Journal of Biomolecular NMR **30:** 367–368, 2004.

Letter to the Editor: Resonance assignments for the endosomal adaptor protein p14

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Received 26 April 2004; Accepted 6 July 2004

Key words: adaptor protein p14, NMR resonance assignments, receptor endocytosis

Biological context

Growth factor receptors (GFRs) utilize a number of mechanisms in the transmission of information from the outside to the inside of the cell. Upon the binding of extracellular stimuli such as hormones, growth factors, cytokines and antigens, the activated GFRs can either recruit and active effector enzymes directly, or alternatively through a plethora of adaptor proteins. Growing evidence shows that internalization of activated receptors, a process named receptor endocytosis plays an important role in the activation of signaling cascades from within the cell (Lemmon and Traub, 2000). It is believed that receptor endocytosis, which for a long time has been envisioned to be largely involved in downregulation and recycling of cell surface receptors, provides another element of specificity by sequestering and localizing components in signaling pathways within the cell. More recently, two novel protein termed p14 and MP1 have been identified to be involved directly in regulating receptor endocytosis through the mediation of mitogen-activated protein (MAP) kinases (Schaeffer et al., 1998; Wunderlich et al., 2001; Teis et al., 2002). MP1 is a scaffolding protein that binds the MAP kinase (MAPK) ERK1 and its upstream kinase activator MEK1. p14 is a peripheral membrane protein associated with the cytoplasmic face of late endosomes involved in receptor endocytosis and directly interacts with MP1. The interaction of p14 with MP1 recruits the latter to late endosomes and the endosomal localization of p14/MP1-MEK1-ERK1 scaffolding complex is required for signaling via ERK MAPK in an efficient and specific manner upon receptor stimulation. In an effort to understand the molecular mechanisms underlying the recruitment of the MP1-MEK1-ERK1 signaling complex to late endosomes, we have undertaken structural study of p14. Here, we report the nearly complete sequence-specific backbone and side-chain ^{1}H, ^{15}N, and ^{13}C resonance assignments of the endosomal adaptor protein p14.

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Methods and results

Mouse p14 (residues 1–125) was subcloned into the pGEX-6P-1 vector (Pharmacia) as a glutathione S-transferace (GST)-fusion protein and expressed in E. Coli BL21(DE3) cells. Uniformly ^{15}N-labeled or $^{13}C/^{15}N$-labeled protein samples were prepared by growing bacteria in minimal media containing $^{15}NH_4Cl$, with or without [U-^{13}C]-glucose (Cambrigde Isotope Labs). Uniformly $^{13}C/^{15}N$-labeled, fractionally deuterated proteins were prepared in a similar fashion by using 75% $^{2}H_2O$. The GST-fusion protein was purified using a glutathione-sepharose column. The GST-tag was removed by the cleavage of the fusion protein with prescission protease to generate p14 protein with the following additional residues GPLGS at the N-terminus. The protein was then subjected to gel filtration chromatography to remove the GST-tag. NMR samples of the protein (typically ~0.5 mM) were prepared in 50 mM Sodium phosphate buffer of pH 6.5, containing 20 mM perdeuterated DTT, in $H_2O/^{2}H_2O$ (90%/10%) or $^{2}H_2O$. All NMR experiments were carried out at 25 °C on Bruker DRX500 and DRX600 spectrometers equipped with four rf channels, and a triple-resonance probe with 3-axis pulsed field gradients. The NMR data were processed and analyzed using programs of NMRPipe (Delaglio et al., 1995) and

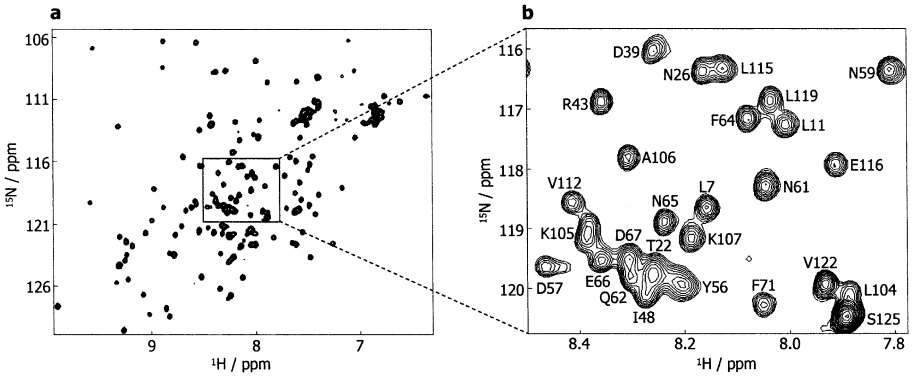

Figure 1. ^{1}H-^{15}N HSQC spectrum for the adaptor protein p14 of pH 6.5 and 25 °C. (a) Full spectrum. (b) An expanded view of the central region of the 2D ^{1}H-^{15}N-HSQC spectrum as indicated. Amide resonances of select residues are labeled.

NMRView (Johnson and Blevins, 1994). Deuterium-decoupled triple-resonance experiments HNCACB, and HN(CO)CACB (Yamazaki et al., 1994) which were recorded with a uniformly ^{13}C/^{15}N-labeled and fractionally (75%) deuterated sample, were used to obtain the backbone resonance assignments. The side chain ^{1}H and ^{13}C atoms were assigned using a 3D (H)C(CO)NH-TOCSY (Logan et al., 1993) experiment recorded on the ^{2}H(75%)/^{13}C/^{15}N-labeled sample. Side chain ^{1}H resonances were assigned with a 3D HCCH-TOCSY spectrum (Clore and Gronenborn, 1994) using a uniformly ^{13}C/^{15}N-labeled sample in $^{2}H_2O$, and were confirmed with a 3D ^{15}N-dispersed TOCSY-HSQC. The side chain ^{1}H and ^{13}C resonances for the aromatic residues were assigned using a combination of experiments, including ^{13}C HSQC, CT-^{13}C HSQC, 3D HCCH-TOCSY recorded in the aromatic carbon region with the double labeled sample.

Extent of assignment and data deposition

Nearly 95% resonance assignment was obtained for backbone atoms ^{1}HN, ^{15}N and ^{13}Cα. The residues for which resonance assignment of backbone atoms was partially or wholly incomplete, due to either spectral overlap or line broadening, include S21, C76, E78, N88, T98, A110 and E117. Figure 1 shows ^{1}H-^{15}N HSQC spectrum of p14 recorded at pH 6.5 and 25 °C. The side chain ^{1}H and ^{13}C resonance assignments were obtained for about 90% of the residues. A total of 45 slowly exchanging amide protons were identified with a series of ^{15}N-HSQC spectra recorded on an uniformly ^{15}N-labeled sample after the H_2O buffer was changed to $^{2}H_2O$ buffer. A total of 126 backbone torsion angle ϕ and ψ were calculated from the chemical shift resonances of Hα and Cα backbone atoms using the semi-empirical program TALOS (Cornilescu et al., 1999). The chemical shift index (CSI) of the Hα and Cα atoms and characteristic pattern of nuclear overhauser effects (NOEs) indicate that p14 is comprised of a mixed αβ fold (data not shown). A table of the ^{1}H, ^{15}N, and ^{13}C chemical shift assignments of p14 has been deposited in the BioMagResBank Database (http://www.bmrb.wisc.edu) under the accession number 6181. The ^{13}C chemical shifts reported are referenced against the protonated sample.

Acknowledgement

This work was supported by a grant from the National Institute of Health to M.-M.Z. (CA80938).

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