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Comparison of the polypeptides of bovine rotavirus serotypes

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RÉSUMÉ

OJEH (C. K.). — Comparaison des polypeptides des sérotypes de rotavirus bovins. *Rev. Elev. Méd. vét. Pays trop.*, 1984, 37 (4) : 406-410.

A partir de 3 sérotypes différents, les polypeptides de 9 souches de rotavirus bovins ont été comparés entre eux et par référence au polypeptide extérieur VP7. On a utilisé l'électrophorèse en gel de polyacrylamide coloré à l'argent.

Le VP7 a été détecté dans 6 des 9 préparations avec une bonne corrélation entre la présence de ce polypeptide dans le gel et la proportion de particules lisses estimées par microscopie électronique après purification sur gradient de sucrose/CsCl.

Dans les prélèvements où il a été détecté, le VP7 des sérotypes a migré différemment. La signification possible de l'absence de VP7 dans quelques préparations fait l'objet d'une discussion.

Mots-clés : Rotavirus bovins - Sérotypes - Polypeptides viraux.

SUMMARY

OJEH (C. K.). — Comparison of the polypeptides of bovine rotavirus serotypes. *Rev. Elev. Méd. vét. Pays trop.*, 1984, 37 (4) : 406-410.

The polypeptides of 9 strains of bovine rotavirus, comparising 3 different serotypes were compared with reference to the outer polypeptide, VP7, in a silver stained polyacrylamide gel electrophoresis (PAGE). VP7 was detected in 6 of 9 preparations, with a good correlation between the presence of this polypeptide in the gels and the proportion of smooth particles estimated by EM after CsCl/sucrose gradient purification. In the samples in which it was detected, VP7 of the serotypes migrated differently. The potential significance of the absence of VP7 in some preparations is discussed.

Key words : Bovine rotavirus - Serotypes - Rotavirus polypeptides.

INTRODUCTION

Rotaviruses are composed of two particle types, the complete, smooth or outer shelled particle, measuring about 70 nm and the incomplete, rough or inner shelled particle, measuring about 60 nm (3). The structure of rotavirus has been the subject of several investigations, resulting initially in a number of conflicting interpretations as regards the total number of polypeptides (3, 12, 21). However, the concensus view is in the range of 8-10 polypeptides (15).

A major glycosylated outer polypeptide, VP7, with MW ranging between 34-37 k has been recognised to elicit neutralising antibodies (1, 8, 10). However it has been suggested that because of the complex nature of rotaviruses, more than one polypeptide may well be involved in neutralisation reaction (1, 2). The outer polypeptides are important not only for neutralisation but are also concerned with infectivity in the complete or double shelled rotavirus (3, 5). Unfortunately, these outer shell polypeptides tend to disintegrate during CsCl/sucrose gradient purification (16), therefore to enhance their stability, divalent cations (Ca^{++} or Sr^{++}) are added to the buffers throughout the purification process (4, 19).

The dsRNA of rotaviruses have been com-

pared in PAGE (9) but not the polypeptides. This investigation compares the polypeptides of the three different serotypes of bovine rotavirus, with reference to their VP7, the polypeptide responsible for infectivity and neutralisation.

MATERIALS AND METHODS

Viruses

The UK (Compton), Northern Ireland, Lincoln and strains 639, all belonging to serotype 1, strain 678 (serotype 2) and strains 411, 683, 1 548 and 2 484 all belonging to serotype 3 (18, 20) were used in this study. All had undergone at least 11 passages in MA 104 cells and with the exception of Lincoln and Northern Ireland strains had been cloned, either by plaque purification or by three passage at terminal dilutions. All viruses were purified through CsCl/sucrose gradient.

Purification of rotaviruses through CsCl/sucrose gradient

Cell cultures infected with different serotypes of rotavirus were frozen and thawed three times to release cell associated viruses. Cellular debris was cleared under low speed centrifugation. Virus was then pelleted at 71,000 × g for 45 min. The pellets were homogenised in 1-2 ml tris buffer, layered onto a discontinuous gradient consisting of 2 ml of a solution containing 1.31 M CaCl and 1.58 M sucrose, overlaid by 1.58 M sucrose in tris buffer and centrifuged at 154,400 × g for 60 min at 5 °C. The opalescent band which appeared just below the interface was harvested, diluted four-fold and pelleted. Pellets were resuspended in 1-2 ml of tris buffer and layered onto a 5-step CsCl/sucrose gradient to which 1.0 µg/ml ethidium bromide had been added and then centrifuged at 50,400 × g for 18 h at 5 °C. The gradient consisted of 1.66 M sucrose/1.49 M CaCl and 1.56 M sucrose/1.49 M CaCl at the extremities. An intermediate density was achieved by mixing equal volumes of the two extremes, and two further steps were achieved by mixing the intermediate solution with the two extremes. The virus band was located by fluorescence under ultraviolet light, harvested with syringe, diluted in tris buffer and pelleted. The pellets were examined by electron microscopy (EM) using negative staining with 1 p. 100 ammonium molybdate (pH 6.0) and the proportion of complete virions estimated.

