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VP6 capsid protein of chicken rotavirus strain CH2: Sequence, Phylogeny and In Silico antigenic analyses

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ABSTRACT

The inner capsid protein VP6 of group A rotavirus possesses group and subgroup epitope specificities. Avian rotaviruses have a unique VP6 that is antigenically different from its mammalian counterpart. The lack of information on the VP6 protein of chicken rotavirus strain, CH2, at the genetic and antigenic level was a major motivation for this work. Sequencing of the complete cDNA of the VP6 gene of CH2, revealed a nucleotide (amino acid) identity that varied from 78.3 to 98.5% (86.4–98.2%) when compared with other avian rotaviruses. Regardless of its host origin dissimilarity, CH2 VP6 showed a close sequence homology (97.4–98.2%) with turkey and pigeon rotaviruses. Homology-based modeling of the CH2 VP6 from the corresponding crystal structure of the bovine rotavirus, RF strain, demonstrated that the hypervariable region (residue 228–240) does have a critical role in strain specific antigenic characteristics of avian and mammalian rotaviruses. A predicted conformational epitope encompasses experimentally characterized group and subgroup epitopes suggesting that it is a major antibody binding site on the VP6 protein. The VP6 structure modeling and conformational epitope prediction together with enzyme immuno assay of SG MAbs placed CH2 in SGI/II. The study may be helpful in designing peptides for group A rotavirus diagnostic assays and to achieve heterotypic protection against rotavirus serotypes.

1. Introduction

Rotavirus, a member of the *Reoviridae* family is a major etiologic agent of acute gastroenteritis in a wide range of mammalian and avian species. The virus constitutes a triple-layered icosahedral protein capsid, and a genome made up of 11 segments of double stranded RNA coding for 6 structural (VP1–4, VP6 and VP7) and 5 non-structural (NSP1–5) proteins (Prasad et al., 1988; Estes and Cohen, 1989). VP6, the major structural protein forms the middle layer of the triple layered concentric protein capsid coat (Lopez et al., 1994). It plays a key role in the morphology of the virion, and acts as a physical adaptor between cell entry (outer layer) and genomic RNA packaging (inner layer) (Prasad and Estes, 1997). During cell entry the virions become trancriptionally active double-layered particles in which mRNAs are synthesized and translocated actively through pores in the capsid. The presence of the VP6 layer has thus been associated with transcriptase activity (Bican et al., 1982). Monoclonal antibodies against VP6 were shown to inhibit the transcription process and protect mice from infection (Burns et al., 1996).

While the two outer capsid proteins, VP7 and VP4 elicit neutralizing antibodies and mediate G and P type specificities, the inner capsid protein VP6 carries the group specificity based on which rotaviruses are classified into seven groups (A–G) (Estes, 1996). Four immunodominant sites (32–64, 155–167, 208–274 and 380–397) that contain group epitopes have been identified on group A mammalian rotavirus (strain RF) VP6 protein on the basis of reactivity with MAbs to VP6 and many consecutive heptapeptides synthesized using the Pepscan method (Kohli et al., 1992, 1993). Along with these common antigenic determinants, two distinct non-overlapping antigenic specificities designated as subgroups I and II (SGI and II) have been identified on the VP6 protein of group A rotaviruses (Greenberg et al., 1983). Accordingly, based on the presence or absence of SG antigens, group A rotaviruses are further classified as SGI, SGII, SGI/II and non-SGI/II. It has been proposed that primarily Ala172, Arg296–Asn299, Ala305 and Asn310 contribute to reactivity to SGI MAbs whereas Phe248, Asn305, Ala306, Gln310 and Gln315 are responsible for reactivity to SGII MAbs (Lopez et al., 1994; Tang et al., 1997; Greig et al., 2006).

The diversity of rotaviruses in different hosts is well known. Despite sharing the common group antigen, group A avian rotaviruses carry a unique VP6 which is immunologically distinct

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from that of mammalian (Theil and McCloskey, 1989). Two group A specific antigenic sites I (45–65) and II (132–142) have been delineated on the VP6 protein of the avian rotavirus PO-13 (Ito et al., 1996). Even though several SG MAbs generated from mammalian rotaviruses have failed to recognize group A avian rotaviruses, SGI antigen has been described on avian rotavirus VP6 with the help of MAbs produced from avian rotavirus strain PO-13 (Minamoto et al., 1993). The nucleotide and deduced amino acid sequences of VP6 segment from earlier reported avian rotaviruses (CH1, 02V0002G3, Ty3, Ty1, PO-13, 993/83 and RK3) exhibit sequence homology with mammalian rotaviruses at a lower degree (67.8–70.7 and 69.8–74.6% respectively) than that within themselves (78.1–93.9 and 86.1–98.7% respectively) (Ito et al., 1997). Among the avian group A rotaviruses chicken strains, CH1 and 02V0002G3 have a genetically and antigenically different VP6 protein having more than 13% amino acid differences in comparison with pigeon and turkey VP6 sequences (Rohwedder et al., 1997). Sequence- and MAb-based analyses of the VP6 protein have emphasized that CH1 and 02V0002G3 do not share the group A specific antigenic site I (45–65) with pigeon, turkey and mammalian rotaviruses. However, neither GenBank sequence data nor antigenic annotations are available for VP6 protein of CH2, the G7 prototype strain of chicken origin. With this background the CH2 VP6 gene was sequenced in this study. Further, based on the knowledge that the SG epitopes are conformational and present only in the trimeric form of VP6 (Lopez et al., 1994), a three-dimensional structure of CH2 VP6 protein was predicted to emphasize and explain antigenic characteristics of the protein in relation to mammalian rotaviruses. To assign the SG specificity to CH2 VP6 protein, we tested its reactivity to SG MAbs, and these could indeed confirm our In Silico findings.

