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# Crystallization and preliminary X-ray analysis of the matrix protein of Borna disease virus

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The matrix protein M of Borna disease virus (BDV) is associated with the inner viral membrane and is thought to be a mediator between the nucleocapsid and the lipid-containing envelope in stabilizing the virus shape. The full-length BDV-M gene encoding a 16 kDa protein was expressed in *Escherichia coli*. M was purified to homogeneity and crystallized by the sitting-drop vapour-diffusion method. The crystals of M belong to the space group $I432$, with unit-cell parameters $a = b = c = 144.6$ Å, and diffract to 3.1 Å.

## 1. Introduction

Borna disease virus (BDV) is a member of the order *Mononegavirales*, which includes the virus families *Filoviridae*, *Paramyxoviridae*, *Rhabdoviridae* and *Bornaviridae*. They are enveloped viruses with a single-stranded non-segmented RNA genome of negative polarity. BDV is a neurotropic virus which causes immune-mediated neurological disease. Borna disease (BD) is characterized by prominent disturbances of movement and behaviour in numerous animals, mainly in horses and sheep, and is often fatal for these animals. Whether BDV also infects humans to cause psychiatric disorders is still unclear (de la Torre, 2001; Richt & Rott, 2001; Schwemmle, 2001).

The BDV genome contains six open reading frames (ORFs). ORF I codes for nucleoprotein (NP), ORF II for phosphoprotein (P), ORF III for matrix protein (M, also known as p16), ORF IV for glycoprotein (gp94), ORF V for RNA-polymerase (L) and ORF VI for protein X. The proteins NP, P, X and L are associated with the RNA genome (Briese *et al.*, 1994; Cubitt *et al.*, 1994; Richt *et al.*, 1998; Schwemmle *et al.*, 1998; Wolff *et al.*, 2000). Until recently, M has been thought to be N-glycosylated and exposed on the virus surface (Kliche *et al.*, 1994; Stoyloff *et al.*, 1997, 1998). However, we have demonstrated that M is not glycosylated and behaves like a matrix protein lining the inner surface of the viral envelope (Kraus *et al.*, 2001).

The general role of viral matrix proteins is to bridge the nucleocapsids and the cytoplasmic parts of the glycoprotein spikes in the inner side of the virus envelope, making matrix proteins essential for virus assembly and budding. Furthermore, matrix proteins may play a role in the regulation of virus replication by ribonucleoprotein formation and in the transport of negative-stranded RNA viruses to the cellular membrane. In the case of influenza viruses, which like BDV replicate in the cell nucleus, nuclear export is directed by the matrix protein (Martin & Helenius, 1991).

With a size of 16.2 kDa, BDV possesses the smallest matrix protein of all negative-stranded enveloped RNA-viruses. As there is no apparent sequence similarity to other viral matrix proteins, it is of interest to know what structural features BDV-M has in common with other matrix proteins. We have obtained crystals suitable for X-ray analysis in order to better understand its functions at a molecular level.

## 2. Materials and methods

### 2.1. Expression and purification

The M gene of BDV strain He/80 was cloned into the expression vector pMAL-c2 (New England Biolabs) using the RT-PCR technique and the primer pairs 5′-CTG GGA TCC ATG AAT TCA AAA CAT TCC TAT-3′ and 5′-GTC GTC GAC CTA AGG CCC TGA AGA TCG AAT-3′, resulting in pMAL/BDV-M. The plasmid carries the maltose-binding protein (MBP) gene connected to the BDV-M gene by an oligonucleotide which codes for the connecting peptide IEGR/ISEFGS, with a factor Xa cleavage site C-terminal to the arginine residue. *Escherichia coli* BL21 (DE3) cells were transformed by pMAL/BDV-M and grown in low-salt LB (10 g peptone, 5 g yeast extract, 5 g NaCl) supplemented with 0.2% glucose. When *E. coli* growth reached an $OD_{595}$ of 0.5, expression of the fusion protein was induced by the addition of 0.3 m*M* isopropyl-$\beta$-D-thiogalactopyranoside (IPTG). Cells were harvested 2 h after induction. *E. coli* cells were resuspended in

amylose resin column buffer (20 m*M* Tris–HCl pH 7.4, 200 m*M* NaCl, 1 m*M* EDTA) and lysed by repeated freeze–thawing and sonification (Branson sonifier; 75 W, 4 × 15 s). Insoluble material was pelleted at 5100*g* for 30 min at 277 K. MBP-BDV-M in the clear supernatant was bound to amylose resin (New England Biolabs) for 1 h at 277 K. The resin was washed with column buffer and MBP-BDV-M was eluted by the addition of 10 m*M* maltose to the column buffer. The cleavage of MBP-BDV-M was performed with factor Xa (Amersham Pharmacia) using 2 U $mg^{-1}$ fusion protein at room temperature for 16 h. The cleaved fusion protein was passed through a HiPrep 26/10 desalting column equilibrated with buffer containing 50 m*M* HEPES, 25 m*M* NaCl pH 7.6. The protein fractions were separated by Mono S HR 5/5 chromatography on a 25 m*M* to 1 *M* NaCl gradient in 20 column volumes of the above-mentioned HEPES buffer and detected by Western blot analysis (Kraus *et al.*, 2001). Finally, M was purified by gel filtration on a HiLoad 16/60 Superdex 75 column (Amersham Pharmacia) in buffer containing 50 m*M* HEPES, 150 m*M* NaCl pH 7.6 and concentrated to 5 mg $ml^{-1}$ using a Centricon-10 filter (Millipore). All chromatography steps were performed with an ÄKTA FPLC instrument (Amersham Pharmacia).

