A Comparison of the Genomes of Bovine Herpesvirus Type 1 and Pseudorabies Virus

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SUMMARY

The DNA sequence homology between bovine herpesvirus type 1 (BHV-1) and pseudorabies virus (PRV) was examined. Reciprocal cross-hybridization of viral DNA labelled by nick translation to Southern blots of restriction endonuclease-digested DNA detected homologous sequences dispersed throughout the genomes of the two viruses. The DNA–DNA hybrids formed were stable under high-stringency wash conditions. Sequences of a 32P-labelled PRV DNA fragment probe were found to hybridize only to a specific region of the BHV-1 genome, suggesting that the detected sequence homology was not due to fortuitous hybridization of G + C-rich sequences. As measured by liquid reassociation kinetics the homology between these two viruses was approximately 8~.

Bovine herpesvirus type 1 (BHV-1) and pseudorabies virus (PRV) are economically important members of the herpesvirus family (Aujeszky, 1902; for review, see Gibbs & Rweyemamu, 1977). BHV-1 has been associated with a variety of clinical conditions in cattle including respiratory, reproductive, ocular, central nervous system and enteric diseases (for review, see Gibbs & Rweyemamu, 1977). Natural as well as experimental infections have also been reported in swine (Woods et al., 1968; Nelson et al., 1972). PRV infection is usually mild or inapparent in adult animals, causing primarily respiratory and reproductive system disease (Gordon & Luke, 1955; Baskerville et al., 1973). In young pigs as well as in other animals including cattle it can be fatal (Dow & McFerran, 1963; Bitsch, 1975).

BHV-1 and PRV resemble each other both morphologically and biochemically (Graham et al., 1972; for review, see Roizman, 1982). The buoyant densities of BHV-1 and PRV in CsCl are 1.731 g/ml (Bowling et al., 1969) and 1.732 g/ml (Ben-Porat & Kaplan, 1962) respectively. The molecular weight of BHV-1 (Cooper strain) DNA is about 84.5 × 10^6 (137 kb) (Mayfield et al., 1983), and that of PRV is approximately 90 × 10^6 (146 kb) (Rubenstein & Kaplan, 1975). Base composition analysis has revealed that the G + C content of these viruses is 72 to 73~ (Graham et al., 1972). Physical maps for BHV-1 and PRV have been established and suggest that the viral genomes are alike in their structural organization (Mayfield et al., 1983; Stevely, 1977; Rixon & Ben-Porat, 1979). These viruses have been classified as having class 2 DNA molecules (Honess & Watson, 1977) which are divided into a long (L) component and a short (S) component. The latter is flanked by inverted repeat sequences and can invert its orientation relative to the L component, thus yielding two isomeric forms.

The antigenic relationship between BHV-1 and PRV has been studied by many researchers with conflicting results. Plummer (1963) reported that there was no cross-neutralization between these two viruses. However, by a one-way complement fixation test he found that PRV did cross-react with BHV-1 antibody at a titre of 1:4. Aguilar-Setien et al. (1979) reported that the serum from 30 of 90 cows naturally infected with BHV-1 neutralized PRV. Conversely, the serum of four swine out of 98 naturally infected with PRV did neutralize BHV-1. In a further
study, Aguilar-Setien et al. (1980) found that cattle immunized with BHV-1 produced antibodies that neutralized both BHV-1 and PRV; however, cattle immunized with PRV produced antibodies that only neutralized PRV. Using hyperimmune rabbit sera, we have recently found that there is a two-way cross-neutralization between BHV-1 and PRV (unpublished results).

Also, employing an ELISA it was found that there was cross-reactivity between these two viruses, with PRV-immunized rabbits producing higher cross-reacting antibody titres than rabbits immunized with BHV-1 (C. E. Bush & R. F. Pritchett, unpublished results).

Due to the biochemical and morphological similarity of these viruses as well as cross-herd infectivity and antigenic cross-reactivity, there has been interest in the study of sequence homology between these two viruses. Studies comparing the genetic relatedness between BHV-1 and PRV by DNA–RNA hybridization (Bronson et al., 1972) showed that there was 6% relatedness when PRV DNA was hybridized with RNA extracted from BHV-1 infected cells, but only 2% homology was detected in the reciprocal cross-hybridization experiment. Since the abundance of transcripts and the extent of viral genome transcription in the infected cells was not determined, the authors stated that complementarity between these two viruses could be more accurately determined by DNA–DNA hybridization techniques. In this paper the question of genetic relatedness between BHV-1 and PRV was re-examined using DNA–DNA reassociation kinetics and the more sensitive method of cross-hybridization of blotted restriction enzyme fragments of one virus with $^{32}$P-labelled DNA of the heterologous virus.

