Characterization of A Canine Rotavirus Hemagglutinin

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ABSTRACT. Hemagglutinin (HAin) of canine rotavirus RS 15 strain was investigated. Polyacrylamide gel electrophoretic and immune-blotting analysis of the virion indicated 5 major rotaviral polypeptides whose molecular weight ranged from 38K to 119K. The HAin of RS 15 strain existed in the outer capsid of virus. Results of hemagglutination-inhibition (HI) test with other rotavirus strains indicated a possibility that the HI test might be of use for detection of type-specific antibody.—**KEY WORDS:** canine rotavirus, hemagglutinin, virus polypeptide.

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Rotavirus is a well-known pathogen to alimentary tract of humans and domestic farm animals, but its clinico-pathological significance in small pet animals has not been ascertained. The authors have already reported isolation of a rotavirus (RS 15 strain) from a dog died of severe diarrhea [16] and some characteristics of the strain [17]. The present communication describes its structural polypeptides, the nature of hemagglutinin (HAin), and antigenic relationship between RS 15 strain and other rotavirus strains determined by hemagglutination-inhibition (HI) test.

MA 104 cells were used for propagation of viruses and plaque assay. The cells were grown in Eagle's minimal essential medium (Eagle's MEM), and canine RS 15, human Wa, bovine Shimane and porcine S80 strains were employed as reported previously [17]. The viruses were cultivated in the cells and Eagle's MEM, which contained no serum but trypsin (SIGMA Chem. Co., type 1) at a final concentration of 10 $\mu\text{g/ml}$, was used as a maintenance medium. The plaque assay was essentially the same as that reported by others [10]. HA test was performed by a microtiter system with pH 7.0 phosphate

buffered saline solution and chicken erythrocytes at room temperature as reported previously [17].

When the cell culture was maintained with Eagle's MEM containing no trypsin after virus inoculation, no HAin but low infectivity was detected in the supernatant. HAin production was maximum when the maintenance Eagle's MEM was supplemented with 5–10 $\mu\text{g/ml}$ of trypsin; the same phenomenon as already reported by others [1, 2, 8]. Addition of more trypsin caused detachment of the cells from the glass surface, so that the yield of HAin was less. Although some strains, canine CU-1 strain [10] for instance, show trypsin independent-growth, RS 15 strain definitely requires the aid of trypsin for its growth [17] and production of HAin in the cell culture.

The viruses were purified by the method of CsCl density gradient centrifugation as described previously [16, 17] and they were applied for sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Virus polypeptides were analysed on 10% SDS-PAGE gels using the discontinuous buffer system of Laemmli [14] under reducing condition. The gel was stained with

Fig. 1. Polypeptide analysis of canine rotavirus RS 15 strain by SDS-PAGE. Lane A: molecular weight marker (205K; myosin, 116K; β -galactosidase, 97.4K; phosphorylase b, 66K; bovine albumin, 45K; egg albumin, 29K; carbonic anhydrase), Lane B: RS 15 strain, Lane C: electrotransferred RS 15 strain from lane B on nitrocellulose paper by immunoblotting. Lanes A and B were stained with Coomassie brilliant blue.

Coomassie brilliant blue and photographed. Molecular weight (MW) of the peptides was determined by comparing their electrophoretic mobilities with those of polypeptides of known MW (Kit for Molecular Weights 30,000–200,000, SIGMA Chem. Co.). After the separation by SDS-PAGE, the polypeptides were electrophoretically transferred to a nitrocellulose paper for 4 hours at 0.36 A under cooled condition. The paper was then examined with the IMMUNE-BLOT ASSAY KIT (Bio-Rad Lab.) with some modification. Since the first antibody, hyperimmune serum against RS 15 strain, had been prepared with specific-pathogen-free (SPF) guinea-pigs [17], rabbit anti-guinea-pig Ig G (Miles-Yeda Ltd.) was additionally applied as the second antibody. At least 11

polypeptide bands were observed in virions on the stained gel (lane B of Fig. 1). Lane C of Fig. 1 shows the result of immunoblotting assay of the virions. As summarized in Table 1, 5 major polypeptides, VP1, VP2, VP3, VP6 and VP7, were identified, but others were not.

The virions were treated with ethylenediaminetetraacetic acid (EDTA), a chelating agent, which changed viral structure from double-shelled to single-shelled [5]. The method was essentially the same as that described by Bastardo *et al.* [4]. By the EDTA treatment of RS 15 strain, HA activity of the virion was abolished and 2 polypeptides, No. 4 (MW 60–61K) and VP7, were missing but no change was observed in other bands when the treated virus was analysed by SDS-PAGE.

