INFECTION AND IMMUNITY, Sept. 1980, p. 1134-1140
0019-9567/80/09-1134/07$02.00/0

Vol. 29, No. 3

# Attachment of SA-11 Rotavirus to Erythrocyte Receptors

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Treatment of human group O and sheep erythrocytes with receptor-destroying enzyme rendered them inagglutinable by simian rotavirus SA-11. The erythrocyte receptors were also removed by periodate oxidation and markedly reduced by incubation with a high concentration of trypsin, but they were not altered by infectivity-enhancing concentrations of trypsin, *p*-hydroxymercuribenzoate, or sodium sulfite ($Na_2SO_3$). Hemagglutinating activity of the virus particles was destroyed by periodate oxidation at 37°C, *p*-hydroxymercuribenzoate, and a high concentration of trypsin and decreased by $Na_2SO_3$ but was not altered by incubation with receptor-destroying enzyme, infectivity-enhancing concentrations of trypsin, or periodate oxidation at 4°C. These results indicate that neuraminic acid-containing receptor substances are involved in the interaction of the virus with human and sheep erythrocytes, and suggest that SA-11-erythrocyte union involves carbohydrate on the surface of erythrocytes but not on the virion. Sensitivities of the SA-11 hemagglutinin to alcohols and repeated freeze-thaw cycles were also investigated.

Among the rotaviruses, Nebraska calf diarrhea virus (7, 11, 21), two Canadian isolates of bovine rotaviruses (20), and simian rotavirus SA-11 (12) have been found to hemagglutinate human and certain animal erythrocytes; in all cases the hemagglutination (HA) activity of the virus strains has been found to be associated with the outer capsid layer of the virions. Recently, Inaba and co-workers (10) reported the presence of calf rotavirus hemagglutinin inhibitors in the infected cell culture fluid, and Bishai et al. (2) reported the physicochemical properties of the Nebraska calf diarrhea virus hemagglutinin. However, as far as we are aware, little is known about the chemical nature of rotavirus hemagglutinin, and the precise mechanism of attachment of rotaviruses to cells is unknown.

The present communication is concerned with the mechanism of attachment of SA-11 to erythrocytes. In particular, we report that pretreatment of human and sheep erythrocytes with receptor-destroying enzyme (RDE) or periodate prevents agglutination by SA-11. This finding illustrates another similarity between rotaviruses and reovirus type 3, which reacts with neuraminic acid-containing receptor substances on ox erythrocytes (8). Experimental results will be described which suggest that a viral protein with accessible or essential sulfhydryl groups appears to be indispensable for SA-11 HA activity.

## MATERIALS AND METHODS

**Virus strain.** Simian rotavirus SA-11 was received from H. Malherbe, Southwest Foundation for Research and Education, San Antonio, Tex. It was propagated in this laboratory in primary cynomolgus monkey (*Macaca fascicularis*) kidney epithelial cells (Commonwealth Serum Laboratories [CSL], Melbourne, Australia) and then passaged five times in MA-104 cells, a continuous line of Rhesus monkey kidney cells obtained from S. Matsuno, National Institute of Health of Japan, Tokyo. Stock virus consisted of a suspension of infected cells and maintenance medium which had been frozen and thawed once.

**Preparation of SA-11 hemagglutinin.** MA-104 cell monolayers were grown in 400-ml bottles with minimum essential medium supplemented with 10% fetal calf serum (CSL). The confluent monolayers were washed once with Dulbecco phosphate-buffered saline and infected with 2-ml inocula of SA-11 virus which contained $10^5$ 50% tissue culture infectious doses per ml. After allowing virus adsorption to cells for 1 h at 37°C, maintenance medium consisting of minimum essential medium with 0.05% bovine serum albumin fraction V (CSL), was added to the bottles. Infected monolayers were incubated at 37°C and harvested when they showed extensive cytopathic effects, approximately 3 days after infection.

The method described by Rodger et al. (18), with minor modifications, was followed for the purification of SA-11 virus. The virus was sedimented through a 35% (wt/wt) sucrose cushion instead of being banded in a sucrose gradient, and, finally, the purified virus was dialyzed against physiological saline (0.14 M NaCl) instead of 0.002 M tris(hydroxymethyl)aminomethane hydrochloride. The virus banding in cesium chloride at a density of 1.36 g/cm$^3$ (double-shelled particles) was used for HA titrations.

