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Acrylamide Concentration Affects the Relative Position of VP7 Gene of Serotype G2 Strains as Determined by Polyacrylamide Gel Electrophoresis

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Abstract

Background: During the course of development and characterization of various rotavirus reassortants, we found that the relative position of the gene encoding neutralization and protective antigen VP7 of certain rotavirus strains in a PAGE gel was influenced by the concentration of acrylamide.

Objectives: We investigated systematically various factors that affected the relative position of the rotavirus VP7 gene in a PAGE gel.

Study design: We analyzed dsRNAs of selected rotavirus strains bearing G1, G2, G3 or G9 specificity by PAGE at varying concentrations of acrylamide.

Results: We demonstrated that the relative position of the VP7 gene of three G2 strains varied depending upon the concentration of acrylamide in a PAGE gel, which occurred not only in a homologous G2 virus gene background but also in a heterologous G3 virus gene background; and the VP7 gene bearing G1, G3, G4 or G9 specificity did not display this phenomenon when the PAGE running conditions were varied.

Conclusions: The concentration of acrylamide in a PAGE gel was the major factor that influenced the relative position of the VP7 gene of G2 rotavirus strains (i.e., VP7 gene coding assignment by PAGE).

Keywords

rotavirus; PAGE; electropherotype; diarrhea; VP7 gene

1. Introduction

The genome of rotavirus (the single most important etiologic agent of infantile diarrhea worldwide) consists of eleven segments of dsRNA numbered 1-11 in order of increasing mobility on polyacrylamide gels, segment 1 being the slowest and segment 11 the fastest (Rodger and Holmes, 1979). Because neutralizing antibodies appear to play an important role

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in protection against many viral diseases, rotavirus outer capsid proteins VP7 (which defines G type) and VP4 (which defines P type), which induce neutralizing protective antibodies independently, have played a central role in research and development of a rotavirus vaccine (Hoshino and Kapikian, 2000). Due to the segmented nature of the genome, when two different rotavirus strains infect the same cell, individual segments reassort at relatively high frequency resulting in the generation of progeny viruses (reassortants) carrying mixed gene constellations from two parental viruses. This technique (genetic reassortment) has been used extensively in rotavirus studies such as constructing selected reassortant vaccine candidates (Clark et al, 1996; Hoshino et al., 2005; Midthun et al., 1985, 1986). Once desired rotavirus reassortants are constructed, the parental origin of each of the 11 segments in each reassortant needs to be assigned. Since, in general, each rotavirus strain displays a dsRNA migration pattern (electropherotype) on polyacrylamide gels distinct from that of other strains (Kalica et al., 1978; Rodger and Holmes, 1979), analysis of such genomic polymorphism as determined by PAGE has been routinely employed to assign the parental gene origin of a reassortant. Such studies have established that the VP7 capsid protein is encoded by genome segment 7, 8 or 9 depending upon the rotavirus strain. For example, the VP7 gene is encoded by the 7th genome segment of rhesus monkey rotavirus MMU18006 strain in a 12% gel (Broome et al., 1993), the 8th genome segment of human rotavirus DS-1 strain in a 7.5% gel (Greenberg et al., 1983) or the 9th genome segment of human rotavirus Wa strain in a 12% gel (Kalica et al., 1981). Such findings have been confirmed by molecular techniques such as RNA-RNA hybridization assay and gene sequencing (Midthun et al., 1985, 1986).

Although the standard PAGE analysis of rotavirus dsRNA is simple, easy and cost-effective to perform, various factors and running conditions of PAGE have been reported to affect the electropherotype of each strain which include the concentration of acrylamide/bisacrylamide, type of running buffer, the magnitude of running voltage and purity of dsRNA analyzed (Espejo and Puerto, 1984; Laemmli, 1970; Loening, 1969; Raymond and Wang, 1960). However, the influence of such factors on the determination of the gene-protein coding assignment of selected rotavirus genome segment(s) has not been investigated systematically. During the course of development and characterization of various rotavirus reassortants including selected vaccine candidates such as *RotaShield*, we found that the relative position of the VP7 gene of certain rotavirus strains in a PAGE gel was influenced by the concentration of acrylamide. Although we noticed that the concentration of polyacrylamide affected the overall electropherotype of the strain, we focused in the present study on various factors that affected the relative position of the VP7 gene in a PAGE gel.

