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Letter to the Editor: ^{1}H, ^{13}C, ^{15}N resonance assignments and fold verification of a circular permuted variant of the potent HIV-inactivating protein cyanovirin-N*

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Biological context

Cyanovirin-N (CV-N) is a 11 kDa protein initially isolated from the cyanobacterium *Nostoc ellipsosporum*, that potently inactivates a wide variety of HIV strains (Boyd et al., 1997). CV-N binds to viral envelope glycoproteins and inhibits crucial biochemical events essential for viral entry and cell-to-cell fusion. It therefore offers considerable promise as a lead in the development of novel treatment strategies for AIDS. Although clearly desirable, specific structural determinants for the anti-HIV activity of CV-N have not been elucidated. In order to address this crucial question, we are involved in structural and functional studies of CV-N. The three-dimensional structure of the protein was determined by NMR (Bewley et al., 1998) and X-ray crystallography (Yang et al., 1999), providing a foundation for a more rational approach to mutational design and analyses. In addition, investigations of the ensuing effects on conformation, stability and biological activity of this interesting molecule can now be carried out in light of firm structural data.

Here we report the complete ^{1}H, ^{13}C and ^{15}N resonance assignments of a circular permutated variant of CV-N (cpCV-N; cf Figure 1B for the amino acid sequence). Circular permuted proteins provide information on different aspects in studies of structure–function relationships, which cannot be achieved through classical single site or deletion mutagenesis. This variant extends the repertoire of already reported CV-N mutants (Mori et al., 1997) and will contribute to a detailed characterization of the pivotal features for activity. In addition to the assignments, we used residual $^{1}D_{NH}$ dipolar couplings measured in liquid crystalline medium to verify the fold of this variant, derived from structure prediction and loop modeling (Fiser et al., 2000).

Methods and experiments

All NMR experiments were carried out at 27 °C on Bruker DMX500, DMX600 and DRX800 spectrometers equipped with x,y,z-shielded gradient triple resonance probes. Spectra were processed with NMRPipe (Delaglio et al., 1995) and analyzed using NMRView (Johnson and Blevins, 1994). ^{1}H, ^{15}N, ^{13}CO and ^{13}C backbone and side-chain resonance assignments were obtained using the following heteronuclear 2D and 3D experiments: ^{1}H-^{15}N HSQC, ^{1}H-^{13}C HMQC, HNHA, HNCACB, CBCA(CO)NH, HCCH-TOCSY, (H)C(CCO)NH-TOCSY, HNCO as well as a 3D ^{15}N separated and a 4D ^{13}C/^{13}C separated NOESY experiment. Residual dipolar couplings ($^{1}D_{NH}$) were obtained by calculating the difference in the $^{1}J_{NH}$ splittings measured in 2D IPAP ^{15}N-^{1}H HSQC spectra (Ottiger et al., 1998) in isotropic and Pf1 liquid crystalline medium.

Extent of assignments and data deposition

The ^{1}H-^{15}N HSQC spectrum is shown in Figure 1A, and all resonance assignments for the backbone amide

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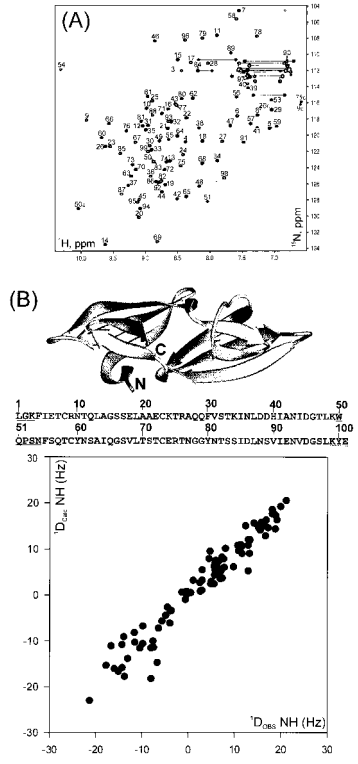

Figure 1. (A) ^{1}H-^{15}N HSQC spectrum of cpCV-N recorded in 20 mM phosphate buffer, pH 6.0, 27 °C. Cross peaks are labeled with the position in the cpCV-N amino acid sequence. (B) Schematic diagram of the overall cpCV-N structure and amino acid sequence (top). The first and last three residues as well as those of the loop are underlined in the sequence. Correlation between the experimentally measured dipolar couplings ($^1D_{obs}NH$) and those calculated ($^1D_{calc}NH$) on the basis of the coordinates of the comparative model (bottom).

groups are indicated. All backbone ^{15}N resonances are assigned, apart from those for the first two and last two amino acids, and only those for Asn81 are missing from the side-chain amino resonances. All other backbone resonances (^{13}Cα, ^{13}CO, ^{1}Hα) are assigned, apart from those for residues 1, 100 and 101, and ^{13}CO frequencies are also missing for residues 51 and 99. Similarly, the side-chain assignments are complete, with only those missing for Leu1, Lys99 and Glu101. All ^{1}H, ^{13}C and ^{15}N chemical shifts for the backbone and side chains of cpCV-N have been deposited at the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 4927.

A comparative model for cpCV-N was built (Šali and Blundell, 1993) assuming that the overall structure is not significantly changed compared to the structure of CV-N (2ezm). Thus, only the loop connecting the two domains needed to be swapped with the N- and C-terminal regions, and residues involved are underlined in Figure 1B. A description of the automated loop modeling methodology is provided in Fiser et al. (2000). The final model was used to best fit the alignment tensor to the observed residual dipolar couplings, $^1D_{NH}$, measured in a colloidal suspension of Pf1, using singular value decomposition (Losonczi et al., 1999) as implemented in the program PALES (Zweckstetter and Bax, unpublished). The correlation between the experimental values of $^1D_{NH}$ and those calculated from the model is shown in Figure 1B. Good agreement between the experimental and predicted values is observed (rmsd = 2.6 Hz, correlation coefficient 0.97), indicating that indeed the overall fold of cpCV-N is very similar to that of the wild-type protein. The present complete resonance assignments as well as sets of residual dipolar couplings will allow the determination of a high-resolution structure of cpCV-N.

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