**Erythrocytes.** Human group O blood was collected in Alsever solution. Sheep erythrocytes were obtained from CSL as a 50% (vol/vol) whole blood in Alsever solution, containing an erythrocyte concentration of

approximately 15% (vol/vol). The erythrocytes were washed in three changes of 0.14 M saline and stored at 4°C, either as packed cells or as 0.5% suspensions in 0.14 M saline.

**Reagents.** The following reagents were dissolved in 0.14 M saline, unless otherwise stated, and then adjusted to the required pH with 0.1 M KOH or 0.1 M HCl: trypsin from bovine pancreas, diphenylcarbamyl chloride treated (Sigma Chemical Co., St. Louis, Mo.); potassium periodate (BDH Chemicals Ltd., Poole, England); sodium sulphite (Standard Laboratories, Melbourne, Australia); RDE, a filtrate of a culture of *Vibrio cholerae*, strain 4Z (CSL); *p*-hydroxymercuribenzoate (PMB; Sigma). This last reagent, obtained as crystalline sodium salt, was dissolved in 0.1 M NaOH. The solution was diluted in saline to the desired concentration and then adjusted to pH 7.0 with 0.1 M HCl. Solutions of $KIO_4$ and $Na_2SO_3$ were made up daily and protected against light to avoid excessive oxidation of the reagents. Ethanol and methanol were analytical reagent grade from Ajax Chemicals (Sydney, Australia).

**Antiserum.** Hyperimmune serum to the SA-11 rotavirus was prepared as follows. Each of two rabbits was injected in multiple sites, including footpads, with 1.0 ml of a suspension containing approximately $10^8$ intact SA-11 particles purified in CsCl (density, 1.36 g/cm$^3$) mixed with an equal volume of Freund complete adjuvant. After 4 weeks, purified virus mixed in a 1:1 emulsion with Freund incomplete adjuvant was injected subcutaneously (multiple sites along the back) and intramuscularly (in each hind leg). After 1 week, a third injection of virus in Freund incomplete adjuvant was administered as above. The animals were bled 7, 14, and 30 days later.

**HA and HAI tests.** HA and HA inhibition (HAI) tests were carried out using microtiter equipment. The diluent used throughout was 0.14 M saline, pH 7.2, containing 1% bovine serum albumin. For the HA tests, doubling dilutions of antigen (0.025 ml) were incubated at 37°C with equal volumes of 0.5% human group O or sheep erythrocyte suspension in 0.14 M saline, pH 7.2. For the HAI test, sera were treated with RDE as explained below. Doubling dilutions of sera (0.025 ml) were incubated at room temperature with equal volumes of antigen containing four hemagglutinating units (HAU). After 1 h, 0.05 ml of a sheep erythrocyte suspension was added to each well. Antigen, cell, and serum controls were included in the test. The plates were then incubated at 37°C for 1 h, and the test was read at the end of the incubation period.

**Treatment of sera with RDE.** The method recommended by the Expert Committee on Respiratory Virus Diseases (6) was followed, with slight modifications. To 0.8-ml samples of RDE diluted 1:5 with 0.14 M saline was added 0.2 ml of each serum sample. The mixture was incubated at 37°C overnight, and after this incubation period, 1 ml of 0.2 M tris(hydroxymethyl)aminomethane, pH 9.0, was added. The enzyme was then inactivated by heating the mixture at 56°C for 30 min, after which time the sample was ready for titration by the HAI test. Controls consisting of 0.8-ml samples of 0.14 M saline instead of the enzyme dilution were included.

**Incubation of erythrocytes with various reagents.** Cells and reagents were mixed and incubated by the method described by Lerner et al. (15). Briefly, packed erythrocytes were suspended in solutions of trypsin, RDE, potassium periodate, PMB, and sodium sulfite; after incubation for 1 h at 37°C with occasional shaking to prevent sedimentation of erythrocytes, cells were washed and diluted to make 0.5% suspensions in 0.14 M saline. HA titrations were carried out with treated and untreated cells. Pretreatment of erythrocytes with periodate was also carried out at 4°C in the dark. HA titrations with RDE- and periodate-treated human erythrocytes were also performed with the Bel strain of influenza virus.

