Acta Crystallographica Section F
Structural Biology and Crystallization Communications
ISSN 1744-3091

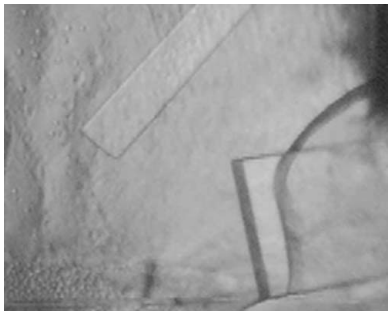
Crystallization and preliminary X-ray diffraction analysis of central structure domains from mumps virus F protein

Yueyong Liu,[a] Yanhui Xu,[b] Jieqing Zhu,[a] Bingsheng Qiu,[a] Zihe Rao,[b] George F. Gao[a] and Po Tien[a]*

[a]Department of Molecular Virology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100080, People's Republic of China, and [b]Laboratory of Structural Biology and MOE Laboratory of Protein Sciences, School of Life Sciences and Bioengineering, Tsinghua University, Beijing 100084, People's Republic of China

Correspondence e-mail: tienpo@sun.im.ac.cn

Received 23 May 2005
Accepted 19 August 2005
Online 31 August 2005



Fusion of members of the *Paramyxoviridae* family involves two glycoproteins: the attachment protein and the fusion protein. Changes in the fusion-protein conformation were caused by binding of the attachment protein to the cellular receptor. In the membrane-fusion process, two highly conserved heptad-repeat (HR) regions, HR1 and HR2, are believed to form a stable six-helix coiled-coil bundle. However, no crystal structure has yet been determined for this state in the mumps virus (MuV, a member of the *Paramyxoviridae* family). In this study, a single-chain protein consisting of two HR regions connected by a flexible amino-acid linker (named 2-Helix) was expressed, purified and crystallized by the hanging-drop vapour-diffusion method. A complete X-ray data set was obtained in-house to 2.2 Å resolution from a single crystal. The crystal belongs to space group $C2$, with unit-cell parameters a = 161.2, b = 60.8, c = 40.1 Å, β = 98.4°. The crystal structure will help in understanding the molecular mechanism of *Paramyxoviridae* family membrane fusion.

1. Introduction

Members of the *Paramyxoviridae* family, as well as of the *Orthomyxoviridae*, *Retroviridae* and *Filoviridae* families, initiate infection by attaching to cell-surface receptors, allowing fusion of the viral-enveloped membrane with host-cell plasma (Bentz, 2000; Eckert & Kim, 2001; Lamb, 1993; Skehel & Wiley, 1998; Weissenhorn *et al.*, 1999). Fusion of members of the *Paramyxoviridae* family involves two glycoproteins: the attachment protein and the fusion (F) protein. The attachment protein is responsible for the initial interaction with the cellular receptors, while the F protein directly mediates membrane fusion. F protein is synthesized as an inactive precursor and is then cleaved to two disulfide-linked subunits by host-cell protease (Lamb, 1993; Morrison, 2003). It has been shown that the majority of the fusion proteins of enveloped viruses contain two highly conserved heptad-repeat (HR) regions: HR1 and HR2. HR1 is located next to the carboxy-terminal fusion peptide and HR2 is located adjacent to the transmembrane domain. It is proposed that HR domains play an important role in the course of virus infection (Lamb *et al.*, 1999; Weissenhorn *et al.*, 1999). Moreover, both HR1 and HR2 are potential inhibitors of viral fusion; this has already been investigated by mutational and functional analysis of HR domains (Lambert *et al.*, 1996; Rapaport *et al.*, 1995; Wild & Buckland, 1997; Young *et al.*, 1997, 1999). The key to understanding the role of the HR domains in viral fusion is the finding that peptides from the HR1 and HR2 domains can form a complex. Biochemical investigation and crystal structures of a number of paramyxoviruses have provided evidence to support the existence of the complex. To date, the crystal structures of the fusion core of the F protein from Newcastle disease virus (NDV), simian parainfluenza virus 5 (SV5) and human respiratory syncytium virus (hRSV) have demonstrated that the HR domains can form a trimeric central coiled coil, which is considered to be the most stable post-fusion structure of F protein (Baker *et al.*, 1999; Chen *et al.*, 2001; Lamb *et al.*, 1999; Morrison, 2003; Zhao *et al.*, 2000).

Mumps virus (MuV) is a member of the genus *Rubulavirus* from the *Paramyxoviridae* family, which is a group of enveloped negative-

stranded RNA viruses that can infect a broad spectrum of species, including humans and domestic and wild animals (Lamb, 1993). In MuV, several lines of experimental evidence suggest that both the F and the attachment protein are required for cell fusion (Merz *et al.*, 1983; Waxham & Aronowsky (1988). In this process, binding of the attachment protein to the sialic acid-containing target-cell receptors triggers conformational changes in the F protein. Conformational changes in the F protein are believed to be the key process in virus fusion (Tanabayashi *et al.*, 1992). We have demonstrated that the HR1 and HR2 peptides from MuV F protein can interact with each other to form a stable six-helix bundle (Liu *et al.*, 2004). However, in order to better understand the molecular mechanism of MuV membrane fusion and to facilitate fusion-inhibitor design, the crystal structure of the six bundle of HR1/HR2 is of great importance. Here, we report the crystallographic study of a single-chain polypeptide (named 2-Helix) containing the two HR regions of the MuV F protein.

