

COMPARISON BETWEEN STRAINS OF INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS (*BOVID HERPESVIRUS 1*), FROM RESPIRATORY AND GENITAL ORIGINS, USING POLYACRYLAMIDE GEL ELECTROPHORESIS OF STRUCTURAL PROTEINS

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ABSTRACT

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The polypeptide composition of three strains of infectious bovine rhinotracheitis virus, isolated from typical respiratory disease (IBR), has been compared with that of three strains isolated from the genital tract of cattle suffering from infectious pustular vulvovaginitis (IPV). All the IBR strains are similar to each other, but different from the IPV strains, which in turn were similar to each other. IBR isolates and IPV isolates differed in three polypeptides.

INTRODUCTION

Many attempts have been made to differentiate strains of infectious bovine rhinotracheitis (*Bovid herpesvirus 1*), especially those of respiratory and genital origins (IBR and IPV). Most attempts revealed that different isolates are quite similar with respect to biophysical and antigenic properties (Gillespie et al., 1959; Wagner and Gillespie, 1959; McKercher et al., 1959; Liess et al., 1960; McKercher, 1963; McKercher, 1964a; Mohanty and Lillie, 1970).

Grinyer et al. (1962) could not observe any difference between the morphology and the intracellular development of the strains they studied; nor did Black and Slack (1972) in comparing the base composition of the desoxyribonucleic acids ($G + C = 72\%$). Plaques produced under agarose, could distinguish strains isolated from encephalitis (Bagust, 1972; Jetteur et al., 1979), whereas the other strains could not be differentiated by this technique (McKercher, 1964b; Buening and Gratzek, 1967; Bagust, 1972; Jetteur et al., 1979).

Bartha et al. (1969) observed differences in the resistance to heat and to trypsin treatments of certain strains. Slight antigenic variations can be observed using neutralization kinetic studies (Gratzek et al., 1966; Buening and Gratzek, 1967; Crandell, 1972; House, 1972; Potgieter and Mare, 1974), as well as biophysical differences by zone-electrophoresis (Straub et al., 1964; Matheka and Straub, 1972).

Reproducible differences in the polypeptide composition have previously been shown to exist between different isolates of herpes simplex type 1 (Heine et al., 1974; Pereira et al., 1976; Cassai et al., 1975/76), but not between different isolates of human cytomegalovirus (Gupta et al., 1977).

To determine whether similar differences occur between IBR and IPV isolates, we compared several strains isolated from the respiratory and the genital tracts of cattle, with the international reference strain (Los Angeles IBRV) (Pastoret et al., 1975, 1978). The present report discusses the differences in polypeptide composition of these IBR-IPV isolates.

MATERIAL AND METHODS

Cells

Maolin Darby Bovine Kidney (MDBK) cells were used for all assays. They were grown in Earle's minimum essential medium (MEM), supplemented with non-essential amino acids (Flow laboratories), 0.85 $\mu\text{g/ml}$ sodium bicarbonate, 10% fetal bovine serum (FBS), 100 units/ml penicillin and 10 $\mu\text{g/ml}$ of streptomycin.

Viruses

Seven different strains of IBR virus were used. They included: the Los Angeles strain (kindly provided by Prof. McKercher, Davis, CA, U.S.A.); strains IBR/Cu5 and IBR/Cu7, isolated in Belgium from respiratory cases; strain IBR/Cu5/TlJ06, isolated from a dexamethasone treated animal which had previously been experimentally infected with IBR/Cu5 (Pastoret et al., 1979); three strains isolated from cattle suffering from infectious pustular vulvovaginitis, i.e. strain IPV/Ak, isolated in The Netherlands (kindly provided by Dr. Akkermans), strain IPV/Wel/3760 and strain IPV/Wel/2144, both isolated in Belgium, and kindly provided by Dr. Wellemans. All isolates were plaque purified three times under agarose.

