TRIPLE RESONANCE ASSIGNMENTS OF HIGHLY-SYMMETRIC CORE MUTANT OF FGF-1, SYM6ΔΔ

J. Lee (FSU, Chemistry and Biochemistry); S. R. Brych (FSU, Chemistry and Biochemistry); M. Blaber (FSU, Chemistry and Biochemistry); T. M. Logan (NHMFL and FSU, Chemistry and Biochemistry)

Introduction

Human acidic fibroblast growth factor (FGF-1) is a member of the heparin-binding mitogen family of proteins and exhibits a characteristic 3-fold tertiary structure symmetry. Despite their structural symmetry, however, the symmetry-related structural subdomains do not exhibit primary sequence identity. In previous studies, we redesigned the hydrophobic core region of FGF-1 to increase the primary sequence symmetry [1]. The final core mutant exhibiting the most symmetric form of FGF-1, called "SYM6ΔΔ", contains six point mutations in the hydrophobic core and two loop deletions. Interestingly, despite the imposed symmetric constraint upon both the primary and tertiary structure, SYM6ΔΔ is substantially more stable than wild type. This mutant also is about 70 times more potent in 3T3 fibroblast mitogenic activity assay than wild type. These results propose that a primary structure symmetric constraint, within a symmetric protein superfold, might represent a solution to, rather than constraint upon, the stability and foldability of the polypeptide.

In spite of its interesting properties, the full understanding about SYM6ΔΔ is hampered by a lack of structural information. The two loop-deletions in SYM6ΔΔ introduce unique problems for crystallographic structure determination. Therefore we turned to NMR to solve the structure of SYM6ΔΔ. For the first step, the ^{1}H, ^{15}N, ^{13}C resonances were assigned with a series of triple resonance NMR experiments. These assignments will be the starting point to study the structure of SYM6ΔΔ.

Experimental

All NMR experiments were performed at 30 °C on a 600 MHz NMR spectrometer in NHMFL. 2D ^{1}H-^{15}N HSQC was acquired on uniformly ^{15}N-labeled protein. ^{1}H-^{13}C CT-HSQC spectra of the uniformly ^{13}C, ^{15}N-labeled protein was acquired. The following 3D spectra were acquired; CBCA(CO)NH, HNCACB, HNHA, HNCO, HCCH-TOCSY, C(CO)NH and H(CCO)NH. 3D ^{15}N-NOESY and ^{13}C-NOESY were acquired on uniformly ^{15}N-labeled protein and ^{13}C, ^{15}N-labeled protein, respectively. All NMR data were processed using NMRPIPE [2] and analyzed with NMRVIEW [3].

Results and Discussion

The sequential resonance assignments of SYM6ΔΔ were achieved based on the analysis of the HNCACB and CBCA(CO)NH spectra. All the main-chain resonances were assigned except for Gly19 and Gln40. Other aliphatic ^{1}H and ^{13}C resonances were assigned using ^{1}H, ^{13}C CT-HSQC, HCCH-TOCSY, C(CO)NH and H(CCO)NH spectra. The side-chain ^{1}H, ^{13}C and ^{15}N resonances are currently assigned for ~70% of all side-chain atoms. The low number of aromatic residues in the SYM6ΔΔ mutant results in extensive methyl group overlap, which is complicating assignments of some amino acids.

Conclusions

Although all of resonances are not assigned yet, we expect to complete the assignments using other complementary NMR experiments. The triple resonance assignments will be a base of the structural study of SYM6ΔΔ.

References

[1] Brych, S. R., *et al*., Journal of Molecular Biology, **344**, 769-780 (2004).
[2] Delaglio, F., *et al*., J. Biomol. NMR, **6,** 277–293 (1995).
[3] Johnson, B. A., *et al.*, J. Biomol. NMR, **4,** 603–614 (1994).