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Sequence homology between human and animal rotavirus serotype-specific glycoproteins

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ABSTRACT

The dsRNA gene segment coding for the major outer shell glycoprotein of a human rotavirus (Hu/Australia/5/77, serotype 2) was converted into DNA and cloned into the PstI site of the plasmid pBR322. The cloned gene was sequenced and found to be 1062 bp long with one long open reading frame capable of coding for a protein 326 amino-acids. When this gene sequence was compared to the published sequences of the corresponding genes of two animal rotaviruses, SA11 (simian) and UK (bovine), all three were found to be closely related (74-78%). The predicted amino-acid sequences of the three genes were also highly conserved (75-86%), despite the fact that the three viruses belong to different serotypes.

INTRODUCTION

Rotavirus is now recognized by the World Health Organization as a major cause of infantile gastroenteritis, and a high priority has been placed on control of this disease by the production of a suitable vaccine (1). Cross-neutralization tests indicate four (or possibly five) (2-4) serotypes of human rotavirus and animal studies appear to show little cross-protection between serotypes (5). Thus a potential vaccine may have to incorporate all the known human serotypes. The virus serotype has recently been shown to be determined by the major outer shell glycoprotein (6-10) (a virus surface protein), and the gene segments coding for this protein from a bovine (UK) and a simian (SA11) rotavirus have recently been sequenced (11,12). To date however, no such gene from a human rotavirus has been analysed. We therefore cloned and sequenced the gene encoding this protein from a human rotavirus, Hu/5 (isolated in Melbourne, Australia) belonging to serotype 2.

MATERIALS AND METHODS

Virus growth and purification

The human rotavirus Hu/5 (Hu/Australia/5/77) (13) was grown in MA104

cells and purified as described previously (14).

Cloning rotavirus cDNA

The procedure for producing cDNA from rotavirus dsRNA, and cloning it into the PstI site of the plasmid pBR322 has been described previously by Dyall-Smith et al. (15).

Identification of cloned copies of the major outer shell glycoprotein gene of Hu/5 rotavirus

Since the UK bovine rotavirus gene encoding the major outer shell glycoprotein (gene 8 of this virus) had previously been cloned (11), this was used to screen the Hu/5 clones. To eliminate pBR322 sequences, the UK gene 8 clone was digested with PstI and the insert separated by agarose gel electrophoresis. The insert was then ^{32}P-labelled by nick translation (16) and hybridized to transformant bacterial colonies which had been lysed on nitrocellulose filters (17).

Northern blot analyses

Hu/5 dsRNA was separated on a polyacrylamide gel and immobilized on aminophenylthioether (APT) paper as described previously (7), except that the RNA was loaded right along the tope of the stacking gel (which was not divided into wells). After transfer, the blot was cut (lengthwise) into strips and hybridized to ^{32}P-labelled cDNA or nick translated DNA probes. Labelled cDNA was prepared from Hu/5 segments 7, 8 and 9 dsRNA (isolated by agarose gel electrophoresis) using reverse transcriptase (Life Sciences Inc. U.S.A.) and random primer DNA (prepared from calf thymus DNA) (18). Hybridization conditions were as follows: blots were prehybridized for 15 min at 60°C in 5 x Denhardt's solution containing 10mM HEPES (pH 7.0), 0.1% SDS, 3 x SSC, 10µg/ml E. coli tRNA, and 18µg/ml herring sperm DNA, and then hybridized (18 hr, 65°C) to the appropriate DNA probe. Blots were washed twice for 15 min at 60°C in 0.2 x SSC containing 0.1% SDS, and exposed to x-ray film.

DNA sequencing

The pBR322 clone was digested with PstI, and the insert subcloned into the PstI site of M13 mp8 (19). Sequences were determined from the M13 ssDNA template by the chain termination method (20) using exonuclease III-treated restriction fragments (except the EcoRI/TaqI fragment) as primers (21). A synthetic primer (5'-dGGTCACAT-3'), complementary to the 3' end of the mRNA-sense strand was also used.

