Location and Characterization of the Bovine Herpesvirus Type 2 Thymidine Kinase Gene

BY MICHAEL SHEPPARD1* AND JOHN T. MAY2

1CSIRO, Division of Animal Health, Animal Health Research Laboratory, Private Bag No. 1, P.O. Parkville, Victoria 3052 and 2Department of Microbiology, La Trobe University, Bundoora, Victoria 3083, Australia

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SUMMARY

The precise genomic location and the nucleotide sequence of the bovine herpesvirus type 2 (bovine herpes mammillitis virus) thymidine kinase (TK) gene have been determined. The genomic location of the TK gene was found to be in a similar position to that of herpes simplex virus. The coding region consists of 918 bases, which is slightly smaller in length than other reported herpesvirus TK genes. However, with an Mr of 38 108 the individual protein is similar in size to other herpesvirus TK enzymes. Despite there being only limited overall sequence homology with the TK genes of other herpesviruses, there are several regions of extensive homology at the amino acid level.

Bovine herpesvirus type 2 (BHV-2) is one of several herpesviruses known to infect cattle. Although BHV-2 is known to cause swelling, inflammation and ulceration of the teats and udder in domestic cattle (Ludwig, 1983; Martin et al., 1964, 1966) it is not a major problem in cattle. BHV-2 can be considered as a potential bovine-specific vector virus as some strains produce no disease and none are transmitted to people or other animals in field situations (Maragos et al., 1986; Maragos & May, 1987). If the virus has a thymidine kinase (TK) gene it can be used for DNA manipulative purposes to produce vaccines with less pathogenicity (Kit, 1985). BHV-2 virus is an alphaherpesvirus with a linear dsDNA genome approximately 135 kb in size and a genomic structure similar to that of herpes simplex virus (HSV; Buchman & Roizman 1978b; Roizman et al., 1982). The overall base sequence homology between HSV-1 and BHV-2, as defined by DNA hybridization studies, is 14% (Roizman et al., 1982; Sterz et al., 1973/74). In this report we describe experiments which have determined the precise location of the TK gene on the physical map of BHV-2 strain BHM-1 (Maragos et al., 1986; Maragos & May, 1987) and the complete nucleotide sequence of this gene. We have also compared this sequence to those of known TK genes from other herpesviruses.

Recently it has been demonstrated that several BHV-2 genes map to a collinear position with equivalent genes in HSV-1 (Maragos & May, 1987; Hammerschmidt et al., 1988a, b). This knowledge was used to determine the approximate location of a BHV-2 TK gene. Based on the known location of the HSV-1 TK gene at map units (m.u.) 30-0 to 31-3 (McGeoch et al., 1988; Reyes et al., 1982) the equivalent region in the BHV-2 genome was determined and found to be contained within the 11-5 kb HindIII I fragment of m.u. 27-1 to 35-8 (Buchman & Roizman, 1978a). Previously we have reported that the HindIII I fragment and a smaller 2-69 kb SalI internal fragment transformed TK- cells to a TK+ phenotype (May et al., 1989). In this case we have mapped this 2-69 kb SalI fragment extensively, and by the use of the calcium phosphate transfection method (Kit et al., 1980) we have determined the precise map position of the BHV-2 TK gene to a 1-2 kb SalI/SphI fragment (Fig. 1). The SalI/SphI fragment was found to transform TK- cells to a TK+ phenotype with an efficiency equal to the HSV-1 1-9 kb PvuII fragment containing the TK gene; the larger fragments were much less efficient. When the SalI/SphI fragment was cleaved with XbaI (Fig. 1) before transfection no TK+ cells were obtained. This
suggestions that the XbaI cleavage site is within the coding sequence or the promoter region of the BHV-2 TK gene.

The positive results in transforming TK- cells to TK+ with the 1-2 kb SalI/SphI fragment led to the complete sequencing of both DNA strands of this region, using the chain termination method of Sanger et al. (1977). The results of this sequence analysis are presented in Fig. 2. With a coding sequence of 918 bp the BHV-2 TK gene is quite clearly smaller than that of other herpesvirus TK genes reported (Kit et al., 1983; Otsuka & Kit, 1984; Sawyer et al., 1988; Swain & Galloway, 1983; Wagner et al., 1981). Although the translation initiation sequence is not ideal as defined by Kozak (1987) there is an A at position -3 which results in 60 to 90% of the translation efficiency obtained by a perfect Kozak sequence (Kozak, 1987).

Apart from the disease caused by BHV-2 this virus is also of interest as it is one of the few non-human herpesviruses whose DNA bears nucleotide sequence homology with the HSV-1 genome (Sterz et al., 1973/74). A direct comparison made between the BHV-2 TK gene nucleotide sequence and that of the HSV-1 TK gene showed a relatively high level of homology (57.4%; data not shown). A comparison of the TK genes at the amino acid level between these viruses reveals a striking level of homology within two distinct regions. These two conserved regions have been identified previously in other herpesvirus TK genes (Kit, 1985). The amino acid sequences of these two conserved regions in the BHV-2 TK gene as well as the corresponding sequences for other known herpesvirus TK genes are presented in Fig. 3. These two regions in the BHV-2 TK gene share the same relative spacing and orientation as that found in other herpesvirus TK genes. Previously, Kit (1985) suggested that these conserved regions are nucleotide (Fig. 3a) and nucleoside (Fig. 3b) binding sites; this suggestion has been further supported by work by Darby et al. (1986) and Liu & Summers (1988). It is of interest to note that these two conserved regions in the BHV-2 TK gene are more closely related to three of the four human herpesviruses, HSV-1, -2 and varicella-zoster virus (VZV) than it is to BHV-1 (Fig. 3).
Fig. 2. DNA nucleotide sequence of the entire BHV-2 TK gene and its translated product. The poly(A) sequence is underlined and the putative CAAT and TATA sequences are enclosed.

The latter exhibits a relatively low level of homology to BHV-2 within these two areas of the TK gene. Recently, the entire sequence of the BHV-1 TK gene has been published (Mittal & Field, 1989). On comparing the BHV-1 and BHV-2 TK coding regions using the LKB DNASIS 2020 program, 50.0% homology was obtained, lower than the sequence homology found between HSV-1 and BHV-2 TK genes.
In conclusion, we have located the precise position of a BHV-2 TK gene to 30.0 to 31.0 m.u., based on the restriction maps of Buchman & Roizman (1978a) of the BHV-2 genome. We have also determined the entire sequence of this gene and have located areas of conserved sequence within this gene. The location and characterization of this TK gene represents a further step towards the full characterization of the BHV-2 genome and facilitates the investigation of BHV-2 as a potential bovine vector virus.

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REFERENCES


Short communication


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