The viruses used in this study were BHV-1, Cooper strain (Zucheck & Chow, 1961) and PRV, Shope strain (Shope, 1931). Virus stocks were all plaque-purified three times and passaged three times prior to propagation. Madin–Darby bovine kidney (MDBK) cells were used to propagate BHV-1, and a continuous rabbit kidney cell line (RK-13) was used to grow PRV. The cells were cultured at 37 °C in 32 oz bottles using Eagle’s MEM, supplemented with 10% foetal bovine serum (FBS), penicillin (100 units/ml) and streptomycin (100 µg/ml). Cell cultures were infected at a m.o.i. of 1. Virus was allowed to adsorb for 1 h at 37 °C. Cultures were replenished with MEM supplemented with 2% FBS and incubated at 37 °C for 24 h. The virus-infected cells and culture fluid were then frozen at −85 °C.

Identical purification schemes were employed for BHV-1 and PRV using a procedure modified from that of Talens & Zee (1976). Virus-infected cells and culture fluid were frozen and thawed three times and clarified by centrifugation at 4°C for 15 min at 3000 g. The virions were subsequently concentrated by centrifuging at 4 °C for 90 min at 35000 g followed by isopycnic centrifugation in linear 20 to 45% (w/w) potassium tartrate gradients. Virions were then pelleted by centrifugation at 4 °C for 90 min at 79000 g.

DNA was released from pelleted virions by Pronase–SDS treatment followed by phenol–chloroform extraction, and purified by centrifugation to equilibrium in CsCl density gradients (Pritchett, 1980). The DNA was digested to completion with the restriction endonucleases (RE) EcoRI, HindIII, BamHI and KpnI (Bethesda Research Laboratories) according to the manufacturer’s suggestions and the fragments were electrophoretically separated in 0.7% agarose gels. The DNA fragments were then stained with ethidium bromide, photographed and transferred to nitrocellulose by the Southern (1975) procedure.

Purified DNAs were labelled in vitro by nick translation (Rigby et al., 1977) with $[^{32}P]$dGTP (800 Ci/mmol) to specific activities of $2 \times 10^8$ to $3 \times 10^8$ c.p.m./µg. Before hybridization, the homologous and heterologous DNA blots were preincubated for 4 h at 68 °C in Denhardt’s solution [0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin in 4 × SSC (1 × SSC is 0.15 M-NaCl, 0.015 M-sodium citrate, pH 7)] (Denhardt, 1966). The $^{32}$P-labelled DNA probes were then denatured with 0.2 M NaOH, neutralized with 0.2 M HCl, and mixed with 2.0 ml hybridization buffer containing 0.9 M-NaCl, 0.03 M-EDTA, 0.02 M-Tris–HCl pH 7.5 and 2 mg of sonicated denatured salmon testes DNA. The DNA probes were then incubated with homologous and heterologous DNA blots (with heterologous blots receiving 10 times as much probe as homologous blots) for 20 h at 68 °C. The blots were then washed under high-stringency conditions: four changes of 2 × SSC containing 0.1% SDS for 10 min at room temperature followed by two 1 h washes in 0.2 × SSC containing 0.1% SDS at 68 °C. The washed blots were then dried and autoradiographed. Autoradiography exposures for heterologous reactions were twice as long as for homologous reactions.
Fig. 1. Demonstration of homologous DNA sequences in BHV-1 and PRV genomes. BHV-1 DNA (5 µg) cleaved with HindIII, BamHI and EcoRI was separated in agarose, stained with ethidium bromide and photographed (lanes 1, 4 and 7). The DNA was subsequently blotted onto nitrocellulose paper which was cut into five 0.5 mm strips. Each strip was then incubated with 10^6 c.p.m. of homologous BHV-1 32P-labelled DNA (lanes 2, 5 and 8) or 10^6 c.p.m. of heterologous PRV 32P-labelled DNA (lanes 3, 6 and 9). Blots were exposed to Kodak X-ray film (XAR-5) with a Cronex intensifying screen for 6 to 8 h for homologous hybridizations and 12 to 18 h for heterologous hybridizations.

