Published in final edited form as:

Biomol NMR Assign. 2008 December ; 2(2): 155–157. doi:10.1007/s12104-008-9109-5.

NMR Assignments of the Sylvatic Dengue 1 Virus Envelope Protein Domain III

David E. Volk^{1,2}, Kurtis M. Anderson¹, Sai H. A. Gandham¹, Fiona J. May³, Li Li³, David W. C. Beasley^{4,5}, Alan D. T. Barrett^{3,4}, and David G. Gorenstein^{1,2,*}

¹The Department of Biochemistry and Molecular Biology, The University of Texas Medical Branch, Galveston, TX, USA 77555-1157

²Sealy Center for Structural Biology and Molecular Biophysics, The University of Texas Medical Branch, Galveston, TX, USA 77555-1157

³Department of Pathology, The University of Texas Medical Branch, Galveston, TX, USA 77555-1157

⁴Sealy Center for Vaccine Development, The University of Texas Medical Branch, Galveston, TX, USA 77555-1157

⁵Department of Microbiology and Immunology, The University of Texas Medical Branch, Galveston, TX, USA 77555-1157

Abstract

Nearly complete backbone and side chain resonance assignments have been obtained for the third domain, residues M289-K400, of the envelope protein from the sylvatic strain (P72–1244) of the dengue 1 virus, containing mutations N366S and E370K, using double- and triple-resonance spectroscopy.

Keywords

dengue; flavivirus; NMR assignments; envelope protein

Biological context

Dengue 1 virus (DEN1V) is a member of the family *Flaviviridae*, genus *Flavivirus*, which contains the dengue viruses (DEN 1–4), West Nile virus, Langat virus, Omsk hemorrhagic fever virus and many others. Receptor binding and membrane fusion of flaviviruses occurs with the envelope (E) protein (Heinz and Allison, 2003). The E protein ectodomain contains three domains, ED1, ED2 and ED3. The highly immunogenic ED3 is a putative receptor-binding domain and many neutralizing antibodies bind to ED3. Thus, ED3 is an attractive target for development of vaccines, anti-viral agents and diagnostic antigens. Chemical shift assignments and/or NMR-based structures for the ED3 proteins of Japanese encephalitis (Wu et al. 2003), West Nile Virus (Volk, Kallick et al. 2004, Volk, Beasley et al. 2004), Omsk hemorrhagic fever virus (Volk, Chavez et al. 2006), Dengue 4 virus (Volk, Lee et al. 2006, Volk, Lee et al. 2007), Langat virus (Mukherjee et al. 2006) and yellow fever virus (Volk, Gandham et al. 2007) have previously been reported. In addition, crystallographic structures for the extra-membrane portion of the E protein have been reported for Tick-borne encephalitis

*To whom correspondence should be addressed. Email:dgorens@utmb.edu, Tel: 409-747-6800, Fax: 409-747-6850.

virus (Rey et al., 1995), Dengue 2 virus (Modis et al. 2003), Dengue 3 virus (Modis et al. 2005) and West Nile virus (Kanai et al. 2006, Nybakken et al. 2006)

Methods and experiments

Uniformly ^{13}C , ^{15}N -labeled recombinant DEN1-ED3 (sylvatic strain P72–1244) protein encompassing residues M289–K400, with mutations N366S and E370K, was expressed using the pET-15 vector (Novagen) in which the N-terminal His-tag sequence encoded in the plasmid was removed. Cells were lysed by sonication in native buffer, the crude cell debris was centrifuged and the supernatant was removed. The pellet was resuspended in denaturing buffer (6 M guanidine HCl) and the solution was pass through a size-exclusion column (G75 Sephadex®) to provide pure protein. Pooled fractions were dialyzed to remove the denaturant. A 3 kDa centricon (Amicon, Inc.) concentrator was used to concentrate the protein. The NMR sample contained 0.25 mM protein in 25 mM Tris-HCl (pH 6.5), 0.1% NaN_3 and 25 mM NaCl in 90% H_2O and 10% D_2O .

