Restriction Endonuclease Analysis of Bovine Herpesvirus 1 DNA and Nucleic Acid Homology between Isolates

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SUMMARY

Isolates of bovine herpesvirus 1 (BHV-1) are associated with a variety of clinical manifestations. To determine if a single form of BHV-1 was responsible for the different virus-associated diseases or whether subpopulations of various isolates produced different clinical symptoms, studies were initiated to examine the DNA restriction enzyme patterns and nucleic acid homology between virus isolates from respiratory infections and other clinical syndromes. Differences between the genomes of several virus isolates were detected using DNA restriction enzyme analyses. However, nucleic acid hybridization studies of the virus DNAs using filter and liquid hybridization indicated at least a 95% genetic homology between the virus isolates from different types of infections. Additionally, these studies demonstrated that the DNA of BHV-1 had an average molecular weight of 84 x 10^6.

Bovine herpesvirus 1 (BHV-1) has been associated with a variety of clinical syndromes. These are primarily respiratory, but may also include genital infections, encephalitis, ocular carcinoma and abortion. The virus associated with respiratory diseases has been referred to as infectious bovine rhinotracheitis (IBR) virus, whereas the virus isolate associated with genital infections has been termed infectious pustular vulvovaginitis (IPV) virus (Kahrs, 1977; Pastoret et al., 1982). Misra et al. (1983) reported that isolates of BHV-1 could be divided into three distinct groups based upon the virus DNA restriction enzyme patterns. As in the situation of herpes simplex virus types 1 and 2 in humans, the possibility exists that respiratory and genital infections may be caused by partially related viruses that appear serologically similar, but by analysis of the virus DNA represent different virus types. Consequently, the investigations reported here were initiated to determine the amount of genetic homology between a respiratory isolate of BHV-1 and other clinical isolates using restriction enzyme analysis and nucleic acid hybridization of virus DNA.

Since reports indicated that DNA from BHV-1 isolates associated with respiratory infections (IBR virus) had different restriction patterns from the DNA isolated from viruses associated with genital infections (IPV virus) (Engels et al., 1981), we initially differentiated virus isolates using restriction enzyme analysis and nucleic acid hybridization. The BHV-1 isolates used in these studies were IBR virus Los Angeles (LA), a respiratory disease isolate, obtained from the American Type Culture Collection and an IPV virus isolate obtained from the Department of Microbiology, School of Veterinary Medicine, University of California, Davis. A variety of isolates from various clinical disease states were also obtained from the School of Veterinary Medicine at Washington State University, Pullman, Washington and from the Agriculture Experiment Station at the University of Nevada. Bovine embryonic lung cells were used for all experiments and cultured in Dulbecco’s MEM with 5% foetal calf serum. Virus DNA for use in restriction enzyme analysis was isolated and purified essentially as described by Huang et al.
Various isolates of BHV-1 representing a variety of clinical syndromes were compared by restriction enzyme analysis and Southern blot hybridization as illustrated in Fig. 1(a, b). Even though there were differences in virus DNA restriction enzyme patterns there appeared to be no correlation between the type of pattern obtained and the corresponding clinical manifestation. The DNA restriction enzyme patterns depicted in Fig. 1 lanes 3, 9, 10, 11 and 13 are from ‘respiratory’ isolates and conform to patterns obtained from vaginal isolates (lanes 2, 4, 5 and 6), an isolate from an aborted foetus (lane 8) or from bovine ocular carcinoma (lane 7). It is of interest to note that the IPV virus isolate illustrated (lane 2) has a pattern exactly like that of a respiratory isolate (lane 13). Additionally, genital isolates (lanes 5 and 6) may have identical patterns to IBR virus LA isolate (lane 3). Another difference is that of a restriction enzyme pattern obtained from the DNA of an encephalitis isolate of BHV-1 (Fig. 1, lane 12). Although very faint in the illustration of the gel it demonstrated a pattern conforming to that of the ‘Cooper’ isolate of BHV-1 (Mayfield et al., 1983).

To initially determine the nucleic acid homology between isolates, the BHV-1 DNA fragments in Fig. 1(a) were transferred to nitrocellulose paper (Southern, 1975) and hybridized (Maniatis et al., 1982) with a nick-translated (Rigby et al., 1977) IBR virus LA isolate 32P-labelled DNA probe (Fig. 1b). In every case the probe DNA hybridized to all the fragments present for each BHV-1 isolate while not hybridizing to the lambda phage DNA used as a control. This demonstrates the extensive homology between DNA sequences present in all the BHV-1 isolates examined. When the IPV virus isolate DNA (lane 2) was used as a probe of an identical gel blotted and hybridized, duplicate results were obtained (data not shown).