2. Methods

2.1. Virus and cell culture

CH2 strain of avian rotavirus used in this study was isolated in chick kidney primary cell line and later adapted to MA104 cells (ATCC, USA) from intestinal contents of diarrheic chicken in Northern Ireland (McNulty et al., 1979). This strain was procured from Poultry Diagnostic and Research Center (PDRC), Venky's India Limited. The virus was pretreated with trypsin (8 μg/ml) and passaged several times in MA104 cell line. The culture grown virus was subjected to Serotec Rota-MA (Japan) ELISA-based on group A specific MAbs and to RNA PAGE to confirm the presence of group A epitopes and RNA migration pattern respectively (Taniguchi et al., 1984; Dolan et al., 1985).

2.2. RT-PCR and sequencing

The sets of primers employed to amplify VP6 gene segment included published (Yolken and Wilde, 1994) and designed sequences as follows:

Primers	Position
5′ GGCTTTTAAACGAAGTCTTC 3′	1–20
5′ TCAACATAATTAGCGTCTAAGTTCA 3′	234–259
5′ GAGAGCAGTTATACCAACAGC 3′	725–745
5′ CCATCAGCTGTTGGTATAACTGC 3′	730–753
5′ GGTCACATCCTCTCACTATACT 3′	1327–1349

The genomic RNA was extracted from 250 μl MA104 cell lysates containing avian rotavirus strain CH2 using Trizol (Invitrogen, USA). Transcription and amplification reactions were carried out as described previously (Wilde et al., 1990). Reverse transcription to cDNA was performed using MMLV expand reverse transcriptase (Roche, USA) at 40 °C for 45 min. Amplification of cDNA was accomplished in 30 cycles with three stepped programme consisting 94 °C for 2 min, 50 °C for 1 min and 72 °C for 1 min. PCR product was visualized on 1.5% agarose gel stained with ethidium bromide under UV illumination.

Amplicons of VP6 gene were purified by mini elute PCR purification kit (Qiagen, USA) and subjected to cycle sequencing using Big Dye Terminator Sequencing kit (Applied Biosystem, USA). Sequences were derived by using ABI PRISM Genetic Analyzer 3100 (USA).

2.3. Alignment and phylogenetic analysis

The VP6 gene and deduced amino acid sequences of CH2 were compared with other group A avian rotavirus VP6 gene and protein sequences available in the GenBank data base. The multiple alignment was generated by Clustal X 1.83 (Chenna et al., 2003). The phylogenetic tree of group A avian rotavirus VP6 genes was constructed using Mega 3.1 (Kumar et al., 2004). The nucleotide sequence of the VP6 gene of CH2 strain of the present study has been deposited in GenBank under the accession no. EF687020.

2.4. Prediction of three-dimensional structure

The three-dimensional structure of the VP6 protein of chicken rotavirus, strain CH2, was predicted by the homology modeling approach. The VP6 protein of group A and SGI bovine rotavirus strain RF having 71% sequence identity was used as a template. The crystallographic atomic coordinates of the template structure (1QHD) solved at 1.95 Å were downloaded from the Protein Data Bank. Initial models were built using Modeller8v1 (Sali and Blundell, 1993). Energy minimization of the molecule using the steepest descents and conjugate gradient algorithms was carried out to remove the geometrical strain. A 13 ns molecular dynamics (MDs) simulation was carried out for a 13-residue segment (Y228–T240) that showed maximum variability at sequence and structure level between the query and template proteins. All MD simulations were done in vacuum using Discover module of InsightII (Accelrys Inc., USA) with the AMBER all atom force field (Cornell et al., 1995). A simulated annealing protocol was adopted wherein the temperature was increased from 300 to 1000 K for a time period of 5 ps followed by a 3 ps equilibration dynamics at 1000 K and subsequent cooling of the system slowly from 1000 to 300 K for 5 ps. The conformers captured every 13 ps were further energy minimized using 500 steps of steepest descent and 1000 steps of conjugate gradient. The structure of the lowest energy conformer after whole molecule unrestrained energy minimization was further validated for satisfaction of stereochemical parameters. Visualization of the molecules and rendering of images was done using Viewer Module of InsightII and Swiss-PdbViewer. Evaluation of the model was done by ProsaII and Procheck (Laskowski et al., 1993). A pseudo-trimer of the template and the model structure was constructed with the transformation matrix of the template using the Swiss-PdbViewer. Antigenic determinants (ADs) and conformational epitopes (CEs) on the trimer models were predicted using an algorithm as implemented in the conformational epitope prediction (CEP) server (Kulkarni et al., 2005).

2.5. Subgroup specific ELISA

To ascertain SG antigen specificity of CH2 VP6, an ELISA was carried out with SG MAbs, SGI (S2–37) and SGII (YO-5) directed to VP6 protein of human rotaviruses S2 (SGI) and YO (SGII) respectively. These MAbs are widely used for SG classification of mammalian rotaviruses in epidemiological studies (Gerna et al., 1990; Urasawa et al., 1990). The procedure for the test and criteria for SG assignment were followed as described earlier (Taniguchi et al., 1984).