	514	V(Val)	T(Thr)	290
R(Arg)	K(Lys)	523	K(Lys)	E(Glu) /G(Gly)	293
A(Ala)	T(Thr)	540	D(Asp)	N(Asn) /S(Ser)	310
D(Asp)	N(Asn)	550	N(Asn)/ T(Thr)	D(Asp)	342
			I(Ile)	V(Val)	390

Isfahan). The samples were grown in SPF embryonated chicken eggs (McGinnes and Morrison, 1995).

RNA extraction. Total RNA was extracted from infected allantoic fluid using an RNX reagent (Cinnagen, Iran). Briefly, allantoic fluid (1 ml) was centrifuged at 35,000 rpm at 4°C for 2 hr. The pellet was resuspended in 200 µl of PBS, 400 µl of RNX reagent was added, mixed well and incubated at room temperature for 5 min. RNA was extracted with 0.2 ml chloroform/ isoamylalcohol (24:1). The RNA, in the aqueous solution, was precipitated by adding an equal volume of isopropanol. The mixture was then centrifuged at 10,000 ×g for 20 min. The pellet was washed with 75% ethanol and dissolved in 20 µl of DEPC water.

RT-PCR and sequencing of sialidase. Reverse transcription followed by PCR was performed using specific primers F (5'-aag tct tgc agt gtg agt gc-3') and R (5'-tca tct ttg agg atc tca ac-3') for amplification of 1000 bp fragments of the sialidase sequence. RT-PCR was

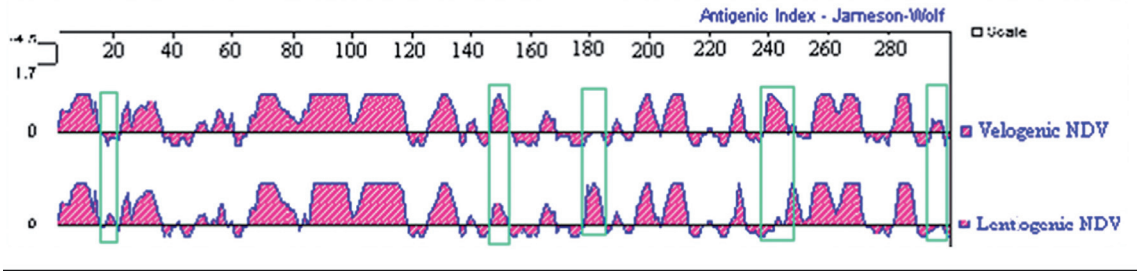

Fig. 2
Antigenic index of the sialidase protein of lentogenic and velogenic NDV strains

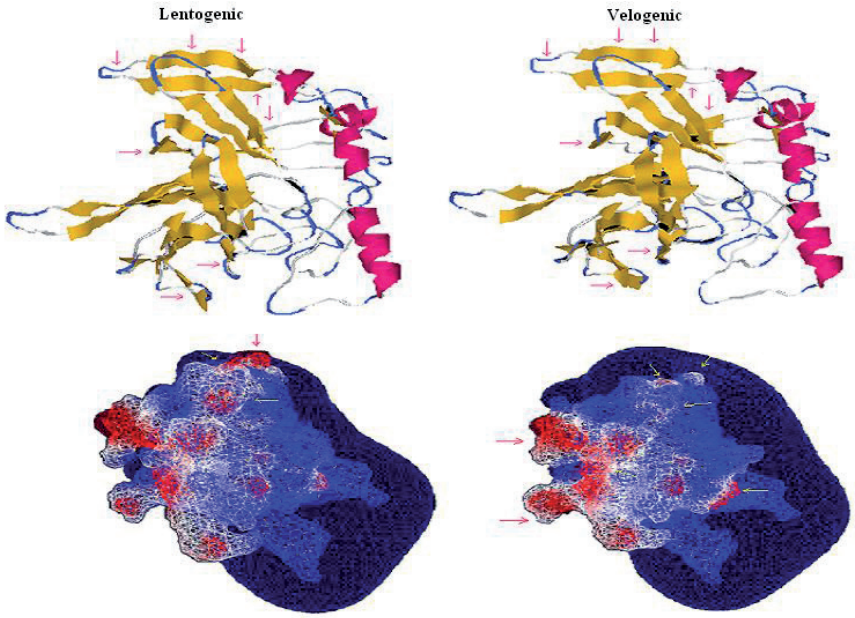

Fig. 3
Tertiary structure of the sialidase protein of velogenic and lentogenic NDV strains
Tertiary structure was predicted by I-TASSER with high score of 1.92. Electrostatic potential of the surface of the sialidase protein was computed by spdbv DeepViewer software in polymorphic residues of the sialidase protein of velogenic and lentogenic strains.

