Short Communication

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Polypeptide analysis of Indian isolates of bovine herpesvirus-1 (BHV-1)

B P SREENIVASA[1], C NATARAJAN[2], M R GAJENDRAGAD[3], V V S SURYANARAYANA[4] and T J RASOOL[5]

Indian Veterinary Research Institute, Bangalore Campus, Hebbal, Bangalore 560 024

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Molecular studies of the bovine herpesvirus 1 (BHV-1) had lead to the classification of the virus into BHV-1.1 (IBR-like), 1.2 (IBR-like), 1.2 (IPV-like) and 1.3 (encephalitic). The present study was undertaken to analyze the polypeptide profile of 2 respiratory isolates and an abortion isolate representing different geographical regions as well as to identify the immunogenic polypeptides of the isolates.

Virus propagation

The virus isolates and a German respiratory isolate as reference strain were propagated using standard cell - culture procedures in Madin-Darby bovine kidney (MDBK) cell monolayers grown in roller bottles in the presence of Eagle's minimum essential medium (EMEM) and 10% inactivated goat serum. The virus from 250 m culture was concentrated by treating with PEG-6000 (8% W/V) and purified by pelleting at 90 000 × g for 2 hr through 30% (W/V) sucrose cushion, and resuspended in TNE buffer. Mock infected culture supernatant was treated similarly and used as control.

Radioactive labelling of infected cells and preparation of cell lysate

Proconfluent monolayers of MDBK cells were infected with the viruses at a multiplicity of 10 TCID 50/cell. After viral adsorption and washing the incubation was continued for 6 hr at 37° C in the presence of methionine-free EMEM. Labelling of the proteins was done by adding approximately 40 pCi/ flask of ^{35} S-methionine (specific activity 300/Ci/mmol). Labelled cells were harvested at 24 hr post-infection.

Cell lysate was prepared by adding radio-immuno-precipitation buffer (RIPB) consisting of 150 mM NaCl, 50 mM Tris (pH 7.4), 1% Triton ×100, 0.1% SDS, 1% sodium dioxycholate, 1 mM EDTA, 0.1% sodium azide and 0.2 mM PMSF to the infected cell pellet. Radioactive counts of the samples were estimated using liquid scintillation counter.

Table 1. Molecular weight of the major virion polypeptides of BHV-1 isolates resolved by 10% SDS- PAGE and coomassie blue staining

Virion polypeptide	Estimated molecular weight (kDa)
1	141.3
2	131.8
3	117.5
4	102.3
5	93.33
6	79.43
7	75.86
8	69.18
9	63.1
10	61.66
11	54.95
12	53.65
13	25.62

Immunoprecipitation

Immunoprecipitation of labelled BHV-1 proteins was carried out with BHV-1 specific antiserum (Central Veterinary Laboratory, Weybridge, UK) as described by Suryanarayana *et al.* (1992). The viral proteins, lysate of the labelled cells and immunoprecipitates were resolved by electrophoresis through 0.1% SDS-10% acrylamide according to the procedure of Laemmli (1970). Electrophoresis was carried out at 100 V for 12 hr. The proteins were stained with 0.25% Coomassie brilliant blue (CBB) and the labelled proteins bands were detected by fluorography.

CBB-stained polypeptide profiles of the 3 isolates and the reference strain are shown in Fig.1. In the viral lanes 13 bands matching with the cellular control (lane C) were considered as cell specific and eliminated. The estimated MW of these 13 bands are presented in Table 1. The bands

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Present address: [1] Ph. D Scholar, [2]Joint Director, [3,4]Senior Scientist. [5]Senior Scientist, Rajiv Gandhi Centre for Biotechnology, Jagathy, Thiruvananthapuram 695014.