2. Methods

2.1. Rotavirus strains, cell culture, and genetic reassortment

Table 1 summarizes human and animal rotavirus strains as well as selected single VP7 gene substitution rotavirus reassortants that were employed in this study. Construction and characterization of a tetravalent *RotaShield* vaccine (i.e., D × RRV, DS-1 × RRV, RRV and ST3 × RRV) have been reported previously (Midthun et al., 1985, 1986). *RotaShield* vaccine consists of rhesus rotavirus (RRV) which provides coverage for VP7 serotype 3, and three human-RRV reassortants each with ten RRV genes and a single human rotavirus gene that encodes VP7 serotype 1, 2, or 4 specificity (Midthun et al., 1985, 1986). Reassortants 1076 × RRV and S2 × RRV were constructed and characterized in this study by a procedure described previously (Hoshino et al., 2005). All rotavirus strains employed in the study were plaque purified three times prior to use.

2.2. Rotavirus RNA extraction and PAGE analysis

The standard phenol-chloroform method was used to extract rotavirus genomic dsRNA (Jones et al., 2003). Analysis of rotavirus dsRNA was performed at room temperature (approximately 26°C) in a discontinuous 5%, 7.5%, 10%, 12%, 15%, 17%, 18% or 19% acrylamide resolving slab gel (acrylamide:bisacrylamide 29:1, Bio-Rad Laboratories, Hercules, CA) with a 4.3% acrylamide stacking gel in the buffer system of Laemmli (Laemmli, 1970) without SDS using a SE600 or SE620 gel apparatus (Amersham Biosciences, San Francisco, CA) and Tris-Glycine running buffer (pH 8.3) (Bio-Rad Laboratories). Since the polymerization temperature of acrylamide/bisacrylamide gels has been reported to affect the tertiary structure of the gel thereby influencing electrophoretic mobilities of selected RNA species (Gressel et al., 1975), the polymerization of the PAGE gels used in this study was performed at a single temperature of 37°C in an incubator. In addition, since heat generated during electrophoresis has been reported to affect the mobilities of rotavirus genomic dsRNA (Espejo and Puerto, 1984), a water chiller (Lauda WKL230, Brinkmann Instruments, Westbury, NY) was used to maintain the desired temperature of running buffer especially when evaluating a gel with a high percentage of acrylamide/bisacrylamide or a long gel. After electrophoresis, viral RNA bands were visualized by staining of the gel with silver nitrate (Herring et al., 1982).

3. Results and Discussion

3.1. Concentration of acrylamide was a major factor that affected the relative position of VP7 gene of certain rotavirus strains in a PAGE analysis

First we determined the relative position of the VP7 gene of each of the four *RotaShield* vaccine components and their respective parental virus strains in a 7.5, 10, 12 or 15% conventional (18 × 16 × 0.075 cm) PAGE gel. As shown in Figure 1, the VP7 gene coding assignment for the strain D (G1, 9th gene), reassortant D × RRV (G1, 9th gene), strain MMU18006 (G3, 7th gene), strain ST3 (G4, 9th gene), and reassortant ST3 × RRV (G4, 9th gene) was not affected by varying the concentrations of acrylamide in the gel. Since segments 7, 8 and 9 of reassortant DS-1 × RRV comigrated in the gel regardless of acrylamide concentration used, the VP7 gene of the reassortant was unassignable. Unexpectedly, although the VP7 gene of the G2 virus DS-1 strain was segment 8 in a 7.5% (Fig. 1A) or 10% (not shown) acrylamide gel, and segment 8 or 9 in a 12% gel (not shown), it was in the 9th position in a 15% (Fig. 1B) acrylamide gel. These findings demonstrated that the DS-1 VP7 gene “flipped over” (i.e., the DS-1 VP7 gene shifted to the 9th position from its previous 8th position) in a PAGE gel containing acrylamide concentration between 10 and 15%. Thus, we confirmed the previous finding in which the 8th gene of the DS-1 was reported to be the VP7 gene in a 7.5% acrylamide gel (Greenberg et al., 1983), and demonstrated that the concentration of acrylamide played a critical role in determining the VP7 gene coding assignment of the DS-1 virus. It is noteworthy in Figure 1 that the different running PAGE conditions affected not only the genes contained in the “triplet” (i.e., genes 7, 8 and 9) but also other genes as well. For example, although segment 2 and 3 of the DS-1 virus comigrated in a 7.5% gel, they were well separated in a 15% gel.

