Journal of Biomolecular NMR **29**: 451–452, 2004.
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Letter to the Editor: Sequence-specific resonance assignments of the tandem SH3 domains in an autoinhibitory form of p47^{phox}

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Received 6 January 2004; Accepted 26 February 2004

Key words: NADPH oxidase, NMR, p47^{phox}, resonance assignments, SH3 domain

Biological context

The NADPH oxidase catalyzes the reduction of oxygen to superoxide using NADPH as an electron donor, which plays a crucial role for killing invading microorganisms in neutrophils and other phagocytic cells. The phagocyte NADPH oxidase is composed of membrane-bound flavocytochrome b_{558}, which is a heterodimer of gp91^{phox} and p22^{phox} and at least four cytosolic regulatory components consisting of p47^{phox}, p67^{phox}, p40^{phox} and Rac (Segal et al., 2000). Upon activation of the cell, the p47^{phox}-p67^{phox}-p40^{phox} complex translocates from the cytosol to the plasma membrane and associates with flavocytochrome b_{558} to assemble the NADPH oxidase subunits into the active complex. p47^{phox} plays a critical role in this translocation process, which contains a PX domain, tandem SH3 domains, a polybasic region/autoinhibitory region (PBR/AIR) and a proline-rich region in order. In resting cells, the tandem SH3 domains of p47^{phox} are masked due to an intramolecular interaction with PBR/AIR, resulting in the autoinhibitory form (Sumimoto et al., 1994; Leto et al., 1994). Upon cell stimulation, some serine residues in PBR/AIR are phosphorylated (El Benna et al., 1994). The phosphorylation induces conformational changes that subsequently lead to rearrangements in intra-molecular interactions and exposure of the tandem SH3 domains to interact with the p22^{phox} subunit in flavocytochrome b_{558} (Sumimoto et al., 1994; Groemping et al., 2003; Yuzawa et al., 2004).

Recently, the structures of the autoinhibitory form of the tandem SH3 domains of p47^{phox} (PDB code: 1NG2 and 1UEC) were determined by X-ray crystallography, which revealed the strand-exchanged dimer in which two monomers are related by crystallographic 2-fold axis. The strand-exchanged portion took a canonical SH3 fold corresponding to the N-terminal SH3 domain. Thus, the split half of the intertwisted dimer in the crystal structure (referred to the globular module) has been considered as physiologically relevant (Groemping et al., 2003; Yuzawa et al., 2004). However, an issue is yet to be elucidated whether the globular module exists in solution and is physiologically relevant. This prompted us to investigate the solution structure of the tandem SH3 domains in the autoinhibitory form, referred to p47^{phox}(151-340). As a step to gain further insight into the masking-unmasking mechanism, we have initiated an analysis of p47^{phox}(151-340) in solution by NMR spectroscopy. Here we report the backbone resonance assignments of p47^{phox}(151-340).

Method and experiments

p47^{phox}(151-340), the truncated form of p47^{phox} from residues 151 to 340, were expressed and purified as previously described (Yuzawa et al., 2003). In order to prepare $^{2}H/^{13}C/^{15}N$ labeled proteins, the *Escherichia coli* BL21(DE3) were grown on M9 minimal medium using $^{15}NH_4Cl$ and [u-^{13}C] glucose in 95% $^{2}H_2O$. A selectively ^{15}N labeled proteins at Arg, Lys or Val residues were prepared by growing the cell in amino acid broth containing 50 mg/L ^{15}N labeled Arg, Lys or Val. All NMR samples were prepared at 1.0 mM protein concentration in 90% H_2O/10% D_2O containing 25 mM BisTris buffer (pH 6.5) and 150 mM NaCl. NMR data were acquired at 25 °C on Varian Unity Plus 600 MHz and Varian Unity INOVA 500 MHz NMR spectrometers equipped with a triple-resonance pulsed field gradient 5 mm probe. After initial ^{1}H-^{15}N HSQC experiments, a series of TROSY-type (Pervushin et al., 1997) three dimen-