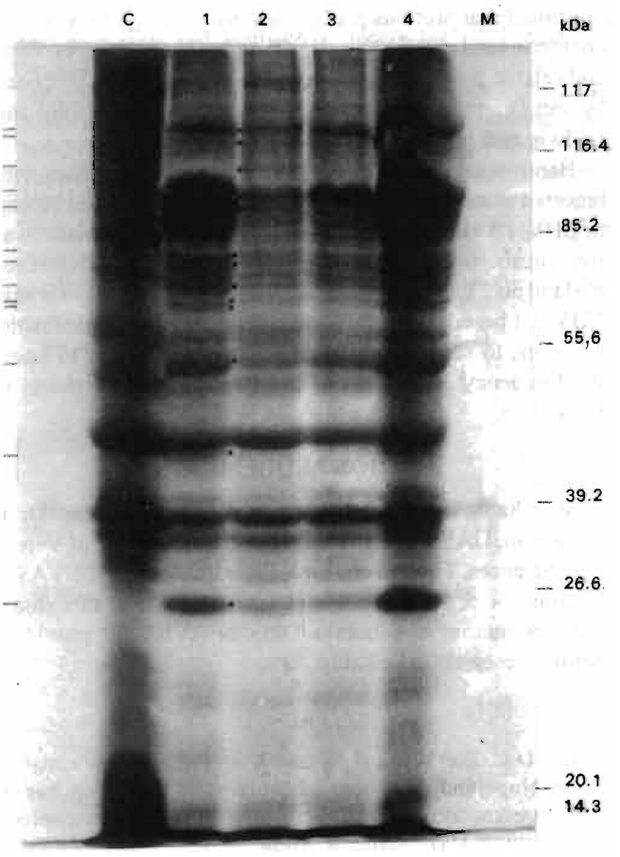

Fig .1. Polypeptides of different BHV1 isolates resolved by SDS-PAGE and Coomassie blue staining. The size of molecular weight markers are shown on the right hand side and the BHV1 polypeptides are marked on left-hand side. Lane C- Mock infected MDBK cell control: 1. BHV1 reference strain: 2. IBRV Hassan isolate: 3 216/IBR-II isolate: 4. IBR/HVS isolate.

ranging in MW from 25 to 141 kDa did not show any variation between the isolates. The bands with MW 25, 54, 102. and 141 kDa were intense showing the comparatively higher concentration of these proteins.

The fluorography of ^{35} S - methionine labelled proteins showed 28 bands which are virus specific (Fig.2). Of these, 9 were major bands and the rest were minor with low intensity. The estimated MW of these bands which ranged between 25 to 158 kDa are shown in Table 2. Comparison of the polypeptide profiles did not show major variation among the isolates. Band no.27, however, showed a marginal decrease in mobility in both the respiratory isolates as compared to the abortion isolate and the reference strain (lanes 4 and 6. Fig. 2).

Using purified virus different authors have reported 20 to 36 virus-specific protein bands for BHV-1 ranging in MW from 12 to 275 kDa (Pastoret *et al.* 1980. Misra *et al.* 1981, Bolton *et al.* 1983, Metzler *et al.* 1985, Scott *et al.* 1988, Trepanier *et al* 1988). While most of these reports,

Table 2. Molecular weight of polypeptides of BHV1 isolates detected by fluorography and immunoreactivity of the polypeptides

Virion polypeptide	Estimated MW (kDa)	Immunoprecipitation
1*	158.5	+++
2*	144.5	++
3	138	++
4	125.9	++
5	114.8	++
6,7*	104.7	+
8	85 11	+
9	75.86	--
10,11	69.18	++
12	66.07	+
13	64 57	+
14	63 1	--
15*	58.88	++
16	57.54	++
17*	54.95	++
18	53.7	--
19	52.48	++
20	46.77	--
21	45.71	--
22	43.65	--
23	41.69	--
24	40.74	--
25	39.81	--
26*	38.02	++
27*	34.67 (35.84) $	--
28	25.62	--

$> MW in parenthesis referes to isolates IBRV/Hassan and 216/IBR-II +,++,+++ , indicate intensity of immunoprecipitated bands. – , no precipitation detected by autography. *, major bands.

exclusively described polypeptide profiles of radioactively labelled virus preparations, Scott *et al.* (1988) and Trepanier *et al.* (1988) reported the polypeptide profiles of unlabelled virus preparations by CBB staining. In the present study using CBB staining, only 13 bands could be identified with clarity. This PAGE profile is comparable with that of Trepanier *et al.* (1988) who reported using a similar approach, 22 polypeptides for a Malaysian isolate.

35 S-methionine incorporation studies showed more number of bands. This may be due to the presence of nonstructural proteins and proteins induced during virus infection, since infected cell lysate was used in the study. It was anticipated that the use of cell lysate could result in more number of bands and hence offer a better chance for detecting variation between the isolates. However, no variation could be detected between the isolates but for band No. 27.