### 2.2. Crystallization and X-ray analysis

Crystals of recombinant M were grown using the sitting-drop vapour-diffusion method at 291 K from a protein solution (5 mg $ml^{-1}$) in 50 m*M* HEPES, 150 m*M* NaCl pH 7.6. The drops contained equal volumes of protein and reservoir solution (2 µl each). Optimization of crystallization parameters resulted in a reservoir solution containing 10% PEG 8000, 0.5 *M* $Li_2SO_4$, 0.1 *M* Tris–HCl pH 6.5 or 7.0. Crystals grew within 3 d to maximum dimensions of 0.1 × 0.1 × 0.1 mm. Crystals diffracted weakly in-house (maximum resolution ∼3.5 Å) using a Rigaku RU-300 rotating-anode generator equipped with an R-AXIS IV image plate at 50 kV and 100 mA and focusing mirrors (MSC, USA). Crystals were frozen (cryobuffer 12.5% PEG 8000, 0.5 *M* $Li_2SO_4$, 0.1 *M* Tris–HCl pH 7.0 and 30% glycerol) in a nitrogen cryostream (X-Stream, MSC, USA) prior to transport to the synchrotron (ESRF, Grenoble, France). A complete native data set was collected at beamline ID14 using a MAR CCD at a distance of 200 mm from the crystal (Table 1). Diffraction data were processed using the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997).

## 3. Results and discussion

Full-length BDV-M was expressed in *E. coli* and purified to homogeneity by affinity, cation-exchange and gel-filtration chromatography. There is strong evidence that BDV-M is able to form homooligomers in solution, which are currently being analyzed. *E. coli* recombinant M crystallized in space group *I*432, with unit-cell parameters $a = b = c = 144.6$ Å (Fig. 1). Assuming one monomer in the asymmetric unit results in a specific volume $V_M$ of 3.89 $Å^3\ Da^{-1}$ (Matthews, 1968), corresponding to a solvent content of 66%. Data collected on beamline ID14 extended to a resolution of 3.1 Å (Table 1). The crystals exhibited a mosaicity of 0.7°. SeMet-labelled protein failed to yield any usable crystals; a search for derivatives is under way.

The recombinant BDV-M isolated from the soluble cytoplasmic fraction of *E. coli* had the same electrophoretic mobility as M of BDV-infected material under denaturing conditions.

Only a few viral matrix proteins have been crystallographically analyzed previously, presumably as a result of their high tendency towards aggregation and insolubility at higher concentrations. Consequently, three-dimensional structures have been determined for a proteolysis-resistant core of VP40 of Ebola virus (Dessen *et al.*, 2000) and of M1 of influenza virus by X-ray diffraction (Sha & Luo, 1997), while that of M1 has also been studied using tritium bombardment (Shishkov *et al.*, 1999). The thermolysin-resistant core of the matrixprotein M of vesicular stomatitis virus has been crystallized and the crystals diffract to 2 Å resolution (Gaudier *et al.*, 2001). Several retroviral matrix proteins have also been analyzed by X-ray diffraction or by nuclear magnetic resonance methods, among them SIV and HIV-1 (Belyaev *et al.*, 1994; Massiah *et al.*, 1994). The crystal structure of a first full-length matrix protein, the M of Borna disease virus, will give a better understanding of the structural elements essential for its biological prerequisites.

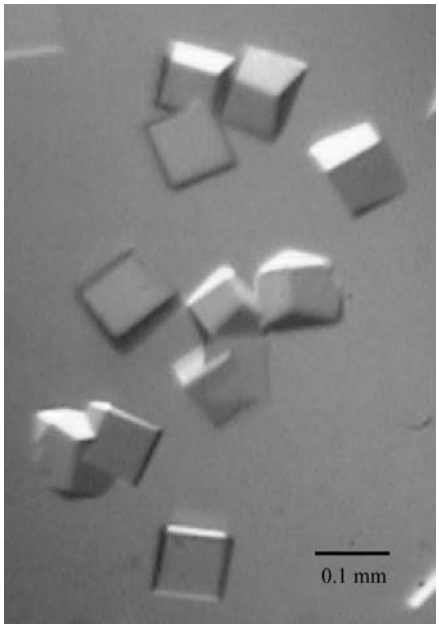


**Figure 1**
Crystals of BDV matrix protein.

**Table 1**
Crystal information and data-collection statistics.

Values in parentheses refer to the highest resolution shell, 3.26–3.15 Å

| | |
|---|---|
| Wavelength (Å) | 0.934 |
| Space group | *I*432 |
| Unit-cell parameters (Å) | 144.6 |
| Resolution range (Å) | 20–3.15 |
| Number of observed/unique reflections | 34175/4501 |
| Completeness (>1$\sigma$) (%) | 98.6 (99.5) |
| $R_{sym}$† (%) | 4.8 (31.8) |

† $R_{sym} = \sum |I - \langle I \rangle| / \sum I$.

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