The 24K protein of Borna disease virus

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Based on partial amino acid sequences obtained from tryptic peptides of the purified 24K antigen of Borna disease virus (BDV), we identified and sequenced four independent cDNA clones established from BDV-infected MDCK cells. Each of the clones encodes a polypeptide of 201 residues (M, 22461) that differs considerably from an amino acid sequence published recently. In vitro transcription/translation of both the wild-type and a 5' truncated clone lacking the first ATG codon yielded a peptide that comigrates on electrophoresis with a polypeptide immunoprecipitated from BDV-infected cells. The deduced amino acid sequence contains a putative signal for nuclear targeting.

Borna disease virus (BDV) causes sporadic neurological diseases in horses and sheep in central Europe. The virus has not been classified, but its genome apparently consists of negative-strand RNA with a size of 8.5 kb (Lipkin et al., 1990; De la Torre et al., 1990) or 10-5 kb (VandeWoude et al., 1990). The virus replicates exclusively in cells of the central nervous system and migrates intraaxonally (Carbone et al., 1987; Morales et al., 1988). In the experimentally infected rat the pathogenetic mechanism has been ascribed to a delayed type hypersensitivity immune reaction induced by BDV-specific CD4+ T cells (Richt et al., 1989, 1990). In infected animals the disease has a biphasic course. The acute phase involves hyperactivity, aggression and ataxia, and then, over a period of several weeks, the animals develop listlessness, blindness and, in some instances, paralysis and obesity (Narayan et al., 1983a, b). These abnormalities have been correlated with alterations in neurotransmitter mRNA levels (Lipkin et al., 1988).

Persistently BDV-infected MDCK cells express at least three virus-specific antigens with Ms of 38/39K, 24K and 14-5K (Haas et al., 1986; Ludwig et al., 1988; Schädler et al., 1985). The 24K antigen was detected in both the cytosol and the nucleus, as shown by indirect immunofluorescence using a monoclonal antibody (MAb) (Fig. 1a). The intranuclear inclusions have a diameter of less than 0.2 μm, as determined by confocal laser scan microscopy (R. Schnabel & H. Niemann, unpublished results). We purified this antigen by affinity chromatography using MAbs and SDS-PAGE. Tryptic fragmentation of the 24K antigen was performed in the polyacrylamide gel matrix for 5 h at 37°C in 100 mM-NH4HCO3 pH 8.5, 0.5 mM-CaCl2, using a protein:trypsin ratio of about 5:1. The resulting peptides were eluted, separated by reversed phase HPLC using a Vydac column C18 and sequenced in the gas phase (underlined in Fig. 2) (Eckerskorn & Lottspeich, 1990). A mixture of 18-mer oligonucleotides [5' (C/T)TC(A/T/G)ATCA-T(A/G)CT(A/G)TT(C/T)TC 3'], corresponding to the amino acid sequence ENSMIE, was used to analyse Northern blots of RNA from BDV-infected and non-infected MDCK cells (Fig. 1b). Prehybridization (18 h) and hybridization (24 h) were performed at 30 °C in 6 × standard saline citrate (SSC), 5 × Denhardt's solution, 10 mM-EDTA, 0.5% SDS and 100 μg/ml of sheared denatured trout sperm DNA. The filters were washed twice for 5 min at 30 °C and once for 10 min at 42 °C in 2 × SSC containing 0.1% SDS. In agreement with previous reports (De la Torre et al., 1990; Lipkin et al., 1990), BDV-specific mRNA species of 0.85, 2.1 and 3.6 kb were detected in both the total RNA fraction (lane 1) and in the poly(A)+ fraction (lane 3). In contrast, no signal was obtained with the poly(A)+ fraction from infected cells (lane 2) or various RNA preparations from non-infected cells (lanes 4 to 6). We then established a cDNA library in a lambda Uni-Zap vector (Stratagene) using the poly(A)+ RNA from BDV-infected cells. About 1% of the 3 × 1010 clones were hybridization-positive with the oligonucleotide mixture. We analysed 26 of these clones in greater detail. The corresponding plasmid clones were generated by in vivo excision and

The nucleotide sequence data reported in this paper have been submitted to the DDBJ, EMBL and GenBank nucleotide sequence databases and assigned the accession number X60701.

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Fig. 1. Intracellular distribution of the 24K antigen and identification of specific RNA. (a) Persistently BDV-infected MDCK cells were fixed 7 days after passage and 24K antigen was visualized by indirect immunofluorescence with an MAb and fluorescamine-conjugated goat anti-mouse IgG. The bar marker represents 20 μm. (b) Northern blot analysis of RNA preparations from BDV-infected (lanes 1 to 3) and non-infected (lanes 4 to 6) MDCK cells. Total RNA (lanes 1 and 4), the poly(A)- fraction (lanes 2 and 5) and the poly(A)+ fraction (lanes 3 and 6) were separated on a 1.0% formaldehyde-agarose gel. The separated material was blotted onto nitrocellulose and hybridized with a mixture of 5’ radiolabelled 18-mer oligonucleotides.

sequenced directly on both strands by the chain termination method (Sanger et al., 1977) according to standard protocols. Four of the clones carried an insert of 700 bp which contained a single open reading frame (ORF) encoding a polypeptide of 201 residues (M, 22461) (Fig. 2).