The single-shelled particles of RS 15 strain did not show HA [17] and the SDS-PAGE analysis revealed that they lacked VP3, VP7 and the MW 60–61K polypeptide. Though the MW 60–61K polypeptide was always detected in the SDS-PAGE analysis of the virion, yet it was not successfully identified by the immunoblotting assay. VP7 of Nebraska calf diarrhea virus (Lincoln strain) or gp34 of SA 11 strain was suggested to be a protein involved in Hain [4, 15]. VP3, trypsin-sensitive outer capsid protein, is cleaved to yield 2 polypeptides, VP5 (MW 60K) and VP8 (MW 28K) by proteolytic enzymes and the cleavage of VP 3 seems to be molecular mechanism in the enhancement of infectivity but not necessary for virus adsorption to cells [6, 7]. It has been subsequently demonstrated by genetic studies and studies with monoclonal antibodies to surface proteins [9, 12], however, that VP3 is responsible for HA of certain animal rotaviruses and VP8 does not contain the HA domain of molecule. Therefore it is provisionally concluded that (1) the Hain of RS 15 strain exists in the outer capsid of virus, (2) the vanishment

Table 1. Summary of polypeptides analysis of canine rotavirus RS 15 strain

Designation of bands detected	Coomassie brilliant blue stain (Mol. wt.)	Immune-blotting assay (Mol. wt.)	Polypeptide designation of SA-11 strain ^{a)} (Mol. wt.)
*1 ^{c)}	ND ^{b)}	D(165K)	
1	D(118K-119K)	D	VP 1(125K)
2	D(94K)	D	VP 2(94K)
3	D(86K)	D	VP 3(88K)
*2	VD	VD(68K-74K)	
4	VD(60K-61K)	ND	
5	D(56K-57K)	D	
6	D(42K)	D	VP 6(41K)
7	D(38K-39K)	SD or ND	VP 7(37K)
*3	ND	D(35K-37.5K)	
8	SD(32K)	VD	
9	D(30K-31K)	VD	
10	SD(29K)	VD	
11	SD(27.5K)	D	
12	SD or ND	D(24K-26K)	
13	ND	D(23K)	

a) Cited from Kapikian, A.Z., and Chanock, R.M. 1985. Rotaviruses. pp. 863-906. *In*: Virology (Fields, B.N. ed.), Raven Press, New York.

b) D: Stably detected, ND: Not detected, VD: Variably detected, SD: Slightly detected.

c) No. 1* was also detected on the paper transferred of non-infected MA 104 cell lysate.

Table 2. Antigenic relationship between canine rotavirus RS 15 strain and human, bovine and porcine rotaviruses by hemagglutination-inhibition (HI) test

Virus strain (species)	HI antibody titer of antisera against			
	RS 15	Wa	Shimane	S80
RS 15 (canine)	160 ^{a)}	160	<10	<10
Wa (human)	10	160	<10	<10
Shimane (bovine)	— ^{b)}	—	—	—
S80 (swine)	<10	10	<10	40

a) Reciprocal of the highest serum dilution which showed absolute hemagglutination-inhibition.

b) No hemagglutinating activity of Shimane strain was obtained in the conditions applied.

of HA might not be correlative with VP7 dropped out, and (3) HA activity might be abolished due to transformation of the HA domain of molecule (possibly VP3) by the

EDTA treatment. Further study by using specific antibodies directed at respective polypeptides will require for identifying the polypeptide(s) responsible for HA of RS 15 strain since the rotavirus HAin is considered to be species-specific marker which could distinguish various isolates into species originated [3].

The immune sera against each rotavirus strain prepared with SPF guinea-pigs [17] were applied for the HI test and the method was the same as that reported previously [17]. Although both human Wa and porcine S80 strains agglutinated human O erythrocytes, no appropriate erythrocytes from animal species was found for bovine Shimane strain in the condition applied so that the strain was not examined for the cross HI test. It may be noteworthy that human Wa strain did agglutinate human O erythrocytes

since HA among rotaviruses has been demonstrated only with animal strains [11, 13, 20] except for one report on human isolates [18]. Table 2 shows antigenic relationships between RS 15 strain and other rotaviruses. There was a one-way antigenic relationship between RS 15 and Wa strains and they were distinct from S80 strain. All strains did not react with the antiserum against Shimane strain. Overall relationship among strains was the same as that obtained by neutralization test reported previously [17], and HI test is considered to be of use for detection of type-specific antibody in place of the neutralization test although the neutralizing antibody might show HI activity partially due to its steric blocking of HA in [9, 19].

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要 約

イヌロタウイルス血球凝集素の性状：望月雅美・阿多瑞代（鹿児島大学農学部家畜微生物学講座）——イヌロタウイルス RS15株について、ポリアクリルアミド電気泳動法と免疫ブロッティング法により、少なくとも5種のウイルスポリペプチドが同定され、血球凝集素はウイルス外殻に存在することが示された。他ロタウイルス株との交差試験結果から血球凝集阻止試験は中和試験に代えて型特異抗体検出法として応用できることが示唆された。