2. Materials and methods

2.1. Protein expression and purification

The construct production and protein expression and purification have been reported previously (Liu *et al.*, 2004). Briefly, the 2-Helix gene, which contains the HR1 and HR genes conjugated by the linker SGGRGG as reported previously (Zhu *et al.*, 2002), was amplified by PCR and then subcloned into pGEX-6p-1, which is a GST gene-fusion expression vector. *Escherichia coli* strain BL21(DE3) transformed with recombinant pGEX-6p-1 plasmid was grown at 310 K to an optical density ($OD_{600\,nm}$) of 0.8 ± 1.0 prior to induction with 1 m*M* isopropyl β-D-thiogalactopyranoside (IPTG) for 4 h. Bacterial cells were harvested by centrifugation at 277 K and lysed by sonication in phosphate-buffered saline (PBS; 10 m*M* sodium phosphate pH 7.3, 150 m*M* NaCl). The supernatant obtained by centrifugation was passed through a glutathione-Sepharose 4B column (Pharmacia) equilibrated with PBS. The GST fusion protein-bound column was washed with ten column volumes of PBS and eluted with three column volumes of reduced glutathione (10 m*M*). The GST fusion proteins were dialysed into cleavage buffer (50 m*M* Tris–HCl pH 7.0, 150 m*M* NaCl, 1 m*M* DTT, 1 m*M* EDTA pH 8.0) and then cleaved using Precission protease (Pharmacia) with 10 cleavage units per milligram of fusion protein at 277 K for 16 h. The Precission protease and GST were removed by passage through a glutathione-Sepharose 4B column. The protein was further purified by gel filtration on a Superdex G75 column (Pharmacia). The column-purified protein, which had a purity of >98%, was dialysed into 20 m*M* Tris–HCl pH 8.0 and concentrated using a 10K ultrafiltration membrane.

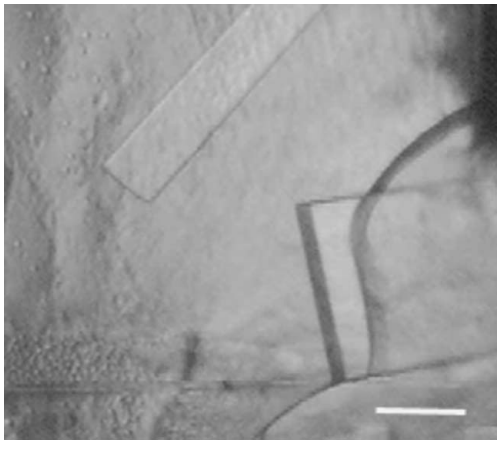


Figure 1
Crystals of 2-Helix grown from 15%(*w*/*v*) PEG 8000, 0.5 *M* lithium sulfate. The scale bar represents 0.1 mm.

Table 1
Diffraction data statistics.

Values in parentheses correspond to the highest resolution shell.

Space group	*C*2
Unit-cell parameters (Å, °)	*a* = 161.2, *b* = 60.8, *c* = 40.1, β = 98.4
Resolution (Å)	50.0–2.2
Measured reflections	187345
Unique reflections	19670
Redundancy	9.5 (4.2)
Average $I/\sigma(I)$	9.2 (2.3)
R_{merge}† (%)	0.122 (0.484)
Completeness (%)	96.3 (94.2)

† $R_{merge} = \sum_{hkl}\sum_i |I(hkl)_i - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I(hkl)_i$, where $I(hkl)_i$ is the *i*th measurement of the intensity of reflection *hkl* and $\langle I(hkl)\rangle$ is the mean intensity of reflection *hkl*.

2.2. Crystallization and X-ray diffraction analysis

Crystallization was conducted at 291 K in 16-well plates using the hanging-drop vapour-diffusion method. Hampton Research Crystal Screen Kits (Hampton Research, Riverside, CA, USA) were used for initial screening. Drops containing 1 µl protein solution and 1 µl reservoir solution were equilibrated against 0.5 ml reservoir solution. A small crystal appeared in 3 d from reservoir solutions consisting of 2%(*w*/*v*) PEG 8000, 1.0 *M* lithium sulfate and 15%(*w*/*v*) PEG 8000, 0.5 *M* lithium sulfate. Crystals with maximum dimensions of 0.5 × 0.10 × 0.05 mm were obtained from a reservoir solution consisting of 15%(*w*/*v*) PEG 8000, 0.5 *M* lithium sulfate after increasing the protein concentration to 20 mg ml^{-1} (Fig. 1).