**Incubation of virus with various reagents.** Equal volumes of a purified virus suspension containing 32 HAU/0.025 ml and solutions of trypsin, $KIO_4$, RDE, PMB, or $Na_2SO_3$ were incubated for 1 h at 37°C, as described previously (15). Incubation of purified virus with periodate was also performed at 4°C in the dark. Treated and untreated virus was titrated for HA with untreated suspensions of human erythrocytes. To avoid the activity of residual enzymes on the erythrocytes during HA titrations, virus samples treated with RDE and with 8,000 μg of trypsin per ml, as well as the corresponding control, were sedimented for 1 h at 30,000 rpm in a Beckman SW65 rotor; and the pellets were suspended to the original volume in saline. HA titrations with the virus samples treated with infectivity-enhancing concentrations of trypsin were done immediately after incubation at 37°C. The other reagent-treated virus samples and virus controls were dialyzed overnight at 4°C against 0.14 M saline.

**Kinetics of inactivation of SA-11 hemagglutinin by PMB, $KIO_4$ and $Na_2SO_3$.** Equal volumes of purified virus suspension (32 HAU/0.025 ml) and of reagent solutions were mixed and incubated at 37°C. The control virus suspension contained saline but no reagents. At intervals, samples were removed, immersed in an ice bath, and HA titrations were done immediately with treated and untreated virus samples.

**Treatment of virus with ethyl and methyl alcohols.** The method described by Bishai et al. (2) was used to test the effect of different alcohol concentrations on the HA activity of SA-11 virus.

**Effect of freezing and thawing on SA-11 hemagglutinin.** The method described by Bishai et al. (2) was used to test the effect of repeated freeze-thaw cycles on the HA activity of the virus.

## RESULTS

**Effect of RDE and periodate on activity of erythrocyte receptors.** Preincubation of erythrocytes with RDE, a filtrate of a culture of *V. cholerae* strain 4Z, rendered human group O and sheep erythrocytes inagglutinable by the simian rotavirus SA-11 (Table 1). Prevention of SA-11 HA occurred up to a 1:20 dilution of RDE in 0.14 M saline. Titers of virus controls showed that sheep erythrocytes were agglutinated by SA-11 rotavirus as well as human group O cells. Pretreatment of cells either at 37 or at 4°C in the dark with potassium periodate at a concen-

tration of 0.25 mM in 0.14 M saline, pH 6.4, also abolished the capacity of human and sheep erythrocytes to be agglutinated by SA-11, whereas HA titrations with periodate-treated human erythrocytes and the Bel strain of influenza virus showed only a twofold decrease in the HA capacity of this virus (Table 1). Potassium periodate at a concentration of 1 mM caused hemolysis of human or sheep erythrocytes during incubation for 1 h at 37°C but not at 4°C, and a 0.1 mM concentration of the reagent did not alter receptors on erythrocytes necessary for HA by SA-11 virus. In the experiments reported here, erythrocytes were never in contact with concentrations of periodate greater than 0.25 mM, and the time of incubation was never greater than 1 h.

**Serum inhibitors.** In microtiter HAI tests employing 4 HAU of the virus, RDE-sensitive inhibitors of SA-11 HA were found at low titer in two preimmunization rabbit sera investigated. As shown in Table 2, treatment of these preimmune rabbit sera with RDE reduced or eliminated their HAI activity for SA-11 virus. A twofold decrease in inhibitor titer of SA-11 hyperimmune serum was also observed after similar treatment with RDE.

**Effect of various reagents on activity of erythrocyte receptors.** Trypsin at a concentration of 8,000 μg/ml destroyed SA-11 receptors of intact human group O erythrocytes, whereas infectivity-enhancing concentrations of the enzyme (5 or 10 μg/ml) had no effect on the ability of the erythrocytes to be agglutinated by SA-11. At the pH and concentrations used PMB or $Na_2SO_3$ treatments of type O red cells did not alter their agglutinability by the virus tested (Table 3).