Virus was produced in MDBK cells as follows. Briefly, confluent cultures were washed and infected at a multiplicity of 5 p.f.u./cell. After 1-h adsorption period at 37°C, unadsorbed virus was removed by one wash in phosphate buffered saline (PBS); the cells were then overlaid with MEM without serum. When the monolayers exhibited extensive cytopathic effect (48 h), the supernatant was collected, and centrifuged for 10 min at 8000 g to remove cellular debris. Labelling was carried out with 5 $\mu\text{Ci/ml}$ of either a [^3H]-amino acid mixture or [^3H]-glucosamine (Amersham), 5 h post-infection.

The virus was purified by pelleting twice (first in MEM, then in PBS) at 120 000 *g* for 60 min each time, and then once at 140 000 *g* in PBS, and resuspended in 3 ml of distilled water. Solid CsCl was added to a final density of 1.26 g/cm³ (Fritsch, 1975). The virus was centrifuged at 100 000 *g* for 30 h at 4°C and fractions collected from the bottom. Virus banding at 1.25 g/cm³ was used for further studies. Purity of the virus samples was assessed by electron microscopy (Smith, 1967; Burton, 1978). Infectivity was titrated under agarose, using standard techniques (Pastoret et al., 1979).

Polypeptide analysis

Purified virus was dialysed overnight at 4°C against distilled water, and denatured by the addition of 8 M urea, 0.5 sodium lauryl sulfate (SLS), 5% mercaptoethanol, and heated for 15 min at 60°C. Following denaturation, bromophenol blue (0.05% in 50% sucrose), was added to each virus preparation, which were then subjected to slab gel electrophoresis (Dual vertical slab gel electrophoresis cell 221, Biorad), as described by Stevely (1975). Twenty-five µg of each virus preparation (in 150 µl) were applied on the stacking gel (3% acrylamide; 0.08% bis-acrylamide; 0.125 M Tris-hydrochloride, pH 7.0; 0.1% SLS; 0.2% (*w/v*) N,N,N',N'-tetramethylethylene-diamine; 0.2% (*w/v*) ammonium persulfate).

The separating gel was composed of: 10% acrylamide + diallyltartardiamide (ratio 27:1); 0.375 M Tris-hydrochloride, pH 8.8; 0.1% SLS; 0.04% (*v/v*) N,N,N',N'-tetramethylethylene-diamine; 0.04% (*w/v*) ammonium persulfate.

The polypeptides were allowed to separate over a distance of 200 mm, at a current of 30 mA per gel. The electrode buffer contained: 0.025 M Tris, pH 8.5; 0.192 M glycine; 0.1% SLS. At the end of the run, the polypeptides were fixed in 7% acetic acid and stained with Coomassie blue. The gels were then destained with 20% methanol in 5% acetic acid.

Molecular weight determinations of the Los Angeles strain polypeptides were conducted in tube gels as described by Spear and Roizman (1972). The separating gels contained either 6%, 8.5%, 10% or 12% acrylamide + bisacrylamide (ratio 40:1); 0.375 M Tris-hydrochloride pH 8.8; 0.1% SLS; 0.03% (*v/v*), N,N,N',N'-tetramethylethylene-diamine; 0.05% (*w/v*) ammonium persulfate.

Following electrophoresis, fixation, staining and destaining, were performed as described above. The molecular weights were calculated according to the method of Weber and Osborn (1969). The relative migration of each subunit was estimated in relation to that of the bromophenol blue, and compared with the relative migration of some chosen proteins of known molecular weights, furnished by Combithek (Boehringer): trypsin inhibitor (M.W. = 21 500 daltons); bovine serum albumin (M.W. = 68 000 daltons); RNA-polymerase from *Escherichia coli*, α subunit (M.W. = 39 000 daltons), β subunit (M.W. = 155 000 daltons), β' subunit (M.W. = 165 000 daltons). We also used swine myosin (M.W. = 220 000 daltons). The mean molecular weight of each differ-

ent structural subunit was calculated from 10 different gels. The amount of protein was estimated by Lowry's method (Lowry et al., 1951), after dialysis if necessary.