Estimations of smooth particles

Purified virions were examined by EM and two hundred particles were counted from five fields and the proportion of smooth to rough particles estimated.

PAGE analysis

The proteins of purified rotavirus samples from infected tissue culture were dissociated to the polypeptide level by the method of TODD and McNULTY (21). Polypeptides, along with reference standard proteins (comprising β-galactosidase, MW 130,000 ; phosphorylase A, 92,00 ; ovotransferin, 76-78,000 ; albumin, 66,200 ; ovoalbumin, 45,000 ; chymotrypsinogen A, 25,700 ; myoglobin, 17,200 and cytochrome C, 12,300) were loaded into the gel tracks and concentrated through a 3 p. 100 stacking gel prior to resolving in a 10 p. 100 separating gel of 0.75 mm thickness, using the discountinuous buffer system of LAEMMLI (11), to which were added Ca^{++} ions. Electrophoresis was preformed at room temperature for approximately 4 h with a constant current of 13 mA.

Silver staining for polypeptides

The silver staining procedure for proteins in polyacrylamide gels as described by MORRISSEY (16) was followed.

RESULTS

The viral polypeptide, VP7, was detected in preparations of UK (Compton), Northern Ireland, Lincoln, 639, 678 and 683 but not in 411, 1 548 and 2 484 (Photo 1). In the preparations in which it was detected, the VP7s migrated between ovoalbumin (MW 45,00) and chymotrypsinogen A (MW 25,000).

The proportion of smooth particles estimated by EM for the different viruses was greater than 50 p. 100 for the 6 viruses in

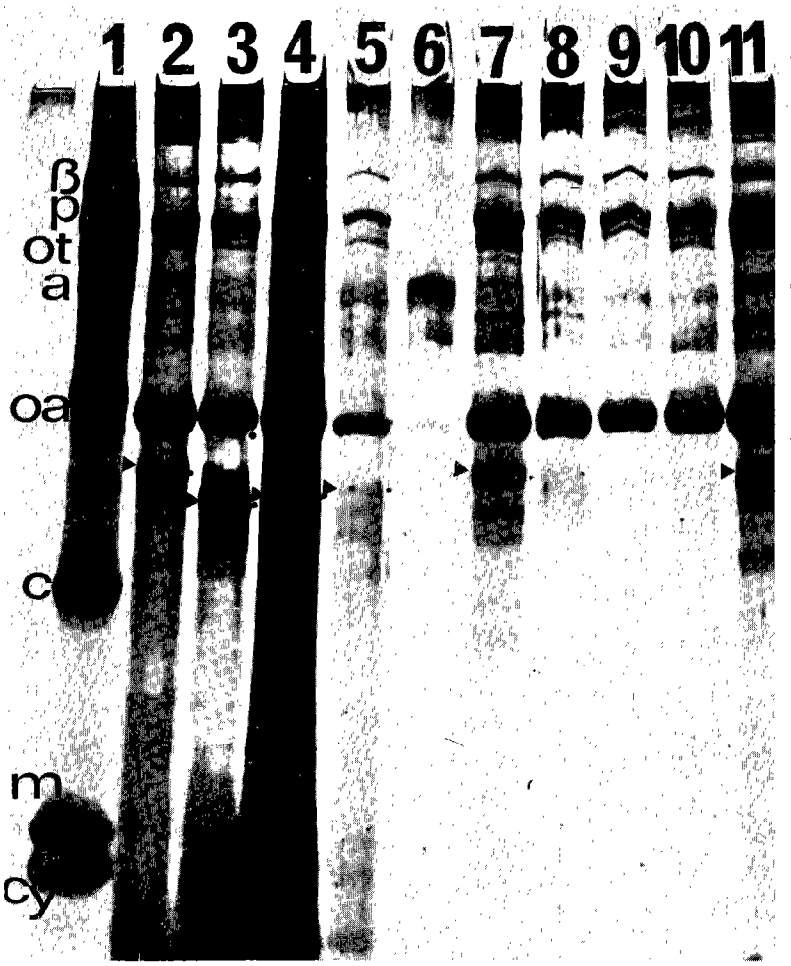

Fig. 1. — Comparison of the polypeptides (VP7) of bovine rotavirus serotypes.