TABLE 1. *Sensitivity of erythrocyte receptors to RDE and periodate oxidation*

| Treatment | Concn | Erythrocytes | HA titer for: | |
|---|---|---|---|---|
| | | | SA-11 | Influenza (Bel) |
| RDE | Undiluted[a] | Human group O | <2 | <12,800 |
| RDE | 1:10 | Human group O | <2 | 204,800 |
| Control | | Human group O | 128 | 204,800 |
| RDE | 1:10 | Sheep | <2 | ND[b] |
| Control | | Sheep | 128 | ND |
| $KIO_4$ | 0.25 mM | Human group O | <2 | 102,400 |
| Control | | Human group O | 512 | 204,800 |
| $KIO_4$ | 0.25 mM | Sheep | <2 | ND |
| Control | | Sheep | 512 | ND |

[a] Undiluted RDE-lysed sheep erythrocytes during pretreatment at 37°C for 1 h.
[b] ND, Not done.

TABLE 2. *Effect of RDE on HAI titer of preimmune and SA-11 hyperimmune rabbit serum*

| Rabbit serum | Treatment | HAI activity[a] |
|---|---|---|
| Preimmune A | 56°C, 30 min | 20 |
| | RDE, 56°C, 30 min | 10 |
| Preimmune B | 56°C, 30 min | 10 |
| | RDE, 56°C, 30 min | <10 |
| Hyperimmune[b] | 56°C, 30 min | 1,280 |
| | RDE, 56°C, 30 min | 640 |

[a] Expressed as the reciprocal of serum HAI titer against 4 HAU of SA-11 virus with sheep erythrocytes.
[b] Prepared as explained in the text by immunizing rabbit B. This serum had a neutralization titer (50% plaque reduction) of $3 \times 10^6$ against SA-11 virus.

TABLE 3. *Effect of various reagents on activity of erythrocyte receptors*

| Treatment | Concn | pH | HA titer[a] |
|---|---|---|---|
| Trypsin | 8,000 μg/ml | 6.5 | 16 |
| Control | | | 1,024 |
| Trypsin[b] | 5 μg/ml | 6.5 | 256 |
| Trypsin | 10 μg/ml | 6.5 | 256 |
| Control | | | 256 |
| PMB | 0.025 mM | 7.0 | 1,024 |
| $Na_2SO_3$ | 0.1 M | 7.6 | 1,024 |
| Control | | | 1,024 |

[a] Human group O erythrocyte HA titer for SA-11 rotavirus.
[b] Infectivity-enhancing concentrations of trypsin were tested with a different virus preparation.

**Effect of preincubation of SA-11 virus with various reagents on HA.** The effect of RDE, trypsin, PMB, $KIO_4$, and $Na_2SO_3$ on the ability of SA-11 virus to agglutinate human erythrocytes was determined. As shown in Table 4, 8,000 μg of trypsin per ml caused a decrease of the HA titer of the virus from 32 to <4, whereas pretreatments with concentrations of trypsin known to increase the virus infectivity or with RDE had no effect on the HA activity of the virus. The treatment of SA-11 virus with PMB and $KIO_4$ at 37°C for 1 h completely destroyed the HA property of the virus, and after treatment with $Na_2SO_3$ the HA titer of the virus was reduced to 12.5%. Preincubation of SA-11 at 4°C in the dark with concentrations of periodate 10 times higher than that used at 37° had no effect on the HA capacity of the virus (Table 4). The reagent-treated virus and control preparations were examined under the electron microscope after negative staining with one-tenth saturated ammonium molybdate. The reagent-treated particles as well as those in control preparations remained intact, which suggested that effects other than uncoating or capsid destruction were produced by these reagents.

**Kinetics of inactivation of SA-11 hemag-**

**glutinin by PMB, $KIO_4$, and $Na_2SO_3$ at 37°C.** Viral HA activity was rapidly inactivated by $KIO_4$ and PMB (Fig. 1). Inactivation by $KIO_4$ was complete within 2 min, and that produced by PMB, also started soon after incubation, was complete within 8 min. The maximum inactivation produced by $Na_2SO_3$ was 87.5%, which was reached within 16 min of incubation at 37°C and remained constant until the experiment was terminated. These data clearly show that $KIO_4$, PMB, and $Na_2SO_3$ at the concentration, temperature of incubation, and pH used reacted with susceptible substrates on the virus particles which are indispensable for the SA-11–erythrocyte interaction.