It was found that the BHV-1 probe hybridized to all RE fragments of the BHV-1 genome (Fig. 1, lanes 2, 5 and 8) and strongly with most of the RE fragments of PRV (Fig. 2, lanes 3 and 6). Conversely, the PRV probe hybridized to all RE fragments of the complete PRV genome (Fig. 2, lanes 2 and 5) and strongly with most of the RE fragments of BHV-1 (Fig. 1, lanes 3, 6 and 9). The small amounts of BHV-1 DNA migrating more slowly than the HindIII and BamHI A fragments and co-migrating with the EcoRI A fragment (Fig. 1, lanes 1 to 9 and Fig. 3, lanes 3 and 5) probably represent defective genomes, since they were still present when digestions were conducted with a threefold increase of RE concentration.

To determine whether the sequence homology between BHV-1 and PRV was restricted to specific genomic sites, the KpnI A fragment of PRV was excised from a 0.7% agarose gel, electroeluted, butanol-extracted and concentrated by ethanol precipitation. The DNA fragment was then 32P-labelled by nick translation and incubated with a blot of PRV DNA digested with KpnI as well as with a blot of HindIII-digested BHV-1 DNA. Fig. 3 (lane 4) shows that sequences of the A fragment of PRV hybridized only to the G/H/I fragment complex of BHV-1. To determine in which fragment of this complex the homologous sequences occurred, the 32P-labelled KpnI A fragment probe of PRV was incubated with a blot of BHV-1 DNA digested with EcoRI. As shown (Fig. 3, lane 6), hybridization was detected to only fragment A. The A fragment includes HindIII fragment G, but not HindIII fragment I (Mayfield et al., 1983); thus, sequences of the KpnI A fragment of PRV are homologous to sequences found only in the BHV-1 HindIII G fragment. The RE linkage maps for the genomes of these viruses show that both the
Fig. 2. Demonstration of homologous DNA sequences in PRV and BHV-1 genomes. PRV DNA (5 μg) cleaved with KpnI and BamHI was separated in agarose, stained with ethidium bromide and photographed (lanes 1 and 4). The DNA was subsequently blotted onto nitrocellulose paper which was cut into five 0.5 mm strips. Each strip was then incubated with 10^6 c.p.m. of homologous PRV 32P-labelled DNA (lanes 2 and 5) or 10^6 c.p.m. of heterologous BHV-1 32P-labelled DNA (lanes 3 and 6) and autoradiographed as for Fig. 1.

Fig. 3. Demonstration of homologous sequence specificity between BHV-1 and PRV. KpnI fragments of PRV DNA blotted onto nitrocellulose paper were incubated with 10^5 c.p.m. of 32P-labelled PRV DNA probe (lane 1) and 10^5 c.p.m. of 32P-labelled KpnI A fragment probe DNA (lane 2). BHV-1 DNA preparations digested with HindIII and EcoRI were blotted onto nitrocellulose paper and incubated with 10^5 c.p.m. of 32P-labelled total BHV-1 DNA probe (lanes 3 and 5, respectively) and 10^5 c.p.m. of labelled KpnI A fragment probe DNA (lanes 4 and 6, respectively). To detect hybridization, the blots were autoradiographed by exposure to X-ray film with a Cronex intensifying screen. The homologous hybridizations between complete DNA blots and total probes were exposed for 18 h. The autoradiograph of hybridization between the A fragment probe and PRV DNA blot was exposed for 3 h. For hybridization of the PRV fragment probe with HindIII and EcoRI BHV-1 DNA blots the exposure was for 18 h.

BHV-1 G fragment (between 0.29 and 0.38 map units) and PRV A fragment (between 0.17 and 0.37 map units) are located in the UL region (Mayfield et al., 1983; Rixon & Ben-Porat, 1979). 32P-labelled PRV and BHV-1 DNAs incubated with companion blots showed that all RE fragments had been transferred (Fig. 3, lanes 1, 3 and 5). Fig. 3 (lane 2) also shows the specificity of the PRV 32P-labelled KpnI A fragment.

