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

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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, a molecular description of the stability and functional properties of 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. Aliphatic side-chain ^{13}C resonance assignments were obtained by analysis of high resolution constant time ^{1}H-^{13}C HSQC, 3D doubly sensitivity-enhanced HCCH-TOCSY and 3D ^{13}C-separated NOESY-HSQC experiment with uniformly ^{13}C, ^{15}N-labeled protein. The methyl groups of Val and Leu were assigned stereo-specifically using fractional ^{13}C labeling of protein [2]. SYM6ΔΔ contains a total of 18 aromatic residues (including histidines) which were assigned using through-bond scalar connectivities coupled with analysis of cross peaks obtained in 3D ^{13}C-separated HMQC-NOESY spectra. All NMR data were processed using NMRPIPE [3] and analyzed with NMRVIEW [4].

Results and Discussion

In the previous study, we assigned the majority of main-chain and the part of aliphatic side-chain resonances of SYM6ΔΔ. To get the complete resonance assignment, other complementary NMR experiments have been carried out. The side-chain ^{1}H, ^{13}C and ^{15}N resonances are currently assigned for ~70% of all side-chain atoms. The chemical shift values of C_{γ}, H_{γ} for Val and C_{δ}, H_{δ} for Leu were stereo-specifically assigned except V86 and L26. The aromatic side chain resonances are currently assigned.

Conclusions

The assignment process has been completed and we are in the process of structure calculation.

References

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