Crystals were picked up using a fibre loop and flash-frozen at 100 K in a stream of cold nitrogen gas without any cryoprotectant. Preliminary X-ray diffraction was performed using a Rigaku rotating-anode Cu $K\alpha$ X-ray generator operating at 48 kV and 98 mA (λ = 1.5418 Å) with a MAR 345 image-plate detector. All intensity data were indexed, integrated and scaled with the *HKL* suite programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997).

3. Results and discussion

To facilitate crystallization, we expressed HR1 and HR2 as single-chain protein (2-Helix, 11.7 kDa; Liu *et al.*, 2004) connected by a flexible amino-acid linker. This strategy has been successfully used in our laboratory for other paramyxoviruses and provided a convenient method to study the crystal structure of the central coiled-coil bundle from viral fusion proteins (Zhu *et al.*, 2002). 2-Helix protein could be expressed in a high yield of up to 20 mg per litre of culture using a GST-fusion system in *E. coli* and could easily be purified using affinity-column and gel-filtration chromatography with high homogeneity. Biochemical and biophysical analysis demonstrated that the 2-Helix protein could form a stable high α-helix content six-helix trimer structure (Liu *et al.*, 2004).

X-ray data were collected from a single crystal and are summarized in Table 1. The crystal belongs to space group *C*2, with unit-cell parameters *a* = 161.2, *b* = 60.8, *c* = 40.1 Å, β = 98.4°. Assuming the presence of three molecules of 2-Helix in the asymmetric unit, the value of the Matthews coefficient V_M (Matthews, 1968) is 3.2 Å^3 Da^{-1}, corresponding to a solvent content of 61.8%. The successful crystallization of 2-Helix to give crystals suitable for

structure determination will help in understanding the molecular mechanism of *Paramyxoviridae* family membrane fusion.

This work was supported by a grant from the National Frontier Research Program (Project 973) of the Ministry of Science and Technology of the People's Republic of China (Grant No. G1999075602).

References

Baker, K. A., Dutch, R. E., Lamb, R. A. & Jardetzky, T. S. (1999). *Mol. Cell*, **3**, 309–319.
Bentz, J. (2000). *Biophys. J.* **78**, 886–900.
Chen, L., Gorman, J. J., McKimm-Breschkin, J., Lawrence, L. J., Tulloch, P. A., Smith, B. J., Colman, P. M. & Lawrence, M. C. (2001). *Structure*, **9**, 255–266.
Eckert, D. M. & Kim, P. S. (2001). *Annu. Rev. Biochem.* **70**, 777–810.
Lamb, R. A. (1993). *Virology*, **197**, 1–11.
Lamb, R. A., Joshi, S. B. & Dutch, R. E. (1999). *Mol. Membr. Biol.* **16**, 11–19.
Lambert, D. M., Barney, S., Lambert, A. L., Guthrie, K., Medinas, R., Davis, D. E., Bucy, T., Erickson, J., Merutka, G. & Petteway, S. R. Jr (1996). *Proc. Natl Acad. Sci. USA*, **93**, 2186–2191.
Liu, Y., Zhu, J., Feng, M. G., Tien, P. & Gao, G. F. (2004). *Arch. Biochem. Biophys.* **421**, 143–148.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–493.
Merz, D. C., Server, A. C., Waxham, M. N. & Wolinsky, J. S. (1983). *J. Gen. Virol.* **64**, 457–1467.
Morrison, T. G. (2003). *Biochim. Biophys. Acta*, **1614**, 73–84.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Rapaport, D., Ovadia, M. & Shai, Y. (1995). *EMBO J.* **14**, 5524–5531.
Skehel, J. J. & Wiley, D. C. (1998). *Cell*, **95**, 871–874.
Tanabayashi, K., Takeuchi, K., Okazaki, K., Hishiyama, M. & Yamada, A. (1992). *Virology*, **187**, 801–804.
Waxham, M. N. & Aronowsky, J. (1988). *Virology*, **167**, 226–232.
Weissenhorn, W., Dessen, A., Calder, L. J., Harrison, S. C., Skehel, J. J. & Wiley, D. C. (1999). *Mol. Membr. Biol.* **16**, 3–9.
Wild, T. F. & Buckland, R. (1997). *J. Gen. Virol.* **78**, 107–111.
Young, J. K., Hicks, R. P., Wright, G. E. & Morrison, T. G. (1997). *Virology*, **238**, 291–304.
Young, J. K., Li, D., Abramowitz, M. C. & Morrison, T. G. (1999). *J. Virol.* **73**, 5945–5956.
Zhao, X., Singh, M., Malashkevich, V. N. & Kim, P. S. (2000). *Proc. Natl Acad. Sci. USA*, **97**, 14172–14177.
Zhu, J., Zhang, C. W., Qi, Y., Tien, P. & Gao, G. F. (2002). *Biochem. Biophys. Res. Commun.* **299**, 897–902.