Electrophoresis of rotavirus dsRNA

dsRNA was extracted from purified virus preparations using a

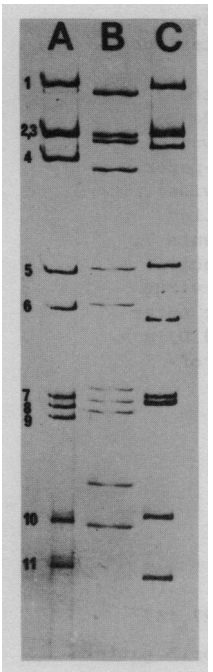

Figure 1. Polyacrylamide gel electrophoresis of rotavirus dsRNA extracted from A, Wa; B, Hu/5; and C, UK viruses. The eleven gene segments of Wa virus have been numbered from largest to smallest.

simplified version of the method of Herring et al. (22). Briefly, 5µl of a purified virus suspension was added to 200µl of 0.1M sodium acetate buffer (pH5.0) containing 1% sodium dodecyl sulphate (SDS) and vortexed for 1 min with an equal volume of 'phenol'/chloroform mixture. The phases were separated by a brief centrifugation (2', 10,000 g) and an aliquot of the aqueous phase (5-20µl) mixed with 20µl of sample buffer (25% (v/v) glycerol, 0.2% bromphenol blue, 0.4M Tris-Cl (pH6.8)) and analysed on a 10% polyacrylamide gel (0.75 mm thick) using the buffer system of Laemmli (23) (but without SDS). The gel was silver stained according to the method of Herring et al. (22), except that the incubation in silver nitrate was for 30 min instead of 2 hr, and sodium borohydride was omitted from the developing solution. Degassing of solutions was also found to be unnecessary.

RESULTS AND DISCUSSION

The rotavirus genome consists of eleven dsRNA segments which upon gel electrophoresis form a characteristic pattern of bands; the virus electropherotype (24). The gel patterns of genomic RNA from the human rotavirus Hu/5 (Hu/Australia/5/77) (13), Wa (25) (human, serotype 1) and UK (26) viruses are shown in Fig. 1, and demonstrate clearly that Hu/5 has a

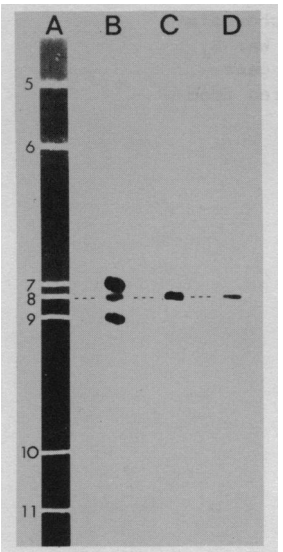

Figure 2. Northern blot hybridizations identifying gene segment 8 of Hu/5 rotavirus as encoding the major outer shell glycoprotein. Track A shows part of the ethidium bromide-stained polyacrylamide gel of Hu/5 dsRNA (only segments 5-11 shown). The RNA bands were transferred to APT-paper and the paper cut into strips (lengthwise). The blots were hybridized to ^{32}P-labelled DNA probes prepared from; B, RNA segments 7, 8 and 9 of Hu/5 virus (to precisely locate these bands); C, a pBR322 clone of UK virus segment 8 (the gene encoding the major outer shell glycoprotein of this virus), and D, a pBR322 clone of the glycoprotein gene of Hu/5 virus.

"short" pattern (due to the positions of segments 10 and 11) (27,14) compared to the "long" gel patterns of the other two. The "short" pattern has previously been associated with serotype 2 human rotaviruses (27-29), and when the Hu/5 virus was serotyped in this laboratory according to the method of Thouless et al. (30) (using typing antisera kindly supplied by M. Thouless and Wa, S2 (31) and SA11 (32) viruses as serotype 1, 2 and 3 reference strains) (4,33) it was indeed found to belong to serotype 2 (data not shown).

Hu/5 genomic dsRNA was converted into DNA and cloned into the PstI site of pBR322 as described previously for UK rotavirus (15). Clones of the major outer shell glycoprotein were identified using a probe (^{32}P-labelled by nick translation) prepared from a cloned glycoprotein gene from UK bovine rotavirus (11). The identity of one of these clones was confirmed by Northern blot analyses which also mapped this gene to segment 8 of Hu/5 rotavirus (Fig. 2). This clone was sequenced according to the strategy shown in Fig. 3 and the full sequence is shown in Fig. 4. The clone is a full-length copy of the glycoprotein gene since a) it is the same length (i.e. 1062 bp) as the corresponding UK and SA11 genes, and b) it has the characteristic conserved 5' and 3' terminal sequences (34,35). It has one open reading frame (the other frames contain multiple stop codons) capable of coding for a protein of 326 amino acids, and 5' and 3' non-coding regions of 48 and 36 bp respectively. In these respects it is identical to