3.2. VP7 gene encoding G2 specificity appeared to be affected most by the concentration of acrylamide in a PAGE gel

To study whether the “flip-over” phenomenon was unique to the G2 rotaviruses, we constructed two additional reassortants (1076 × RRV and S2 × RRV) each of which had only the VP7 gene of Swedish G2 strain 1076 (Hoshino et al., 1985) or Japanese G2 strain S2 (Urasawa et al., 1982) and the remaining 10 genes of the RRV (Stuker et al., 1980). The VP7 gene of the 1076 strain was in the 7th position in a 5% (not shown) or 10% (Fig. 2A) gel, the 7th or 8th position in a 12% (not shown) gel, however, it was in the 8th position in a 15% (Fig. 2B) gel. Thus, the VP7 gene of Swedish G2 virus 1076 strain also displayed a “flip-over” phenomenon between 10 and 15% acrylamide concentration.

Previously, the VP7 gene of the S2 strain was assigned to genome segment 8 in a 5% PAGE gel (Herring et al., 1982; Kobayashi et al., 1993). In this study, the VP7 gene of the S2 was in the 8th position in a 5% (not shown), 10% (Fig. 3A) or 15% (not shown) gel, and in the 8th or 9th position in a 17% (not shown) gel. However, it was in the 9th position in an 18% gel (Fig. 3B) indicating that the VP7 gene of Japanese G2 virus S2 strain also displayed a “flip-over” phenomenon between 15 and 18% acrylamide concentration. Thus, these findings clearly demonstrated that the migration in a PAGE gel of the VP7 gene bearing G2 specificity was markedly influenced by the concentration of acrylamide in the gel.

3.3. VP7 gene of G2 virus DS-1 strain displayed a “flip-over” phenomenon not only in a homologous gene background but also in a heterologous gene background

Next we studied whether or not this “flip-over” phenomenon of the G2 VP7 gene could be observed in a heterologous virus gene background by analyzing DS-1 × RRV reassortant which had 10 genes from rhesus rotavirus and only the VP7 gene from human rotavirus DS-1 virus (Midthun et al., 1985). As we observed in Figure 1, in a 7.5% or 15% gel, the VP7 gene of DS-1 × RRV could not be assigned, because segments 7, 8, and 9 were inseparable in such gels. However, in a 5% regular (18 × 16 × 0.075cm) gel, the VP7 gene was shown in position 7 (Fig. 4A) whereas it was in the 9th position in a 5% long (18 × 32 × 0.075cm) gel (Fig. 4B). Thus, these results demonstrated that the VP7 gene of the DS-1 virus exhibited a “flip-over” phenomenon in a heterologous rhesus rotavirus gene background; it was the VP7 gene (not the gene 9 encoding NSP3 protein) of the DS-1 virus that displayed a “flip-over” phenomenon; and the length of PAGE running time in a given acrylamide concentration influenced the relative position of the VP7 gene of G2 DS-1 virus.