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Figure 1. ^{1}H-^{15}N HSQC spectrum of ^{2}H/^{13}C/^{15}N labeled p47^{phox}(151-340). The 2D ^{1}H-^{15}N TROSY HSQC spectrum of 1.0 mM ^{2}H/^{15}N/^{13}C labeled p47^{phox}(151-340) in 90% H_2O/10% D_2O at 25 °C and pH 6.5, recorded on a Varian Unity plus 600 MHz NMR pectrometer. The peaks were labeled according to the full-length p47^{phox} sequence using the one letter amino acid codes.

sional triple resonance experiments with deuterium decoupling were carried out including 3D-HNCA, 3D-HN(CO)CA, 3D-HNCACB, 3D-HN(CO)CACB, 3D-HN(CA)CO and 3D-HNCO recorded using uniformly ^{2}H/^{13}C/^{15}N-labeled protein. As an aid for the backbone resonance assignments, the ^{1}H-^{15}N HSQC spectra were recorded for specifically ^{15}N-labeled protein at Arg, Lys or Val residues. Pulse programs were a modified version of the Varian Protein Pack sequences (http://www.varianinc.com). All the data were processed with nmrPipe (Delaglio et al., 1995). The $^{1}H_{N}$, ^{15}N and ^{13}C backbone resonances and those of the β carbons of p47^{phox} (151-340) were assigned using the Olivia program (Yokochi and Inagaki, 2003) from http://fermi.pharm.hokudai.ac.jp

Extent of assignments and data deposition

The sequential assignments of the backbone $^{1}H_{N}$, ^{13}Cα, ^{13}C′ and ^{15}N and the side chain ^{13}Cβ resonances were made by the combination of TROSY-type 3D-HNCA, 3D-HN(CO)CA, 3D-HNCACB, 3D-HN(CO)CACB, 3D-HN(CA)CO and 3D-HNCO experiments recorded on the uniformly ^{2}H/^{13}C/^{15}N labeled p47^{phox}(151-340) sample. p47^{phox}(151-340) gave spectra of high quality as was demonstrated by the ^{1}H-^{15}N TROSY HSQC spectrum shown in the Figure 1. The backbone resonance assignments were further confirmed by the 2D ^{1}H-^{15}N HSQC spectra recorded on the protein samples with selectively ^{15}N labeled amino acid residues at Arg, Lys or Val. Total 179 out of 182 possible backbone resonances (193 residues minus 10 proline residues and the amino terminal residue) were observed in the ^{1}H-^{15}N HSQC spectra and were assigned. No assignments have been made for residues Ala149 derived from a cloning artifact, Gly172 and Ser173 in the RT-loop of the N-SH3 domain. Double peaks for Gly154, Ile156 and Ile157 were observed due to conformational heterogeneity around Pro155. Approximately, 98% of the backbone $^{1}H_{N}$, ^{13}Cα, ^{13}C' and ^{15}N and the side chain ^{13}Cβ resonances were assigned sequence specifically. Assignments of the side chain protons and aromatic ring carbons were not made. A list of the chemical shifts for the resonances of p47^{phox}(151-340) has been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 6057.

Acknowledgements

This work was supported by CREST of Japan Science and Technology (JST) and in part by the National Project on Protein Structural and Functional Analyses from the Japan Ministry of Education, Science, Sports and Culture of Japan to FI.

References

Delaglio, F. et al. (1995) *J. Biomol. NMR*, **6**, 277–293.
El Benna, J. et al. (1994) *J. Biol. Chem.*, **269**, 23431–23436.
Groemping, Y. et al. (2003) *Cell*, **113**, 343–355.
Leto, T.L. et al. (1994) *Proc. Natl. Acad. Sci. USA*, **91**, 10650–10654.
Pervushin, K. et al. (1997) *Proc. Natl. Acad. Sci. USA*, **94**, 12366–12371.
Segal, B.H. et al. (2000) *Medicine*, **79**, 170–200.
Sumimoto, H. et al. (1994) *Proc. Natl. Acad. Sci. USA*, **91**, 5345–5349.
Yokochi, M. and Inagaki, F. (2003) Olivia: Objective, chemical shift library based and stereo view assignment software, http://fermi.pharm.hokudai.ac.jp
Yuzawa, S. et al. (2003) *Acta Crystallogr. Sect. D*, **59**, 1479–1480.
Yuzawa, S. et al. (2004) *Genes Cells*, in press.