It was apparent from the initial restriction enzyme analysis and hybridization data (Fig. 1a, b) that a high percentage of homology existed between isolates of BHV-1 regardless of the clinical manifestation. Therefore, more extensive studies were conducted using the LA isolate (Fig. 1, lane 3) and an IPV isolate (Fig. 1, lane 2), two isolates from different clinical syndromes which show the greatest variation in restriction enzyme pattern. One µg of DNA isolated from the IBR LA and IPV virus was digested with 5 units of the restriction enzymes HindIII, EcoRI or BamHI (Bethesda Research Laboratories), end-labelled with [α-32P]dATP, using the Klenow fragment of DNA polymerase I in the presence of unlabelled dGTP, dCTP and dTTP (Maniatis et al., 1982) and electrophoresed (Fig. 2). Molecular weights of the resultant fragments were determined by comparison with phage lambda DNA standards (Bearden, 1979). Molar amounts of the virus DNA restriction fragments were calculated from DNA obtained from an in vitro infection using 32P-labelled H2PO4 to label the virus DNA uniformly. Following electrophoresis and extraction of the virus DNA fragments from agarose gel slices, molarities were determined as described by Wharton et al. (1981) and the data are presented in Table 1. The sum of the molecular weights of individual restriction fragments indicated average molecular weights for IBR virus and IPV virus DNA to be 84 × 106. Genome molecular weights for both BHV-1 isolates obtained by summation of the molecular weights of virus DNA fragments were lower than that reported by Engels et al. (1981), but concurred with that of Mayfield et al. (1983) and Misra et al. (1983).

Although the results of blot hybridizations indicated homology between all DNA fragments

Fig. 1. Comparison of HindIII restriction enzyme cleavage patterns and Southern blot hybridization of clinical isolates of BHV-1. Following digestion with HindIII 10 µl of tracking dye (50% Ficoll, 0.75% bromophenol blue, 0.1% EDTA) was added to the reaction mixture and electrophoresed in a horizontal 0.7% agarose slab gel for 14 h at 75 mA in Tris-acetate buffer (40 mM-Tris, 20 mM-sodium acetate, 18 mM-NaCl, 2 mM-EDTA, pH 8.4). The gel was stained with ethidium bromide and photographed with Polaroid type 667 film during u.v. transillumination (a). The DNA fragments were transferred to nitrocellulose and hybridized with a nick-translated 32P-labelled IBR LA virus DNA probe (b). Lane 1, lambda phage DNA restricted with HindIII; lane 2, IPV; lane 3, IBR LA isolate; lane 4, vaginal isolate following vaccination; lanes 5 and 6, vaginal isolates from genital infections; lane 7, isolate from bovine ocular carcinoma; lane 8, isolate from an aborted foetus; lane 9, Colorado isolate obtained from U.S.D.A., National Veterinary Service, Ames, Iowa; lanes 10 and 11, respiratory disease isolates; lane 12, isolate from an encephalitis infection; lane 13, respiratory disease isolate.
Table 1. Sizes* of bovine herpesvirus 1 virus isolate DNA fragments produced by restriction endonuclease cleavage

<table>
<thead>
<tr>
<th></th>
<th>HindIII</th>
<th>EcoRI</th>
<th>BamHI</th>
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<tr>
<td></td>
<td>IBR</td>
<td>IPV</td>
<td>IBR</td>
</tr>
<tr>
<td>A</td>
<td>14.3</td>
<td>14.3</td>
<td>28.4</td>
</tr>
<tr>
<td>B</td>
<td>12.9</td>
<td>12.9</td>
<td>15.0</td>
</tr>
<tr>
<td>C</td>
<td>11.3†</td>
<td>11.3†</td>
<td>12.4</td>
</tr>
<tr>
<td>D</td>
<td>10.4‡</td>
<td>10.4‡</td>
<td>11.8</td>
</tr>
<tr>
<td>E,F</td>
<td>8.6‡</td>
<td>8.6‡</td>
<td>8.9</td>
</tr>
<tr>
<td>G,H</td>
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<td>8.0‡</td>
<td>5.8</td>
</tr>
<tr>
<td>I</td>
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<tr>
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<tr>
<td>L</td>
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<td>P</td>
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* Molecular weights ($\times 10^{-6}$) were determined by regression analysis of fragments electrophoresed with bacteriophage lambda DNA fragments.