Fluorography

Autoradiography was conducted according to the technique described by Bonner and Laskey (1974), and Laskey and Mills (1975). Gels were washed three times in dimethylsulfoxide (DMSO), placed in a solution containing 20% 2,5-diphenyloxazole (PPO) dissolved in DMSO (*w/v*) then in distilled water to precipitate the PPO. Drying of the gels was carried out under vacuum in a gel slab dryer (Biorad, 224).

Autoradiographic images were obtained by exposing the gels to Agfa-Gevaert Curix RP2 (X-Ray film), sensitized by exposure to a brief flash of monochromatic light. The contact time between the gel and the film was 120 h at -70°C .

RESULTS

The numbers and molecular weights of the polypeptides of the Los Angeles strain of IBRV are given in Table I. Twenty-one different polypeptides were found to be present in purified enveloped extracellular virus and to differ from those present in non-infected MDBK cells (Fig. 1). Ten of them appeared to be glycosylated as determined by incorporation of [^3H]-glucosamine.

The molecular weights ranged from 31 000 to 275 000 daltons. Similar molecular weights were observed for other IBRV isolates, but there appeared to be distinct differences between the IPV and IBR isolates (Fig. 2), although there were always equal numbers of polypeptides in IBR and IPV.

The major differences between the IPV and IBR isolates are that VP7 is consistently of lower molecular weight in the IPV strains than in IBR strains. In contrast, the glycoproteins VP 12 and VP 13 are larger in the IPV strains.

CONCLUSION

The molecular weights of the structural polypeptides of *Bovid herpesvirus 1* (BHV1) are distributed in the same way as those present in five other herpesviruses studied to date (Killington et al., 1977). However, according to the polypeptide and nucleic acid composition (G + C), BHV1 strains appear to be more closely related to pseudorabies virus (Goodheart and Plummer, 1975; Stevely, 1975; Sklyanskaya et al., 1977; Pastoret et al., 1978).

Although there are these general similarities between IBR-IPV and other herpesviruses, we could distinguish IBR from IPV by polyacrylamide gel electrophoresis of their polypeptides. Thus, the glycosylated VP 12 and VP 13, are of higher molecular weights in the IPV isolates, than in IBR. The reverse is true for VP 7. The fact that there are only small differences between these

TABLE I

Molecular weights of the polypeptides of extracellular enveloped IBRV (Los Angeles strain)

Polypeptide	Molecular weight (daltons)	Glycosylation
VP1	275 000	+
VP2	256 000	
VP3	180 000	
VP4	150 000	
VP5	139 000	
VP6	120 000	+
VP7	99 000	
VP8	90 000	
VP9	86 000	+
VP10	83 000	+
VP11	76 000	+
VP12	68 000	+
VP13	66 000	+
VP14	62 000	
VP15	54 000	+
VP16	53 000	+
VP17	43 000	+
VP18	41 000	
VP19	38 000	
VP20	33 000	
VP21	31 000	

Sum of the molecular weights: 2 043 000 daltons.

strains supports the previous observations that it is difficult, but not impossible to differentiate the isolates. Since the major variation appears to be associated with glycoproteins (envelope proteins), it may explain why subtle differences could be detected by serum neutralization and zone-electrophoresis.