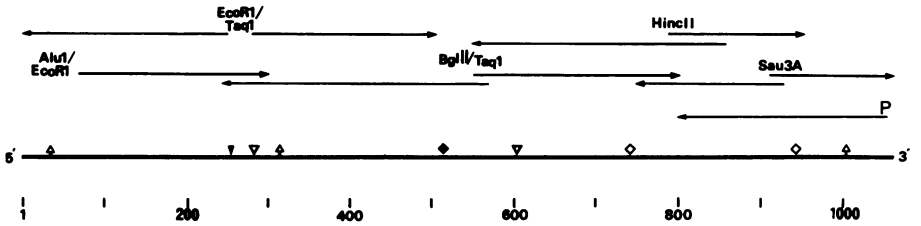

Figure 3. Summary of the sequencing strategy used to determine the nucleotide sequence of the cloned DNA copy of dsRNA gene segment 8 of Hu/5 rotavirus. The number of nucleotides are indicated below the line representing the clone, and the restriction sites used to generate sequencing primers are shown immediately above (⍋ , AluI; ▼ ,EcoRI; ▽ ,TaqI; ◆ , BglII; ◇ , HincII). A synthetic primer (5'-dGGTCACAT-3') complementary to the 3' end of the mRNA-sense strand was also used (primer P). The orientation of the clone is such that the mRNA-sense DNA strand is in the indicated 5' to 3' direction.

UK and SA11 glycoprotein genes (11,12). The base sequence homologies of the Hu/5, SA11 and UK glycoprotein genes are as follows; Hu/5:UK or SA11 = 74% and UK:SA11 = 77.6%. They are obviously closely related.

When the predicted amino-acid sequence of the Hu/5 virus glycoprotein gene was compared to those of UK and SA11 (Fig. 5) an even greater degree of similarity was observed. In pair-wise comparison the amino-acid sequence homologies are; Hu/5:UK = 75.8%, Hu/5:SA11 = 75.2% and UK:SA11 = 85.6%. Studies with UK and SA11 viruses have shown that the glycosylation of these proteins is asparagine-linked and consists of simple ("high mannose") oligosaccharide moieties (36-38). Fig. 5 shows that all three proteins retain a potential glycosylation site (of the type Asn-X- $\begin{matrix}\text{Ser}\\\text{Thr}\end{matrix}$) at residue 69, which for SA11 is the only such site. The Hu/5 and UK proteins also have potential sites at residues 238 (both), 146 (Hu/5) and 318 (UK), however the distribution of carbohydrate in these proteins is not known.

All glycoproteins of eukaryotic cells require a signal sequence for vectorial transport across the endoplasmic reticulum (39). Using the general rules proposed by Perlman and Halvorson (40) a typical signal sequence can be discerned in the first 25 residues of the 3 rotavirus glycoproteins. Their putative hydrophobic core sequences (res. 6-19) are preceded by the charged residue Glu^{-} (res. 5). The likely cleavage sites are after serine at position 15, or after position 25 (Ser/Thr). Recent studies with SA11 virus (41) have demonstrated a cleaved signal sequence for this protein with a molecular weight (1,500MW) consistent with the earlier predicted cleavage site. It is interesting that the first 25

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                                Hu/5 Segment 8      5'-GGCTTTAAAAACGAGAATTTCCGTCTGGCTAGCGGTTAGCTCTTTTTA[48]

ATG TAT GGT ATT GAA TAT ACC ACA ATT CTG ACC ATT TTG ATA TCT ATC ATA TTA TTG AAT TAT ATA TTA AAA ACT[123]
Met Tyr Gly Ile Glu Tyr Thr Thr Ile Leu Thr Ile Leu Ile Ser Ile Ile Leu Leu Asn Tyr Ile Leu Lys Thr
                                                                                                  25

ATA ACT AAT ACG ATG GAC TAC ATA ATT TTC AGG TTT TTA CTA CTC ATT GCT TTA ATA TCA CCA TTT GTA AGG ACA[198]
Ile Thr Asn Thr Met Asp Tyr Ile Ile Phe Arg Phe Leu Leu Leu Ile Ala Leu Ile Ser Pro Phe Val Arg Thr
                                                                                                  50