3.4. Why VP7 gene of G2 viruses displays a “flip-over” phenomenon

We analyzed by using the GeneQuest program a folding structure of mRNA of the rotavirus VP7 gene belonging to various G types and noticed that the G2 virus VP7 gene was predicted to display a unique large loop structure (Fig. 5) which was not observed in VP7 gene encoding G1, G3-G8, G10-G15 specificity. Interestingly, G9 virus VP7 gene mRNA was also predicted to display a similar large loop structure, which might be responsible for a “flip-over” phenomenon that was observed in G2 virus VP7 gene. In order to test this hypothesis, we analyzed dsRNA of a single VP7 gene substitution reassortant rotavirus 116E × UK (Hoshino et al., 2004) and its two parental rotaviruses, human G9 rotavirus 116E strain (Das et al., 1993) and bovine rotavirus UK strain (Woode et al., 1975), in a 5, 10, 17 or 19% gel. The VP7 gene of the 116E virus was in the 9th position in each of the four different acrylamide concentrations tested in a PAGE gel (data not shown) indicating that the VP7 gene of G9 virus 116E strain did not display the altered mobility phenomenon that was observed on G2 virus VP7 gene. The conformation of dsRNA of rotavirus VP7 gene is unknown, however, dsRNA of the G2 virus VP7 gene may have a unique conformation which could be responsible for an observed “flip-over” phenomenon in a PAGE gel.

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Abbreviations

PAGE, polyacrylamide gel electrophoresis; dsRNA, double-stranded RNA.

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Figure 1.

Electrophoretic migration patterns of each of four components of *RotaShield*TM and their parental rotavirus strains in 7.5% (A) and 15% (B) PAGE gels. Lane 1, D strain; lane 2, D × RRV; lane 3, RRV; lane 4, DS-1 strain; lane 5, DS-1 × RRV; lane 6, RRV; lane 7, RRV; lane 8, ST3 strain; lane 9, ST3 × RRV; lane 10, RRV. Arrowheads indicate the VP7 genes.

Figure 2.

Electrophoretic migration patterns of Swedish G2 strain 1076, 1076 $\times$ RRV reassortant and D $\times$ RRV in 10% (A) and 15% (B) PAGE gels. Lane 1, 1076 $\times$ RRV; lane 2, D $\times$ RRV; lane 3, 1076 strain; lane 4, 1076 $\times$ RRV. Arrowheads indicate the VP7 genes.

Figure 3. Electrophoretic migration patterns of Japanese G2 strain S2, S2 x RRV and D x RRV in 10% (A) and 18% (B) PAGE gels. Lane 1, S2 x RRV; lane 2, D x RRV; lane 3, S2 strain. Arrowheads indicate the VP7 genes.

Figure 4.

Electrophoretic migration patterns of DS-1 strain, DS-1 $\times$ RRV and RRV in a 5% regular ($18 \times 16 \times 0.075\text{cm}$) (A) or 5% long ($18 \times 32 \times 0.075\text{cm}$) (B) PAGE gel. Lane, 1, DS-1 strain; lane 2, DS-1 $\times$ RRV; lane 3, RRV. Arrowheads indicate the VP7 genes.

Figure 5.

Secondary structure of VP7 gene mRNA of strain D (G1); strains DS-1 (G2) and S2 (G2); strain RRV MMU18006 (G3); and strain 116E (G9) as predicted by GeneQuest program.

Table 1

Rotavirus strains employed in this study

Strain	Rotavirus		Species of origin	Country of origin	Reference
	G type	P type [genotype]			
<i>Rotashield</i> ™					
D × RRV	1	5B[3]	reassortant	USA	Midhun et al., 1985
DS-1 × RRV	2	5B[3]	reassortant	USA	Midhun et al., 1985
RRV (MMU18006)	3	5B[3]	rhesus monkey	USA	Stuker et al., 1980
ST3 × RRV	4	5B[3]	reassortant	USA	Midhun et al., 1985
D	1	1A[8]	human	USA	Wyatt et al., 1982
DS-1	2	1B[4]	human	USA	Wyatt et al., 1982
1076	2	2A[6]	human	Sweden	Hoshino et al., 1985
S2	2	1B[4]	human	Japan	Urasawa et al., 1982
ST3	4	2A[6]	human	UK	Wyatt et al., 1983
116E	9	8[11]	human	India	Das et al., 1993
UK	6	7[5]	bovine	UK	Woode et al., 1975
1076 × RRV	2	5B[3]	reassortant	USA	this study
S2 × RRV	2	5B[3]	reassortant	USA	this study
116E × UK	9	7[5]	reassortant	USA	Hoshino et al., 2004