The percentage homology between BHV-1 and PRV was determined by liquid reassociation kinetics using S1 nuclease to distinguish between double-stranded and single-stranded DNA as described earlier (Pritchett, 1980). The hybridization rates between 32P-labelled PRV DNA and unlabelled BHV-1 DNA (Fig. 4) and 32P-labelled BNV-1 DNA and unlabelled PRV DNA (Fig. 5) were accelerated for the first 60 min to a (C0/S)^2 value of 1.2, corresponding to
Fig. 4. DNA-DNA reassociation kinetics between \(^{32}\text{P}\)-labelled PRV DNA in the presence of BHV-1 DNA. PRV \(^{32}\text{P}\)-labelled DNA (\(2 \times 10^8\) c.p.m.) was mixed separately with 12 \(\mu\)g/ml unlabelled PRV DNA (■), 22 \(\mu\)g/ml unlabelled BHV-1 DNA (▲) or 250 \(\mu\)g/ml unlabelled RK-13 cell DNA (●). The mixtures were heat-denatured and reassociated in hybridization buffer at 68 °C. Samples were taken at time periods up to 24 h and reassociated DNA was assayed by S1 nuclease. Data were then analysed by the equation \(\left(\frac{C_s}{S}\right)^{2.22} = 1 + kC_ot\) (Smith et al., 1975), where \(C_0\) is the initial concentration of \(^{32}\text{P}\)-labelled PRV DNA, \(S\) is the amount of S1 nuclease-sensitive DNA at time \(t\), and \(k\) is the kinetics constant.

Fig. 5. DNA-DNA reassociation kinetics between \(^{32}\text{P}\)-labelled BHV-1 DNA in the presence of PRV DNA. \(^{32}\text{P}\)-labelled BHV-1 DNA (\(2 \times 10^8\) c.p.m.) was mixed separately with 22 \(\mu\)g/ml unlabelled BHV-1 DNA (■), 12 \(\mu\)g/ml unlabelled PRV DNA (▲), and 250 \(\mu\)g/ml unlabelled MDBK cell DNA (●). Further details as for Fig. 4.

approximately 8% reassociation. The rates then decreased to that observed for the reassociation of \(^{32}\text{P}\)-labelled viral DNAs in the presence of either MDBK or RK-13 cell DNA. These results were found to be reproducible in two separate experiments.

Hybridization of RE-digested DNA to Southern blots provides a very useful technique for determining the homology relationship between DNAs. It was concluded that BHV-1 DNA and PRV DNA have common sequences which are distributed throughout the viral genomes. The finding that sequences of the PRV KpmI A fragment hybridized only to the BHV-1 HindIII G fragment suggests that the homology detected is not simply fortuitous hybridization of G + C-rich sequences. It is possible that these common sequences represent highly conserved regions of DNA. Since BHV-1 and PRV have been shown to cross-react serologically it is possible that some of these sequences code for common antigenic determinants.

Molecular hybridization techniques have been widely employed in the study of genetic relatedness of both human and animal herpesviruses. Kieff et al. (1972) showed that the genomes of herpes simplex virus type 1 (HSV-1) and HSV-2 have approximately 50% common sequences; it was later shown by Wilkie et al. (1979) that the genomes of these two viruses are colinear. Recently, Davison & Wilkie (1983) showed that the genomes of equid herpesvirus-1 (EHV-1) and varicella-zoster virus (VZV) contain sequences homologous to HSV-1 and HSV-2. When the homologous regions of the genomes were oriented, it was found that the genomes of EHV-1 and VZV were colinear with the L inverted or L and S inverted genome arrangements of HSV-1 and HSV-2. PRV DNA has been compared with HSV-1 and HSV-2 DNAs (Ludwig et al., 1972; Rand & Ben-Porat, 1980). It was found by using DNA–DNA cross-hybridization that PRV shared 8% homology with HSV-1 and HSV-2 and that the homologous sequences were spread throughout the genomes of the viruses. The positions on the genomes of sequence homology between PRV and HSV-1 have been recently examined (Ben-Porat et al., 1983). It was found that there was partial colinearity between the genome of PRV and the IL (inversion of L) form of the HSV-1 genome. The colinearity was specifically shown for the genes coding for the major immediate-early protein, the major capsid protein and the thymidine kinase. It would be of future interest to compare the exact genomic locations of the homologous sequences found to be common between BHV-1 and PRV.
REFERENCES


Short communication


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