The deduced amino acid sequence shown in Fig. 2 differs significantly from that of the 24K antigen of BDV reported previously (VandeWoude et al., 1990). The protein we identified contains a different N terminus involving residues 1 to 66, as well as different residues in a domain from positions 122 to 133. As in this study, the previous sequence was also deduced from a cDNA clone established from BDV-infected MDCK cells. Unfortunately, the nucleotide sequence was not included in the earlier report (VandeWoude et al., 1990). However, an inspection of our cDNA sequence revealed that the previous peptide sequence could be explained if translation initiation occurred at the first ATG codon (indicated by the horizontal arrow in Fig. 2) and if at least three frameshift mutations had taken place.

Several lines of evidence indicate, however, that the sequence presented here is indeed correct. First, four independent clones yielded the same protein sequence. The different lengths of the individual 5’ non-coding sequences (Fig. 2) indicated that these clones had not been generated during the single amplification step.
Second, partial sequences of tryptic peptides match the sequence deduced from the nucleotide sequence. The sequence N^{37}ALTQPVD ... was detected as a minor sequence together with the peptide K^{37}LVTLEAESMI{89}. The presence of this first peptide argues against the possibility that the reading frame beginning at the first ATG codon is used for translation of the 24K antigen ORF. In addition, the presence of isoleucine in position 133 is supported by sequence data obtained at the peptide level. This suggests that the sequence of residues A_{122} to A_{133} in Fig. 2, which again differs from the sequence published previously, is indeed present in the 24K protein.

Third, to prove that translation of the gene encoding the 24K antigen started only at the second ATG codon (Fig. 2), we deleted the first by cleavage with SacI and religation. The deletion was verified by DNA sequencing. We then performed combined in vitro transcription/translation reactions according to standard protocols (Mayer et al., 1988). mRNA was generated with T3 RNA polymerase and translated in reticulocyte lysate in the presence of 75 μCi [35S]methionine (1000 Ci/mmol). To obtain 24K antigen from BDV-infected MDCK cells, we grew the cells on 4-5 cm Petri dishes in Dulbecco's MEM in the presence of 10% foetal calf serum. The cells were then metabolically labelled for 6 h with [35S]methionine (1000 Ci/mmol, 100 μCi/dish), lysed in RIPA buffer (Kessler, 1975) and aliquots were used for immunoprecipitation reactions. The results of the combined in vitro transcription/translation reactions are summarized in Fig. 3. As expected, polypeptides with an M_{r} of 24K were generated from both the wild-type B6 clone (lanes 2 and 3) and its 5' truncated form (lanes 5 and 6). Both peptides were precipitated either with a polyclonal rabbit serum (lanes 2 and 5) or with an MAb raised against purified 24K antigen (lanes 3 and 6). In addition, some minor peptides with M_{r}s of about 20K, 16K and 14K were obtained. It has been suggested previously (VandeWoude et al., 1990) that the mRNA that encodes the 24K antigen also encodes the 14K antigen. However, none of the four clones sequenced contained a second ORF that would be large enough to allow expression of a 14K peptide. It is likely that the smaller peptides obtained in the translation reactions were generated by premature termination of translation, as is commonly observed in the process of in vitro translation. The major 24K translation product comigrated with a peptide immunoprecipitated from infected cell lysate with the polyclonal BDV-specific serum (lane 8) or the MAb (lane 9).

Fourth, a cluster of basic amino acid residues R^{23}RKRSGSPPRK^{33} shows similarity to a sequence within the C-terminal domain of the core proteins of several hepatitis B virus strains. The related sequence motif RRRSGSPRRRR has been shown recently to determine the nuclear localization of the core antigen (Eckhardt et al., 1991). Whether this basic motif does provide the target signal for nuclear sorting (Niemann et al., 1989) remains to be shown. Site-directed mutagenesis of this sequence and its insertion into foreign proteins will provide further insights into its biological function.

Finally, it will be interesting to determine whether the methionine-rich region involving residues at positions 145, 148, 149, 152, 155 and 156 serves a specific biological function. Computer predictions of the secondary structure using the methods of Chou & Fasman (1978) or Garnier et al. (1978) suggest that residues M_{145} to D_{160} form an amphipathic α-helix in which all the methionine residues line up on one side of the helix. The hydrophobic side groups could, therefore, be involved in stabilizing the tertiary structure of the 24K antigen. Alternatively, these residues could be involved in an interaction with other viral or, perhaps more interestingly, unknown cellular proteins.

Fig. 3. Characterization of the gene product by combined in vitro transcription/translation. mRNA transcribed from linearized B6 DNA (lanes 1 to 3) and B6 DNA carrying a 5' deletion to the SacI site (lanes 4 to 6) was translated in a reticulocyte lysate in the presence of [35S]methionine. In addition, cell lysates were prepared from persistently BDV-infected MDCK cells that had been metabolically labelled with [35S]methionine (lanes 7 to 9). The products were subjected to immunoprecipitation using a polyclonal BDV-specific rabbit serum (lanes 2, 5 and 8), a monoclonal mouse IgG (lanes 3, 6 and 9) or, as a negative control, rabbit IgG from non-immunized animals (lanes 1, 4 and 7). The samples were analysed together with M_{r} markers (lane M) on a 12% SDS-polyacrylamide gel and then autoradiographed.

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References


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