performed in 50 µl reaction mixture containing 10 µl of 5× reaction buffer, 2.5 mmol/l each dNTPs, 1 µl of AMV enzyme (Titan one tube RT-PCR system kit, Roche Diagnostic, Germany), 10 pmol/l each primer, 1 µg total RNA, 2.5 µl DTT (0.1 M), 1.5 mmol/l $MgCl_2$, and 23.5 µl of H_2O. PCR program was 42°C for 1 hr, 94°C for 3 min, 30 cycles of 93°C for 30 sec, 52°C for 30 sec, 72°C for 1 min, followed by 72°C for 5 min. The PCR products were purified using PCR product purification kit (Roche). Sixteen PCR products (16 of 27 isolates) were purified from the 1% agarose gel and sequenced in both directions with forward and reverse primers (MWG Co.). These sequences were analyzed by MegAlign software from DNAstar Package.

Secondary and tertiary structure prediction. Secondary structure of sialidase protein and other biochemical parameters such as antigenic index, hydrophobicity and isoelectric point were compared in velogenic and lentogenic strains of NDVs by DNAstar and DNAsis software. Three-dimensional structure of sialidase protein was predicted using the homology-based method and this structure was viewed by spdbv DeepViewer (Fig. 3). Electrostatic potential was computed and compared between velogenic and lentogenic NDVs.

Results

Based on multiple alignment we identified seventeen amino acid residues in sialidase protein, Asn_{263}→Lys, Ala_{265}→Val, Ile/Val_{266}→Thr, Val_{288}→Thr, Thr_{290}→Val, Glu/Gly_{293}→Lys, Asn/Ser_{310}→Asp, Asp_{342}→Asn/Thr, Val_{390}→Ile, Val_{395}→Ile, Ser_{432}→Tyr, Val_{466}→Ile, Tyr_{479}→H, Ile_{514}→Val, Lys_{523}→Arg, Thr_{540}→Ala and Asn_{550}→Asp, that differed between known lentogenic strains and velogenic NDVs (Iranian velogenic isolates), respectively (Fig. 1).

Ten specific amino acids (Arg in residue 269, Ser in residue 315, Val in residue 329, Ile in residue 369, Ile in residue 404, Ile in residue 453, Ile in residue 477, Gly in residue 494, Ala in residue 502 and Thr in residue 509) were identified in lentogenic strains (Lasota, B1 and other Lentogenic field isolate) but in velogenic NDV these amino acids were: Ser, Pro, Ala, Val, Val, Tyr, Val, Asp, Val and Ile (Fig. 1).

In lentogenic NDVs, one amidation pattern X-G[RK]-[RK] at the position 365–368, four myristoylation sites G-{EDRKHPFYW}=X(2)-[STAGCN]-{P}, nine CK2-glycosylation sites [ST]-(2)-[DE] and one TYR-phosphorylation [RK]-X(2,3)-[DE]-X(2,3) were identified. No differences in numbers and positions of amidation, myristoylation and CK2-phosphorylation sites between velogenic and lentogenic NDVs were observed. But, eight and five PKC-phosphorylation sites were observed in sialidase protein of lentogenic and velogenic NDV strains, respectively. Lentogenic NDVs have three more phosphorylation sites than velogenic isolates at positions 432–434, 509–511, and

Table 2. Posttranslational modification sites in sialidase protein of lentogenic and velogenic NDVs