**Effect of ethyl and methyl alcohols on HA activity of SA-11 rotavirus.** Equal volumes of a purified virus suspension dialyzed against 0.14 *M* saline and containing 32 HAU/0.025 ml, were mixed with either ethanol or methanol. After 5 min at 4°C the virus-alcohol mixtures were dialyzed against saline at 4°C with constant stirring. Control samples were processed in the same manner with 0.14 M saline only. Treated virus and control preparations were titrated for HA activity. The results showed that a final concentration of 25% ethanol or 35% methanol completely inactivated the SA-11 hemagglutinin (Table 5). The alcohol-treated virus particles and those in control preparations were examined under the electron microscope. After treatment with absolute and 70% (initial concentrations) alcohols most of the particles appeared broken down, and those that remained intact were all single shelled (Fig. 2). This observation suggested that alcohols at those concentrations uncoated the virus particles and often fragmented even the inner capsid. However, ethanol at a final concentration of 25% under the same experimental conditions inhibited the HA activity of the virus without uncoating the SA-11 virions but produced clumping of the particles.

**Effect of freezing and thawing on HA.** The effect of repeated quick-freezing (−70°C) and thawing on the HA activity of SA-11 virus was investigated. The repeated freeze-thaw cycles inactivated the SA-11 hemagglutinin without noticeable changes in virus morphology as observed under the electron microscope. The hemagglutinin titer decreased from 512 to <2 after the virus had been frozen and thawed 14 times (Fig. 3).

TABLE 4. *Effect of pretreatment of SA-11 rotavirus with various reagents on HA*

| Treatment | Concn | pH | HA titer[a] |
|---|---|---|---|
| Trypsin | 8,000 μg/ml | 6.5 | <4 |
| Trypsin | 10 μg/ml | 6.5 | 32 |
| RDE | 1:5 | 6.8 | 32 |
| $KIO_4$ (37°C) | 0.1 mM | 6.4 | <4 |
| $KIO_4$ (4°C) | 1 mM | 6.4 | 32 |
| PMB | 0.025 mM | 7.0 | <4 |
| $Na_2SO_3$ | 0.1 M | 7.6 | 4 |
| Control | | | 32 |

[a] Human group O erythrocyte HA titer for SA-11 rotavirus.

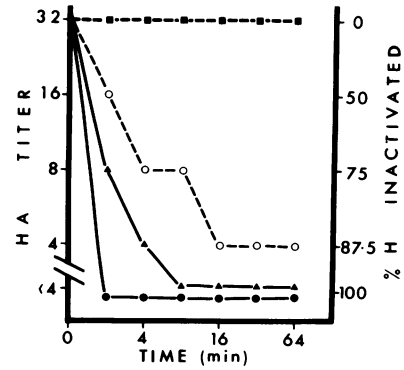


FIG. 1. *Kinetics of inactivation at 37°C of SA-11 virus hemagglutinin by 0.1 mM $KIO_4$ (●), 0.025 mM PMB (▲), and 0.1 M $Na_2SO_3$ (○). Controls (■) which consisted of virus treated with 0.14 M saline instead of reagent were carried through the entire procedure. HA titrations were carried out with sheep erythrocytes. The hemagglutinin (H) inactivated is expressed as percentage of the control.*

TABLE 5. *Effect of ethyl and methyl alcohols on HA activity of SA-11 rotavirus*

| Treatment | Final concn (%, vol/vol) | HA Titer[a] |
|---|---|---|
| Ethanol | 50 | <2 |
| | 35 | <2 |
| | 25 | <2 |
| | 10 | 8 |
| | 5 | 8 |
| Control | | 8 |
| Methanol | 50 | <2 |
| | 35 | <2 |
| | 25 | 8 |
| | 10 | 8 |
| | 5 | 8 |
| Control | | 8 |

[a] Sheep erythrocyte HA titer for SA-11 virus.

