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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignments for domain III of the West Nile Virus envelope protein

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Biological context

West Nile virus (WNV) is a member of the family *Flaviviridae*, genus *Flavivirus* and in 2002 was responsible for the largest outbreak of arthropod-borne encephalitis recorded in the Western hemisphere, with 4156 human infections and 284 deaths reported in the United States (CDC, 2003). In 2003, WNV has continued to spread and cause human and animal disease across North America, and it has now been detected in Central America (Estrada-Franco et al., 2003). Currently, there are no approved vaccines or therapeutic treatments for WN encephalitis.

The envelope (E) protein is the major virion surface protein of the flaviviruses. The E protein is also the primary immunogen and it plays a central role in virus attachment and entry to cells via membrane fusion. The X-ray crystallographic structures for the ectodomain of the E protein of tick-borne encephalitis virus (Rey et al., 1995) and the mosquito-borne dengue-2 virus (Modis et al., 2003) have been solved and both resolve the protein into three distinct structural domains that correspond to previously-characterized antigenic domains. Domain III (D3) has been proposed to be the receptor-binding domain and, as for other flaviviruses, domain III of West Nile virus (WND3) contains multiple surface-exposed epitopes recognized by virus neutralizing antibodies (Beasley and Barrett, 2002). As such, WND3 represents an attractive target for development of vaccines, antiviral agents and/or diagnostic antigens. Here we report the nearly complete sequence-specific backbone and side-chain ^{1}H, ^{13}C, and ^{15}N resonance assignments of domain III of the West Nile virus envelope protein.

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Methods and experiments

Uniformly ^{13}C, ^{15}N-labeled WND3 from neuroinvasive lineage I WN virus strain 385-99 was overexpressed as a MBP fusion using the pMal c2x vector (Beasley and Barrett, 2002) and the 115-residue WND3 was purified using size-exclusion chromatography. The NMR sample contained 0.7 mM protein in 50 mM K_2HPO_4 (pH 6.8), 100 mM NaCl, 10 mM NaN_3 and 0.1 mM EDTA in 90% H_2O/10% D_2O. All NMR experiments were acquired on a Varian UnityPlus 750 MHz spectrometer at 25 °C. Sequence-specific backbone chemical shifts were obtained from 3D HNCA, HNCACB (Sattler et al., 1999) and HNCO (Ikura et al., 1990) experiments. The backbone assignments (Figure 1a) were verified through sequential NH-NH and NH-Hα NOEs in the ^{15}N-edited 3D NOESY-HSQC spectrum (Marion et al., 1989). Side chain chemical shifts were obtained from 3D H(CCO)NH-TOCSY (Sattler et al., 1999), CC(CO)NH-TOCSY, TOCSY-HSQC{^{1}H,^{15}N} and HCCH-TOCSY (Clore and Gronenborn, 1994) experiments. The CC(CO)NH-TOCSY experiment also aided in the assignments of the Cα and Cβ carbons in cases of degeneracy in the HNCACB experiment, and in the assignments of the ASN and GLN side chain amide groups. Aromatic chemical shifts were assigned based on a CT-^{1}H,^{13}C-HSQC spectrum (Vuister and

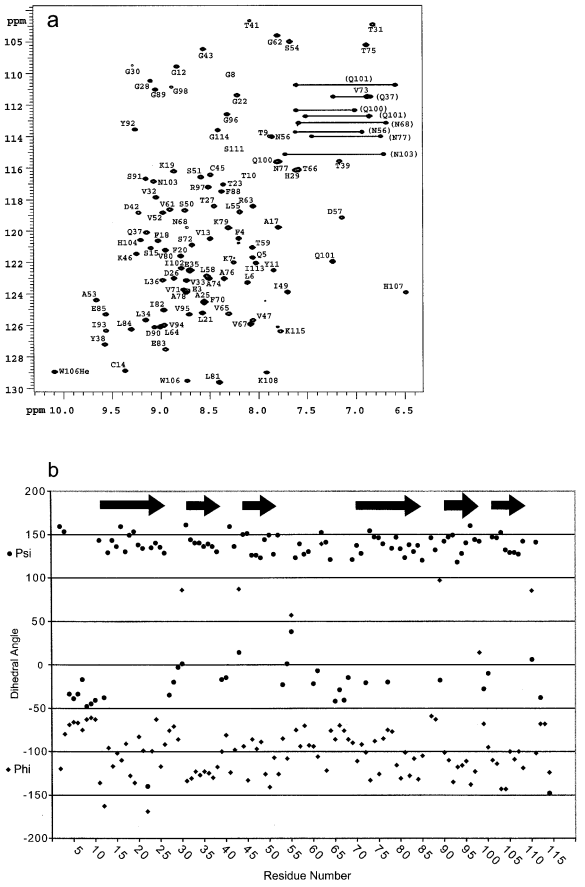

Figure 1. (a) Assigned ^{1}H-^{15}N-HSQC spectrum of WND3. The side chain amide groups are indicated with horizontal lines and their assignments are shown in parenthesis. (b) Dihedral angles calculated by TALOS and the predicted secondary structure of WND3.

Bax, 1992) and NOE data. Spectra were processed using Varian or Felix software.

Backbone-backbone NOEs from ^{15}N- and ^{13}C-edited 3D NOE experiments, as well as TALOS chemical shift analysis, indicate the presence of six β-strands, as indicated in Figure 1b. Residues within the six β-strands are : 13–26, 31–38, 44–52, 70–85, 90–97, 101–108.

Extent of assignment and data deposition

The backbone resonances of WND3 were fully assigned with the exception of the amide proton and nitrogen of six residues: I1, S2, E99, H105, S109, G110, and S112. Complete side chain assignments were obtained for all residues with the exceptions of I1, P96, S109-S111, and several aromatic protons. The amide proton of gly 40 is shifted significantly upfield and resonates at 4.46 ppm. A table of the ^{1}H, ^{13}C, and ^{15}N chemical shift assignments of domain III of the West Nile Virus envelope protein have been deposited (accession number 6046) in the BioMagResBank (http://www.bmrb.wisc.edu).

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