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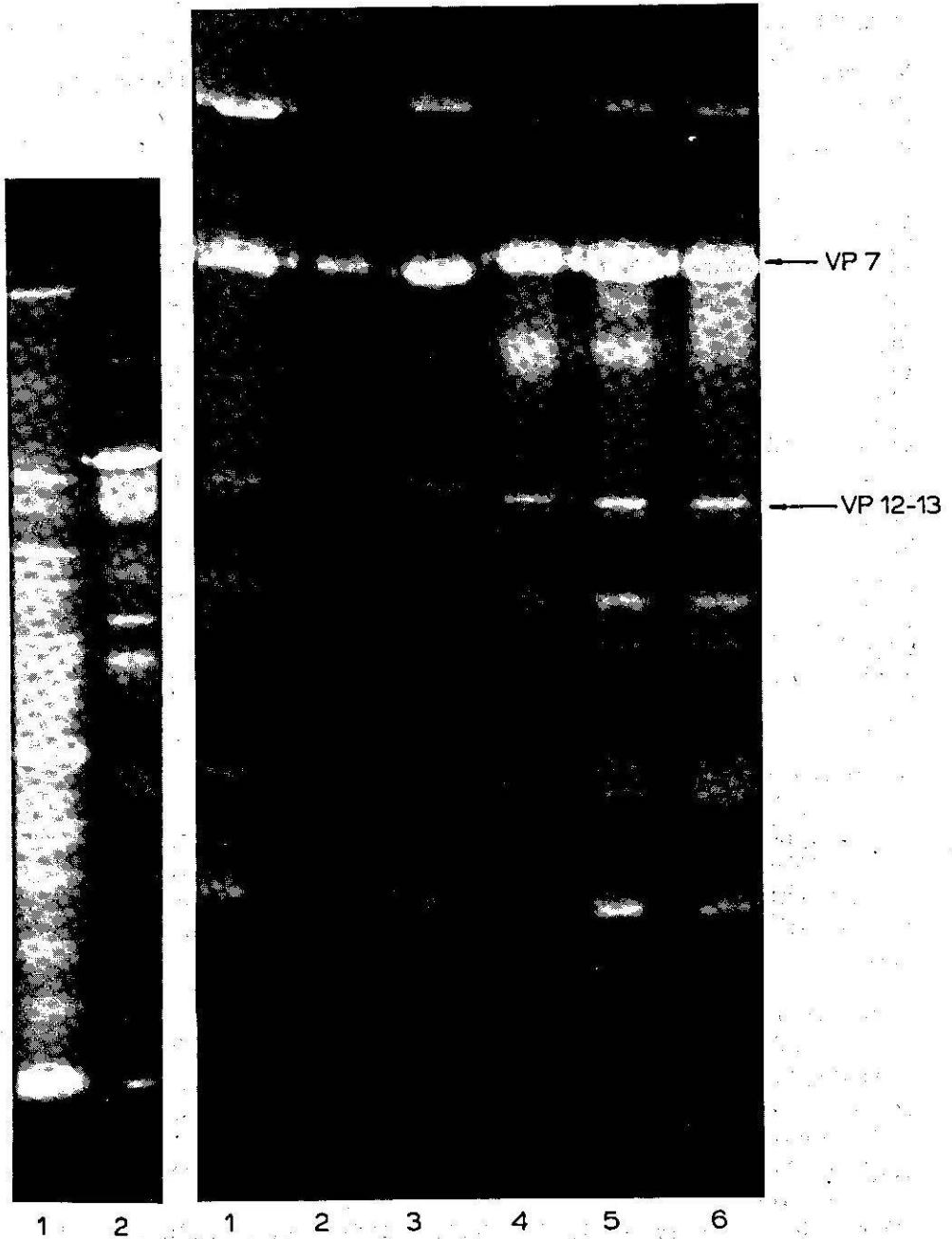


Fig. 1. Electrophoretic comparison between Los Angeles strain of IBRV and uninfected MDBK cells. Labelling with [^3H]-amino acid mixture. 10% acry-diallyl.
(1) IBR/Los Angeles.
(2) Uninfected MDBK cells.

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Fig. 2. Electrophoretic comparison between IPV and IBR strains of *Bovid herpesvirus 1*. Labelling with [³H]-amino acid mixture; 10% acrylamide-diallyltartardiamide slab gel and fluorography.

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|------------------|-------------------|
| (1) IPV/Wel/3760 | (4) IBR/Cu5 |
| (2) IPV/Ak | (5) IBR/Cu5/T1J06 |
| (3) IPV/Wel/2144 | (6) IBR/Cu7 |

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