CAA AAT TAT GGC ATG TAT TTA CCA ATA ACG GGA TCA CTA GAC GCT GTA TAT ACG AAT TCT ACT AGT GGA GAG CCA[273]
Gln Asn Tyr Gly Met Tyr Leu Pro Ile Thr Gly Ser Leu Asp Ala Val Tyr Thr Asn Ser Thr Ser Gly Glu Pro
                                                                                                  75

TTT TTA ACT TCG ACG CTG TGT TTA TAC TAT CCA GCA GAA GCT AAA AAT GAG ATT TCA GAT GAT GAA TGG GAA AAT[348]
Phe Leu Thr Ser Thr Leu Cys Leu Tyr Tyr Pro Ala Glu Ala Lys Asn Glu Ile Ser Asp Asp Glu Trp Glu Asn
                                                                                                 100

ACT TTA TCA CAA TTA TTT TTA ACT AAA GGA TGG CCA ATT GGA TCA GTT TAT TTT AAA GAC TAC AAT GAT ATT AAT[423]
Thr Leu Ser Gln Leu Phe Leu Thr Lys Gly Trp Pro Ile Gly Ser Val Tyr Phe Lys Asp Tyr Asn Asp Ile Asn
                                                                                                 125

ACA TTT TCT GTG AAT CCA CAA CTA TAT TGT GAT TAT AAT GTA GTA TTG ATG AGA TAT GAC AAT ACA TCT GAA TTA[498]
Thr Phe Ser Val Asn Pro Gln Leu Tyr Cys Asp Tyr Asn Val Val Leu Met Arg Tyr Asp Asn Thr Ser Glu Leu
                                                                                                 150

GAT GCA TCA GAG TTA GCA GAT CTT ATA TTG AAT GAA TGG CTG TGC AAT CCT ATG GAT ATA TCG CTT TAC TAT TAT[573]
Asp Ala Ser Glu Leu Ala Asp Leu Ile Leu Asn Glu Trp Leu Cys Asn Pro Met Asp Ile Ser Leu Tyr Tyr Tyr
                                                                                                 175

CAA CAA AGT AGC GAA TCA AAT AAA TGG ATA TCG ATG GGA ACA GAC TGC ACG GTA AAA GTT TGT CCA CTC AAT ACA[648]
Gln Gln Ser Ser Glu Ser Asn Lys Trp Ile Ser Met Gly Thr Asp Cys Thr Val Lys Val Cys Pro Leu Asn Thr
                                                                                                 200

CAA ACC CTA GGG ATT GGA TGC AAA ACT ACG GAT GTA AAC ACA TTT GAG ATT GTT GCG TCG TCT GAA AAA TTA GTA[723]
Gln Thr Leu Gly Ile Gly Cys Lys Thr Thr Asp Val Asn Thr Phe Glu Ile Val Ala Ser Ser Glu Lys Leu Val
                                                                                                 225

ATT ACT GAC GTT GTA AAT GGT GTT AAC CAT AAC ATA AAT ATT TCA ATA AAT ACG TGC ACT ATA CGC AAC TGT AAT[798]
Ile Thr Asp Val Val Asn Gly Val Asn His Asn Ile Asn Ile Ser Ile Asn Thr Cys Thr Ile Arg Asn Cys Asn
                                                                                                 250

AAA TTA GGA CCA CGA GAA AAT GTT GCT ATA ATT CAA GTT GGT GGA CCG AAC GCA TTA GAT ATC ACT GCT GAT CCA[873]
Lys Leu Gly Pro Arg Glu Asn Val Ala Ile Ile Gln Val Gly Gly Pro Asn Ala Leu Asp Ile Thr Ala Asp Pro
                                                                                                 275

ACA ACA GTC CCA CAA GTT CAA AGA ATC ATG CGA ATA AAT TGG AAA AAA TGG TGG CAA GTA TTT TAT ACA GTA GTT[948]
Thr Thr Val Pro Gln Val Gln Arg Ile Met Arg Ile Asn Trp Lys Lys Trp Trp Gln Val Phe Tyr Thr Val Val
                                                                                                 300