Velogenic NDV			Lentogenic NDV			Posttranslation modification sites
Pattern	Position	No.	Pattern	Position	No.	
X-G[RK]-[RK]	365-368	1	X-G[RK]-[RK]	365-368	1	AMIDATION
N-{P}-[ST]-{F}	341-344	3	N-{P}-[ST]-{P}	341-344	4	*ASN_GLYCOSYL
	433-436			433-436		
	481-484			481-484		
				538-541		
[ST]-(2)-[DE]	255-258	9	[ST]-(2)-[DE]	255-258	9	CK2_PHOSPHO
	257-260			257-260		
	306-309			306-309		
	324-327			324-327		
	328-331			328-331		
	343-346			343-346		
	381-384			381-384		
	490-493			490-493		
	545-547			545-547		
G-{EDRKHP	301-306	4	G-{EDRKHP	301-306	4	MYRISTYL
FYW}=X(2)-[STAGCN]-{P}	319-324		FYW}=X(2)-[STAGCN]-{P}	319-324		
	451-456			451-456		
	486-491			486-491		
[ST]-X-[RK]	358-360	5	[ST]-X-[RK]	358-360	8	*PKC_PHOSPHO
	375-377			375-377		
	458-460			432-434		
	483-485			458-460		
	537-539			483-485		
				509-511		
				521-523		
				537-539		
[RK]-X(2,3)-[DE]-X(2,3)	274-281	1	[RK]-X(2,3)-[DE]-X(2,3)	274-281	1	TYR_PHOSPHO

521–523. On the other hand, lentogenic NDVs have one glycosylation site more than velogenic isolates in sialidase protein, at the position 538–541.

Discussion

Our results showed different pattern in amino acid sequences of sialidase protein between iranian velogenic isolates and known lentogenic NDV strains (B1, Lasota and V4). Iranian isolates showed low similarity (~82 %) with all vaccine strains and high similarity (~92-95 %) with other velogenic strains in GenBank. The HN protein of NDV is an important multifunctional envelope protein, which plays key roles in virus attachment, neuraminidase and fusion promotion activities. The HN gene of NDV has six glycosylation sites, two of which are not used for addition of carbohydrates. The exact role of the four functional glycosylation sites in NDV pathogenesis is unknown. Amino acid sequence alignment showed 17 amino acid variations in sialidase protein in residues Asn_{263}→Lys, Ala_{265}→Val, Ile/Val_{266}→Thr, Val_{288}→Thr, Thr_{290}→Val, Glu/Gly_{293}→Lys, Asn/Ser_{310}→Asp, D_{342}→N/T, Val_{390}→Ile, Val_{395}→Ile, Ser_{432}→Tyr, Val_{466}→Ile, Tyr_{479}→H, Ile_{514}→Val, Lys_{523}→Arg, Thr_{540}→Ala and Asn_{550}→Asp in lentogenic and velogenic NDVs, respectively. Three of sixteen amino acid variations in residues 432, 479, 549 were located near the three glycosylation sites of the sialidase protein. On the other hand, sialidase protein has seven active catalytic sites at the residues 174, 198, 401, 416, 498, 526 and 547. Interestingly, the three amino acid residues 395, 523 and 550 were located in catalytic sites of sialidase protein.

We have also found differences in the number of PKC-phosphorylation and Asn-glycosylation sites in two groups of NDVs. Lentogenic NDVs have three more phosphorylation sites than velogenic isolates, at positions 432–434, 509–511, and 521–523. Lentogenic also NDVs have one more glycosylation site than velogenic isolates in sialidase protein at the position 538-541.

The seventeen differing amino acids, especially six residues 395, 432, 479, 523, 540 and 550, might have an effect in the pathogenesis of NDV. Previous studies showed that the HN protein along with the F protein is the main target of immune response against NDV (Meulemans *et al.,* 1986, Morgan *et al.*, 1992). A recent report (Zeng *et al.,* 2002) indicated that the HN protein of NDV was responsible for induction of interferon-alpha and tumor necrosis factor. Since HN possesses immunogenic properties, studies on this protein may prove to be useful for the development of NDV vaccines.

The bioinformatic analysis of velogenic and lentogenic strains showed differences in the secondary structure of the sialidase protein and other biochemical properties such as hydrophobicity and isoelectric point. Also, these two groups of NDVs showed different pattern in posttranslational modification sites and electrostatic potential in the tertiary structure of the sialidase protein. These results indicated that the sialidase protein may have an effective role in the pathogenesis due to differences in amino acid sequences between velogenic and lentogenic strains. These results might be useful in designing molecular techniques for differentiation of velogenic and lentogenic isolates based on the differences in the sialidase protein.

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