NMR experiments were performed at 25 °C on Varian Inova 800 MHz (UTMB & Rice Univ.) or 600 MHz (Rice Univ.) spectrometers with triple resonance coldprobes (Rice Univ.) and proton frequencies were referenced to internal DSS. The ^{13}C and ^{15}N dimensions were referenced indirectly using frequency ratios. Sequence-specific backbone assignments (Figure 1) were obtained using the 2D ^1H , ^{15}N -HSQC, 3D HNCACB (Sattler et al., 1999), 3D CBCA (CO)NH, and 3D HNCO (Ikura et al., 1990; Bax and Grzesiek, 1993) experiments. Non-aromatic side chain assignments were obtained using the TOCSY- ^1H , ^{15}N -HSQC, H(C)CH-TOCSY (Clare and Gronenborn, 1994), H(CCO)NH and C(CO)NH (Sattler et al., 1999) experiments. Aromatic proton assignments were obtained from the (HB)CB(CGCD)HD and (HB)CB(CGCDCE)HE experiments (Yamazaki et al., 1993). A NOESY- ^1H , ^{15}N -HSQC experiment (Marion et al., 1989; Wüthrich, 1986) provided several missing side chain assignments. The NMR spectra were processed in VNMRJ (Varian Inc.) or Felix2000 (Felix, Inc.) software.

Extent of assignments and data deposition

For the backbone atoms, 95% of the resonances have been assigned. The missing backbone atom resonances consist of one alpha carbon (N60), three carbonyl carbons (M1, N60 & P83) and eleven amide nitrogen atoms, corresponding to the six prolines, the N-terminal residues M1 and D2, and exposed residues Q28, G40 and N60.

The non-exchangeable proton side chain resonance assignments are nearly complete (99%), but many of the exchangeable proton/carbon pairs, such as the lysine $\text{H}^\epsilon\text{C}^\zeta$ pairs and the HIS29 $\text{H}^{\delta 1}/\text{N}^{\epsilon 1}$ and $\text{H}^{\epsilon 1}/\text{N}^{\epsilon 1}$ pairs were not assigned. The aromatic carbon atoms were not assigned. The ^1H , ^{13}C , and ^{15}N chemical shifts have been deposited in the BioMagResBank (<http://www.bmrb.wisc.edu>) under the BMRB accession number 15782.

Acknowledgments

This work was by the Pediatric Dengue Vaccine Initiative, the Centers for Disease Control (U90CCU618754), the National Institute for Allergic and Infectious Diseases (U01 AI054827), the Welch Foundation (H1296), and the State of Texas Advanced Technology Program (004952-0038-2003). We thank Sean Moran for assistance with the Rice University spectrometer whose Carbon-Enhanced HCN Cold Probe was obtained through the Strategic Partnership for Research in Nanotechnology Grant AFRL (AFOSR) FA9550-04-1-0328.

References

Bax A, Grzesiek S. Methodological advances in protein NMR. *Acc. Chem. Res* 1993;26:131–138.