GAC TAT ATT AAC CAA GTT ATA CAA GTC ATG TCC AAA CGA TCA AGA TCA TTA GAC GCA GCT GCT TTT TAT TAT AGA[1023]
Asp Tyr Ile Asn Gln Val Ile Gln Val Met Ser Lys Arg Ser Arg Ser Leu Asp Ala Ala Ala Phe Tyr Tyr Arg
                                                                                                 325

ATT TAG ATATAGATTTGGTCAGATTTGTATGATGTGACC-3'[1062]
Ile
326
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Figure 4. Nucleotide sequence and predicted amino-acid sequence of the mRNA-sense DNA strand of the segment 8 clone of Hu-5 rotavirus. In phase termination codons are indicated by solid bars.

residues of all three glycoproteins show relatively greater conservation than the subsequent 25.

While the glycoproteins of Hu/5, UK and SA11 are very similar in amino-acid sequence, they must differ in antigenically significant regions since the three viruses are serotypically different, i.e. Hu/5 is a human serotype 2 virus, UK belongs to a bovine serotype (33), and SA11 although

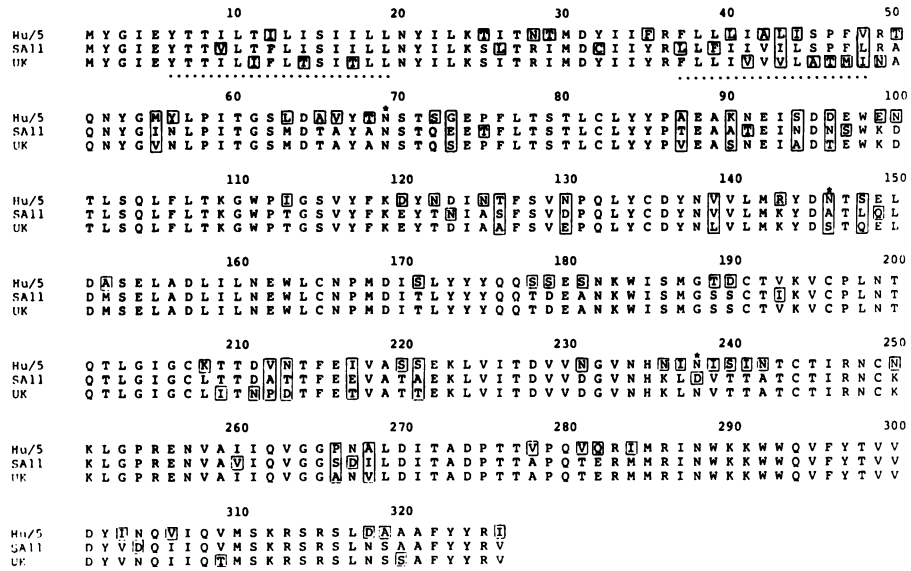

Figure 5. Comparison of the predicted amino-acid sequence of the major outer shell glycoproteins of Hu/5, SA11 and UK rotaviruses. Data for the SA11 and UK protein sequences are from refs. 11 and 12. Amino-acid substitutions are boxed, and where all three amino-acids differ at the same position, all three have been outlined. Asterisks show potential glycosylation sites in the Hu/5 sequence, and dotted lines indicate highly hydrophobic regions near the N-terminus.

of simian origin is serologically human type 3 (33). Results of competition experiments using monoclonal antibodies to SA11 virus have demonstrated only one or possibly two epitopes involved in neutralization (S. Sonza and A. Breschkin, manuscript submitted for publication), and future work in this laboratory is aimed at precisely identifying these regions.

The sequence data (above) support the wealth of serological evidence (42-44) that rotaviruses are a closely related group. Indeed they appear to be much more closely related than the three serotypes of mammalian reovirus, which are structurally and epidemiologically similar to rotaviruses (45). The genes encoding the serotype-specific protein of the three reovirus serotypes are related only to the extent of 1-12% as estimated by nucleic acid hybridization (46). The fact that two simian rotaviruses, SA11 and rhesus (MM18006) are serologically closely related (33) yet were isolated over 20 years apart (47,48) also suggests that rotavirus serotypes are fairly stable antigenically, unlike influenza A

subtypes which show antigenic drift (49). While many more rotavirus glycoprotein genes need to be studied, the limited number of human serotypes so far detected and the apparently low level of antigenic drift look encouraging for the development of human rotavirus vaccines.

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