† 0.5 M amount approximately. All other values are approximately 1.0 M. The molar abundance of each fragment was determined by the formula $M = (\text{Fragment c.p.m.}/\text{total c.p.m.})/(\text{Fragment mol. wt.}/\text{mol. wt. of intact molecule})$.

‡ Present in 1.5 M amount.

§ Present in 2.0 M amount.
Short communication

Fig. 3. Comparison of IBR and IPV virus DNA by DNA-DNA reassociation. (a) Hybridization of $^{32}$P-labelled IBR virus DNA in the presence of unlabelled DNA from IBR virus (●), IPV virus (○) and lambda phage (■). (b) Hybridization of $^{32}$P-labelled IPV virus DNA in the presence of unlabelled DNA from IPV virus (●), IBR virus (○) and lambda phage (■). Following nick translation (Rigby et al., 1977) and heat denaturation, single-stranded probe DNA was collected by hydroxylapatite column chromatography (Martinson, 1973). The labelled probe, 4 μg unlabelled virus DNA and 20 μg salmon sperm DNA were sonically disrupted to approximately 5S in size (Cedar, 1976), heat-denatured at 100 °C for 15 min and then brought to 1 ml in 1 × PIPES buffer (0.72 M-NaCl, 0.01 M-PIPES, 0.001 M-EDTA, pH 7.0). The DNA was allowed to reassociate at 61 °C (T_m - 25 °C) and following initiation of the reaction, samples were taken at 0.5, 1, 2, 5, 8, 16 and 24 h. The fraction of reassociated $^{32}$P-labelled DNA was analysed by $S_1$ enzyme differential digestion (Vogt, 1973). C_{ot} (mol. s/l deoxynucleotide) was calculated on the basis of the total amount of virus DNA present in each assay. Background values were subtracted and the data normalized following five separate hybridizations with three samples taken per time point.

of the isolates examined, partial homology between a specific fragment and the probe DNA could produce a similar pattern by autoradiography, as would complete hybridization to the same fragment. To quantify differences in genetic composition between the isolates of BHV-1, which exhibited the greatest variation in restriction pattern, liquid hybridization between the DNA of the IBR LA and IPV virus isolates was conducted and the results plotted as the percent DNA reassociated versus C_{ot} and incubation time (Britten & Kohne, 1968). In both sets of hybridizations, the $^{32}$P-labelled virus probes did not reassociate significantly in the presence of lambda phage DNA used as a control. As illustrated in Fig. 3(a) there were no differences in the rates of reassociation of $^{32}$P-labelled IBR LA virus DNA with unlabelled IBR LA virus DNA or with unlabelled IPV virus DNA. Conversely, $^{32}$P-labelled IPV virus DNA reannealed at the same rate with unlabelled IPV virus DNA as it did with unlabelled IBR LA virus DNA (Fig. 3b). The C_{ot}t_{1/2} value of $4 \times 10^{-2}$ was the same in both sets of reactions. Consequently, there is very little detectable quantitative difference in sequence homology between these isolates of BHV-1.

The results of the liquid hybridization studies indicate a high degree of homology between the DNA of IBR and IPV virus isolates examined. Although a 5% mismatch during DNA hybridizations may occur (Sugino & Kingsbury, 1976), the conditions used for both the Southern blot hybridizations and the liquid hybridizations were relatively stringent. Southern blots were hybridized at 15 °C below the T_m of BHV-1 DNA in 50% formamide followed by extensive washing at 60 °C in 0.1 × SSC, while the liquid hybridizations were conducted at 25 °C below the T_m of BHV-1 DNA in 0.72 m-NaCl.

The results presented indicate that, although the DNA from the IBR LA virus isolate, IPV and other BHV-1 virus isolates may vary in their restriction pattern, the DNA sequences of these isolates are at least 95% homologous. Differences in restriction enzyme patterns are probably due to specific point mutations resulting in the loss or gain of certain restriction endonuclease sites. Considering the high degree of DNA homology between various isolates, the reason for differences in clinical syndromes is still not well understood. In agreement with Misra et al. (1983), it may be that routes of infection and/or other environmental factors determine whether a particular isolate causes a typical respiratory tract infection or some other type of disease such as an encephalitis or genital infection. Also, the relationship between
BHV-1 isolates does not appear analogous to that of human herpes simplex virus types 1 and 2 where there is a 50% nucleic acid homology difference (Kieff et al., 1972; Ludwig et al., 1972).

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REFERENCES


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