## DISCUSSION

Sheep erythrocytes were found to be agglutinated by SA-11 to the same titer as human group O cells, and the ability of either human or sheep erythrocytes to be agglutinated by the virus was inhibited by pretreatment of the cells with RDE. This observation suggested that simian rotavirus SA-11 interacts with neuraminic

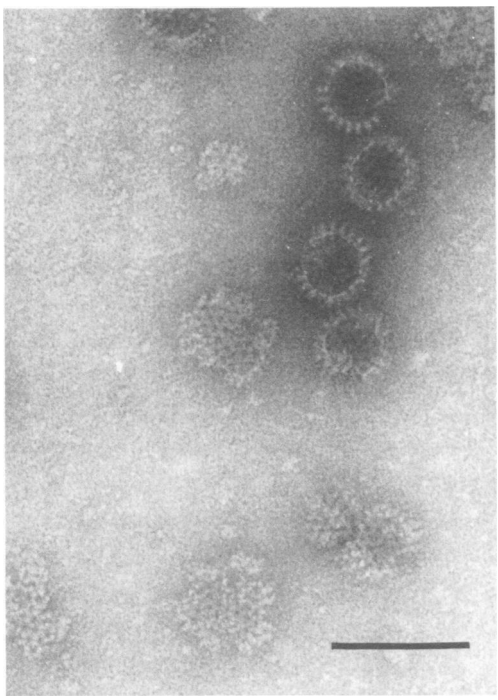

FIG. 2. *Effects of alcohol on SA-11 rotavirus morphology. Virus particles after treatment with a final concentration of 50% ethanol. Bar represents 100 nm.*

acid-containing receptors and that some inhibition of HA could be caused by nonantibody, neuraminic acid-containing molecules in sera or secretions. To support this view, HAI experiments with normal rabbit sera and 4 HAU of the virus were carried out, but the low inhibitor titers obtained (Table 2) with the preimmunization rabbit sera investigated did not permit a definite conclusion although the treatment of these sera with RDE reduced or eliminated their HAI activity for the virus. Low titers of RDE-sensitive inhibitors have been reported for polyoma virus HA, and it was found that the titer of nonspecific serum inhibitor in occasional sera from uninoculated mice was inversely proportional to the test dose of hemagglutinin used (9). Titers were higher with 1 or 2 HAU than with 4 or more HAU of virus. The present data was obtained with 4 HAU of the virus, which could explain the variations between sera and the low titers obtained. Gomatos and Tamm carried out similar experiments with reovirus type 3, and they found that treatment with *V. cholerae* filtrate rendered ox erythrocytes inagglutinable by the virus (8). They also reported that the agglutination of ox erythrocytes by reovirus type 3 was inhibited by normal mouse, rabbit, and rat sera, and that treatment of the sera with *V. cholerae* filtrate destroyed the inhibitory activity.

RDE-removable erythrocyte receptors have been described for two other viruses which are apparently biologically different from members of the myxovirus group, namely, encephalomyocarditis virus and the rat virus of Kilham (13).

Additional evidence for glycoprotein (probably sialoglycoprotein) erythrocyte receptors in HA SA-11 virus was obtained by gentle periodate oxidation of erythrocytes, either at 37°C or at 4°C in the dark. HA by SA-11 virus was even more sensitive to oxidation of the cellular receptors by 0.25 mM periodate than that due to influenza virus. The effects of periodate oxidation on *N*-acetylneuraminic acid in glycoproteins and on the HA activity of influenza virus have been reported (22), and they were found to vary with the amount of periodate used.

PMB, which reacts with —SH groups (16) and $Na_2SO_3$, which is known to split S—S bonds (4), did not diminish the agglutinability of erythrocytes by SA-11 virus, whereas pretreatment with high concentrations of trypsin greatly decreased SA-11 receptors on human group O erythrocytes. These results indicated that a protein component of the erythrocyte surface, without accessible or essential sulfhydryl groups and which may be a blood group substance (15), is necessary for virus-erythrocyte interaction.

The effects on HA titers of preincubation of either erythrocytes or SA-11 virus with various reagents are summarized in Table 6. Treatments of SA-11 virus with RDE or infectivity-enhancing concentrations of trypsin had no effect on

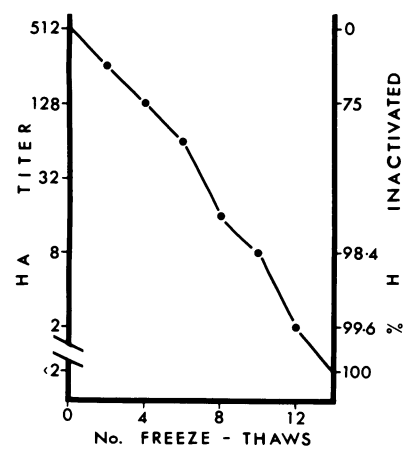


FIG. 3. *Effect of freezing and thawing on SA-11 rotavirus hemagglutinin. Repeated freeze-thaw cycles of purified virus in 0.14 M saline were carried out. HA titrations were performed with sheep erythrocytes. The hemagglutinin (H) inactivated is expressed as percentage of the control.*