Track 1 contains standard reference proteins comprising of :

B	=	β-galactosidase	MW 130,000
P	=	Phosphorylase A	92,000
ot	=	ovotransferin	76-78,000
a	=	albumin	66,200
oa	=	ovoalbumin	45,000
c	=	chymotrypsinogen A	25,000
m	=	myoglobin	17,200
cy	=	cytochrome C	12,000

Track 2	UK (Compton)	Serotype 1
3	Northern Ireland	Serotype 1
4	Lincoln	Serotype 1
5	639	Serotype 1
6	Accidental loading	
7	678	Serotype 2
8	411	Serotype 3
9	1 548	Serotype 3
10	2 484	Serotype 3
11	683	Serotype 3

Note : Arrow heads points to VP7 of different strains migrating differently.

which VP7 was detected and 15 p. 100 for the 3 viruses in which VP7 was not detected (Table I). There was good correlation between the presence of VP7 in the gels and the proportion of smooth particles.

DISCUSSION

Genetic analysis of rotaviruses had identified dsRNA segments 9 for human and monkey and 8 or 9 for calf strains as coding for the protein that elicits neutralising antibodies (7, 8, 14). This protein has been recognised as the outer polypeptide, VP7 (1, 13). Because of the importance of this polypeptide in eliciting neutralising antibodies and infectivity, its presence in the different serotypes of bovine rotaviruses was studied.

The migration pattern of the VP7 in the different serotypes varied from strain to strain. This was not surprising, as it had already been shown that the dsRNA of these serotypes migrated differently (OJEH, to be published).

TABLE N°I-Estimation of Smooth Particles of the serotypes of bovine rotavirus after CsCl/sucrose gradient purification

Virus	Serotype	Percentage Smooth Particles
UK (Compton)	1	68
Northern Ireland	1	60
Lincoln	1	70
639	1	54
678	2	90
683	3	63
411	3	10
1548	3	11
2484	3	15

However, the presence of VP7 in some strains and not in others (Fig. 1), after CsCl/sucrose gradient purifications, despite the use of divalent cations (Ca^{++}), in the buffers was not clear. The most likely explanation was that some strains of calf rotavirus loose their outer capsid more readily than others. NOVO and ESPARAZA (17) reported similar observations with isolates of bovine rotavirus, while the outer shell capsid of mouse rotavirus were equally easily lost with handling (22). The good correlation between VP7 and the presence of smooth particles confirm that CsCl/sucrose could be detrimental to the complete structure of rotavirus.

It was interesting to observe that those strains lacking VP7 were difficult to adapt to tissue culture propagation, requiring higher concentrations of trypsin and longer incubation periods (data not shown). But from other reports (6), VP4 and *not* VP7 is responsible for growth restrictions in human rotavirus strains. So rather than associate difficulty to propagate *in vitro* with VP7, it could be that VP4 which is an outer shell polypeptide as well, was equally disintegrated by CsCl/sucrose handling. Although these viruses belonged to serotype 3, it would require comparison of more isolates of this serotypes to ascertain whether the lack VP7 in tissue culture preparations is a constant feature of this serotypes. Nevertheless, this underscores a potential problem if vaccines with this serotypes is contemplated.

RESUMEN

OJEH (C. K.). — Comparación de los polipéptidos de los serotipos de rotavirus bovinos. *Rev. Elev. Méd. vét. Pays trop.*, 1984, **37** (4) : 406-410.

A partir de 3 serotipos diferentes, se compararon los polipeptidos de 9 cepas de rotavirus bovinos entre ellos y por referencia al polipéptido exterior VP7.

Se utilizó la técnica de electroforesis en gel de poliacrilamida coloreado con plata.

Se descubrió el VP7 en 6 de las 9 preparaciones con una buena correlación entre la presencia de este polipéptido en el gel y la proporción de partículas lisas estimadas por microscopia electronica después de purificación sobre gradiente de azucar/CsCl.

En las mucstras dónde se lo descubrió, el VP7 de los serotipos migró diferentemente. Se discute de la significación posible de la ausencia de VP7 en algunas preparaciones.

Palabras claves : Rotavirus bovinos - Serotipos - Polipéptidos virales.

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