- Clare GM, Gronenborn AM. Multidimensional heteronuclear nuclear magnetic resonance of proteins. *Meth. Enzymol* 1994;239:249–363.
- Heinze FX, Allison S. Flavivirus structure and membrane fusion. *Adv. Virus Res* 2003;59:63–97. [PubMed: 14696327]
- Ikura M, Kay LE, Bax A. A novel approach for sequential assignment of ¹H, ¹³C, and ¹⁵N spectra of proteins: heteronuclear triple-resonance three-dimensional NMR spectroscopy. *Biochem* 1990;29:4659–4667. [PubMed: 2372549]
- Kanai R, Kar K, Anthony K, Gould LH, Ledizet M, Fikrig E, Koski RA, Modis Y. Crystal structure of West Nile virus envelope glycoprotein reveals viral surface epitopes. *J. Virol* 2006;80:11000–11008. [PubMed: 16943291]
- Modis Y, Ogata S, Clements D, Harrison SC. A ligand-binding pocket in the dengue virus envelope glycoprotein. *Proc. Natl. Acad. Sci. U.S.A* 2003;100:6986–6991. [PubMed: 12759475]
- Modis Y, Ogata S, Clements D, Harrison SC. Variable surface epitopes in the crystal structure of dengue virus type 3 envelope glycoprotein. *J. Virol* 2005;79:1223. [PubMed: 15613349]
- Mukherjee M, Dutta K, White MA, Cowburn D, Fox RO. NMR solution structure and backbone dynamics of domain III of the E protein of tick-borne Langat flavivirus suggests a potential site for molecular recognition. *Prot. Sci* 2006;15:1342–1355.
- Nybakken GE, Nelson CA, Chen BR, Diamond MS, Fremont DH. Crystal structure of the West Nile virus envelope glycoprotein. *J. Virol* 2006;80:11467–11474. [PubMed: 16987985]
- Rey FA, Heinz FX, Mandl CW, Kunz C, Harrison SC. The envelope glycoprotein from tick-borne encephalitis virus at 2 Å resolution. *Nature* 1995;375:291–198. [PubMed: 7753193]
- Sattler M, Schleucher J, Griesinger C. Heteronuclear multidimensional NMR experiments for the structure determination of proteins in solution employing pulsed field gradients. *Prog. NMR Spectrosc* 1999;34:93–158.
- Volk DE, Beasley DWC, Kallick DA, Holbrook MR, Barrett ADT, Gorenstein DG. Solution structure and antibody binding studies of the envelope domain III from the New York strain of West Nile virus. *J. Biolog. Chem* 2004;279:38755–38761.
- Volk DE, Chavez L, Beasley DWC, Barrett ADT, Holbrook MR, Gorenstein DG. Structure of the Envelope Protein Domain III of Omsk Hemorrhagic Fever Virus. *Virology* 2006;351:188–195. [PubMed: 16647096]
- Volk DE, Gandham SA, May FJ, Anderson A, Barrett ADT, Gorenstein DG. NMR Assignments of the Yellow Fever Virus Envelope Protein Domain III. *Biomol. NMR Assign* 2007;1:49–50. [PubMed: 19636823]
- Volk DE, Kallick DA, Holbrook MR, Beasley DWC, Barrett ADT, Gorenstein DG. ¹H, ¹³C, and ¹⁵N resonance assignments for the domain III of the West Nile Virus envelope protein. *J. Biomol* 2004;29:445–446.
- Volk DE, Lee Y-C, Li X, Barrett ADT, Gorenstein DG. NMR Assignments of the Yellow Fever Virus Envelope Protein Domain III. *J. Biomol. NMR* 2006;36:52.
- Volk DE, Lee Y-C, Li X, Thivyanathan V, Gromowski GD, Li L, Lamb A, Beasley DWB, Barrett ADT, Gorenstein DG. Structure of the Envelope Protein Domain III of Dengue-4 Virus. *Virology* 2007;364:147–154. [PubMed: 17395234]
- Wu K-P, Wu C-W, Tsao Y-F, Kuo T-W, Lou Y-C, Lin C-W, Wu S-C, Cheng J-W. Structural basis of a flavivirus recognized by its neutralizing antibody. *J. Biol* 2003;278:46007–46013.
- Wüthrich, K. *NMR of Protein and Nucleic Acids*. New York, NY: Wiley; 1986.
- Yamazaki T, Forman-Kay JD, Kay LE. Two-dimensional NMR Experiments for Correlating ¹³Cβ and ¹Hδ/ε Chemical Shifts of Aromatic Residues in ¹³C-Labeled Proteins via Scalar Coupling. *Am. Chem. Soc* 1993;115:11054–11055.