The major bands observed in the study (Table 2) corresponded with VP4, VP11, VP24, VP25, VP30 and VP32/33 of Metzler *et al.* (1985). Proteins 2 and 19 appear to be virus-induced and analogous to 'd' and 'I' of Metzler *et al.* (1985). The protein No. 27 which showed marginal mobility shift in this study corresponded to VP32/33 of

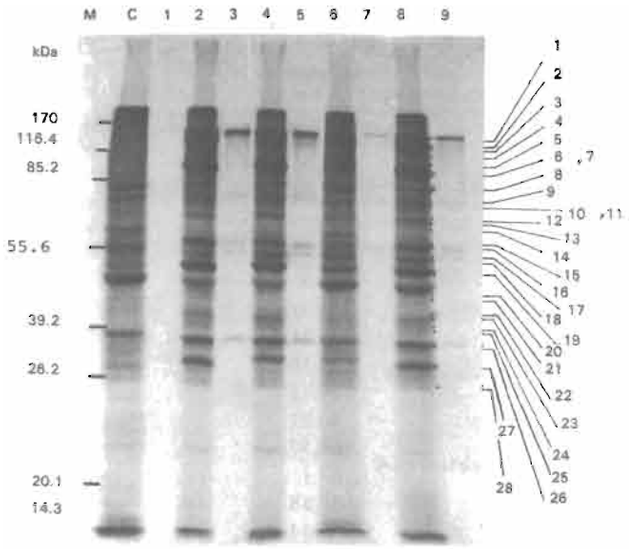

Fig. 2. 35S-Methionine labelled and immunoprecipitated BHV-1 polypeptides. Molecular weight markers are marked on the left hand side and the viral polypeptides are marked on the right hand side. Immunoprecipitated polypeptides of BHV are marked with asterisks. Lane C- Mock infected-cell control; 1, immunoprecipitate of the cell control; 2,4,6 and 8 -virus isolates as described in Fig. 1 in that order; 3,5,7 and 9 immunoprecipitates of the same in the same order.

Metzler *et al.* (1985) which did not show any variation among 11 European and 2 reference strains (Cooper and Los Angeles). Since a comparison of protein profiles reported in the literature for different BHV-1.1 isolates did not reveal any shift in the mobility for any of the other polypeptides, this variation is presumed to be a minor intratypic variation.

Fluorography of the immunoprecipitated virion polypeptides with BHV-1 specific polyclonal antisera showed 12 viral polypeptides under reducing conditions (Fig. 2 : lanes 3,5,7 and 9). One of the striking observations was the lack of precipitation of polypeptide No.27 which showed an intense band in fluorography and differences in mobility between the isolates. It may be logical to speculate that this protein may not be of immunological significance and hence the virus could have tolerated a minor change in its size.

The prominent immunoprecipitated proteins were 1,10/11, 15/16, 17, 19 and 26 (Fig.2, Table 2). It is worth mentioning that polypeptide 26 with a MW of 38 kDa was very intense in spite of its low MW. It could be due to high methionine content in this polypeptide. Polypeptide 10/11 with an estimated MW of 70 kDa could be the reduced form of glycoprotein gIV as reported by van Drunen Littel-van den Hurk and Babiuk (1986) and Collins *et al.* (1983) Multiplicity of immunogenic proteins, existence of precursors, individual bias in estimation of MW etc., make it difficult to compare the equivalent precipitated proteins with the earlier reports. Disregarding some minor differences in precipitation patterns and molecular weights, it could be presumed that proteins precipitated by the polyclonal serum comprise viral proteins gI,gII,gIII and gIV, their components and related polypeptides, as per the nomenclature proposed by van Drunen Littel-van den Hurk and Babiuk (1986) and Ludwig and Letchworth (1987).

Band -wise comparison of the polypeptide profiles with reported standard pattern showed that all the isolates belong to BHV-1.1 (IBR-like), even though one of the isolates was from an abortion case. The variation observed with reference to Band no.27 appears to be the first report of variation within BHV-1.1 based on polypeptide profile. It is highly desirable, however, to screen significant number of isolates to know the frequency and extent of polypeptide polymorphism in BHV-1.1.

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