TABLE 6. *Summary of effects of reagents and freezing and thawing on HA of SA-11 rotavirus*

| Treatment | Effect on HA of pretreating: | |
|---|---|---|
| | Erythrocytes | SA-11 |
| RDE | Decreased | No effect |
| Trypsin (8,000 μg/ml) | Decreased | Decreased |
| Trypsin (5 to 10 μg/ml) | No effect | No effect |
| $KIO_4$ (37°C) | Decreased | Decreased |
| $KIO_4$ (4°C) | Decreased | No effect |
| PMB | No effect | Decreased |
| $Na_2SO_3$ | No effect | Decreased |
| Ethanol | Not done | Decreased |
| Methanol | Not done | Decreased |
| Freeze-thaw | Not done | Decreased |

HA, but preincubation with trypsin at concentrations higher than those used either for enhanced growth in cell cultures (1) or for the formation of plaques (17) eliminated the HA activity of the virus. The fact that the HA activity of SA-11 virus could also be inactivated by PMB and decreased by $Na_2SO_3$ has provided suggestive evidence that sulfhydryl groups or disulfide bonds in virus surface proteins may be essential for HA with this virus, but the erythrocyte receptors are insensitive to sulfhydryl reagents.

Previous studies have shown that at 37°C periodate can also react with proteins and oxidizes several amino acids whenever they occur in the peptide chain (5), whereas, at 0 or at 4°C in the dark, periodate is considered to react specifically with carbohydrates and oxidizes α-glycols, α-hydroxyaldehydes, α-Ketols, α-Ketoaldehydes, and α-diketones to split C—C bonds (3, 23). In the present study, inhibition of SA-11 virus HA occurred when the purified virus was treated with potassium periodate at 37°C, but at 4°C the reagent, even at a concentration 10 times higher, had no effect on the HA activity of the virus. This finding suggests that a protein on the virus surface determines or is necessary for HA, but that carbohydrate is not directly involved. Rodger et al. (19) recently reported that the major outer shell polypeptides of calf, human, and simian rotaviruses are glycosylated, and the same is true of type 3 reoviruses (14). However, recent studies of the gene coding for the hemagglutinin of reovirus demonstrated that in type 3 reoviruses the hemagglutinin is not the glycoprotein but the minor outer capsid protein σ1 (24). Similarly, in the light of the present results the glycosylated major outer capsid protein of SA-11 seems less likely to be the hemagglutinin although its polypeptide moiety could still be involved. Our data suggest that erythrocyte-SA-11 virus union involves a polypeptide sequence on the viral surface containing accessible —SH groups and a glycoprotein, possibly free of —SH groups on the erythrocyte.

We do not yet understand the mechanisms of inactivation of SA-11 hemagglutinin by freezing and thawing or exposure to high dilutions of alcohols. Bishai et al. (2) obtained similar results with bovine rotavirus, except that they observed only uncoating of double-shelled particles by alcohols, whereas most of the inner capsids of SA-11 virus were fragmented under similar conditions.

In conclusion, the results of this study have shown that the simian rotavirus SA-11 possesses most of the HA properties already reported for reovirus type 3 (8, 15, 24). This may seem surprising since the range of cells susceptible to rotavirus infection is much more limited than in the case of reoviruses. Even though infectivity-enhancing concentrations of trypsin had no effect on either the agglutinability of erythrocytes or the HA activity of the virus, it remains to be seen whether the receptors involved in the infection of cells by rotaviruses resemble those detected in this study of HA.

## ACKNOWLEDGMENTS

We thank Linda Mercer for assistance with electron microscopy and photography, C. Adeney for technical assistance, and Thea Webb for typing of the manuscript. We also acknowledge the helpful suggestions of the referee who pointed out that periodate reacts with proteins at 37°C and enabled us to correct an initial erroneous conclusion.

This work was supported by the National Health and Medical Research Council of Australia. J. Bastardo is the holder of a postgraduate fellowship from the Universidad de Oriente, Cumana, Venezuela.

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